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The Role of Ovarian Steroids on Pituitary Ovulating Hormone in PMS-treated Immature Female Rats

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PMS 처리한 미성숙 쥐의 腦下垂體 排卵호르몬에 미치는 卵巢스테로이드의 影響

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摘 要

본인은 생후 24일 된 쥐에 PMS와 더불어 estradiol을 처리하면 排卵이 예정보다 24시간 앞당겨 일어남을 보고한 바 있다. 본 실험은 이의 계속 연구로 腦下垂體前葉에서 분비되는 preovulatory LH surge가 PMS 처리 후 51~56시간에 일어남을 보았다. 그러나 estradiol을 PMS와 동시에 처리하면 예정보다 24시간 단축된다.

卵巢를 제거한 쥐에 0, 24, 48시간에 2.5~40 μg의 estradiol을 처리하면 51~56시간에 腦下垂體의 LH가 증가되지 않는다. 그러나 estradiol을 처리하고 이어 48시간에 1 mg의 progesterone을 처리하면 卵巢를 제거한 쥐에서도 腦下垂體의 LH level은 증가된다.

본 실험의 결과로 estradiol처리가 PMS로 유도한 排卵을 24시간 앞당기는 사실은 estrogen이 progesterone과 길항작용으로 腦下垂體에서 LH 분비를 24시간 앞당겨 일으키고, 따라서 排卵도 24시간 단축되는 것이라고 생각된다.

INTRODUCTION

Evidence has accumulated to show that the ovulatory surge of luteinizing hormone (LH) at proestrus depends on the prior increase in estrogen secretion in the rat (Miyake, 1969; Brown-Grant *et al.*, 1970). Experiments using ovariectomy (Schwartz, 1964), estrogen antagonist (Shirly *et al.*, 1968) or antibody to 17β-

estradiol (Ferin *et al.*, 1969 a) attest to the essential role of estrogen in the ovulatory surge of LH. Release of the ovulating hormone can be induced in the immature female rat by pregnant mare serum (PMS) (Zarrow and Quinn, 1963; McCormack and Meyer, 1964). Ferin *et al.* (1969 b) blocked PMS-induced ovulation with antibody to 17β-estradiol administered preceding the expected time of LH release, confirming the concept that the LH release in PMS-treated animals resulted from a positive feedback of circulating estrogen. Exogenous estrogen also stimulated LH secretion by a positive feedback action in the cycling rat as well as in the immature female rat. In normal five day cycling rats the administration of estrogen on the second day of diestrus advanced ovulation 24 hours (Everett, 1948; Brown-Grant, 1969; Krey and Everett, 1973). Furthermore, a marked rise in the plasma LH was shown following estrogen implantation in the median eminence (Weick and Davidson, 1970), or daily injections of estradiol (Callantine *et al.*, 1966). Administration of small doses of estrogen to immature rat was demonstrated to induce ovulation (Ying and Greep, 1971 a, b).

In the previous study (Park and Zarrow, 1972) it has been shown that estradiol simultaneously treated with PMS advanced ovulation 24 hours in immature female rat. This premature ovulatory response following estradiol was blocked by pentobarbital at 27~32 hours following PMS. It suggests that estradiol might advance PMS-induced ovulation, by facilitating LH release 24 hours prior to the normal release time for LH following PMS. Consequently, the following experiments were conducted to determine the role of estradiol in advancing PMS-induced ovulation and in the regulation of pituitary ovulating hormone in the immature female rat.

MATERIALS AND METHODS

Twenty four-day old female rats of the Purdue Wistar strain were used in this study. All rats used were kept in a controlled environment at 21°C, with a relative humidity of 55%. The light-dark cycle consisted of 13 hours of light and 11 hours of darkness with the light starting at 7 : 00 A.M. Food and water were given *ad libidum*. PMS was dissolved in saline at a concentration of 30 I.U./0.1 m*l* and injected at 10 : 00 A.M. (time zero). Both steroid hormoes, 17β-estradiol and progesterone, were dissolved in peanut oil and injected in a volume of 0.1 m*l*. All injections were made subcutaneously. Ovariectomy was performed on day 23 or day 24 of age using dorsolateral approach.

