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Serum Luteinizing Hormone Response in Pregnant Mare Serum Gonadotropin-treated Rats

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PMSG처리한 래트에 있어서 혈청 LH의 반응

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초 록

미성숙 래트의 외경정맥에 카테타를 장착하고, 다음날 (28일령) 대조군에는 4IU, 다배란 처치군에는 20IU의 PMSG를 피하주사하였다. 각 실험동물은 혈중의 LH농도 변화를 측정하기 위하여 PMSG 투여 직전 (0시간), 투여후 12시간, 그 이후 6시간 간격으로 혈액을 채취하고 72시간에 희생시켰다.

그 결과 다배란 용량의 PMSG 투여는 먼저 배란반응 및 난소중량을 대조군에 비하여 각각 4.7배 및 2.1배나 현저하게 ($P<0.05$) 증가시켰다. 그리고 혈청 LH농도는 Radioimmunoassay (RIA)에 의하여 결정되었는데, 먼저 두 군 모두 두 개의 분명한 peak을 가진 경시적 변화관계를 보였다. 즉 이들 두 군에 있어서 LH농도 변화는 0~18시간대에 처음으로 완만한 증가와 54~60시간대에 두번째의 급격한 증가(surge)를 보였다. 그러나 두 군간에 LH농도의 크기는 현저하게 달라, 다배란처치군의 동물에 있어서는 두번째의 LH peak에 앞서 전반적인 LH농도가 대조군보다 현저하게 ($P<0.001$) 높았으며, 반대로 PMSG 투여후 60시간에 일어나는 peak에 있어서는 LH농도가 대조군보다 현저하게 ($P<0.001$) 54%나 낮았다. 덧붙여 두 peak간의 증가폭은 대조군에 비하여 다배란 처치군에서 훨씬 낮았다.

본연구 결과는 PMSG 처치된 래트에 있어서 두 가지의 분명한 LH peak의 존재를 정의하며, 다배란처치에 따른 난소과잉자극과 내인성 LH surge의 감소와의 연관성을 밝힌다.

INTRODUCTION

A single injection of pregnant mare serum gonadotropin(PMSG) is known to initiate induction and synchronization of the ovulatory response in immature rats. Low doses of PMSG elicit a preovulatory gonadotropin surge to generate a "physiological" number of ovulatory follicles wherein a pattern of circulating steroid hormones is similar to that seen in adult rats with spontaneous cycles (Wilson *et al.*, 1874; Parker *et al.*, 1976). Time-course studies of the ovulatory response employing the low doses of PMSG in immature rats demonstrated an integration of sequential changes in the endocrine response associated with a synchronized process of oocyte maturation: critical time of the luteinizing hormone (LH) surge at 52~57hr (Sorrentino *et al.*, 1972; Costoff *et al.*, 1974; Moon *et al.*, 1990), meiotic resumption of follicular oocytes 2~3hr thereafter (Hillensjo *et al.*,

1974) and ovulation at 60~72hr after PMSG (Walton *et al.*, 1983; Yun *et al.*, 1987). However, administration of superovulatory doses of PMSG to immature rats produces precocious or multiple waves of ovulations presumably by intrinsic LH activity of high doses of PMSG (De La Lastra *et al.*, 1972; Miller and Armstrong, 1981; Yun *et al.*, 1987). This atypical ovulation accompanies oviductal recovery of meiotically aberrant oocytes which has been associated with disruption of normal follicular steroidogenesis (Yun *et al.*, 1989).

In view of above observations, the present study was designed to compare the features of serum LH between two different groups of animals receiving control and superovulatory treatment regimens. To determine more specifically the sequential changes of circulating LH levels after PMSG, a model of chronically catheterized immature rats has been introduced.

MATERIALS AND METHODS

Immature female Sprague-Dawley rats at 22 days of age were initially housed at a constant temperature of 21℃ with lights on between 0700 and 1900hr and were provided free access to standard rat chow and water. One day before the experiment, the animals were installed with chronically indwelling catheters as described by Harms and Ojeda(1974). Briefly, a catheter made of silastic tubing (Dow-Corning Corp., Midland, MI) was inserted into the external jugular vein to approach or enter the right atrium under pentobarbital anaesthesia (35mg/kg body wt) and connected with a flexible piece of heparinized polyethylene tubing (Fisher Co., PE50) for withdrawal of blood samples.

