COMPARISON OF THE EFFECT OF NEONATAL ADMINISTRATION OF TESTOSTERONE AND DIHYDROTESTOSTERONE IN THE FEMALE RAT

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Summary. Female rats were given a single subcutaneous injection of either 10 or 100 μg testosterone propionate (TP) or dihydrotestosterone propionate (DHTP) on Day 1 or 5 of life (Day 1 = day of birth). Both TP and DHTP delayed vaginal opening on Day 1 and advanced vaginal opening on Day 5. Testosterone propionate, but not DHTP, induced constant vaginal cornification and polyfollicular ovaries in the majority of rats. Dihydrotestosterone propionate had no effect on the Receptivity Quotient (lordosis/mount ratio $\times 100$), whereas 100 μg TP on Day 1 or 5 significantly lowered the Quotient compared to DHTP treatment. Both TP and DHTP were equally potent in preventing ovarian compensatory hypertrophy in unilaterally ovariectomized female rats. In addition, treatment with 200 μg DHTP daily did not prevent the ovarian weight increase in response to exogenous gonadotrophin. The significance of these observations are discussed with respect to the physiological rôle of DHT.

INTRODUCTION

During the early postnatal period in the rat, the central nervous system is sensitive to the 'organizing' action of steroid hormones (Barracrough, 1961; Gorski & Barracrough, 1963). In the female, the neonatal injection of androgen induces an acyclic hypothalamus and an acyclic pattern of gonadotrophin secretion (Gorski, 1966). The extent of the effects are both dose- and age-dependent (Barracrough, 1968; Arai & Gorski, 1968; Gorski, 1968). In addition to altering the pattern of hormone secretion, early androgen treatment also induces changes in the behavioural response to oestrogen and progesterone in the adult (Barracrough & Gorski, 1962; Levine & Mullins, 1966; Whalen, 1968; Clemens, Hiroi, Gorski, 1969). Recent work on the mechanism of action of androgens suggests that the Ring A saturated steroid, 17 β -hydroxy-5 α androstan-3-one (DHT), may be the active form of testosterone in some peripheral target organs (Anderson & Liao, 1968; Bruchovsky & Wilson, 1968; Liao & Fang, 1969). By contrast, it has been shown that, when compared with testosterone, DHT is ineffective in inducing sexual behaviour in the female rabbit (Beyer, McDonald & Vidal, 1970), or restoring sexual behaviour in the castrate male rat (McDonald & co-authors, 1970; Whalen & Luttge, 1971). These results suggest that DHT is not involved in androgen-mediated behavioural responses. In order to determine whether DHT could mimic the

'organizational' effect of testosterone, it was administered to neonatal female rats and the effect on vaginal opening and cyclicity, ovarian compensatory hypertrophy and sexual behaviour was studied. In addition, the gonadotrophin-inhibiting effect of DHT in normal female rats was compared to that of testosterone. Some of these data have been previously published in abstract form (McDonald, 1971).

MATERIALS AND METHODS

Experiment 1

Groups of neonatal Sprague Dawley female rats were injected with oil alone, or 10 or 100 μg testosterone propionate (TP) or dihydrotestosterone propionate (DHTP) on Day 1 or 5 of life. The day of birth was designated Day 1. Animals in any one litter were given identical treatments; the males were killed at birth and, if necessary, the number of females reduced to eight. All the rats were weaned at 21 days and housed two to three per cage in a light- (14 hr light/10 hr dark) and temperature-controlled room. They were examined daily for vaginal opening and the examination of daily vaginal smears was begun immediately. On Day 60, one ovary was removed from each female and 10 days later, the remaining ovary was removed. Representative ovaries from different treatment groups were sectioned at 10 μm , stained with haematoxylin and eosin and examined histologically.

Not less than 2 weeks after removal of the second ovary, the females were treated with two injections of 10 μg oestradiol benzoate followed 24 hr later by 0.5 mg progesterone. The animals were tested for receptivity beginning 4 hr after progesterone treatment. Sexually active male rats were placed in the observation cages for 5 min before the introduction of the female. Tests lasted 10 min or until each female had been mounted eight times. The number of times the female was mounted and the number of lordoses were recorded for each animal. The lordosis/mount ratio $\times 100$ was used to calculate the Receptivity Quotient (R.Q.). Testing normally took place during the second half of the light period (15.00 to 17.00 hours). Each female was tested on two separate occasions at an interval of 10 days.

