A Sex Difference in the Rate of Rise of Plasma LH in Rats Following Gonadectomy[1] (35181)

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(Introduced by Roger Guillemin)
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It has been reported that gonadectomy in mammals leads to an increase in plasma and urinary LH concentrations, an increase in the quantity of pituitary LH stores, and the appearance of "castration cells" in the pituitary. These events are thought to occur because of the removal of inhibitory influences of gonadal steroids over the brain–pituitary axis. This postulate is supported by the observations that (a) injection of estradiol into ovariectomized rats causes a fall in "plasma LH levels" as measured by OAAD assay (1), (b) stereotaxic implantation of estrogens into the hypothalamus blocks the hypertrophy of pituitary gonadotropic cells following ovariectomy (2, 3), and (c) implantation of ovarian tissue into hypothalamus induces uterine atrophy (4).

In the past, lack of sensitive and specific methods has prevented investigators from obtaining information about levels of plasma LH as they vary within hours after gonadectomy. Recent availability of radoimmunoassays for rat LH has provided such appropriate methodology; the present study was thus undertaken to evaluate, by means of a solid phase radioimmunoassay technique, the rate of change in plasma LH levels following gonadectomy in male and female rats.

Material and Methods. Male and female rats (Holtzman derived) were obtained from Cheek Co., Houston, Texas. They were housed 3 to 4/cage, fed and watered *ad libitum*, and maintained on a schedule of 14 hr light and 10 hr darkness. Vaginal smears were taken daily for 3 weeks prior to gonadectomy. Gonadectomy was performed under ether anesthesia. All blood samples were taken during midmorning.

Design of Experiments. Expt. 1. Plasma LH levels in male rats following castration. Eighteen 150 g male rats were divided into 4 groups: Group I was composed of six animals and served as the control, while groups II, III, and IV contained 4 animals each, and served as experimental subjects. A 2-ml blood sample was taken from the external jugular vein of each member of group I at the start of the exeriment (0 hr). Similar blood samples were withdrawn from members of groups II, III, and IV at 6, 12, 18, 24, 30, 36, 42, and 48 hr postcastration. Members of groups II, III, and IV were bled alternately during the experiment in order to maintain fluid and electrolyte balance.

Expt. 2. Plasma LH levels in male and female rats following gonadectomy. Twelve 200 g male rats were divided into 3 groups containing four animals each. A sample of blood (0 hr) was taken from all of the animals at the time of gonadectomy. Subsequently, 2-ml blood samples were taken at 6, 12, 18, 24, 36, 72, 96, 120 hr and 10, 20, 30 days following surgery. Again, in order to maintain fluid and electrolyte balance, animals from alternate groups were bled during the interval 6–120 hr. Animals from all groups were bled at 10, 20, and 30 days.

Twelve 200 g female rats were studied with the same experimental design. In this experiment, the rats were not grouped according to

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TABLE I. Change in Plasma LH Levels in 150 g Male Rats Following Castration.

Treatment (hr after castration)	N	LH (ng/ml; mean ± SE)[a]	p[b]
Control	6	1.48 ± 0.59	
6	4	4.16 ± 0.79	NS
12	4	7.02 ± 1.26	*
18	4	12.38 ± 1.90	**
24	3	16.81 ± 2.31	**
30	4	16.90 ± 1.49	**
36	3	19.44 ± 1.73	**
42	3	13.03 ± 2.36	**
48	3	13.51 ± 0.53	**

[a] Expressed in terms of rat LH (DNW-8-37) with an OAAD potency of 2.6 × NIH-LH-S11 reference standard.

[b] Level of statistical significance of the difference from control as determined by the one sided multiple comparison test of Dunnett: NS = not significant; * 95%; ** 99%. See text for discussion of results of the multiple range test of Duncan.

their stage in the estrous cycle.

Expt. 3. Plasma LH levels in female rats gonadectomized at known stages of the estrous *cycle.* Groups of six 200 g rats, representing each stage of the estrous cycle, were gonadectomized as described earlier. A 2-ml sample of blood was taken from each animal immediately prior to surgery (0 hr). Thereafter, similar blood samples were taken at 1, 2, 3, 4, 5, and 15 days postgonadectomy. The members of each group were bled alternately on days 1–5; all of the animals were bled on days 10 and 15.