On the following day (Day 28 of age), to provide the basal level of serum LH, 0.5ml of whole blood was collected from individual rats via the catheter immediately before administration of PMSG (Eqinex, Ayerst). The rats then received a single subcutaneous dose of PMSG for control (4IU/0.4ml saline) or superovulation (20IU/0.4ml saline) treatment between 0830 and 0900hr. All rats in both groups were bled 0.5ml whole blood at 12hr and subsequently at 6hr intervals until sacrifice at 72hr after PMSG. To alleviate anemia from blood loss and to prevent clot formation in the catheter, each blood sample was followed by replacement of an equal volume of a dilute heparin-saline solution (25 IU/ml) as described elsewhere (Waynforth, 1980). Serum samples were separated by centrifugation and stored at −20℃ until LH radioimmunoassay (RIA).

Animals were sacrificed by cervical dislocation at 72hr. Ovaries were dissected and weighed. Ovulation was assayed by counting oocytes flushed out from oviducts as described previously (Yun *et al.*, 1987).

Serum LH concentrations were measured by RIA using the procedure outlined by NIADDK, and expressed in terms of ng NIH-rat-LH-RP2/ml. All samples were measured in a single assay, in which a pool of serum from intact cycling adult rats on the day of diestrus had a level of 0.98ng/ml with the intra-assay coefficient of variation of 6.4%. The minimum detectable value of LH was 40pg per tube. Serum samples were assayed in duplicate in 100μl aliquots. PMSG was found to cross-react with the LH antiserum, although the binding was not parallel to that of NIH-LH-RP2. The addition of 0.125 IU PMSG to 100μl serum from intact rats raised the apparent level of "LH" in this assay by about 300pg NIH-LH-RP2 equivalent. This is approximately equal to the increase in serum immunoreactive LH that was measured in the rats of the superovulation group following injection of 20IU PMSG (Fig. 1, 12hr vs. 0hr).

Experimental data were evaluated statistically by Student's t-test, or when appropriate, by

analysis of variance followed by Fisher's PLSD test. Comparisons with $P<0.05$ were considered to be significant.

RESULTS

The time course and patterns of LH release in blood for control(4IU PMSG) and superovulated(20IU PMSG) rats are illustrated in Fig. 1. Both groups showed a similar time relationship of biphasic LH response to PMSG, However, a great difference in the magnitude of serum LH levels between the two groups was noted.

Mean basal levels of serum LH prior to administration of PMSG were 0.37 and 0.50

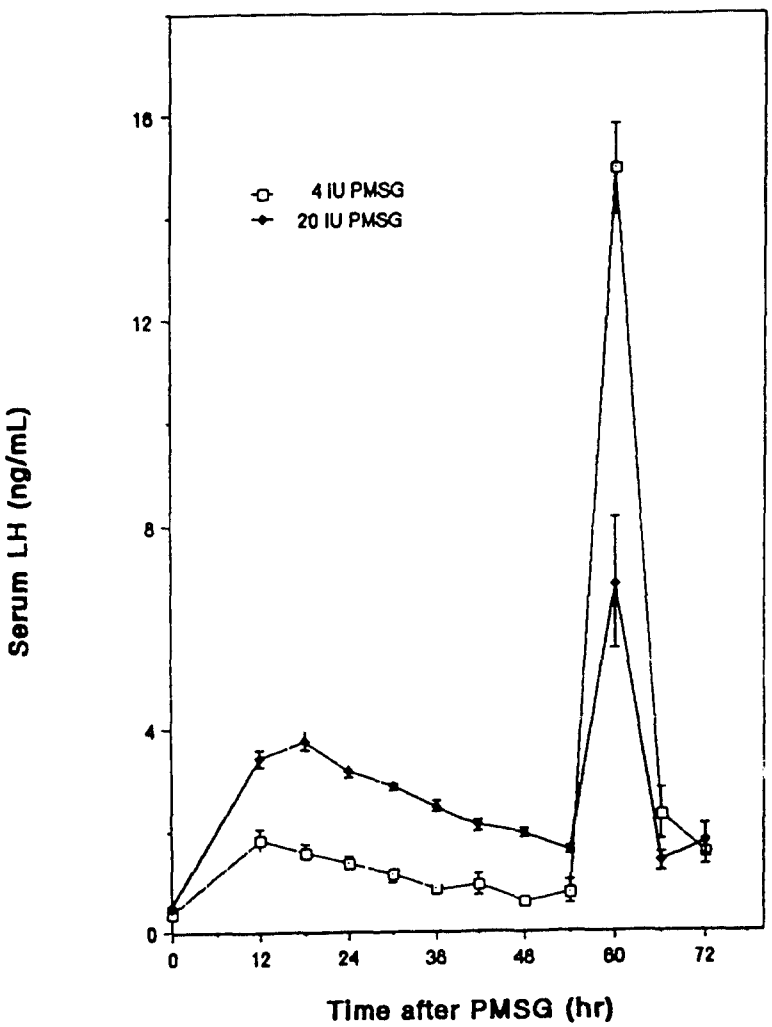

Fig. 1. Changes in serum luteinizing hormone(LH) concentrations after administration of 4IU or 20IU PMSG to immature rats. Sequential blood samples were taken from the same individual using chronic catheterization into external jugular vein. Values at each point represent the means ± SE (n=7)

