

## Effect of Gonadotropins on Neonatal Testicular Activity and Sexual Differentiation of the Brain in the Rat<sup>1</sup> (37385)

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(Introduced by C. H. Sawyer)

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It is generally believed that the normal mechanism of gonadotropin (GTH) regulation in the male rat is organized by the internal secretion of the neonatal testis during the first few days of life. Removal of the neonatal testes during these few days produces "feminine males" which are capable of secreting GTH in a cyclic or female pattern (1, 2). Testicular androgen is presumed to be the actual organizing factor.

Recently evidence suggesting the existence of pituitary testicular feedback regulation in the neonatal male rat has been presented. Yaginuma *et al.* (3) reported that unilateral orchidectomy of the newborn rat is followed by significant hypertrophy of the remaining testis within 3 days. Goldman and Gorski (4) found that serum GTH levels are low in the neonatal male rat but increase rapidly after castration. This response to castration is effectively inhibited by exogenous androgen or estrogen. In the present study, as one step to elucidate a possible role of GTH in the sexual differentiation of the hypothalamus, it was attempted to determine whether or not exogenous GTH could stimulate the neonatal testes to secrete androgen and advance the masculinization of the GTH regulatory mechanism in the rat.

*Materials and Methods.* Wistar male rats were raised in our vivarium and were treated with GTH preparation twice daily from Day 1 (= the day of birth) to Day 2, or once on Day 2. Subcutaneous injections of HCG (Gonatropin, Teikoku Hormone Mfg., Co., Ltd., Tokyo), ovine LH (NIH-LH-S18) or ovine FSH (NIH-FSH-S9) were given in

0.04 ml saline. Neonatal castration was performed under cold anesthesia on Day 3. Removed testes were weighed, fixed in Bouin's fluid and then studied following routine histologic preparation and H-E staining. At approximately 30 days of age, half of an ovary from a littermate was transplanted subcutaneously on the ventral body wall. At 60 days of age, these males were autopsied and body and organ weights were recorded. Ovarian histology was used as the criterion to estimate the pattern of GTH release. The presence of numerous corpora lutea (CL) of normal size and healthy appearance was considered evidence of the female pattern of cyclic release of GTH. The absence of CL in an ovarian graft which contained numerous vesicular follicles was considered to be evidence of the masculine pattern. Since it has been reported that polyfollicular ovaries, which are indicative of masculine pattern of GTH release, may show local luteinization or occasional CL formation (5, 6), this type of polyfollicular ovaries was included in the masculine type of ovaries in the present study. Animals with a degenerate ovarian graft were not incorporated in this report.

*Results. Effects of GTHs on 3-day-old testes.* There was no significant difference in body weight among the GTH-treated rats and saline controls (Group 1) when measured upon orchidectomy on Day 3 (Table I). Testes of the rats treated with 10 or 40 IU of HCG, 20 or 40  $\mu$ g of LH or 40  $\mu$ g of FSH (Groups 2-6) were significantly heavier than those of the controls (Group 1) although the testicular weights of rats receiving a single injection of 40 IU HCG only on Day 2 (Groups 8 and 9) were not significantly different from those of the control group (Group 1).

<sup>1</sup> Supported by grants from the Ministry of Education of Japan. The authors have received bibliographic aids from UCLA Brain Information Service which is supported by the NINDB grant.

TABLE I. Body and Testicular Weights on Day 3 in Rats Injected with GTH for the First 2 Days of Life.

Group	Treatment	No. of rats	Body wt (g)	Testicular wt (mg/100 g body wt)
1	Saline (0.4 ml $\times$ 2/day)	21	7.87 $\pm$ 0.16 <sup>a</sup>	81.61 $\pm$ 1.49
2	40 IU HCG (20 IU $\times$ 2/day) from Days 1-2	12	7.88 $\pm$ 0.15	98.92 $\pm$ 3.45
3	10 IU HCG (5 IU $\times$ 2/day) from Days 1-2	13	8.01 $\pm$ 0.21	93.02 $\pm$ 2.95
4	40 $\mu$ g LH (20 $\mu$ g $\times$ 2/day) from Days 1-2	14	7.75 $\pm$ 0.24	103.07 $\pm$ 3.31
5	20 $\mu$ g LH (10 $\mu$ g $\times$ 2/day) from Days 1-2	13	7.95 $\pm$ 0.18	89.77 $\pm$ 2.45
6	40 $\mu$ g FSH (20 $\mu$ g $\times$ 2/day) from Days 1-2	18	7.26 $\pm$ 0.23	96.07 $\pm$ 10.50
8	40 IU HCG (40 IU $\times$ 1/day) on Day 2, 24 hr before castration	14	7.83 $\pm$ 0.44	80.48 $\pm$ 2.99
9	40 IU HCG (40 IU $\times$ 1/day) on Day 2, 12 hr before castration	13	7.42 $\pm$ 0.26	81.61 $\pm$ 1.44