Assay method for plasma LH: Each blood sample was centrifuged at 1000*g* for 30 min and plasma was decanted and frozen for subsequent use. LH concentration in 100-μl sample was determined by the solid phase radioimmunoassay method as described by Amoss and Guillemin (5). The rat LH standard, which was used in these experiments, is DNW-8-37, with an OAAD potency of 2.6 × LH-S11. (DNW-8-37 was kindly furnished by Dr. D. N. Ward).

Results. Expt. 1. Although a rise in plasma LH was discernible at 6 hr postcastration, it did not become significantly different from the control level until 12 hr after surgery. High levels were recorded by 36 hr after the operation (Table I). It is interesting to note that after the peak has been reached at 36

TABLE II. Change in Plasma LH Levels Following Gonadectomy in 200 g Rats.

Treatment (time after gonadectomy)	Male			Female		
	N	LH (ng/ml)[a]	p[b]	N	LH (ng/ml)[a]	p[b]
Control	12	0.54 ± 0.04	NS	12	0.49 ± 0.01	NS
hr						
6	4	1.08 ± 0.03	*	4	1.56 ± 0.62	**
12	4	2.07 ± 0.68	**	4	0.65 ± 0.09	NS
18	4	3.14 ± 0.97	**	4	0.83 ± 0.09	NS
24	4	7.18 ± 1.22	**	4	0.90 ± 0.07	NS
36	4	4.55 ± 0.35	**	4	1.55 ± 0.64	**
48	4	4.11 ± 0.83	**	4	0.73 ± 0.09	NS
72	4	4.82 ± 0.78	**	4	1.02 ± 0.22	NS
96	4	5.44 ± 1.26	**	3	1.26 ± 0.34	*
120	4	8.80 ± 0.88	**	4	1.23 ± 0.58	NS
days						
10	10	12.90 ± 1.43	**	12	3.86 ± 0.47	**
20	10	20.99 ± 1.76	**	11	13.89 ± 1.02	**
30	11	21.94 ± 1.93	**	11	17.75 ± 1.62	**

[a] See Table I.

[b] Level of statistical significance of the difference from control as determined by the one sided multiple comparison test of Dunnett on $\log_{10}$ transformed data: NS = not significant; * 95%; ** 99%.

TABLE III. Changes in Plasma LH Levels Following Ovariectomy Performed at Known Stages of the Estrous Cycle.

Treatment (days after ovariectomy)	Levels of plasma LH (ng/ml) at various stages of the estrous cycle[a]											
	Proestrus			Estrus			Metestrus			Diestrus		
	Mean ± SE	*N*	*p*[b]	Mean ± SE	*N*	*p*[b]	Mean ± SE	*N*	*p*[b]	Mean ± SE	*N*	*p*[b]
Control	0.64 ± 0.14	6	NS	0.34 ± 0.56	6		0.60 ± 0.06	6		0.50 ± 0.00	6	
1	0.77 ± 0.12	3	NS	0.58 ± 0.13	3	NS	0.59 ± 0.09	2	NS	0.84 ± 0.16	3	NS
2	0.83 ± 0.11	3	NS	1.14 ± 0.39	3	*	2.05 ± 0.73	3	NS	0.82 ± 0.32	3	NS
3	0.64 ± 0.14	3	NS	0.59 ± 0.09	3	NS	1.08 ± 0.10	3	*	3.29 ± 0.15	3	**
4	3.81 ± 1.16	3	**	3.44 ± 0.41	3	**	3.15 ± 0.56	3	NS	1.95 ± 0.19	3	*
5	1.80 ± 0.82	3	NS	1.31 ± 0.35	3	**	0.60 ± 0.10	3	NS	0.50 ± 0.00	3	NS
10	5.21 ± 0.61	6	**	7.74 ± 2.07	5	**	8.05 ± 3.08	5	**	8.68 ± 2.29	5	**
15	9.98 ± 2.16	6	**	9.97 ± 1.07	5	**	10.59 ± 3.32	6	**	10.73 ± 3.71	5	**

[a] See Table I.

