PROGESTERONE LEVELS AFTER INDUCTION OF OVULATION IN DIOESTROUS RATS

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SUMMARY

Maximal levels of progesterone in the plasma after premature ovulation induced by either the administration of human chorionic gonadotrophin (HCG) or LH-releasing hormone (LH-RH) to dioestrous (day 0) rats were observed from 33 to 45 h but decreased 3 h earlier than after spontaneous ovulation. This suggested an earlier decline in the secretory activity of corpora lutea formed from premature ovulations than that of corpora lutea formed during a normal oestrous cycle.

The next spontaneous ovulation occurred 4 days (day 5) after premature ovulation induced by LH-RH on day 0. A single s.c. injection of 2.5 μg oestradiol-17β (OE2) at 10.00 h on day 2 to these animals advanced the next spontaneous ovulation by 1 day. A normal number of oocytes was shed, indicating that earlier secretion of oestrogen on day 2 had advanced the next spontaneous ovulation. A single injection of 2.5 μg OE2 to normal 4-day cyclic rats at metoestrus failed to advance the next ovulation. An earlier decline of progesterone levels in the plasma of rats after premature ovulation as compared with spontaneous ovulation may explain the greater effectiveness of oestrogen in the former group.

The progesterone surge was observed during the period of premature ovulation in both HCG- and LH-RH-treated groups. This progesterone release in the periovulatory period may be responsible for the inhibition of gonadotrophin surges on the expected day of pro-oestrus (day 1).

INTRODUCTION

A selective surge of FSH occurs in dioestrous rats during the period of premature ovulation induced either by human chorionic gonadotrophin (HCG) or luteinizing hormone releasing hormone (LH-RH; Sasamoto, Harada & Taya, 1977, 1979) and is thought to be responsible for the initiation of follicular maturation of the new set of follicles for the succeeding oestrous cycle (Schwartz, 1969; Welschen & Dullaart, 1976). When premature ovulation is induced by LH-RH in dioestrous rats, the next spontaneous ovulation occurs 4 days later. After premature ovulation induced by HCG in dioestrous rats, however, the next spontaneous ovulation occurs 3 days later, probably because of the advancement of oestrogen secretion by 1 day in the succeeding oestrous cycle in HCG-treated animals (Sasamoto et al. 1979).

The present study is concerned with the role of oestrogen and progesterone in determining the day of the next spontaneous ovulation after premature ovulation in dioestrous rats. Secretion of progesterone during the period of premature ovulation was also examined.

MATERIALS AND METHODS

Virgin female rats of Wistar strain, weighing 200–280 g, were used after showing at least three consecutive 4-day oestrous cycles. The cycles were monitored by daily vaginal smears.

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The animals were maintained on a schedule of 14 h light : 10 h darkness (lights on at 05.00 h).

Stock solutions of HCG (2200 i.u./mg; Sankyo Zoki Co., Tokyo, Japan) and LH-RH (Takeda Chemical Industries Ltd, Osaka, Japan) were prepared as described previously (Sasamoto et al. 1979). To induce premature ovulation, rats were given 10 i.u. HCG i.v. at 17.00 h or 1 μg LH-RH i. v. three times at intervals of 1 h from 17.00 to 19.00 h on the day of dioestrus (day 0). To examine progesterone levels in the plasma, animals were killed at various times after the administration of HCG or LH-RH by bleeding from the abdominal aorta whilst under ether anaesthesia using a heparinized syringe. Normal 4-day cyclic rats were also bled in a similar manner at various times from oestrus to dioestrus to determine plasma levels of progesterone. Individual plasma samples were obtained after centrifugation and stored at −20 °C until assayed.

To examine the role of oestrogen in the advancement of the next spontaneous ovulation, animals treated with LH-RH on day 0 were given a single s.c. injection of 2·5 μg oestradiol-17β (OE2; Sankyo Zoki Co.) in 0·2 ml sesame oil at 10.00 h on day 2. Changes in the vaginal smears of these animals were noted and the rats killed on the morning of day 4, the expected day of pro-oestrus. The oviducts were examined for the presence of oocytes by the method of Burdick & Whitney (1941). As a control experiment, normal 4-day cyclic rats were also given 2·5 μg OE2 s.c. at 10.00 h on the day of metoestrus and killed on the morning of the expected day of pro-oestrus to check for the presence of oocytes in the oviduct.

