Masculinization in male rats is inhibited by neonatal injections of dihydrotestosterone

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Masculinization of the brain of the neonatal male rat is known to be dependent on testicular androgen, and leads to an absence of cyclicity in ovarian transplants in adulthood and inability to show lordosis in response to appropriate stimuli. While both brain and pituitary may be involved in neonatal testicular activation (Kawashima, 1964; Yaginuma, Matsuda, Murasawa, Kobayashi & Kobayashi, 1969; Goldman & Mahesh, 1970; Goldman, Quadagno, Shryne & Gorski, 1972; Arai & Serisawa, 1973; Arai, 1974), blood concentrations of gonadotrophins in male neonates are low or undetectable (Goldman, Grazia, Kamberi & Porter, 1971; Döhler & Wuttke, 1974). Dihydrotestosterone propionate (DHTP) is known to stimulate differentiation and growth of male accessory sex organs (Schultz & Wilson, 1974), and inhibit gonadotrophin secretion (Korenbrod, Paup & Gorski, 1975) but does not masculinize the brain (Luttge & Whalen, 1970; McDonald & Doughty, 1972). We therefore used this substance to determine whether gonadotrophins are required for the testicular activation which brings about masculinization of the neonatal male rat brain.

Locally bred ($R \times U$) F_1 hybrid rats derived from two inbred Wistar substrains were used. In these rats masculinization of the brain in males occurs on the first day of life with respect to cyclicity in ovarian transplants (Day 1, 100%, $N = 30$; Day 2, 23%, $N = 17$; Day 5, 0%, $N = 16$). However, masculinization with respect to lordosis with intact male rats in response to 0.05 mg oestradiol benzoate + 1 mg progesterone 48 and 4 hr respectively before testing has not occurred by Day 5 (lordosis quotient 95 ± 3 (S.E.M.), $N = 6$, on Day 1; 95 ± 4 , $N = 12$, on Day 5).

Intact male and female rats were injected within 4 hr after birth (= Day 1 of life) with 1 mg DHTP dissolved in 0.05 ml arachis oil or with oil only. The injections were repeated on Days 3, 5, 10 and 15. Testosterone concentrations were determined as described by Verjans, Cooke, De Jong, De Jong & Van der Molen (1973), except that silica gel plates were used to separate completely testosterone and dihydrotestosterone. Serum concentrations of FSH and LH were determined by radioimmunoassays (Welschen *et al.*, 1975) and expressed in ng NIAMDD-rat-FSH-RP1/ml and ng NIAMDD-rat-LH-RP1/ml, respectively. Neonatal injections of DHTP interfered with the occurrence of cyclic ovarian activity in 3/11 female rats but lordosis behaviour with intact males seemed unchanged.

The effects of DHTP injections in male rats are summarized in Table 1. There was a marked reduction in adult testicular weight, a decrease in testicular testosterone concentrations on Day 5 and almost complete suppression of gonadotrophin secretion after castration on Day 1. Cyclicity in ovarian transplants was observed in two rats but lordosis behaviour occurred consistently.

In a separate experiment the possibility was examined that DHTP could act competitively with testosterone and thus inhibit masculinization. Neonatal female rats were therefore injected with testosterone propionate combined with oil or DHTP in oil. As shown in Table 2 DHTP did not prevent testosterone propionate-induced blockade of ovulation and decrease of the lordosis quotient.

The present experiments demonstrate that masculinization of the neonatal male rat brain can be inhibited by neonatal injections of dihydrotestosterone propionate. The inhibition of increased gonadotrophin secretion after castration and the reduction of testicular testosterone concentrations in intact rats after administration of DHTP suggest that peripheral concentrations of gonadotrophin and testosterone in intact DHTP-treated male rats are very low. While, therefore, inhibition of masculinization by DHTP may be attributed to suppression of the secretion of gonadotrophins (and,

Table 1. The effect of repeated injections of dihydrotestosterone propionate during the neonatal period in male rats (results given as means $\pm$ SEM; no. in parentheses)

Treatment	Testicular wt (mg) (Days 80–270)	Testicular testosterone conc.* (ng/g testis)	Serum conc.†		Cyclicity in ovarian transplants‡	Lordosis behaviour§ (quotient)
			LH	FSH		
Oil	1250 $\pm$ 35 (10)	179 $\pm$ 75 (4)	240 $\pm$ 50 (8)	2350 $\pm$ 130 (8)	0/18	0/6 (0–4)
DHTP	830 $\pm$ 35 (11)	25 $\pm$ 12 (5)	<20 (9)	<110 (8)	2/9	13/13 (72–100)
Significance	$P < 0.001$	$P < 0.05$	$P < 0.001$	$P < 0.001$	—	$P < 0.001$

* Exp. design: injection of 1 mg DHTP or oil on Days 1 and 3; autopsy on Day 5.

† Exp. design: castration on Day 1; injection of 1 mg DHTP or oil on Days 1, 3, 5, 7; autopsy on Day 9.

‡ Ovarian and vaginal tissue grafted at the time of castration at 30–50 days of age; autopsy 30 days later.

§ Oestradiol benzoate (0.05 mg) + progesterone (1 mg) given 48 and 4 hr respectively before testing with intact male rats.

Table 2. The absence of a protective action of dihydrotestosterone propionate (1 mg on Days 4 and 6, 0.6 mg on Day 5) on the effects of an injection of 0.25 mg testosterone propionate (on Day 5) in female rats

Treatment	No. of rats	Oestrous cycles Days 40–60 and CL on Day 60	Lordosis behaviour when ovaries <i>in situ</i> contained follicles only	Ovulation induction*	Lordosis behaviour† (quotient)
DHTP + testosterone	12	1/12	1/6	0/5	5/9 (43 $\pm$ 5)
Oil + testosterone	11	1/11	1/5	0/5	9/9 (53 $\pm$ 9)

* Examination 24 hr after a single injection of 5 mg progesterone.

† Oestradiol benzoate (0.05 mg) + progesterone (1 mg) given 48 and 4 hr respectively before testing with intact male rats.

secondarily, of testosterone), it cannot be due to direct inhibition of the action of testosterone on the brain. It is clear that circulating gonadotrophins, although low (Goldman *et al.*, 1971; Döhler & Wuttke, 1974), in neonatal male rats are essential for masculinization of the brain.

There may be several reasons why complete inhibition was not obtained in the present study. Firstly DHTP itself may have a weak masculinizing effect on the brain (Gerall, McMurray & Farrell, 1975) as suggested also by the finding that it interfered with the development of cyclic ovarian activity in some female rats. Secondly, there must be a latency between the time of injection of DHTP and suppression of testicular testosterone production. Production is highest on the 1st day of life (Resko, Feder & Goy, 1968; Döhler & Wuttke, 1974; Warren, Haltmeyer & Eik-Nes, 1975) and the delay might be sufficient to produce masculinization to a degree comparable to that found in male rats castrated on the 2nd day of life. Thirdly, the possibility should be considered that testicular testosterone secretion in the perinatal period is independent of stimulation by gonadotrophins (Price & Ortiz, 1965; Noumura, Weisz & Lloyd, 1966; Wilson & Siiteri, 1973; Warren *et al.*, 1975). It would then follow from the present data that the hypothalamo-hypophysial system gains control over testicular activity during the 1st or 2nd day of life.

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