Animals were killed by decapitation and their pituitaries were removed. Immediately after the removal, pituitaries were weighed, lyophilized and stored in a desiccator. At the time of assay, pituitaries of each treatment group with six animals were pooled and homogenized in physiological saline. The homogenate

was centrifuged at 2,000 rpm for 10 minutes and the supernatant was diluted to contain the equivalent of 1.0 mg (wet weight) pituitary/m*l* saline. Aliquots of the homogenate, sufficient for one assay, were stored separately at −20°C until used. The concentration of LH in the anterior pituitary gland was measured by the ovarian cholesterol depletion assay (Zarrow and Clark, 1969).

Thirty two-day old rats were used as assay animals. The rats were killed by cervical dislocation at 5 hours after the injection of pituitary homogenate and both ovaries were analyzed for cholesterol content. In each assay, two dose levels of pituitary homogenate were used and control ovarian cholesterol values were determined in saline injected rats. The ovarian cholesterol depletion induced by the pituitary homogenate was compared to the standard curve and the pituitary LH concentration was expressed in equivalent of NIH-LH-BI. Assay was considered valid when the slope of the line for each assay was not statistically different from the slope of the standard curve as determined by the "t" test.

RESULTS

1. Effect of estradiol given simultaneously with PMS on the pituitary LH level

This experiment was designed to determine if the premature ovulatory response following estradiol in PMS-treated immature rats is due to a premature release of LH from the pituitary gland. Twenty four-day old rats were injected with 30 I.U. PMS and estradiol at time zero. Pituitaries were removed at 24, 48, 51 and 56 hours in PMS treated groups and at 24, 27 and 32 hours in PMS and estradiol-treated groups. Pituitaries were also removed from untreated animals at time zero. PMS-treated animals showed a gradual increase in the pituitary LH concentration from 13.7 μg at time zero to 18.0 μg at 24 hours, 22.1 μg ($p.<0.05$) at 48 hours and 26.5 μg ($p.<0.01$) at 51 hours. By 56 hours LH level (16.8 μg) had significantly dropped from 26.5 μg at 51 hours ($p<0.01$, Fig.1). Forty μg of estradiol given at time zero in PMS-treated rats elevated the pituitary LH level 24 hours earlier prior to pituitary LH level normally observed in PMS-treated rats (Fig.1). The level of pituitary LH (25.3 μg) at 27 hours was significantly greater than that of pituitary LH (16.3 μg) at 32 hours ($p<0.01$). Following the treatment with 2.5 μg estra-

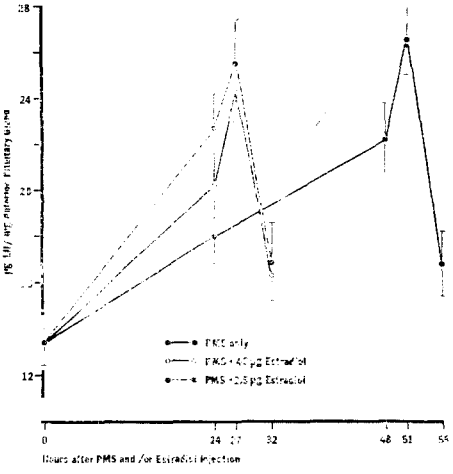

Fig.1. LH levels in μg equivalent of NIH-LH-BI per mg fresh anterior pituitary gland following treatment with PMS or PMS and estradiol in 24-day old female rats. Each point consists of five groups of six animals.

diol at time zero, a similar result was obtained. The pituitary LH level dropped from 25.5 μg at 27 hours to 16.9 μg at 32 hours ($p<0.01$, Fig.1).