**Radioimmunoassay for progesterone**

An antiserum to progesterone-3-oxime-bovine-serum albumin (14-5(AL-1)), obtained from Teikoku Zoki Pharmaceutical Co., Kawasaki, Japan, was used as a 1:18 000 dilution in gelatin buffer (0·1 M-phosphate buffer (pH 7·0) containing 0·9% NaCl solution and gelatin (1 g/l; Merck, Darmstadt, Germany)). The specificity of this antiserum to progesterone has been described previously (Makino, 1973). [1,2-3H]Progesterone (55-7 Ci/mmol; New England Nuclear Co., Boston, U.S.A.) was dissolved in gelatin buffer to give about 2000 counts/min per 0·1 ml. Dextran (ICN Pharmaceutical Inc., Cleveland, U.S.A., mol. wt, 15 000–20 000; 1 g/100 ml)-coated charcoal (2 g/l) was prepared as a suspension in gelatin buffer.

Plasma samples (0·1 or 0·2 ml) were adjusted to a final volume of 0·4 ml by the addition of distilled water. The plasma was extracted with 4 ml hexane (Wako Pure Chemical Industries Ltd, Osaka, Japan) and 0·2 and 0·1 ml of the extracts were prepared in duplicate for the assay. Progesterone was measured using the radioimmunoassay procedures of de Villa, Kenneth, Wiest, Mikhail & Flickinger (1972). The lower limit of sensitivity was 12·5 pg/tube. At mean concentrations of 29·25 ± 2·45 (s.e.m.) nmol/l plasma at 11.00 h on pro-oestrus, intra- and interassay coefficients of variation were 6·0 and 19·8% (n = 6) respectively. At those of 94·90 ± 9·61 nmol/l plasma at 20.00 h on pro-oestrus, intra- and interassay coefficients of variation were 4·6 and 23·0% (n = 5) respectively.

Significance of differences between two means was determined by Student’s t-test, but when more than two means were compared, an analysis of variance was carried out and the significance of difference between means was determined by Duncan’s new multiple range test (Steel & Torrie, 1960).

**RESULTS**

**Secretion of progesterone from day 2 to 3 after premature ovulation or from metoestrus to dioestrus after spontaneous ovulation**

Changes of progesterone levels in the plasma from the evening of oestrus until the morning of dioestrus in five normal 4-day cyclic rats are shown in Fig. 1a. Plasma concentrations of progesterone began to increase on the day of metoestrus and reached the maximal values of
78.16 ± 5.35 nmol/l at 05.00 h of dioestrus. This was followed by a marked decrease to 20.40 ± 3.10 nmol/l by 11.00 h on the day of dioestrus.

As shown in Fig. 1b and c, changes in plasma concentrations of progesterone from the morning of day 2 to the morning of day 3 in the HCG-treated group were similar to those in the LH-RH-treated group. In both HCG- and LH-RH-treated groups (n = 5 in both groups), plasma concentrations of progesterone began to increase on the afternoon of day 2 and reached plateau levels of 55.34 ± 3.71 or 59.10 ± 3.94 nmol/l by 17.00 h on day 2 respectively. These levels were maintained during the next 12 h and then declined to basal levels at 11.00 h on day 3 (HCG-treated group, 24.56 ± 3.24 nmol/l; LH-RH-treated group, 23.80 ± 4.44 nmol/l). Furthermore, the changes in progesterone levels from 11.00 h on day 2 until 11.00 h on day 3 in both HCG- and LH-RH-treated groups were similar to the changes

