Role of Luteinizing Hormone-Releasing Factor (LH-RF) and LH on Maintenance of Early Gestation in Rats

TSUNEHISA MAKINO1, MASAKI SHIINA, SUSUMU NAKASHIMA1
RIHACHI IIZUKA1, BUN-ICHI TAMAOKI2 AND ROY O. GREEP3

1Department of Obstetrics and Gynecology, School of Medicine, Keio University, Shinjuku-ku, Tokyo 160, Japan, 2National Institute of Radiological Sciences, Chiba-shi 280, Japan and 3Laboratory of Human Reproduction and Reproductive Biology, Harvard Medical School, Boston, Massachusetts 02115, U.S.A.

Synopsis

Levels of serum progesterone (P) and LH were determined daily in pregnant rats from Day 4 through parturition. In other pregnant rats the effect of antisera to LH(LH-A/S) and to LRF(LRF-A/S) on serum P, LH and fetal development were determined. These animals were treated with antisera on Days 8 through 10. Samples of blood were obtained on Day 8 before treatment started and again on Days 11 and 15. The animals were sacrificed on Day 15 and examined for fetal development and the ovaries weighed.

In untreated pregnant rats, the level of serum P showed two peaks, one on Day 7 and the other on Day 16. Serum P dropped acutely shortly before delivery. Serum LH showed no significant peaks through pregnancy but fluctuated between 30 and 160 ng/ml.

In rats receiving 0.2 ml LH-A/S s.c. on Days 8-10, the serum P was reduced to nondetectable levels on Day 11 and had recovered only slightly by Day 15. Pregnancy was terminated in all instances and the ovarian weights were reduced. Lower doses of LH-A/S were ineffective. Rats receiving 1.3 ml of LRF-A/S on Days 8-10 showed no effect on serum P, or LH and no injurious action on fetal development. Lesser amounts of this LRF-A/S (0.8 ml) were known to block the proestrous gonadotropin surge and inhibit ovulation in cycling rats. These findings confirm that LH is an essential component of the luteotropic complex during early pregnancy in the rat. They also suggest that a basal of LH and P can be to some extent maintained in rats treated with LRF-A/S during early gestation.

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Address reprint requests to: Dr. Tsunehisa Makino, Department of Obstetrics and Gynecology, School of Medicine, Keio University, 35 Shinanomachi, Shinjuku-ku, Tokyo 160, Japan.

It is well known that the levels of estrogen and progesterone secretion throughout the rat estrous cycle depend on the plasma level of gonadotropins. Luteinizing hormone (LH) has been demonstrated to induce a significant acute increase in net progesterone synthesis of rat ovaries in vitro and in vivo (Armstrong et al., 1964; Armstrong, 1968; Major et al., 1967). By employing LH-antiserum (LH-A/S) as a useful immunobiological tool, the luteotropic action of LH...
has been clearly demonstrated from the day of ovo-implantion to Day 11 of pregnancy, and during lactation (Hayashida and Young, 1963; Madhwa Raj and Moudgal, 1970; Yoshinaga et al., 1971).

Since our previous study (Makino et al., 1973; Makino et al., 1974) demonstrated that the administration of the specific antiseraum for LH-releasing factor (LRF-A/S) significantly decreased serum level of LH during the critical period of proestrous rats and blocked ovulation on the next estrous morning, it was of interest to investigate by utilizing this immunobiological technique how the hypothalamo-pituitary function influenced blood progesterone levels and ovo-development during early gestation of rats. This study was designed to reduce serum LH and/or progesterone levels by administration of different amounts of LRF-A/S and LH-A/S in pregnant rats and then to examine the effects of the treatment on the development of implanted embryos.

Materials and Methods

Animals.