These data demonstrated that the ovulatory release of LH occurred between 51 and 56 hours following PMS injection, and the estradiol injected simultaneously with PMS advanced LH release 24 hours.

2. Effect of ovariectomy on estradiol-advanced LH release

Twenty four-day old rats were injected with 30 I.U. PMS and 40 μg estradiol at time zero. They were then ovariectomized within 1 hour of PMS and estradiol injection. Pituitaries were removed at 27 or 32 hours. Pituitary LH levels were 15.5 μg and 16.8 μg at 27 hours following PMS or PMS with 40 μg estradiol injection in ovariectomized rats (Table 1). No significant rise in the pituitary LH was observed at 27 hours following treatment. Thus, the elevation in the pituitary LH following estradiol in PMS-treated rat was not observed if animals were castrated.

Table 1. Effect of castration on estradiol-induced premature LH release in PMS-treated immature rats.

Treatment			Number of separate pools assayed	LH (μg equiv./mg wet pituitary)	
				Time of post-mortem after initial treatment (hrs.)	
PMS (I.U.)	Estradiol (μg)	Ovariectomy		27	32
30	—	—	3	16.7±4.0	18.5±3.7
30	40	—	6	25.3±1.4	16.3±1.8
30	—	Ovariectomized	4	15.5±2.3	17.2±1.2
30	40	Ovariectomized	4	16.8±2.0	14.2±3.1

3. Pituitary LH levels in the ovariectomized immature rats following treatment with estradiol and/or progesterone

The failure to observe estrogen effect in ovariectomized rats led to the following experiment. Rats were castrated on day 23 of age and treated with estradiol at 0, 24 and 48 hours. One mg of progesterone was injected at 48 hours. All pituitaries were removed at 51 hours following the first injection of estradiol. Different doses of estradiol (2.5~40 μg) given at 0, 24 and 48 hours failed to increase pituitary LH levels by 51 hours in the ovariectomized rats (Table 2). Pituitary LH concentrations were 13.9, 14.8, 15.0 or 15.2 μg respectively following the injection of 40, 10, 5 or 2.5 μg estradiol, all of which were not significantly different from 14.5 μg of ovariectomized-oil treated rats. However, LH concentration in the pituitary gland significantly increased from 15.0 μg to 25.2 μg following 5 μg estradiol at 0, 24 and 48 hours followed by 1 mg progesterone at 48 hours ($p<0.01$). This LH concentration of 25.2 μg was not significantly different from 26.5 μg at 51 hours follow-

ing PMS treatment in intact rats (Fig. 1).

These data suggest that both estrogen and progesterone are synergistically involved in the regulation of pituitary LH.

Table 2. Concentration of LH in the anterior pituitary gland of the immature ovariectomized rats following treatment with 17β-estradiol and/or progesteron

Treatment after ovariectomy				Number of separate pools assayed	LH (μg equiv. /mg wet pituitary) at 51 hrs. after initial treatment
Estradiol		Progesterone			
μg	Time of treat. (hrs.)	mg	Time of treat. (hrs.)		
—	—	—	—	5	*14.5±4.1
40	0, 24, 48	—	—	6	13.9±2.3
10	0, 24, 48	—	—	6	14.8±2.6
5	0, 24, 48	—	—	6	15.0±2.5
2.5	0, 24, 48	—	—	6	15.2±2.2
5	0, 24, 48	1	48	6	25.2±2.2

*Animals were ovariectomized and injected with peanut oil.

