The Influence of Thymectomy and Steroid Hormones in Neonatal Rats (35162)

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Treatment of neonatal rats of either sex with androgens or estrogens results in alterations of the hypothalamo–hypophyseal–gonadal interrelationship leading to sterility in the adult animal. It has generally been considered that this deleterious effect is due to a "damaging" influence on those hypothalamic centers which in later life are destined to control the synthesis and/or release of pituitary gonadotropins (1).

We have noted that neonatal animals are less susceptible to hormonal treatment if they were previously injected with a suspension of thymic cells (2). Since a dependence between the thymus and brain centers has not been established to date, the above hypothesis may not be entirely tenable.

The basic function of the thymus is to establish initially immunological competence in rodents (3). The gland may also produce humoral factors which control lymphoid proliferation and differentiation during ontogenesis (4), and may mediate trophic hormone stimulation of adrenal, thyroid, and gonadal endocrine systems (5).

In view of the paucity of information concerning a possible relationship between the thymus and the gonads, we have undertaken a series of experiments to study this problem. The present communication describes the effect of injecting steroid hormones into 5-day-old male and female rats thymectomized within 24 hr after birth.

Materials and Methods. Sprague-Dawley rats (Holtzman Laboratories, Madison, Wisconsin) bred in our laboratories were thymectomized within 24 hr after birth under hypothermia. The sternum was cut with small scissors, thymus was removed by suction (glass pipette about 2.5 mm in diameter) and the wound was closed by one or two sutures. Sham-operated animals were handled similarly. Several groups were injected subcutaneously at the age of 5 days with the following hormones, dissolved in 0.05 ml of sesame oil: testosterone propionate (TP), 10 or 100 μg; estradiol benzoate (EB), 10 or 100 μg. The pups were weaned at the age of 25–27 days and were maintained under standard laboratory conditions until autopsy which was performed at the age of 56 days. At autopsy, completeness of thymectomy was checked macroscopically (animals with residual thymus tissue were discarded from the study). Selected organs were weighed, gonads were fixed in Bouin's solution, sectioned at 6 μ, and stained in hematoxylin–eosin. The status of seminiferous epithelium development was evaluated as described previously (6) by examining five fields in each slide. In each field about 50 tubules were graded according to the development. The following stages were recognized: (i) normal tubules with free sperm in the lumen; (ii) absence of free sperm but presence of spermatids in various stages of development; and (iii) absence of spermatids, presence of spermatocytes or spermatogonia.

Results. Mortality during thymectomy was about 10%. The thymectomized (Tx) animal survival rate was lower than sham-operated (SO) pups and was lowest in animals injected with estradiol benzoate. Mortality in estrogen treated (both doses) Tx groups was 45% for males and 29% for females; whereas in estrogen treated sham-operated animals it was 22% for males and 0% for females.

The effect of different treatment on body and organ weights of 56 day old males is summarized in Table I. Thymectomy (or sham-operation) performed within 24 hr after birth had no significant influence on any of

TABLE I. Influence of Thymectomy and Steroid Hormone Treatment on Body and Organ Weights of 56-Day-Old Male Rats.

Group no.	Treatment[a] (μg total dose)	No. of rats	Body wt (g ± SE)	Organ wt (mg ± SE)				
				Testes	Ventral prostate	Seminal vesicles	Adrenals	Pituitary
1	0	9	218 ± 13	2905 ± 133	166 ± 20.2	226 ± 19.0	36.3 ± 2.1	9.0 ± 0.6
2	Sham-operated (SO)	8	189 ± 2[7b]	2815 ± 33	117 ± 12.7	175 ± 14.3[7]	35.5 ± 1.2	7.0 ± 0.4
3	SO + EB, 10	8	239 ± 8	2379 ± 84[8]	127 ± 15.3[8]	134 ± 11.6[8]	48.2 ± 1.6[2]	9.8 ± 0.4[2]
4	100	10	187 ± 7	1698 ± 40[2]	63.9 ± 4.8[2,6]	37.4 ± 5.8[2]	47.4 ± 1.8[2]	7.4 ± 0.3
5	SO + TP, 10	7	188 ± 11	2448 ± 45	123 ± 18.6	94.4 ± 11.6[2]	35.9 ± 1.5	7.4 ± 0.4
6	100	6	206 ± 15	2160 ± 151[2]	158 ± 19.7	156 ± 17.4	35.8 ± 3.4	8.3 ± 0.3
7	Thymectomized (Tx)	9	235 ± 6	3012 ± 65	173 ± 22.7	233 ± 8.3	38.9 ± 2.1	9.1 ± 0.5[2]
8	Tx + EB, 10	7	192 ± 14	1826 ± 126[7]	51.9 ± 5.9[7]	66.7 ± 10.2[7]	49.6 ± 2.3[7]	9.7 ± 0.7
9	100	8	165 ± 19	1332 ± 151[7]	24.0 ± 4.0[7]	18.0 ± 3.9[7]	54.3 ± 3.2[7]	7.2 ± 0.6
10	Tx + TP, 10	3	222 ± 13	2785 ± 66	127 ± 5.7	186 ± 12.0[5]	47.0 ± 5.7	8.5 ± 0.9
11	100	5	201 ± 16	2399 ± 125[7]	122 ± 4.9	109 ± 16.5[7]	42.8 ± 2.8	9.0 ± 0.4

[a] EB = estradiol benzoate; TP = testosterone propionate.
[b] Indicates the group from which it is significantly different ($p<0.05$) by analysis of variance.