Eighty- to 90-day-old cycling female rats of Sprague-Dawley strain were given Purina chow and water ad libitum, and maintained under artificial lighting 14 hours and 10 hours of darkness. At least, two estrous cycles were checked before the females were caged with the males. The day on which sperm was found in the vagina was designated as Day 1 of pregnancy. Blood samples were collected daily from tail veins of 4-7 untreated pregnant rats under light ether anesthesia (10:00-12:00 a.m.) from Day 4 of pregnancy to the day of parturition. Another 49 pregnant rats were distributed into 7 groups and the blood samples were collected on Day 8 of pregnancy. Beginning on Day 8 these rats were injected for 3 days either with normal rabbit serum (NRS), 4 different doses of LH antiserum or 2 different doses of LRF antiserum. Normal rabbit serum and LH were injected subcutaneously and LRF antiserum was injected intravenously. On Day 11 of pregnancy blood samples were again collected. On Day 15 the rats were sacrificed by decapitation, the blood collected and each reproductive organ examined. All blood samples were allowed to clot at 4°C; the serum was removed and kept frozen at -20°C until assayed.

Preparation and characterization of LRF-A/S and LH-A/S.

The production of LRF-A/S and its immunobiological character were reported earlier (Makino et al., 1973; Makino et al., 1974; Takahashi et al., 1975). Synthetic LRF was deamidated without significant peptide bond cleavage by incubation in 0.5 N HCl solution with continuous stirring for 24 hours at 23°C. The deamidated LRF was then conjugated to bovine serum albumin (BSA, Sigma, Cohn fraction V) by the methods described in our previous reports (Makino et al., 1973 and 1974; Takahashi et al., 1975). New Zealand female rabbits (4-7 months old) were immunized by repeated dorsal intradermal injections of LRF-BSA conjugate emulsified in Freund's complete adjuvant (Difco). Three booster injections were given at 2-week intervals before blood was collected from the ear vein. The antiserum at different dilutions and 125I-labelled-LRF (15,000 cpm/tube, specific activity 140-160 ƒÊCi/ƒÊg) were incubated in 0.2 M Tris acetate buffer (pH 7.3) at 4°C. Thirty to 40% binding between these reagents was obtained at 1:3,000 dilution of the antiserum. Rat LH and FSH, substance P, oxytocin, lysine- and arginine-vasopressin, and synthetic TRF showed less than 0.03% displacement of LRF under the employed conditions. The minimal i.v. dose of LRF-A/S needed to inhibit ovulation in 4-day cycling female rats was 800 ƒÊl.

The procedure for preparing LH-A/S was identical with that reported earlier (Makino et al., 1972). New Zealand white female rabbits were immunized with ovine luteinizing hormone (NIH-LH-S17). Non-specific antibodies were removed with 1:10 diluted normal sheep serum and the LH-A/S was characterized. The antibody content of A/S as determined by the quantitative precipitin test was between 1 and 2 mg/ml of the serum. The diluted LH-A/S was also incubated with 125I-labelled ovine LH (specific activity 374.7 ƒÊCi/µg, 15,000-20,000 cpm) at 4°C to examine the specific binding to ovine LH. The biological ability of this LH-A/S to neutralize endogenous LH was tested by determining the minimum dose that would induce resorption of fetuses when given on the eighth day of pregnancy. The minimal dose of LH-A/S which consistently terminated pregnancy was 200 ƒÊl. This LH-A/S reacted with rat LH but did not show cross-reaction with sheep or rat prolactin by Ouchterlony agar gel double diffusion test.

Radioimmunoassay of rat LH.

Two ƒÊg of purified rat LH (NIAMD-rat LH-I-1) was radioiodinated with 1.5 mCi of 125I by a modified method of Greenwood et al. (1963). After 4 days
incubation at 4°C, the antigen-antibody complex was separated by adding excess amount of anti rabbit γ-globulin. The serum LH values were expressed in terms of ng of NIAMD-rat-LH RP-1 (biological potency=0.03×NIH-LH-S1 by OAAD assay).

Radioimmunoassay of progesterone.

One half ml of serum samples were extracted with 3 volumes of petroleum ether by constant shaking for 20 minutes at 4°C. After centrifuging, the upper phase was evaporated to dryness under a stream of nitrogen. The residue was dissolved into 0.3 ml of iso-octane, benzene and methanol (90:5:5, v/v, solvent I) and chromatographed through Sephadex LH-20 column (0.9×15 cm) by elution with solvent I.

