Induction of Ovulation in Rats Treated Neonatally with Androgen[1] (37690)

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It is well-known that the hypothalamic regulation of reproductive function in newborn female rats can be modified by exposure to androgen before ten days of age. When neonatal female rats receive a single injection of testosterone propionate (TP), they enter into a permanent anovulatory condition. Immediately after puberty, these animals show permanent vaginal cornification, and their ovaries contain follicles with no corpora lutea. As the animals become older, their ovaries again show numerous vesicular follicles and some interstitial tissue hypertrophy, but still lack corpora lutea. The follicles of these animals are not cystic, but consist of a well-defined theca interna and externa, with well-organized granulosa cell layers.

Abnormalities in the secretion of gonadotropins, as well as in the histological composition of the ovary of androgenized rats, have also been reported. Barraclough (1) has observed that the pituitary LH concentration in androgen-sterilized rats was significantly lower than that in normal estrous rats. Correspondingly, the plasma LH levels of these animals were high when compared with undetectable levels in the normal rat at estrus. Such abnormalities may, in turn, be due to a blockade of the preovulatory discharge of LH-releasing hormone (LHRH) from the hypothalamus by androgen which has interfered with the sexual differentiation of the brain.

The preovulatory surge of LH in cyclic rats occurs on the afternoon of proestrus as repeatedly demonstrated by neuropharmacologic blockade experiments, hypophysectomy, and direct measurement of plasma LH. Intravenous injections or LH to hypophysectomized proestrus rats, rats blocked neuropharmacologically, or intact rats on the second day of diestrus have proven effective in inducing ovulation. Presumably, the preovulatory surge of LH is mimicked as a means of testing the follicular response to gonadotropins. In the present experiment, the responsiveness of the follicles of androgenized rats to gonadotropins and gonadotropin-releasing hormones was investigated.

Methods. Female rats born in our laboratory of Holtzman Co. rats (Madison, WI) were used. The day of birth was designated as day 1. On day 5, female pups received a single sc injection of 1.0 mg testosterone propionate in 0.1 ml sesame oil, and litter sizes were adjusted to six young. The animals were kept in a temperature-controlled room with the light schedule of 14 hr light and 10 hr darkness. The midpoint of the dark period is considered as "midnight, colony time", and all times are reported in relation to that, employing the convention of Everett and Sawyer (2). Approximately 2 weeks before autopsy, daily smears were taken to confirm the persistent cornification of the vagina induced by TP injection. Follicle-stimulating hormone, 200 μg, or LH, 25 μg dissolved in 0.1 ml saline, was injected intravenously via the tail vein at 1:30 PM on different days of age. Normal 4-day cyclic rats exhibiting the second day of diestrus at the same days of age served as controls and were given FSH or LH in the same manner. On the following day, the animals were killed, and the oviducts were examined under a light microscope for the presence of ova.

In another series of experiments, the follicular response of the androgenized females

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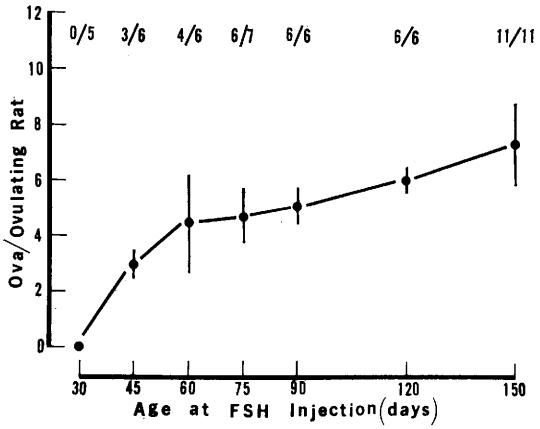

FIG. 1. Effect of FSH injection on ovulation in TP-treated rats on different days of age. The fractions at the top of the figure indicate the number of animals which ovulated per number of androgenized females injected with FSH at a given age.

to endogenous gonadotropins was examined by giving 3.0 μg LHRH subcutaneously at 1:30 PM on different days of age. Synthetic LHRH obtained from Beckman Co. (Palo Alto, CA) was given in a single injection in 16% gelatin.

Results. Figure 1 shows the effect of FSH on ovulation in rats treated neonatally with TP. In control animals (not shown), the doses of gonadotropins (FSH and LH) given at different days of age resulted in ovulation in all animals with an average number of eight ova. When 200 μg FSH were given to rats treated neonatally with TP, the incidence of ovulation was observed in all animals, but the ovaries of these animals were less responsive. However, it appears that with the increase of age, such animals show a better response to exogenous gonadotropin. At 150 days of age, the androgenized females closely resemble normal females in this respect. Figure 2 indicates that the same was true with 25 μg of LH. The androgenized rats are ready to respond to LH, but are less responsive than the controls. Figure 3 shows that injection of LHRH induced ovulation in all androgen-sterilized rats except those given LHRH on day 30 of age. In general, TP-treated rats are more sensitive to LHRH than FSH or LH.

Table I indicates the effect of varying doses of LHRH in 16% gelatin on cyclic rats and TP-treated rats at 75 days of age. All cyclic rats ovulated. Testosterone propionate-treated rats receiving the lowest dose of LHRH showed partial incidence of ovulation. One hundred percent of the animals receiving the high doses of LHRH ovulated. The number of ova in each treatment was related to the dose of LHRH injected.