<sup>a</sup> M  $\pm$  SE.

Histological study showed that the hyperplasia and hypertrophy of the interstitial cells were striking in the rats injected with HCG or LH from Days 1 to 2 (Groups 2-5). Eosinophilic hypertrophied interstitial cells were found in a massive cluster in intertubular spaces (Figs. 2 and 3), in contrast to the control testes in which interstitial cells were present only in a small group (Fig. 1), but no marked change was detected in the tubular elements. The stimulation of interstitial elements was also recognized, but less in degree in the rats of Groups 8 and 9. In these rats, a single injection of 40 IU HCG (12 or 24 hr prior to the removal of the testes) did not increase their testicular weights significantly. However, administration of 40  $\mu$ g of FSH (Group 6) from Days 1 to 2 did increase the testicular weight markedly but it was ineffective in stimulating the interstitial cells (Fig. 4).

*Influence of exogenous GTHs on sexual differentiation of the hypothalamus of the male rat.* Table II shows the effects of neonatal treatment with GTHs on postpubertal pattern of GTH release. Most of ovarian grafts in the male rats treated with saline alone and castrated on Day 3 (Group 1) were characterized by numerous CL and an irregular number of developing follicles. Only 2 of 16 rats had polyfollicular masculine type of ovarian grafts: 87.5% of rats became "feminine males." However, in the male rats

pretreated with 40 or 10 IU HCG for the first 2 days of life, castration on the third day failed to induce feminine males. Ovarian grafts of all rats treated with 40 IU HCG (Group 2) or 9 out of 11 rats treated with 10 IU HCG (Group 3) were of the masculine type. Similar results were obtained by pretreatment with 40 or 20  $\mu$ g of LH (Groups 4 and 5): 60.0 and 66.6% of the rats had a masculine type of ovarian graft, respectively. In contrast, the pretreatment with 40  $\mu$ g of FSH (Group 6) did not alter the incidence of feminine males significantly, compared with that of the saline controls (Group 1). Only 2 of 11 rats were masculinized. In the rats which were castrated on the day of birth and treated with 40 IU HCG from that day to Day 2, none of the animals was masculinized (Group 7).

In another series of the experiments, effects of a single injection of HCG and the intervals between the injection and orchidectomy were examined. A single injection of 40 IU HCG 24 hr prior to castration on Day 3 (Group 8) was found to be effective in initiating the masculinization of the postpubertal GTH release pattern. Only 3 grafts of those removed from Group 8 rats contained CL and the other 10 grafts were polyfollicular. Furthermore, when the interval between the injection and castration was reduced to 12 hr, 70.0% of rats of Group 9 were still masculinized.