[b] Level of statistical significance of the difference from control as determined by the one sided multiple comparison test of Dunnett on $\log_{10}$ transformed data: NS = not significant; * 95%; ** 99%.

hr, there was a significant decline in plasma LH concentrations at both 42 and 48 hr when compared to the 36 hr level as determined by the multiple range test of Duncan.

Expt. 2. Plasma LH levels began to rise soon after gonadectomy in both male and female rats. However, the rate of elevation was different in the two sexes (Table II). Male rats showed a rapid rise during the period 0 to 24 hr. Subsequently, the plasma level rose slowly over the next 5 days, and by day 20, it had reached a level of 20 ng/ml which remained the same at day 30.

Female rats, which had been gonadectomized at a random phase of their ovarian cycle, showed no rapid rise in plasma LH levels between 0 and 24 hr; in fact, the concentration remained near the 0 hr level for 5 days. It was not until day 10 that plasma LH concentration started rising; by day 30, plasma LH levels were similar to those observed in the castrated males.

Expt. 3. Female rats showed little deviation from the pattern described above (Expt. 2), whatever the stage in the estrous cycle during which gonadectomy was performed (Table III). Each group demonstrated a slow rise in plasma LH levels between 0 and 5 days; plasma LH levels had increased to 10 ng/ml by day 15.

Discussion. The results show that male rats respond to orchidectomy by elevating their plasma LH concentration manyfold between 0 and 36 hr postgonadectomy, that this initial elevation is followed by a plateau, and subsequently, that the plasma LH levels continue to rise slowly over the next 30 days. This is in contrast to the response seen in female rats, where one finds that plasma LH levels do not begin to rise appreciably until 10 days postgonadectomy, regardless of when ovariectomy is performed during the estrous cycle. By 30 days postgonadectomy, there appears to be no significant difference between the plasma LH levels found in male and female rats. These observations are in accord with those seen by Gay and Midgley (6) and Yamamoto *et al.* (7).

The different rate at which male and female rats elevate their plasma LH levels following gonadectomy may result from the conversion of extragonadal androgens to estro-

gens, which have different effects on the brain–pituitary axis of each sex. That such conversions can occur in normal (8, 9) and gonadectomized (10, 11) animals has been reported; as has the observation that removal of the gonads and the adrenals leads to a fall in urinary estrogen concentration below the level of detectability. Thus, the adrenals can produce androgen substrates, which are acted upon by enzyme systems, of adrenal or extra-adrenal origins (12) to form estrogens.

Whatever the source of the converting system, it appears that androgens can be altered to form estrogens in both sexes. This might account for the rapid rise in plasma LH levels that is seen in the male following castration, since the level of circulating androgens is lowered both as a result of the surgical removal of the testes and the conversion of extragonadal androgens to estrogens. Since the gonadotropin secretory capacity of the male brain–pituitary complex is influenced primarily by androgens (13, 14), the low androgen level present after castration would be insufficient to depress LH output.

The mechanism appears to be different in the case of the female: Here, estrogens and progesterone are the major hormonal regulators of brain–pituitary gonadotropin secretion (15–17), and it has been shown that ovariectomy leads to an increased sensitivity of this axis to the effects of progesterone (18). Therefore, it may be that the converting system maintains plasma estrogen at a level which allows it to synergize with progesterone of adrenal origin in acting on a sensitized brain–pituitary complex to retard, but not inhibit, a rise in plasma LH after ovariectomy.

Summary. Plasma LH levels were measured in male and female rats soon after gonadectomy by means of a solid phase radioimmunoassay method. Male rats responded to gonadectomy by elevating their plasma LH concentrations within 12 hr postcastration; a continuous rise in plasma LH concentration was observed up to day 20 at which date a plateau level was reached. Female rats showed little elevation in plasma LH concentrations up to 10 days after gonadectomy; at this time, the plasma LH level was considerably lower than that found in the male; the stage of estrus during which gonadectomy was performed had no major effect on the animal's ability to elevate plasma LH concentration. At day 30 following gonadectomy, similar levels of plasma LH were observed in both sexes.

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