Experiment 2

Eleven groups of mature female rats with regular 4-day oestrous cycles were unilaterally ovariectomized without regard to the day of the cycle. They were injected once daily beginning on the day of hemiovariectomy, with oil alone, 1, 10, 50, 100 or 200 μg TP or DHTP. All the rats were killed 11 days later and the weight of the remaining ovary was recorded. In order to determine whether the influence of DHTP was mediated centrally or peripherally, three further groups of intact mature rats received either 200 μg DHTP daily for 10 days or 200 μg DHTP plus either 10 or 50 i.u. PMSG daily. All rats were killed on Day 11 and the ovarian and uterine weights compared to those of oil-treated control rats. In all the experiments, TP and DHTP were administered subcutaneously in 0.1 ml corn oil.

RESULTS

Administration on Day 1

The data shown in Table 1A indicate that treatment with either TP or DHTP on the day of birth significantly delayed the time of vaginal opening at the lower dose level and prevented vaginal opening at the 100 μ g level. In spite of the delay in opening, animals receiving 10 μ g DHTP had normal oestrous cycles, whereas those receiving TP had constantly cornified vaginal smears except for an occasional leucocytic one.

TABLE 1

THE EFFECT OF NEONATAL TREATMENT WITH TESTOSTERONE PROPIONATE OR DIHYDRO-TESTOSTERONE PROPIONATE ON VAGINAL OPENING, CYCLICITY AND OVARIAN WEIGHT IN THE RAT

Treatment	No. of rats	Day of vaginal opening (mean $\pm$ S.E.)	Vaginal smear pattern	No. of rats	Ovarian weight (mg)	
					Initial (mean $\pm$ S.E.)	Final (mean $\pm$ S.E.)
(A) DAY 1						
Oil	14	39.0 $\pm$ 0.4	C	6	28.4 $\pm$ 1.4	45.8 $\pm$ 1.5
10 μ g TP	10	45.7 $\pm$ 1.2**	CC	7	25.3 $\pm$ 3.5	34.6 $\pm$ 5.1
10 μ g DHTP	8	42.1 $\pm$ 1.3**	C	8	32.9 $\pm$ 1.6**	48.0 $\pm$ 2.3
100 μ g TP	14	VC	—	8	16.5 $\pm$ 1.3**	23.6 $\pm$ 1.9
100 μ g DHTP	13	43†, VC	—	6	29.5 $\pm$ 2.3	38.9 $\pm$ 2.3
(B) DAY 5						
Oil	12	42.5 $\pm$ 1.1	C	7	33.7 $\pm$ 2.0	50.9 $\pm$ 1.6
10 μ g TP	13	38.5 $\pm$ 0.8**	CC	6	20.3 $\pm$ 2.1**	29.8 $\pm$ 3.3
10 μ g DHTP	15	38.4 $\pm$ 0.5**	C	7	32.1 $\pm$ 1.1	46.4 $\pm$ 1.7
100 μ g TP	14	34.5 $\pm$ 0.8**	CC	5	16.1 $\pm$ 3.1**	22.7 $\pm$ 1.2
100 μ g DHTP	15	35.1 $\pm$ 0.5	C	7	39.1 $\pm$ 2.2	56.4 $\pm$ 2.5

TP = Testosterone propionate; DHTP = dihydrotestosterone propionate; C = cyclic; CC = constant cornification; VC = vagina closed.

† Only one animal showed vaginal opening. Regular cycles shown.

** $P < 0.01$ versus oil-injected controls.

It is of interest that the single rat receiving 100 μ g DHTP, whose vagina opened on Day 43, had ovaries which appeared indistinguishable from those of the controls, i.e. contained both recently formed corpora lutea and developing follicles.