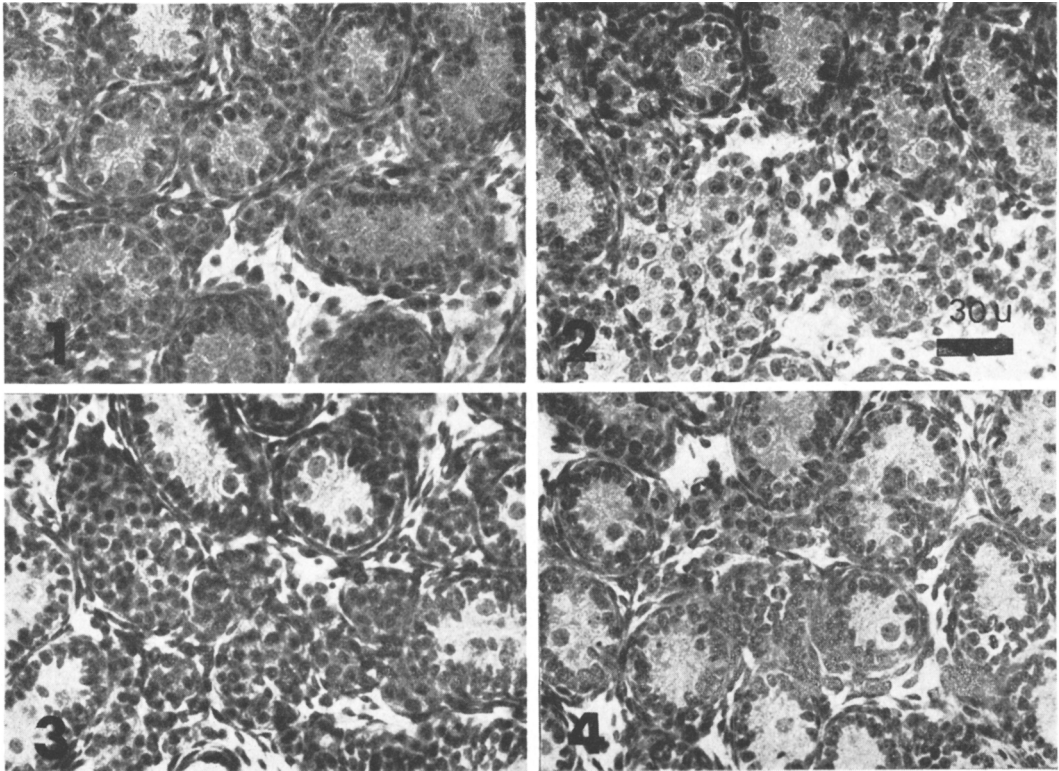


FIG. 1. A section of testis of 3-day-old rat treated with saline.

FIG. 2. A section of testis of 3-day-old rat treated with 40 IU HCG from Days 1-2. Note that interstitial cells are markedly increased.

FIG. 3. A section of testis of 3-day-old rat injected with 40  $\mu$ g LH from Days 1-2. Note hypertrophy of interstitial cells.

FIG. 4. A section of Day 3 rat testis treated with 40  $\mu$ g FSH for the first 2 days of life. No marked change in the interstitial tissue. H-E staining.

*Discussion.* The results of the present study clearly indicate that HCG or LH can accelerate the masculinizing process in the neonatal male rat hypothalamus, when given prior to the neonatal castration on Day 3. Since the treatment with HCG was not effective in the rats castrated before HCG injection started on Day 1, the presence of testes seems to be essential for the initiation of the masculinization of GTH regulatory mechanisms. Adrenal cortex may not be primarily involved in the process. Price and Ortiz (7) and Bishop and Leathem (8) noted interstitial cell stimulation in neonatal rodent testes in response to PMS. Similar stimulation of the interstitial cells was histologically confirmed in testes obtained from 3-day-old rats pretreated with HCG

or LH. This finding is closely correlated with high incidence of the rats with masculine type of ovarian graft in HCG- or LH-treated groups postpubertally. Recently it has been reported that testicular tissues from newborn rats convert approximately half of progesterone to testosterone *in vitro* (9). Resko, Feder and Goy (10) demonstrated that testosterone actually exists not only in the testis but also in the blood of neonatal male rats. Although androgen levels of the testis and the blood were not measured in the present study, increased production and secretion of androgen, which was responsible for the initiation of the masculinization of the hypothalamus, would be assumed in the rats receiving GTH treatments neonatally. Failure of FSH to secure the masculine type

TABLE II. Effects of Neonatal Treatment with GTH on Body Weight and Organ Weights and Differentiation of the Secretory Pattern of GTH in the Male Rat.

