Ovulation induction with LH-RH in rats rendered acyclic by hypophyseal grafts

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That the rat hypophysis is dependent on hypothalamic support was established several decades ago by shrewd transplantation studies (Greep, 1936; Harris & Jacobsohn, 1952). An hypophysis removed from contact with the median eminence area loses the capacity to synthesize and release most of its trophic hormones. Since prolactin is controlled primarily by a factor which inhibits its release, its serum concentration is elevated in hypophysectomized rats bearing hypophyseal homografts in their kidneys (Chen, Amenomori, Lu, Voogt & Meites, 1970). As a model for the study of the correlation between control of prolactin and gonadotrophin secretion we have adopted the intact female rat bearing a second pituitary beneath the kidney capsule and investigated the ability of exogenous LH-RH to induce ovulation in such animals.

The rats were from an outbred colony of Wistar origin. Donors of hypophyses were not litter-mates of the recipients. In our experience with this model to date, some 70% of animals so prepared exhibit vaginal dioestrus from the day of surgery, and the condition is maintained for at least 8 weeks, the longest period studied so far. Concentrations of serum prolactin in these animals are markedly elevated but LH and FSH are only minimally altered (Table 1).

Table 1. Mean $\pm$ S.E.M. serum hormone concentrations (ng NIAMDD-RP1/ml serum) of female rats (8/group) killed at different times after hypophyseal grafting

Weeks after grafting	Prolactin	LH	FSH
Controls	19.9 $\pm$ 3.5	19.8 $\pm$ 2.6	329.4 $\pm$ 21.3
1	119.0 $\pm$ 17.4**	23.4 $\pm$ 1.9	235.6 $\pm$ 9.2**
2	61.5 $\pm$ 13.6	25.5 $\pm$ 5.6	251.6 $\pm$ 14.5
3	73.4 $\pm$ 15.7*	34.8 $\pm$ 3.2**	241.3 $\pm$ 23.0
4	82.2 $\pm$ 14.7**	34.6 $\pm$ 8.5	287.5 $\pm$ 34.6
5	73.5 $\pm$ 16.8*	34.3 $\pm$ 5.2	245.4 $\pm$ 22.0
6	56.1 $\pm$ 7.8**	21.0 $\pm$ 1.9	313.2 $\pm$ 41.5
7	49.6 $\pm$ 5.2*	25.6 $\pm$ 2.9*	234.7 $\pm$ 28.8*

The control values were obtained from cyclic rats in dioestrus bearing skeletal muscle grafts for 6 weeks.

Significantly different from control values by the Kolmogorov-Smirnov test (Darling, 1957): * $P < 0.05$; ** $P < 0.005$.

After rats with a grafted hypophysis had been dioestrous for 6 weeks, exogenous LH-RH was administered intravenously in single doses ranging from 0.1 to 10 μ g between 10.00 and 14.30 hours. These rats did not ovulate. Similar rats were therefore treated with 2 or 3 doses of 10 μ g LH-RH at 1½-hr intervals. Serum LH concentrations were measured in blood samples obtained by retro-orbital puncture 15 min after each injection. As shown in Table 2, serum LH levels were always elevated, regardless of the time of injection, but reliable ovulatory responses, determined from the number of eggs found in the oviduct after killing the next morning, were recorded only with a regimen involving at least three injections.

Our model confirms the observation of Quilligan & Rothchild (1960) that a foreign hypophysis implanted into an intact female rat is capable of exerting an influence on the animal's own hormonal balance. The result is a cessation of cyclic hormone changes and an elevation of serum prolactin concentrations, i.e. a state of pseudopregnancy. This condition was manifested not only by constant

Table 2. The effect of 10 µg LH-RH on ovulation and mean $\pm$ S.E.M. serum LH levels (ng NIAMDD-RP1/ml serum) 15 min after injection of intact rats rendered dioestrous for 6 weeks by hypophysial grafts (combined results of two separate experiments)

No. of rats	Time of LH-RH injections (hours)				No. of rats ovulating	Mean $\pm$ S.E.M. no. of eggs/ovulating rat
	10.00	11.30	13.00	14.30		
8	431 $\pm$ 44	3842 $\pm$ 587	2564 $\pm$ 257	1676 $\pm$ 177	8	9.4 $\pm$ 1.6
10	487 $\pm$ 61	4370 $\pm$ 488	2682 $\pm$ 226	—	10	7.6 $\pm$ 1.5
9	—	550 $\pm$ 72	2719 $\pm$ 456	1613 $\pm$ 284	7	7.4 $\pm$ 0.9
5	—	—	540 $\pm$ 49	2989 $\pm$ 616	2	3.0 $\pm$ 1.0
5	574 $\pm$ 131	2352 $\pm$ 585	—	—	3	1.7 $\pm$ 0.7

vaginal dioestrus but also by the anatomical changes in the mammary glands, ovaries, uteri and vaginae observed in histological studies (unpublished results). The induction of ovulation in such animals with LH-RH was surprising, because a single LH-RH injection resulted in normal ovulatory plasma LH elevations but did not cause ovulation. The resistance of the histologically mature Graafian follicles to these LH elevations strongly suggests that an unidentified inhibitory factor is involved; the requirement of three successive LH-RH applications for ovulation would then be best explained by assuming the need for a priming effect at the hypophysis (compare the increment between first and second plasma LH concentrations) and the ovary before an ovulatory response becomes possible.

Our experience to date has convinced us that the simple model described is ideally suited to studies for clarifying the inadequately understood relationships between prolactin and the gonadotrophins.

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