ng/ml. Serum LH levels began to rise significantly ($P<0.01$) between 0hr and 12hr, and reached the first peaks of 1.81 ± 0.21 ng/ml at 12hr and 3.78 ± 0.18 ng/ml at 18hr in control and superovulated rats, respectively. Thereafter, the LH levels in both groups gradually tapered off by 54hr. The first peaks were followed by enormous and significant ($P<0.001$) elevations of serum LH with the second peaks synchronized at 60hr in both groups. The peak values of second elevations were 14.98 ± 0.92 ng/ml and 6.85 ± 1.29 ng/ml in control and superovulated rats, respectively. Subsequently, the LH levels in both groups rapidly fell by 66hr with no further changes apparent at 72hr.

As compared to controls, between 12hr and 54 hr, cumulative levels of serum LH in superovulated rats were significantly ($P<0.001$) elevated. This elevation was consistent at each time point. However, at 60hr, the mean peak value of the second elevation in superovulated rats was markedly reduced by 54% below that of control rats. Similarly, a maximum increase of mean Δ LH between the two peaks was much lesser in superovulated than that in control rats. The mean Δ LH after subtraction of the first peak from the second peak was 3.07ng/ml in superovulated rats and 13.17ng/ml in control rats.

After final collection of blood samples at 72 hr, the ovulatory response and changes of ovarian weight in response to two different doses of PMSG were examined(Table 1). In control rats, the total mean number of oocytes recovered was 7.6 ± 0.5 oocytes per rat, and mean wet weight of paired ovaries was 32.8 ± 1.9 mg per rat. On the other hand, a superovulatory dose of PMSG was shown to significantly increase the ovulatory response (36.0 ± 8.5 oocytes per rat, $P<0.05$) and ovarian wet weight (68.7 ± 8.2mg per rat, $P<0.01$) above those obtained by a control dose of PMSG.

DISCUSSION

In the present study, both a control dose (4IU) and a superovulatory dose (20IU) of PMSG elicited two distinct LH peaks in rats: a first slight rise at 0~18hr and a second sharp rise at 54~60hr after treatment. This result, in general, agrees with the patterns produced by a different dose (10IU) of PMSG (Hillensjo *et al.*, 1974). Together, these findings indicate a similar and dose-independent time relationships of PMSG to induce LH surge. However, by analyzing the magnitude of the LH response, a superovulatory dose of PMSG was associated with a significant attenuation of the endogenous LH surge accompanied by prolonged elevations of serum LH, as compared to that corresponding to control regimen.

The present observation of two LH increases coupled with previous findings of two distinct ovulations after superovulatory treatment in rats (De La Lastra *et al.*, 1972; Yun *et al.*, 1987) suggests that the LH response to PMSG has

Table 1. Ovulations and ovarian weights 72hr after administration of PMSG to immature rats

Treatment	No. of animals[a]	No. of oocytes (mean±SE)	Ovarian weight(mg) (mean±SE)
4IU PMSG	7	7.6 ± 0.5	32.8 ± 1.9
20IU PMSG	7	36.0 ± 8.5^{b}	68.7 ± 8.2^{c}

[a]All individuals in each group exhibited ovulations.

[b]$p<0.05$ and [c]$p<0.01$, statistically significant compared to 4IU PMSG-treated group.