Group	Treatment	No. of rats	Body wt (g)	Organ wt (mg/100 g body wt)				Description of ovarian graft at autopsy <sup>a</sup>		Males with masculine pattern of GTH secretion
				Pituitary	Adrenals	Seminal vesicles	Ovarian graft <sup>e</sup>	With CL follicular	Poly-	
1	Saline	16	212 ± 18	6.9 ± 0.3	28.8 ± 2.6	23.8 ± 6.9	48.7	14	2	2/16 12.5
2	40 IU HCG, Days 1-2	8	228 ± 9	7.0 ± 0.4	34.9 ± 1.7	31.3 ± 1.7	35.9	0	8	8/8 100.0
3	10 IU HCG, Days 1-2	11	206 ± 8	8.3 ± 0.3	34.0 ± 1.9	31.4 ± 3.3	47.5	2	9	9/11 81.8
4	40 µg LH, Days 1-2	5	226 ± 21	7.6 ± 0.5	34.7 ± 2.1	28.7 ± 5.8	26.4	2	3	3/5 60.0
5	20 µg LH, Days 1-2	9	231 ± 14	7.4 ± 0.4	35.0 ± 1.9	31.2 ± 4.1	35.4	3	6	6/9 66.6
6	40 µg FSH, Days 1-2	11	202 ± 5	7.2 ± 0.3	33.9 ± 1.5	32.0 ± 4.8	47.5	9	2	2/11 18.2
7	Castration on Day 1 + 40 IU HCG, Days 1-2	4	275 ± 8	6.3 ± 0.5	32.3 ± 1.3	14.9 ± 2.1	67.0	4	0	0/4 0.0
8	40 IU HCG, Day 2, 24 hr before castration	13	221 ± 18	8.1 ± 0.6	34.7 ± 1.9	32.9 ± 4.8	38.0	3	10	10/13 77.9
9	40 IU HCG, Day 2, 12 hr before castration	10	205 ± 7	10.0 ± 1.0	36.1 ± 1.4	30.6 ± 1.9	44.9	3	7	7/10 70.0

<sup>a</sup> Transplantation of ovarian tissue was performed on Day 30, and autopsy on Day 60.

of GTH release in Group 6 rats may be explained by the lack of the stimulatory effect on the interstitial cells of Day 3 rat testes.

Goldman *et al.* (11) have reported that GTH antiserum given neonatally disturbed the normal development of male external genitalia and male sexual behavior. These GTH antiserum-treated males retained the ability to display female sexual behavior but failed to display a capacity for cyclic release of GTH. Since there is evidence that the system of GTH regulation is more sensitive to the neonatal effect of androgen than that for feminine sexual behavior (2, 12), it seems possible that the treatment with GTH antiserum could not inhibit completely the activity of the neonatal testis in these rats. These findings together with our results indicate that GTH plays a significant role in the sexual differentiation of the hypothalamus via control of the neonatal testicular activity.

The fact that a single injection of HCG 12 hr before castration on Day 3 was capable of initiating the masculinization of GTH release pattern indicates that Day 2 rat testes can secrete physiological levels of androgen within a short period of time in response to a single dose of GTH. This additionally suggests that if a sufficiently increased amount of endogenous androgen exists in the blood for less than 12 hr before castration, the hypothalamus of Day 3 rat can be masculinized. Cumulative influence of subphysiological amount of testicular androgen possibly secreted before GTH injection must be considered as a kind of priming factor. The results of the present study are still consistent with the concept of our previous findings that androgen can exert its masculinizing action on the developing brain during a short period of time after its injection in the female rat (13-15).

**Summary.** Neonatal treatment of male rats with HCG or LH caused marked hyperplasia and hypertrophy of the interstitial cells when the testicular histology was examined upon castration at 3 days of age. In these males, advancement of differentiation of the masculine pattern of GTH release apparently

occurred. Most of the rats which received HCG or LH injection(s) neonatally had a polyfollicular ovarian graft when examined at autopsy on Day 60, whereas castration on Day 3 still produced "feminine males" at high incidence in rats injected with FSH or saline alone neonatally. Only a small number of rats (2/11, 2/16, respectively) were masculinized. These results suggest possible participation of GTH in the regulation of the neonatal testicular activity and the masculinization of the rat brain.

We express our appreciation to Dr. C. H. Sawyer for his valuable criticism. We also express our thanks to the Hormone Distribution Section, NIAMD, Bethesda, MD for the LH and FSH used in this study.

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Received Jan. 2, 1973. P.S.E.B.M., 1973, Vol. 143.