The first 3-ml fraction contains cholesterol and the second 3-ml fraction contains progesterone. Tracer amounts of 3H-progesterone were used to correct for recovery. The standard steroid or 50 μl of this second fraction was incubated with 3H-labelled progesterone and antibody for 2-24 hours at 4°C. The antigen-antibody complex was separated by adding 1 ml of charcoal suspension (1:10 weight/0.01 M phosphate buffer, pH 7.4) and radioactivity was counted in a liquid scintillation spectrometer.

Student’s t test was used to examine the level of significance of difference between the groups.

Results

Progesterone levels in intact pregnant rats from Day 4 to parturition are shown in Fig. 1. The range of serum progesterone was 50 to 250 ng/ml serum. Two peaks of this steroid were observed; the first was on Day 7 and the second on Day 16. The latter was statistically significant as compared to the value at mid-pregnancy on Day 11 (p<0.025). Serum progesterone dropped acutely just before parturition. The mean values of serum LH concentrations from Day 4 to parturition were between 30 and 160 ng/ml (Fig. 2). Serum progesterone was reduced to an undetectable level on Day 11 by the subcutaneous injection of 0.2 ml of LH-A/S on Days 8 to 10 (Fig. 3); although it was again detectable on Day 15 the value was extremely low. Complete fetal absorption of implanted embryos was observed in all animals. In contrast, 0.025 ml to 0.1 ml of LH-A/S failed to decrease serum progesterone significantly as compared to the NRS treated control group. The serum progesterone in these three groups was also higher on Day 15 than on Day 8 and 11. Serum LH was technically immeasurable in the group treated with LH-A/S. Ovarian weights of Day 15 were also markedly decreased but weights of the pituitary glands were not significantly changed (Table 3). Intravenous administration of large amounts of LRF-A/S (0.8 ml and 1.3 ml) for three days did not change serum progesterone concentration (Fig. 4) and induced no significant effect on fetal devel-
Table 1. Effects of LH-A/S and LRF-A/S on rat fetal development.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>No. animals</th>
<th>Implantation/No. C. L. (R)</th>
<th>(L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>(NRS, 0.2 ml on Day 9–10) (7)</td>
<td>5.2±0.2/6.6±0.4*</td>
<td>5.4±0.5/6.2±0.5*</td>
</tr>
<tr>
<td>LH-A/S</td>
<td>(0.2 ml on Day 8–10) (7)</td>
<td>0±0/5.1±0.8</td>
<td>0±0/5.0±0.5</td>
</tr>
<tr>
<td>LRF-A/S</td>
<td>(1.3 ml on Day 8–10) (7)</td>
<td>4.7±0.7/5.9±0.9</td>
<td>4.3±0.7/5.6±0.9</td>
</tr>
</tbody>
</table>

* Mean±S.D.

Table 2. Effects of prior administration of LH-A/S and LRF-A/S on rat serum LH on Day 15 of pregnancy.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>No. animals</th>
<th>Serum LH (ng/ml serum)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>(NRS, 0.2 ml on Day 8–10) (7)</td>
<td>55.4±18.0*</td>
</tr>
<tr>
<td>LH-A/S</td>
<td>(0.2 ml on Day 8–10) (7)</td>
<td>Unmeasurable</td>
</tr>
<tr>
<td>LRF-A/S</td>
<td>(1.3 ml on Day 8–10) (7)</td>
<td>51.5±18.7</td>
</tr>
</tbody>
</table>

Reference Preparation= NIAMD Rat LH-RP-1
* Means±S.D.

Table 3. Effects of administering LH-A/S or LRF-A/S on Days 8, 9 and 10 of pregnancy on the wet weights of the ovary and anterior pituitary on Day 15.

<table>
<thead>
<tr>
<th>Group</th>
<th>No. animals</th>
<th>Ant. pit. (mg) (R)</th>
<th>Ovary (mg) (L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>(NRS, 0.2 ml on Day 8–10) (7)</td>
<td>9.02±0.14*</td>
<td>42.25±5.58*</td>
</tr>
<tr>
<td>LH-A/S</td>
<td>(0.2 ml on Day 8–10) (7)</td>
<td>9.52±0.15</td>
<td>30.63±0.04</td>
</tr>
<tr>
<td>LRF-A/S</td>
<td>(1.3 ml on Day 8–10) (7)</td>
<td>9.34±0.95</td>
<td>40.54±0.08</td>
</tr>
</tbody>
</table>

* Mean±S.D.
Development as shown in Table 1. Similarly, thirteen hundreds μl of undiluted antiserum to LRF injected intravenously did not alter serum LH levels on Day 15 as compared to those of the control group (Table 2).

