PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 158, 179–182 (1978)

Effect of Parturition Time on the Response to Neonatal Androgen in Female Rats[1] (40166)

GEORGE M. BUTTERSTEIN AND EDMOND S. FREIS

Department of Biological Sciences, Union College, Schenectady, New York 12308

The sterilization of female rats through the neonatal administration of androgen, specifically testosterone propionate (TP), has been reported extensively (review, 1). Persistent vaginal cornification and ovarian tissue devoid of corpora lutea at adulthood characterize the anovulatory or androgenized female rat. Both the timing (2) and the dosage (3) of TP administration are important in defining the "critical period" during which androgens interfere with the sexual differentiation of the brain. In the rat, unlike primates (4), the critical period in which the hypothalamic neural mechanism is susceptible to androgen influence extends beyond gestation for a period of 10 days. The "neuro-competent period", or the total time during which androgens exert an organizing effect on the central nervous system (2), may begin prior to parturition. Doses of 10 and 25 mg TP given to pregnant rats induced the androgen syndrome in the offspring, while 20 to 100 μg directly into the fetus 1–4 days before birth caused both external masculinization and anovulatory ovaries (5). The present study examines the critical period for androgenization for female rats delivered after a shortened or lengthened period of gestation in rats delivered by cesarean section on days 22 and 24 of gestation.

Methods. Sprague-Dawley female rats (Charles River) were mated and day 1 of pregnancy determined by sperm in the vaginal lavage. Progesterone (P) treatment (4 mg/day) to certain mothers was initiated on day 20 to prolong pregnancy to day 24 and to examine the influence of prior P treatment to the mother on the subsequent effectiveness of TP administration to the offspring. The groups consisted of non-injected mothers with female rats cesarean delivered on days 22 and 23 and injected rats with animals delivered on days 22, 23 and 24. Following cesarean delivery the animals (number = 382) were given to foster mothers in litters of 8. On either day 3, 5, 7, 8, 9 or 10 of age (measured from time of birth) animals were given a subcutaneous injection of 100 μg TP in oil. After weaning, the littermate controls and injected animals were caged together. The animals were examined for vaginal opening and thereafter vaginal smears taken daily until day 70 of age, at which time the animals were autopsied. The ovaries were weighed and subjected to routine histological procedures. Androgen sterilization was confirmed by at least 10 consecutive days of vaginal cornification and the lack of corpora lutea in the ovaries at day 70. Statistical significance of all data was determined by analysis of variance and the Wilcoxon two sample rank test (6).

Results. Vaginal opening was delayed in animals delivered on day 22 (42.0 ± 0.7 days, $P < 0.001$) when compared to rats delivered on days 23 or 24 (37.7 ± 0.6 days, 36.7 ± 0.5 days) (Table I). Testosterone treatment on day 3 resulted in a failure of vaginal opening in all of the 22 day delivered animals, a phenomenon which decreased to 82 and 12% in 23 and 24 day animals respectively (Table II). Progesterone treatment of the mother had no effect on vaginal opening in 22 or 23 day animals treated on day 3 with TP. Testosterone propionate treatment (days 3–10) significantly advanced the onset of vaginal opening in 22 and 23 day animals when compared to non-treated controls ($P < 0.05$ for 22 day animals; $P < 0.01$ for 23 day animals) but day of administration of TP within that period was not important (Table I). Moreover, prenatal P treatment of the mother prevented the TP induced earlier vaginal opening for all delivered age groups except 22 day animals treated on day 7. A significant overall effect of P administration to the mother on vaginal opening was demonstrated when all TP treat-

[1] This study was supported by Grant No. 09058 from NIH-NICHD.

0037-9727/78/1582-0179$01.00/0
Copyright © 1978 by the Society for Experimental Biology and Medicine
All rights reserved.

TABLE I. THE EFFECTS OF TP[a] TREATMENT ON DIFFERENT POSTNATAL DAYS ON THE TIME OF VAGINAL OPENING (DAYS) IN RATS CESAREAN DELIVERED ON DAYS 22, 23, AND 24 OF PREGNANCY.[d]