Fig. 1. Concentrations of progesterone in the plasma of (a) normal cyclic rats and of female rats induced to ovulate by (b) human chorionic gonadotrophin (HCG), or (c) LH-releasing hormone (LH-RH). An injection of HCG (10 i.u.) was given i.v. at 17.00 h of dioestrus (day 0) and LH-RH (1 µg) was given i.v. three times at intervals of 1 h from 17.00 to 19.00 h of day 0. All points are the mean ± S.E.M. of five observations.
occurring in intact rats from 11.00 h of metoestrus until 11.00 h of dioestrus. With one exception, the values at 05.00 h on day 3 after HCG (51.54±4.22 nmol/l) or LH-RH (51.58±4.27 nmol/l) were significantly lower (P<0.01) than those found at 05.00 h of dioestrus in intact rats (78.16±5.35 nmol/l).

**Effects of injection of OE₂ on the advancement of ovulation**

When 2.5 µg OE₂ was given s.c. to four rats at 10.00 h on day 2 after premature ovulation induced by LH-RH on day 0, all rats ovulated on day 4 (24 h before the expected day of the next spontaneous ovulation), with comparable numbers of oocytes to those found in normal spontaneous ovulation (Table 1). A single s.c. injection of the same amount of OE₂ given to normal cyclic rats at metoestrus could not, however, advance their next ovulation, although vaginal smears of the pro-oestrous type were noted the next morning (expected day of dioestrus) and these were followed by an oestrous smear on the expected day of pro-oestrus.

Table 1. Effect of 2.5 µg oestradiol-17β (OE₂) given s.c. at 10.00 h on the advancement of ovulation after premature ovulation induced by LH-releasing hormone (LH-RH) given to rats on the day of dioestrus (day 0). (Values are mean±S.E.M.)

<table>
<thead>
<tr>
<th>Treatment day</th>
<th>LH-RH*</th>
<th>OE₂</th>
<th>Day of autopsy</th>
<th>Rats ovulating/ rats examined</th>
<th>No. of oocytes in ovulating rats</th>
</tr>
</thead>
<tbody>
<tr>
<td>None (control)</td>
<td>None</td>
<td>Metoestrus</td>
<td>Expected pro-oestrus</td>
<td>0/5</td>
<td>—</td>
</tr>
<tr>
<td>None (control)</td>
<td>None</td>
<td>Metoestrus</td>
<td>Expected pro-oestrus</td>
<td>5/5</td>
<td>12.8±1.0</td>
</tr>
<tr>
<td>Dioestrus (day 0)</td>
<td>Dioestrus</td>
<td>Day 2</td>
<td>Day 4</td>
<td>4/4</td>
<td>14.0±0.6</td>
</tr>
<tr>
<td>Dioestrus (day 0)</td>
<td>—</td>
<td>Day 4</td>
<td>0/5</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Dioestrus (day 0)</td>
<td>—</td>
<td>Day 5</td>
<td>5/5</td>
<td>13.6±1.0†</td>
<td>—</td>
</tr>
</tbody>
</table>

* LH-RH (1.0 µg) was given i.v. three times at intervals of 1 h from 17.00 to 19.00 h on day 0. Premature ovulation occurred the following morning.
† Results from a previous experiment (Sasamoto et al. 1979).

**Secretion of progesterone during the period of premature ovulation**

Changes in plasma levels of progesterone after i.v. administration of either HCG or LH-RH on day 0 are shown in Fig. 1b and c. Plasma progesterone increased threefold within 3 h of the injection of HCG but this was followed by a small decline at 23.00 h. These high levels were maintained until 02.00 h on the next morning. Thereafter plasma levels of progesterone gradually decreased to basal levels which were reached by 08.00 h on day 1. After injection of LH-RH, plasma levels of progesterone increased twofold by 20.00 h on day 0 and increased levels persisted until 02.00 h on day 1, followed by a decline to basal values 3 h later. Plasma concentrations of progesterone of HCG-treated rats were significantly greater than those of LH-RH-treated animals at 20.00 h (P<0.01) and 23.00 h (P<0.01) on day 0 and at 02.00 (P<0.01), 05.00 (P<0.01) and 11.00 h (P<0.05) on day 1.