DISCUSSION

In the previous paper (Park and Zarrow, 1972), estradiol given simultaneously with PMS was demonstrated to advance ovulation 24 hours. Based on experiments in which pentobarbital blocked this response, it was hypothesized that such advanced ovulation was resulted from a premature release of LH. The present study confirms this. Estradiol induced a significant release of LH between 27 and 32 hours following PMS. This time period corresponded to the time of day of the critical period for the release of LH normally observed 24 hours later, since the ovulatory surge of LH in this strain was shown to occur between 51 and 56 hours after the injection of PMS (Fig.1). This result is consistent with the findings that estrogen injections to 4 day cycling rats on day 1 advanced ovulation 24 hours (Everett, 1948; Krey and Everett, 1973) inducing LH release on day 2, which is normally observed on proestrus in regularly cycling rats (Krey *et al.*, 1973; Ying and Greep, 1972). It is not still clear how estrogen advanced LH release in PMS-treated rat. Estrogen might act directly on the pituitary to stimulate LH release (Schneider and McCann, 1970). Estrogen could promote LH secretion by increasing sensitivity of the pituitary gland to endogenous hypothalamic LH-releasing hormone (LH-RH, Arimura and Schally, 1971). Estrogen might also stimulate LH-RH release by a positive feedback on the hypothalamus (Antunes-Rodrigues, 1966). However, the diminished LH release in PMS-treated rats acutely ovariectomized within one hour of estradiol injection indicates that the stimulatory feedback action of exogenous estrogen on LH secretion depends on the secretion of ovarian hormones (Table

1). Similarly, acute ovariectomy immediately following estrogen injection in either immature rat or adult pseudopregnant rat also prevented any consequent LH surge (Bradbury, 1947; Ramirez and Sawyer, 1965; Caligaris *et al.*, 1972). Even large doses of estradiol benzoate (EB) did not stimulate LH surge in chronically ovariectomized rats unless supplemented with additional estrogen or progesterone injections some days later (Caligaris *et al.*, 1971 a; Caligaris *et al.*, 1971 b; Swerdloff *et al.*, 1972). Krey *et al.* (1973) presented the hypothesis that ovarian progesterone secretion plays an integral role in advancing ovulatory LH surge in cycling rat. In particular, EB injection on day 1 which consistently advanced ovulation also stimulated an increase of ovarian progesterone on the morning of day 2, prior to the initiation of the ovulatory LH surge.

In ovariectomized immature rat estradiol alone has no effect on the levels of LH in the pituitary gland (Table 2). This is not in agreement with the previous finding that injection of EB could lead to an increase in pituitary LH level in ovariectomized immature female rats. (Zarrow and Dinius, 1971). Such a discrepancy may be due to the type of estrogen used i.e., the benzoate form *v.s.* the free form. However, estradiol given at 0, 24, and 48 hours followed by progesterone at 48 hours increased pituitary LH level at 51 hours after the first injection of estradiol in ovariectomized immature rats. It is well established that progesterone can influence on LH release, subsequently ovulation in many species. The increase in progesterone precedes LH surge in the preovulatory adult rat (Goldman *et al.*, 1969). In immature rat, a significant rise in the plasma progesterone is obtained at 48~52 hours after PMS, which is prior to the time of onset of LH release (Zarrow *et al.*, 1971). Furthermore, progesterone injection at 24 hours after PMS advanced LH release 24 hours (Zarrow and Dinius, 1971). Ample evidence is also available for progesterone to increase pituitary LH in female rat (Labhsetwar, 1969).

The present work together with earlier work (Park and Zarrow, 1972) indicates that estrogen advanced PMS-induced ovulation by means of inducing premature release of LH and estrogen might synergize with progesterone in the regulation of LH in the pituitary gland.

SUMMARY

The release of ovulatory level of LH from the pituitary gland occurred between 51 and 56 hours after PMS treatment in 24-day old female rats. Estradiol given simultaneously with PMS advanced LH release 24 hours. Injections of estradiol (2.5~40 μg.) at 0, 24 and 48 hours failed to increase pituitary LH level by 51 hours after the first injection in ovariectomized rats. However, 5 μg estradiol at 0, 24 and 48 hours followed by 1 mg progesterone at 48 hours elevated pituitary L

level by 51 hours in ovariectomized rats.

These results indicate that advancement of PMS-induced ovulation by estradiol in the previous study occurred by means of inducing premature release of LH and estrogen might synergize with progesterone in the regulation of LH in the pituitary gland.

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