Discussion

Our previous report demonstrated that antiserum to LRF generated in female rabbit by LRF-BSA conjugate was specific to synthetic LRF and applicable to a radioimmunoassay for LRF (Makino et al., 1973 and 1974; Takahashi et al., 1975). This antiserum inhibited ovulation on the day of proestrous in mature cycling female rats (Makino et al., 1973) and postpartum ovulation was also blocked by treatment with LRF-A/S beginning on Day 20 of pregnancy (Ford et al., 1975). Since LRF-A/S interrupts LH and FSH surges in cycling and postpartum rats (Makino et al., 1974; Ford et al., 1975; Koch et al., 1973; Arimura et al., 1974), presumably by immunobiological neutralization of endogenous LRF, it was of interest to observe serum levels of LH in pregnant rats after treatment with LRF-A/S. Serum LH concentrations from Day 4 of pregnancy to parturition were between 30 and 160 ng/ml. No significant peaks were observed indicating that the anterior pituitary secretes a basal level of LH during pregnancy. This is in good agreement with the report by other investigators, except that we did not observe a gradual increase of serum LH levels after Day 21 of gestation (Linkie and Niswender, 1972). Our data also show that treatment with LRF-A/S on Days 8 to 10 of gestation failed to alter serum LH concentration as compared to that of NRS treated control animals; serum progesterone in the LRF-A/S-treated animals also showed no marked changes and normal fetal development was observed on Day 15. This is of interest in terms of termination of pregnancy as compared to another report (Nishi et al., 1976) and different biological potency of LRF-A/S may draw these different results.

In contrast, small amounts of LH-A/S lowered or diminished serum progesterone levels in pregnant rats when administered between Days 8 and 10. Fetal resorption was observed consistently in these animals, confirming the previous report by Madhwa Raj et al. (1970). Although serum LH was not detectable in LH-A/S-treated rats by radioimmunoassay, our data indicate that considerably reduced levels of serum LH can maintain serum progesterone levels; 0.025 ml to 0.1 ml LH-A/S which neutralized part of the endogenous LH, did not alter the serum progesterone level as shown in Fig. 3.

It can be estimated from the specific activity of 125I-LRF and its binding ratio with the diluted antiserum that one μl of LRF-A/S can neutralize approximately one ng of LRF in vitro. Although only pg values of endogenous LRF have been reported in serum and there are ng amounts in the hypothalamus, it is hard to transfer this in vitro data directly to immunobiological phenomena in vivo, since endogenous LRF was immuno-technically imm measurable in LRF-A/S-treated rats. However, it appears likely that the biological activity of endogenous LRF is considerably inhibited by large amounts of exogenously administered LRF-A/S.

Our previous data (Makino et al., 1974) and those of others (Ford et al., 1975; Koch et al., 1974; Arimura et al., 1974) have demonstrated that LH and FSH surges in proestrous and postpartum rats can be inhibited by LRF-A/S. It is of interest that these investigators have demonstrated that the basal levels of serum gonadotropins were maintained during and after LH and FSH surges in non-pregnant rats treated with LRF-A/S. Furthermore, basal levels of LH were detectable in rats and rabbits actively immunized against LRF (Fraser et al., 1974;
Shiina, 1976). Ford et al. (1975) demonstrated that normal parturition was also observed in pregnant rats treated with LRF-A/S on Days 20 to 23, indicating that normal labor did not require endogenous LRF action. Our present data confirm again that the basal level of endogenous LH may play an important role as one component of the luteotropic complex during the early stages of gestation in the rat. These results also suggest that the basal level of serum LH, and presumably FSH also, was maintained in early gestation even after considerable amounts of endogenous LRF were neutralized by LRF-A/S.

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References