TABLE II. Development of Seminiferous Epithelium in 56-Day-Old Male Rats Injected with Various Steroids at 5 Days of Age.[a]

Steroid treatment	Sham-operated			Thymectomized			
	No. of observations[b]	Stage 1	Stage 2	Stage 3	No. of observations	Stage 1	Stage 2	Stage 3
EB 10	18	63.5 ± 1.9	24.5 ± 2.3	12.0 ± 1.6	18	65.7 ± 1.9	18.9 ± 1.3	15.4 ± 1.9
100	18	59.5 ± 1.8	28.0 ± 2.3	12.5 ± 1.7	18	49.6 ± 3.8	22.6 ± 2.3	27.8 ± 4.6
TP 10	12	69.0 ± 1.9	25.7 ± 1.8	5.3 ± 1.3	12	69.4 ± 1.9	23.2 ± 1.2	7.4 ± 1.5
100	18	58.0 ± 1.8	25.4 ± 1.5	16.6 ± 2.2	18	59.5 ± 1.6	29.5 ± 1.8	11.0 ± 1.4

[a] Percentage of tubules ± SE.
[b] Number of fields.

TABLE III. Influence of Thymectomy and Steroid Hormone Treatment on Body and Organ Weights of 56-Day-Old Female Rats.

Group no.	Treatment (μg total dose)	No. of rats	Body wt (g $\pm$ SE)	Organ wt (mg $\pm$ SE)			
				Ovaries	Uterus	Adrenals	Pituitary
1	0	7	145 $\pm$ 10	38.5 $\pm$ 5.4	170 $\pm$ 33.5	45.6 $\pm$ 3.0	7.8 $\pm$ 0.8
2	Sham-operated (SO)	8	163 $\pm$ 2	48.2 $\pm$ 2.7	227 $\pm$ 17.6	46.1 $\pm$ 1.8	7.9 $\pm$ 0.4
3	SO + EB, 10	10	168 $\pm$ 4	26.6 $\pm$ 3.0[2b]	183 $\pm$ 20.9	53.0 $\pm$ 2.0	8.1 $\pm$ 0.3
4	SO + EB, 100	13	157 $\pm$ 6	16.3 $\pm$ 1.2[2]	121 $\pm$ 22.0	49.1 $\pm$ 1.5	6.3 $\pm$ 0.3
5	SO + TP, 10	13	169 $\pm$ 14	40.5 $\pm$ 4.2	292 $\pm$ 16.3	48.1 $\pm$ 1.3	9.2 $\pm$ 0.5
6	100	8	146 $\pm$ 9	26.8 $\pm$ 1.3[2]	241 $\pm$ 12.7	45.7 $\pm$ 2.1	10.4 $\pm$ 0.5
7	Thymectomized (Tx)	9	170 $\pm$ 3	51.4 $\pm$ 4.4	241 $\pm$ 25.5	50.6 $\pm$ 1.9	8.7 $\pm$ 0.8
8	Tx + EB, 10	17	142 $\pm$ 9	21.9 $\pm$ 3.8[7]	121 $\pm$ 22.0[7]	50.6 $\pm$ 3.1	6.1 $\pm$ 0.5[7]
9	100	14	146 $\pm$ 11	13.0 $\pm$ 1.6[7]	86 $\pm$ 17.0[7]	51.2 $\pm$ 3.0	6.2 $\pm$ 0.5[7]
10	Tx + TP, 10	7	176 $\pm$ 10	41.7 $\pm$ 9.7	255 $\pm$ 34.5	51.9 $\pm$ 3.4	9.3 $\pm$ 0.9
11	100	7	132 $\pm$ 15	18.3 $\pm$ 1.5[7]	150 $\pm$ 37.1	54.5 $\pm$ 2.0	6.4 $\pm$ 0.7[6]

[a] EB = estradiol benzoate; TP = testosterone propionate.

[b] Indicates the group from which it is significantly different ($p < 0.05$) by analysis of variance.

the modalities studied as compared to untreated controls. Injection of increasing amounts of estradiol benzoate to sham-operated animals resulted in significant atrophy of testes and accessory sex organs (ventral prostate and seminal vesicles) and hypertrophy of the adrenals. When the same amount of this steroid was injected into thymectomized rats the degree of atrophy of the gonads and of the accessory sex organs was significantly higher (analysis of variance). In the SO group injected with 10 μg of estradiol benzoate, testes atrophy was 15%; ventral prostate, 0%, and seminal vesicles, 23%. For Tx animals injected with the same dose these values were 39, 70, and 71%, respectively. For animals injected with 100 μg of the estrogen the values for sham-operated animals and thymectomized groups (in parenthesis) were: testes 40% (56%); ventral prostate 45% (86%); and seminal vesicles 79% (92%). Injection of 10 μg of testosterone propionate had no influence on organ weights. A dose of 100 μg produced a modest atrophy of the testes in both Tx and SO groups. Seminal vesicles were atrophied only in Tx animals but not in the SO group. Table II lists the results of histological evaluation of the testes. There was a significant increase in the average number of tubules lacking spermatids in Tx groups injected with 100 μg of EB (27.8 $\pm$ 4.6) as compared to the SO group (12.5 $\pm$ 1.7).