As expected, 100 μ g TP led to a significant decrease in ovarian weight by Day 60 when compared to controls. All groups showed a significant ($P < 0.01$) increase in the weight of the remaining ovary following unilateral ovariectomy. Treatment with 10 μ g DHTP resulted in a larger ovarian weight than that of the control rats on Day 60. The significance of this is not readily apparent since 100 μ g DHTP given on the same day had no significant effect on ovarian weight.

Administration on Day 5

In contrast to the Day-1 treatment, administration of TP or DHTP on Day 5 significantly advanced the time of vaginal opening (Table 1B). As was the case on Day 1, treatment with TP led to the development of constant vaginal

cornification with polyfollicular ovaries and an absence of corpora lutea, while DHTP did not affect vaginal cyclicity nor did it alter the histological appearance of the ovaries.

TEXT-FIG. 1. Receptivity Quotients of female rats treated with oil, TP or DHTP on Day 1 or 5 of life. Bars are means of two separate tests conducted 10 days apart.

TEXT-FIG. 2. Inhibition of ovarian compensatory hypertrophy in unilaterally ovariectomized rats receiving different doses of DHTP daily. The weight of the initial ovary was taken as 100%. For significant differences, see text.

Treatment with TP led to a significant reduction in the initial ovarian weight when compared to other groups. All groups, however, showed significant compensatory hypertrophy following unilateral ovariectomy.

Mating behaviour

Text-figure 1 shows the R.Q. for all treatment groups. There were no significant differences between the TP- and DHTP-treated animals at the lower dose level, but treatment with 100 µg TP on Day 1 or 5 significantly lowered the R.Q. when compared to DHTP treatment. This was especially noticeable on Day 5, where the R.Q. was also significantly less than the control value ($P < 0.01$). These data indicate that neonatal treatment with DHTP does not suppress lordosis in the adult female.

Inhibition of ovarian compensatory hypertrophy

The data in Text-fig. 2, expressed as a percentage of the initial ovarian weight, show that DHTP, like TP, could prevent the increase in weight of the remaining ovary following unilateral ovariectomy. It is also clear that DHTP was as

TABLE 2

THE INFLUENCE OF DAILY INJECTIONS OF DIHYDROTESTOSTERONE PROPIONATE WITH AND WITHOUT PREGNANT MARES' SERUM GONADOTOPHIN ON OVARIAN AND UTERINE WEIGHT IN FEMALE RATS

Organ weight	Treatment			
	Oil	200 µg DHTP	200 µg DHTP 10 i.u. PMSG	200 µg DHTP 50 i.u. PMSG
Ovarian weight (mg)	74.7 ± 4.6	52.2** ± 5.7	155.7* ± 33.9	364.3*** ± 36.7
Uterine weight (mg)	465.5 ± 60.8	200.7** ± 16.5	582.8 ± 37.0	709.0* ± 68.4

Figures represent mean ± S.E. DHTP = dihydrotestosterone propionate.

*** Significantly different from oil-injected control $P < 0.01$.

** Significantly different from oil-injected control $P < 0.02$.

* Significantly different from oil-injected control $P < 0.05$.

potent as TP in suppressing this response since there were no significant differences between TP and DHTP at any dose level tested. The effect of DHTP in intact rats (Table 2) shows that 200 µg DHTP could significantly reduce the ovarian and uterine weights compared to treatment with oil alone ($P < 0.02$). The fact that daily injections of 10 and 50 i.u. PMSG could reverse this effect suggests that the response of the ovary to gonadotrophin had not been significantly affected by the DHTP treatment.

DISCUSSION

Those results of the present study using TP, largely confirm the observations made previously by numerous investigators (see Barraclough, 1968, for review). Neonatal treatment with TP prevents the development of normal cyclicity within the female hypothalamus leading to an acyclic pattern of gonadotrophin secretion which is most clearly manifested by the occurrence of continuous vaginal cornification. This effect was absent in the rats treated with DHTP and is in agreement with the recent findings of Luttge & Whalen (1970).

