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Prepubertal Change in Ovulating Response to LH-RH in PMS-Nembutal-Primed Rats

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Summary

Ovulating response with exogenous LH-RH was examined in PMS-Nembutal primed rats.

Three IU of PMS was injected into female rats of 21–31 days of age followed by Nembutal and various doses of LH-RH injections 51 and 52 hours after PMS treatment.

Animals were killed to check for ovulation 72 hours after PMS injection. The minimal doses of LH-RH to induce the high rate of ovulation were 5.0, 0.5, 0.5, 0.5 and 0.1 μg at 21, 25, 27, 29 and 31 days respectively, indicating that the ovulating response to LH-RH was hightened with development.

The result suggests that the pituitary response to LH-RH in rats may be enhanced with approaching puberty.

A remarkable functional change in the hypothalamo-pituitary axis in female rats occurs at puberty, indicated by the abrupt discharge of gonadotrophins in the afternoon preceeding the first ovulation (1–3).

As a means to clarify the mechanism of the maturational change in the axis, the effects of LH-RH (luteinizing hormone-releasing hormone) on the pituitary function were examined in developing rats.

So, several experiments were made on the pattern of the ovulating response and of gonadotrophin levels after LH-RH treatment (2, 4–8).

It is known that ovulation usually does not occur in prepubertal rats even near puberty with only a single treatment of LH-RH (4) or of HCG (9, 10) unless a follicle stimulator such as PMS (pregnant mare serum gonadotrophin) was pretreated.

This indicated that prepubertal ovarian follicles were too undeveloped to respond to the ovulating hormone which was released by the LH-RH.

This experiment was designed to know if the ovulating response to LH-RH is changed with prepubertal development after a proper stimulus for follicular maturation with PMS in rats.

Materials and Methods

Female immature rats of the Wistar-Imamichi strain were purchased from Dobutsu-Hanshoku-Kenkyujo (Oomiya-shi) at 21 days of age and given a pellet diet (Piglet: Zenno). The day of vaginal opening was 35.8±0.3, S.E. (n=90) according to the data from the supply center.

Three I.U. of PMS (P-mex: Sankyo Zoki) was injected s.c. at 10:00 on each of the following days (21, 25, 27, 29 and 31) and Sodium-pentobarbital (Nembutal: 6 mg/100 g. b.w.) was injected s.c. 51 hours after the PMS injection to inhibit ovulation.

This PMS dose is known in our laboratory to induce a high rate of ovulation with 6–10 ova through the prepubertal age (10).

One hour after Nembutal injection, LH-RH (Synthetic decapeptide: Takeda) was injected through the femoral vein with various doses (0, 0.05, 0,1 0.2, 0.5, 1.0, 2.0 and 5.0 μg) in 0.5 ml saline.

Seventy-two hours after the PMS injection, the rats were killed to check for ovulation under a dissecting microscope. When the vaginal opening occurred before the preceeding day of the autopsy, the record was discarded.

Results

The result was shown in Table 1.

TABLE 1. *Ovulating Response to LH-RH in PMS-primed Rat*

The day of PMS injection	LH-RH dose (μg/0.5 ml)							
	0	0.05	0.1	0.2	0.5	1.0	2.0	5.0
	Ovulation rate in rats with Nembutal 51 hrs. after PMS injection (number of ova)							
21	—	—	0/3	0/5	2/4 (2, 4)	1/5 (5)	0/4	5/5 (7.0±1.3)*
25	—	—	1/4 (3)	1/4 (8)	4/4 (6.0±0.9)	4/4 (7.0±1.1)	2/2 (6, 7)	—
27	—	—	1/3 (2)	1/4 (6)	2/3 (7, 6)	2/2 (7, 5)	—	—
29	1/4 (11)	0/3	2/7 (1, 7)	3/7 (8.0±0.6)	4/5 (10.5±0.7)	4/5 (7.3±0.3)	1/1 (9)	—
31	1/3 (6)	2/5 (13, 4)	5/5 (10.0±1.3)	5/5 (8.6±0.4)	3/3 (10.0±0.6)	2/2 (11, 6)	—	—

* Mean±S.E.

At 21 days, 2.0 μg or less of LH-RH did not fully induce ovulation. Ovulation occurred by elevating the dose to 5 μg. At 25 days, 0.2 μg or less of LH-RH induced some ovulation (25%) while more than 0.5 μg resulted in a 100% response.

At 27 days, the ovulating response was almost similar to that of 25 days. At 29 days, the ovulation rate was low with 0.1 μg or less of LH-RH while a full response was seen with 0.1 μg or more at 31 days.

There was a trend that the number of ova was smaller with a lower dose of LH-RH at 21, 25 and 27 days.

Discussion

The result shows that the ovulating response to LH-RH was hightened with pubertal development in the PMS-Nembutal primed rats.

This might suggest that pituitary responds with lower doses of LH-RH to release the ovulating hormone sufficient to rupture the matured follicles as animals grow to puberty. When this ovulating response is regarded as an index of pituitary response to LH-RH, the responsibility seems to be enhanced near puberty.

The alteration of pituitary response to LH-RH with development differd with the results of previous works on the levels of gonadotrophins after LH-RH injection where the enhanced pituitary response was seen to be around 10–15 days of age followed by decreased response approaching puberty (2, 5, 8). The difference seems due to the pretreatment to mature follicles or to elevate the endogenous estrogen (11), which might change the pituitary responsibility to LH-RH as in the adult female rat (12, 13).

Although the blood levels of estrogen are not estimated in this experiment, the secreted levels after PMS treatment seem not to be so varied throughout the pubatal stage because of the similar number of follicles matured to ovulate.

Therefore, it is presumed that the sensitivity of the hypothalamo-pituitary axis to estrogen is enhanced with development near puberty.

This idea may be supported by the report of Ying & Greep (14) that a single injection of estradiol benzoate can induce ovulation in prepubertal rat and the response is enhanced near puberty.

Also, it might be possible that the pituitary easily responds with lower doses of LH-RH in the change of the functional condition in the axis.

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