Results obtained in 56-day-old females are summarized in Table III. The data show that steroid hormone treatment produced atrophy of the ovaries in all the groups with the exception of animals injected with 10 μg of testosterone propionate. The decrease in the weights of ovaries in Tx females injected with 100 μg of TP (18.3 $\pm$ 1.5) compared to SO group (26.8 $\pm$ 1.3) is significant when analyzed by Student's t test. The uterine weight difference between the Tx and SO groups and pituitary weight differences in the group injected with both doses of EB and 100 μg of TP are also significant. Corpora lutea (CL) formation was prevented in groups treated with the high dose of both steroids. In the groups injected with 10 μg of EB, 6 of 17 animals had luteinized ovaries (Tx groups) as compared to 5 of 10 in SO group. Corresponding values for TP treated animals were 5/7 (Tx) and 9/13 (SO group), respectively.

Microscopic examination of atrophied ovaries confirmed the absence of luteinized tissue. Secondary, Graafian, and regressing follicles were, however, present in all animals. In 2 animals, a few large cyst-like follicles were noted. The endometrial lining in the uterus consisted only of low columnar cells without proliferation, consistent with the pic-

ture of moderate estrogenic stimulation.

Discussion. The effect of steroid hormones injected into neonatal male (7) and female rats (8) was shown to vary with the age of the animal at the time of the injection and the dose. The increased atrophy in groups injected with higher doses of steroid hormones observed in the present study is in agreement with these previous observations.

Ueda *et al.* (9) observed that administration of 100 μg EB to 8-day-old rats produced anovulatory sterility in thymectomized female rats, but not in control animals. The authors suggested that "neonatal thymectomy seemed to prolong the postnatal steroid sensitive periods to induce permanent sterility" and added that the action of estrogen may not be specific in respect to the neonatal hypothalamus.

Our study shows that neonatal thymectomy influences the sensitivity of gonads to steroid treatment in rats of both sexes. This is apparent by comparing the weights of testes, ventral prostate, and seminal vesicles in SO and Tx rats injected with 10 μg of estradiol benzoate and ventral prostate weights in the groups injected with 100 μg of this estrogen. In thymectomized females injected with estradiol benzoate, uterine weights were significantly decreased whereas no atrophy was seen in sham-operated animals. Thymectomy alone had no apparent effect on sexual maturation when the animals are sacrificed at the age of 56 days. This is in agreement with the observation made by Ueda and collaborators (9). In contrast, neonatal thymectomy in mice was reported to result in developmental arrest of the ovary, but not of the testis (10) possibly due to a viral infection affecting mostly the ovary. Should this be the cause in thymectomized mice our results would indicate that rats may be more resistant to such an infection.

Our present data are not sufficient to permit speculation concerning the mechanism by which thymectomy increases the sensitivity of rats to steroid hormones. Additional studies are needed to clarify possible relationships between the thymus and neonatal sterilization induced by steroids.

Summary. Extirpation of thymus within 24 hr after birth increased the sensitivity of rats to steroid hormones injected at the age of 5 days. In 56-day-old males the atrophy of testis and of accessory sex tissues produced by a single injection of estradiol benzoate was more severe in thymectomized animals than in sham-operated controls. In female rats the atrophy produced by injecting the same steroid resulted in greater atrophy of the uterus in thymectomized animals than in controls.

1. Barraclough, C. A., *in* "Advances in Reproductive Physiology" (A. McLaren, ed.), Vol. 1, p. 81. Academic Press, New York (1968).

2. Kincl, F. A., Oriol, A., Folch Pi, A., and Maqueo, M., Proc. Soc. Exp. Biol. Med. **120**, 252 (1965).

3. Miller, J. F. A., *in* "The Thymus; Ciba Foundation Symposium" (G. E. W. Wolstenholme and R. Porter, eds.), p. 153. Little, Brown, Boston (1966).

4. Klein, J. J., Goldstein, A. L., and White, A., Proc. Nat. Acad. Sci. U.S.A. **53**, 812 (1965).

5. Comsa, J., Arzneim-Forsch. **16**, 18 (1966).

6. Maqueo, M., and Kincl, F. A., Acta Endocrinol. **46**, 25 (1964).

7. Kincl, F. A., Folch Pi, A., and Herrera Lasso, L., Endocrinology **72**, 966 (1963).

8. Gorski, R. A., and Barraclough, C. A., Endocrinology **73**, 210 (1963).

9. Ueda, G., Katsu, N., and Nagai, K., Wakayama Med. Rep. **13**, 71 (1968).

10. Nishizuka, Y., and Sakakura, T., Science **166**, 753 (1969).

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