two different components: a slight and prolonged elevation with the first peak independent of pituitary secretion and a precipitous second elevation of the pituitary-dependent surge. PMSG is known to possess an exceptionally high sialic acid content and a consequent slow clearance rate as well as a predominant LH-like activity when measured by bioassay (Schams *et al.*, 1978). The circulating half life of PMSG varies from about 6 to 26hr depending upon species (Parlow and Ward, 1961; Sasamoto *et al.*, 1972; Aggarwal and Papkoff, 1981). This estimation is considerably longer than the half life of endogenous gonadotropins which were reported to as 2.5hr for FSH and 0.5hr for LH in rats (Bogdanove and Gay, 1969). A study of neutralization with anti-PMSG demonstrated the active biological life of PMSG of 54hr to 60hr in mice (Sasamoto *et al.*, 1972) and its circulating inactivation time of 36hr in rats (Sasamoto and Kennan, 1973). On the basis of these chemical properties of PMSG and a cross reaction between PMSG and rat LH antibody in the current assay system, it is concluded that the elevated serum LH around the time of the first peak is actually an intrinsic component of PMSG. Additionally, the timing of the surge between 54 and 60hr in the present study is well in agreement with the critical period of endogenous LH secretion established in numerous time-inhibition studies using various neuropharmacologic central depressants (Sorrentino *et al.*, 1972; Hillensjo *et al.*, 1974) and hypophysectomy (Strauss and Meyer, 1962; Zarrow and Quinn, 1963).

It has been previously reported that in immature rats, a low dose of PMSG (3 IU) initially stimulates a rapid follicular development during the first 36hr prior to its inactivation in blood, and then by the next 25hr, endogenous gonadotropin secretion is responsible for the maintenance of follicles to ensure final ovulation (Sasamoto and Kennan, 1973). In the present study, a superovulatory dose of PMSG resulted in the initial prolonged elevations of serum LH around the first peak and subsequent suppression of the second, endogenous, LH surge as compared to control levels of serum LH. Therefore, in superovulated rats, the current observation of increased ovulatory response and ovarian weights as well as the previous findings of precocious ovulation as early as 24hr (De La Lastra *et al.*, 1972; Yun *et al.*, 1987) reflect the effectiveness of PMSG-derived intrinsic gonadotropin for ovarian hyperstimulation. However, this interpretation does not rule out the involvement of endogenous gonadotropin secretion in the process of follicular maturation and ovulations even in superovulated rats, since the second elevation of serum LH remains higher than the preceding peak.

There recently have been several lines of evidence that superovulatory regimens using exogenous gonadotropins inhibit the onset of endogenous LH surge and attenuate its magnitude in humans (Ferraretti *et al.*, 1983; Messinis and Templeton, 1986) and monkeys (Littman and Hodgen, 1984). The results of the present study are consistent with these findings, and provide further evidence on a nonprimate model. In rats, a superovulatory dose of PMSG significantly suppressed the endogenous LH surge without affecting the timing of its onset. This suppression has previously been attributed to the action(s) of inhibin-like protein and/or nonsteroidal ovarian factor(s) produced from hyperstimulated follicles which mediates directly a negative feedback of LH secretion (Ferraretti *et al.*, 1983) or interferes with estrogen-mediated positive feedback (Littman and Hodgen, 1984). The substance responsible for this effect was further shown to be a factor of ovarian origin with a short circulating half-life, since bilateral ovariectomy restored normal pitu-

itary responsiveness within 30 min. (Schenken and Hodgen, 1983). Therefore, it seems likely that a significant suppression of endogenous LH surge in superovulated rats is a reflection of hyperstimulation of multiple follicles by a superovulatory dose of PMSG. The factor(s) and its regulatory mechanism for the endogenous LH secretion in superovulated rats remain to be further clarified.

SUMMARY

Catheters were placed into the external jugular veins of immature female rats. On the following day (Day 28 of age), the animals were injected subcutaneously with pregnant mare serum gonadotropin (PMSG): 4IU (control) or 20IU (superovulation). Each animal was sequentially bled at 0hr and 12hr and subsequently at 6hr intervals until sacrifice at 72hr after PMSG.

The superovulatory dose of PMSG significantly ($P<0.05$) increased the ovulatory response by 4.7 fold and ovarian weight by 2.1 fold above controls. Serum luteinizing hormone (LH) levels were determined by radioimmunoassay. Both groups exhibited a similar time relationship with two distinct peaks: an initial slight rise at 0~18hr and a second sharp rise at 54~60hr. However, there was a marked change in the magnitude of LH levels between the two groups. In superovulated animals, prior to the second peak, overall LH levels were significantly ($P<0.001$) higher than controls. In contrast, at the peak occurring at 60hr, LH concentrations were significantly ($P<0.001$) reduced by 54% below that of control. Additionally, a maximum increase of mean ΔLH between the two peaks was much less in superovulated as compared to control rats.

This study defines two distinct LH peaks in PMSG-treated rats, and illustrates the possible association between ovarian hyperstimulation and attenuation of the endogenous LH surge after superovulatory treatment.

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