**DISCUSSION**

Changes in plasma levels of progesterone from metoestrus to dioestrus are thought to be derived from the changes in the secretory activity of the set of corpora lutea formed by the most recent ovulation (Uchida, Kadowaki & Miyake, 1969; Smith, Freeman & Neill, 1975). In both the HCG- and LH-RH-treated groups (Fig. 1b and c), plasma concentrations of progesterone also began to increase on day 2 (1 day after premature ovulation) and fell on
the morning of day 3 (2 days after premature ovulation). This demonstrated the capacity of corpora lutea formed by premature ovulation to secrete progesterone.

In contrast with progesterone levels in the plasma, a larger amount of oestrogen seemed to be secreted on day 2 in the rats induced to ovulate by HCG than in those treated with LH-RH as inferred from changes in uterine weight (Sasamoto et al. 1979). Table 1 shows that a single s.c. injection of 2.5 µg OE₂ on the morning of day 2 advanced the next spontaneous ovulation by 1 day with normal numbers of oocytes being present in the animals induced to ovulate by LH-RH on day 0. These results indicated that administration of oestrogen on day 2 advanced the next spontaneous ovulation after induced premature ovulation in rats.

Although a single injection of 2.5 µg OE₂ on day 2 (the day after premature ovulation induced by LH-RH) invariably advanced the next spontaneous ovulation by 1 day, the same amount of OE₂ given on the day of metoestrus (the day after spontaneous ovulation) to 4-day cyclic rats did not have the same effect (Table 1). Previous studies indicate that injection of oestradiol benzoate on the day of metoestrous advances ovulation by 1 day in some 4-day cyclic rats, producing a small number of oocytes (Brown-Grant, 1969; Krey & Everett, 1973). When OE₂ (200 µg) was given on the day of metoestrous, seven out of 16 rats ovulated with four oocytes (mean) on the expected day of pro-oestrous (Kobayashi, Hara & Miyake, 1971). The present results indicated that either an endogenous or exogenous source of oestrogen advances the next spontaneous ovulation more effectively after premature ovulation than after normal spontaneous ovulation.

Under present conditions, ovulation in our colony of Wistar rats ends by 05.00 h on the morning of oestrus (Sasamoto & Johke, 1975). As shown in Fig. 1a, the increase of progesterone in the plasma of normal cyclic rats continued until 05.00 h of dioestrus (48 h after the last ovulation). When animals were treated with either HCG or LH-RH on day 0, premature ovulation was completed by 08.00 h on day 1. Though maximal levels of progesterone in the plasma after premature ovulation were maintained up to 05.00 h on day 3 (45 h after premature ovulation) in either HCG- or LH-RH-treated groups, the values at this time were significantly lower than those of intact rats at 05.00 h of dioestrus (48 h after spontaneous ovulation). Six hours later a marked decrease was observed in all the groups. These results suggest that in 4-day cyclic rats progesterone secretion by corpora lutea formed from premature ovulation may decline at least 3 h earlier than the secretion of corpora lutea resulting from spontaneous ovulation. This earlier decline of progesterone secretion after premature ovulation might be responsible for the greater effectiveness of oestrogen in advancing the next spontaneous ovulation. The measurement of levels of OE₂ and progesterone in the serum of 4- and 5-day cyclic rats (Nequin, Alvarez & Schwartz, 1979) supports this assumption; these authors reported that while OE₂ levels begin to increase at approximately the same time in both types of rat, prolongation of secretion of progesterone occurs in the 5-day cyclic rat. This prolonged secretion of progesterone is thought to be involved in the delay of ovulation by 24 h.

As shown in Fig. 1b and c, a large amount of progesterone was released during the period of premature ovulation, though no increase in concentrations of progesterone in the plasma was noted during the night between dioestrus and pro-oestrus in normal 4-day cyclic rats (Horikoshi & Suzuki, 1974; Smith et al. 1975; Nequin et al. 1979). This progesterone surge during the period of premature ovulation may itself inhibit the gonadotrophin surge on the following afternoon of the expected day of pro-oestrous, as suggested by Freeman, Dupke & Croteau (1976).

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