Parturition time (days)	Control	Day of injection of TP[b]					
		3	5	7	8	9	10
22	42.0 ± 0.7 (18)	—	37.8 ± 1.1 (12)	39.2 ± 1.2 (10)	38.8 ± 1.3 (6)	39.6 ± 0.7 (12)	39.0 ± 1.1 (11)
22 + Progesterone[c]	43.4 ± 1.1 (22)	—	41.0 ± 0.6 (5)	38.8 ± 1.1 (10)	40.7 ± 1.6 (9)	41.4 ± 0.9 (9)	40.2 ± 1.3 (11)
23	37.7 ± 0.6 (22)	35.6 ± 0.6 (3)	34.4 ± 0.4 (8)	35.4 ± 0.8 (11)	33.8 ± 0.6 (7)	35.7 ± 0.6 (7)	35.6 ± 0.7 (9)
23 + Progesterone[c]	36.8 ± 0.7 (11)	36.9 ± 0.9 (7)	37.1 ± 0.8 (9)	37.2 ± 0.7 (9)	36.3 ± 1.0 (9)	36.5 ± 1.6 (7)	37.2 ± 1.9 (6)
24 + Progesterone[c]	36.7 ± 0.5 (20)	36.2 ± 1.3 (15)	36.8 ± 1.4 (6)	36.2 ± 0.9 (10)	36.7 ± 0.9 (4)	36.8 ± 0.3 (5)	34.5 ± 1.3 (8)

[a] 100 μg testosterone propionate.
[b] Measured from day of birth.
[c] 4.0 mg/day to the mother from day 20; ( ) number of animals.
[d] Statistical evaluation: Day effect: P; 22 control vs. 23, 24 controls <0.001; TP effect: 22 control vs. 22 with TP combined <0.05; 22 + Progesterone control vs. 22 + Progesterone with TP combined N.S.; 23 control vs. 23 with TP combined <0.01; 23 + Progesterone control vs. 23 + Progesterone with TP combined N.S.; 24 + Progesterone control vs. 24 + Progesterone with TP combined N.S.; Progesterone effect: 22 with TP combined vs. 22 + Progesterone with TP <0.05; 23 with TP combined vs. 23 + Progesterone with TP <0.01.

TABLE II. THE EFFECT OF TP[a] TREATMENT AT 3 DAYS OF AGE ON VAGINAL OPENING IN RATS CESAREAN DELIVERED ON DAYS 22, 23, AND 24 OF PREGNANCY.

Parturition Time (days)	Animals failing to exhibit vaginal opening by day 70	
	Number	%
22	18/18	100
22 + Progesterone[b]	10/10	100
23	14/17	82
23 + Progesterone[b]	11/18	61
24 + Progesterone[b]	2/17	12

[a] = 100 μg testosterone propionate.
[b] = 4.0 mg/day to the mother from day 20 of pregnancy.

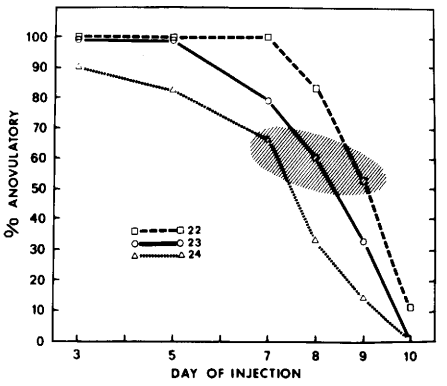

FIG. 1. The incidence of the anovulatory syndrome at day 70 following treatment of 100 μg TP at various neonatal days for animals delivered at different parturition times. The shaded area represents identical TP treatment days relative to the biological age of the hypothalamus. The same number of animals as in Table I.

ment days were combined and compared to nontreated controls (22 day animals, $P < 0.05$; 23 day animals, $P < 0.01$).

The critical period for androgen sterilization was of longer duration for animals delivered on day 22 of gestation than for animals delivered on day 24. For example, 53% of the 22 day delivered animals injected with TP on day 9 demonstrated the anovulatory syndrome by day 70, while only 33 and 14% of the 23 and 24 day delivered animals exhibited persistent estrous (Fig. 1). For all 6 injection days, we find a consistent pattern in which the highest anovulatory percentages are found in 22 day animals, and lowest in 24 day animals. The probability that such a consistent pattern could be the result of chance alone is less than 1.2×10^{-4}. The hypothalamic responsiveness to androgen was dependent on the biological age of the hypothalamus, for when TP treatment was considered relative to the chronological age from conception (treatment on day 9 for 22 day animals = treatment on day 7 for 24 day animals), the number of animals exhibiting

the anovulatory syndrome was essentially identical (shaded area in Fig. 1). In addition, prior P treatment of the mother had no overall influence on the induction of anovulation in the TP treated animals (Table III).

Discussion. These studies have shown that (1) early androgen treatment of the prematurely delivered rat resulted in a failure of vaginal opening, (2) the responsiveness of the female hypothalamus to androgen is related to the biological age of the hypothalamus, and (3) TP administration advanced the time of vaginal opening in the premature and 23 day delivered rat, while P administration to the mother prevented this effect.