Discussion. It has been well-established in our laboratory that when female rats are treated neonatally with a high dose of TP, a persistent estrus, anovulatory state ensues. In such animals, a single dose of pregnant-mare serum gonadotropin (PMSG) at 28 days of age does not induce ovulation. Presumably, this is due to a lack of release of "ovulation-inducing hormone" by the pituitary gland, as reported by Brown-Grant *et al.* (3) and Uilenbroek and van der Werff ten Bosch (4). However, Barraclough (1) has observed ovulation in 90% of the androgen-sterilized rats following a single iv injection of 2 international units of HCG.

Our findings with exogenous and endogenous gonadotropins confirm that follicles in the androgen-sterilized rats are ready to respond to gonadotropin. Furthermore, at every age tested, the androgen-sterilized rats shed fewer ova per ovulating rat than do the controls. This indicates that either less preovulatory LH is released so that fewer ova

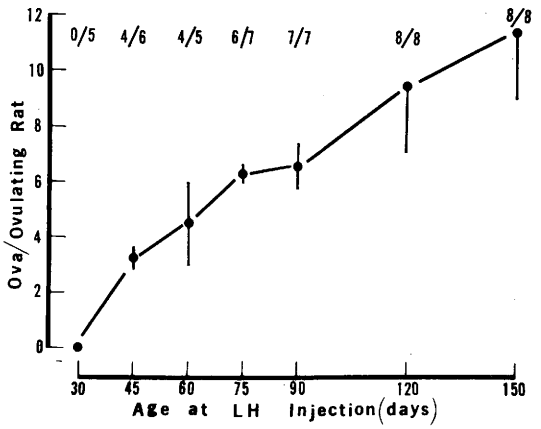

FIG. 2. Effect of LH injection on ovulation in TP-treated rats on different days of age. The fractions at the top of the figure indicate the number of animals which ovulated per number of androgenized females injected with LH at a given age.

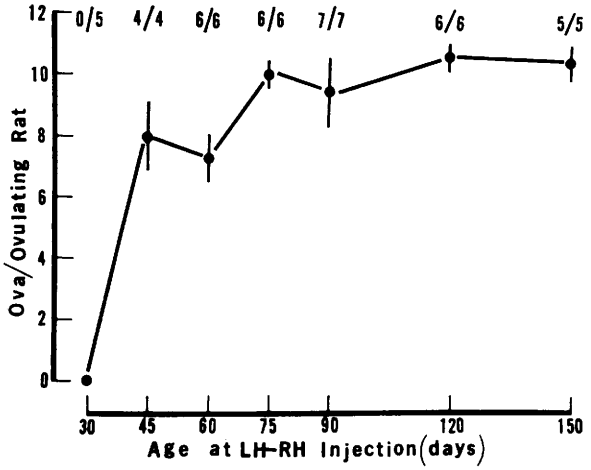

FIG. 3. Effect of LHRH injection on ovulation in TP-treated rats on different days of age. The fractions at the top of the figure indicate the number of animals which ovulated per number of androgenized females injected with LHRH at a given age.

are shed by the ovaries, or that fewer mature follicles ready to rupture are available. It is also possible that the limited ovarian responsiveness in these animals is secondary to deficient gonadotropic stimulation, because abnormal levels of serum FSH and LH have been reported by Weisz and Ferin (5), Johnson (6), and Gorski and Barraclough (7). Nevertheless, induction of ovulation with synthetic LHRH showed that the pituitary of the androgen-sterilized rat could be induced to release a substantial amount of "ovulation-inducing hormone." While ovulation could be induced in this manner, the number of ova found in TP-treated rats was smaller than that in the control animals. Our findings indicate that follicles in androgenized rats are ready to respond to both exogenous and endogenous gonadotropins, and that the ovarian threshold to gonadotropin decreases with age. Androgen-sterilized rats are, however, less responsive than cyclic rats of the same age receiving the same treatment.

Summary. Female rat pups injected sc with 1.0 mg testosterone propionate in 0.1 ml sesame oil on day 5 of life were given a single dose of FSH (200 μg) or LH (25 μg) iv or LHRH (3.0 μg) sc at 1:30 PM on day 30, 45, 60, 75, 90, 120, or 150. Normal cyclic rats were given FSH or LH in the same manner on the second day of diestrus at the same days of age. The data indicate that follicles in androgenized rats are ready to respond to exogenous and induced-endogenous gonadotropin, and that the ovarian threshold to gonadotropin decreases with age. However, the androgenized rats require more gonadotropin than their cyclic rat contemporaries receiving exogenous gonadotropin in order to produce the same ovulatory response.

TABLE I. Effect of LHRH on Ovulation in Control and TP-Treated Rats.

Doses of LHRH (μg)	Control		TP-treated	
	Ovulating rats/total rats	Ova/ovulating rat ± SE	Ovulating rats/total rats	Ova/ovulating rat ± SE
3.000	5/5	12.0 ± 2.0	6/6	10.5 ± 1.2
1.500	7/7	11.5 ± 3.5	6/6	10.1 ± 0.5
0.750	8/8	8.8 ± 1.2	4/4	4.5 ± 1.1
0.375	6/6	6.0 ± 0.6	4/6	3.8 ± 0.6

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