Although DHTP did not lead to disruption of the oestrous cycle in those animals from which smears were obtained, it clearly had an effect on the time of vaginal opening which was similar to that of TP itself. Bradbury (1941) and Swanson & van der Werff ten Bosch (1963, 1964, 1965) have shown that TP administration during either late gestation or the early postnatal period can induce masculinization of the genital tract with inhibition of vaginal development and opening. In the present experiment, the lower doses of TP and DHTP given on Day 1 significantly delayed vaginal opening and the higher doses prevented vaginal opening in twenty-six out of twenty-seven cases. The fact that the ovaries from rats treated with 100 μ g DHTP on Day 1 appeared histologically and morphologically normal in spite of the closed vaginae is of particular interest in this context. A somewhat similar finding was reported earlier by Swanson & van der Werff ten Bosch (1965), who noted that doses of androgen which were too small to prevent later ovarian cyclicity often induced genital masculinization. However, in the present experiments, the 100- μ g dose of TP given on Day 1 had obvious effects on ovarian weight and morphology as well as on the vagina. It is also of interest that DHTP like TP caused vaginal opening to occur significantly earlier when given on Day 5 although, in the case of DHTP, no ovarian effects were seen. The TP data are in agreement with earlier studies (Gorski 1966).

The fact that DHTP does not activate or restore sexual behaviour (Beyer *et al.*, 1970; McDonald *et al.*, 1970; Whalen & Luttge, 1971) or appear to affect differentiation of the hypothalamus (Luttge & Whalen, 1970) suggests that the effects noted on vaginal opening are the result of a direct action of the steroid on the vagina. The data on ovarian weights and vaginal cyclicity further support this view; if the effects of DHTP on vaginal opening were in any way centrally mediated, changes in ovarian histology and smear patterns might have been expected. Such changes clearly did not occur. Our observations on the effect of TP and DHTP on vaginal opening contrast with those of Luttge & Whalen (1970). This may be due to the fact that the longer acting propionate was used in this study as opposed to the natural hormone used by Luttge & Whalen since Alkint & Norgren (1971) have shown testosterone (T) to be less effective than TP in inducing masculinization.

The data in Text-fig. 1 show that treatment with DHTP did not significantly affect the display of female mating behaviour when compared to that of the oil-treated animals, a finding which agrees with the observations of Luttge & Whalen (1970). Treatment with 100 μ g TP on Day 1 significantly depressed behaviour when compared to DHTP treatment but was not different from control values. However, 100 μ g TP on Day 5 significantly depressed behaviour below both control and DHTP values. This finding is similar to that reported by Clemens *et al.* (1969), who found a decrease in sensitivity to progesterone in female rats treated with TP. The effect was most noticeable when the androgen was given on Days 4 to 6 of life.

The lack of effect of DHTP on central mechanisms may be due to the failure of the compound to penetrate the blood-brain barrier. However, Whalen & Luttge (1971) have shown that labelled DHT can be accumulated by the brain and peripheral target tissues. There was no evidence from the unilateral

ovariectomy study that either TP or DHTP had affected the sensitivity of the oestrogen feedback mechanism, but the parameter used in this study was fairly crude. Other workers, using radioactive oestradiol, have reported decreased uptake by hypothalamic tissue following neonatal TP treatment (Flerko, Mess & Illei-Donhoffer, 1969).

Concerning the inhibition of ovarian compensatory hypertrophy (Text-fig. 2), both TP and DHTP appear to be equally effective in preventing increased gonadotrophin secretion and the data in Table 2 suggest that this effect of DHTP is mediated centrally rather than by way of a direct effect on the ovary. A similar finding in the case of TP was reported by Bottomley & Folley (1938). This agrees with the recent findings of Kingsley & Bogdanove (1971) who have shown that intrapituitary implants of either TP or DHTP can prevent the appearance of castration cells in gonadectomized male rats. In addition, Perez-Palacios, Castaneda, Gomez-Perez, Perez & Gual (1970) have shown that T can be converted to DHT by hypothalamic and pituitary tissue *in vitro*, again suggesting that DHT may be involved in the central control of gonadotrophin release.

In conclusion, DHT does not appear to be involved in the differentiation of the hypothalamus or in the induction of sexual behaviour. However, in the adult it is possible that DHT may play a rôle in controlling gonadotrophin secretion.

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