Neonatal TP treatment of rats (7) and mice (8) has been previously shown to advance the onset of puberty, a finding also substantiated by this study. The delay in vaginal opening characteristic of the prematurely delivered rat (9) was partially negated by TP treatment, regardless of day of TP treatment. Dorfman (10) and Arai and Gorski (11) have shown that P administration simultaneously with TP can inhibit to some degree the androgenization effects on the female reproductive cycle, at least at 45 days of age. Although P administration to the mother from day 20 of gestation in the present study had no effect on vaginal opening in control animals, it blocked the effects of TP induced accelerated time of vaginal opening. It must be emphasized, however, that prematurely delivered rats received prenatal P exposure for only 2 days, whereas 24 day delivered animals resulted from mothers receiving P treatment for 4 days. The results, however, were pronounced in both cases. Also, TP treatment on day 3 in animals delivered on day 22 resulted in a failure of vaginal opening. Masculinization of the external genitalia of the female following androgen treatment during development is a well known phenomenon (5), however previous studies on the "critical period" in 1- to 3-day TP injected normally delivered animals have not disclosed failure of vaginal opening as a predominant effect of early TP treatment. Thus, the prematurely delivered animal appears to be more sensitive to the masculinization effects of early TP treatment.

TABLE III. THE EFFECTS OF TP[a] TREATMENT ON DIFFERENT POSTNATAL DAYS FOLLOWING PROGESTERONE ADMINISTRATION TO THE MOTHER ON THE INCIDENCE OF PERSISTENT ESTROUS (%) AT DAY 70 OF AGE.[d]

Parturition time (days)	Control	Day of Injection of TP[b]					
		3	5	7	8	9	10
22	0	100	100	100	83	53	9
22 + P[c]	0	100	100	90	63	66	9
23	0	100	100	79	60	33	0
23 + P[c]	0	100	100	90	60	29	0
24 + P[c]	0	90	83	66	33	14	0

[a] = 100 μg testosterone propionate.
[b] = measured from time of birth.
[c] = 4.0 mg/day to the mother from day 20 of pregnancy.
[d] The same number of animals/group as in Table I.

Exposure of the developing brain to androgen has profound effects on hypothalamic maturation and differentiation. A high dose of androgen prevents ovulation from ever occurring, while low doses (10 μg of TP) can induce a delayed anovulation syndrome (2, 3). Although it has been previously shown that androgen treatment of either the mother or fetus can induce an early androgen syndrome (5), the present study relates definitive parturition times with exposure to 100 μg neonatal androgen. Since the critical period was longer in 22 day delivered animals and shorter in animals delivered on day 24, the critical exposure period of androgen appears to represent an intrinsic characteristic of brain differentiation related to biological hypothalamic age. However, the beginning period in development during which androgens first begin to influence hypothalamic differentiation remains to be determined.

Summary. Female rats were cesarean delivered on days 22, 23 and 24 of gestation and treated with 100 μg testosterone propionate on various neonatal days. Certain mothers were administered progesterone prior to delivery of the offspring. Prematurely delivered rats had a longer critical period for androgenization than animals delivered on day 24 indicating that the responsiveness of the hypothalamus to androgens is age dependent. Although early testosterone treatment of the prematurely delivered rat resulted in a failure of vaginal opening, androgen treatment on subsequent days (5–10) advanced the timing of vaginal opening, while progesterone administration to the mother prevented this effect.

The authors thank Dr. John Boyer for assistance in statistical analyses.

1. Gorski, R. A., *in* "Frontiers in Neuroendocrinology" (L. Martini and W. F. Ganong, eds.) Oxford Press, New York, 257 (1971).
2. Gorski, R. A., Endocrinology **82,** 1001 (1968).
3. Swanson, H. E., and van der Werff ten Bosch, J. J., Acta Endocrinol. (Kobenhavn) **45,** 1 (1964).
4. Treloar, O. L., Wolf, R. C., and Meyer, R. K., Endocrinology **90,** 281 (1972).
5. Swanson, H. E., and van der Werff ten Bosch, J. J., Acta Endocrinol (Kobenhavn) **50,** 379 (1965).
6. Wilcoxon, F., Biomet. Bull. **1,** 80 (1947).
7. Barraclough, C. A., *in* "Hormonal Steroids" (L. Martini and W. F. Ganong, eds.) Academic Press, New York, Vol. II, 61 (1967).
8. Doeg, L. H., and Leathem, J. H., Proc. Penn. Acad. Sci. **32,** 202 (1958).
9. Butterstein, G. M., and Garone, M., Biol. Reprod. **16,** 479 (1977).
10. Dorfman, R. I., Anat. Rec. **157,** 547 (1967).
11. Arai, Y., and Gorski, R. A., Endocrinology **82,** 1005 (1968).

Received October 14, 1977. P.S.E.B.M. 1978, Vol. 158.