ROLE OF THE PITUITARY AND CONCEPTUSES IN THE
REGULATION OF THE LUTEAL STEROIDOGENESIS
IN THE MID-PREGNANT RAT

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Synopsis  Effects of hypophysectomy or reduction of the conceptus number on luteal steroidogenesis were studied in the pregnant rat. On day 7 of pregnancy (day 1 = day of insemination), the number of conceptuses was adjusted to one by aspirating all but one conceptus from the uterus. Another group of rats received hypophysectomy on day 12 of pregnancy. On day 15 of pregnancy, the weights of the corpora lutea (CL), serum progesterone levels, and luteal concentrations of cholesterol, pregnenolone, and progesterone were determined. The aspiration group showed significant a decrease in the weight of the CL and in the serum progesterone level compared with those of the hypophysectomy or control group. However, there were no significant changes in the luteal concentrations of cholesterol, pregnenolone or progesterone. On the other hand, hypophysectomy caused a significant decrease in the luteal concentration of cholesterol, whereas no change was observed in the weight of the CL, serum progesterone level or the concentrations of pregnenolone or progesterone in the CL.

These results indicated that in mid-pregnancy the pituitary regulated the uptake or storage of cholesterol of the CL, while the placental hormones regulated the serum progesterone level mainly by affecting the growth of the CL.

Key words:  Rat • Pregnancy • Cholesterol • Pregnenolone • Progesterone

Introduction

In pregnant rats, the luteal function is mainly maintained by the placental hormones during the second half of pregnancy[10]. It is well known that rapid increases in the growth of the corpora lutea (CL) and the progesterone secretion begin after day 12 of pregnancy[11,14], and both the rate of progesterone secretion and the growth of the CL on day 15 of pregnancy are directly proportional to the number of conceptuses in rats[11]. However, it is not clear at present how the placental hormones influence the luteal function.

On the other hand, there is some evidence that the pituitary also affects the luteal function after day 12 of pregnancy in rats. In vitro study, Toyoshima found in pregnant rats that LH could stimulate the estrogen production of the CL on day 15 of pregnancy, which in turn stimulated the progesterone production[11,22]. Rodway et al. showed that the CL function could be maintained by the presence of prolactin and estrogen after day 12 of pregnancy in rats[19]. Further, the pituitary, probably prolactin, also stimulated cholesterol accumulation in the CL[13-15], which is essential for membrane biosynthesis as well as for progesterone production. Although there is some contradictory evidence that the pituitary would exert the luteolytic effects during the mid-pregnancy in rats[48], it is reasonable to suppose that both the placental hormones and the pituitary play important roles in the regulation of the luteal function during the second half of pregnancy in rats. In order to investigate how the pituitary and the placental hormones influence the luteal function, the present study was conducted in order to test the effects of hypophysectomy or the reduction of the placental hormones on the steroidogenesis of the CL in pregnant rats.

Material and Methods

Animals and treatment:
Sprague-Dawley female rats, weighing 210 to 280 g., were housed with free access to ordinary rat chow and water. Day 1 of pregnancy was taken as the day that sperm was found in the vaginal smear. Pregnancy was confirmed at laparotomy on day 7 by the presence of embryonic swelling in the uterus. Ether was used as anesthesia for all operations.

Hypophysectomy was done by parapharyngeal approach on day 12 of pregnancy. Sham hypo-
physcctomy was done in exactly the same way but without aspirating the pituitary. Completeness of hypophysectomy was evaluated by observation of the pituitary removed at operation and by examination of the pituitary fossa at autopsy.

The number of conceptuses was adjusted to one on day 7 of pregnancy according to the method of Kato et al.\textsuperscript{11}. In other words, all of the conceptuses except the one nearest the left ovary were aspirated through a Pasteur pipette attached to a suction line. The sham aspiration group received an operation which was performed similarly but the aspiration was done between the embryonic swellings.

The control group consisted of 2 groups: One with sham hypophysectomy and another with sham aspiration. Since there were no significant differences in any parameters tested in this study between these 2 groups, they were combined and regarded as the control group.

All the rats were autopsied on day 15 of pregnancy. The ovaries were removed and the CL was separated from the remaining ovarian tissue. The isolated CLs were immediately weighed, homogenized by a glass-homogenizer, and extracted with a cold mixture of acetone: ethanol (1:1) in a concentration of 10mg wet tissue/mL\textsuperscript{13}. The homogenate was centrifuged at 1500\times g for 10 min at 4°C. Five hundred \( \mu l \) of the supernatant was placed into each tube and evaporated to dryness under a stream of nitrogen. These tubes were used for the determinations of cholesterol, pregnenolone and progesterone as described below.

Determination of cholesterol:

To each of the duplicate tubes which contained the CL extract were added 0.3ml of 33% (W/V) potassium hydroxide and 3ml of 95% ethanol. They were placed in a 60°C heating block for 15 min. After being extracted with a 10ml volume of hexane, cholesterol was determined according to the method of Rudel et al.\textsuperscript{20}.

Determination of pregnenolone and progesterone:

To each of the duplicate tubes which contained the CL extract was added 0.5ml of benzene: ethanol solution (benzene: ethanol = 97:3). The solution was applied on a 1\times12cm column of Sephadex LH-20, equilibrated and eluted with benzene: ethanol solution. The fractions containing pregnenolone were collected and pooled. Pregnenolone concentration was determined by radioimmunoassay as described by Nishida et al.\textsuperscript{19}, using the antiserum against pregnenolone-3-hemisuccinate-BSA which was kindly donated by Dr. M. Horino. Coefficients of variation of intra and interassay were 6.8% and 11.6%, respectively. Following acetone: ethanol extraction and chromatography, the recovery of \(^3\)H-pregnenolone was 64.5\pm 5.9%.

In order to determine progesterone concentration in the CL, 5ml of n-hexane was added to each tube which contained the CL extract. The tubes were centrifuged and 0.5ml of the supernatant was placed into a 1\times7cm tube and was evaporated under a stream of nitrogen. The determination of progesterone was performed in duplicate by a radioimmunoassay as described previously\textsuperscript{12}.

Blood samples were also obtained at autopsy, and serum progesterone levels were determined by radioimmunoassay\textsuperscript{12}.

Protein concentration was determined by the method of Lowry et al.\textsuperscript{13}.

Statistical evaluation:

The statistical significance between the experimental groups was evaluated by ANOVA and Duncan’s multiple range test. AP value of less than 0.05 was considered as significant.

Results

The weights of the CL and serum progesterone levels were significantly (p<0.05) lower in the aspiration group than in the control or hypophysectomy group (Fig. 1).

Fig. 2 shows the luteal concentrations of cholesterol, pregnenolone and progesterone in these 3 groups of rats. The hypophysectomy group showed a significantly (p<0.05) lower concentration of cholesterol than the other 2 groups of rats. There were no significant differences in the pregnenolone and progesterone concentrations between the hypophysectomy group and the control group.

Neither were there any significant differences in any concentration of cholesterol, pregnenolone or progesterone between the aspiration group and the control group. However, since the weights of the CL were significantly lower in the aspiration group (Fig. 1), the total contents of cholesterol or progesterone in the CL were significantly lower (p<0.05) in the aspiration group than in the control group (Table 1). The total content of pregnenolone was also lower, although not statistically significant, in the aspiration group than in the other 2 groups (Table 1).
Fig. 1. The weights of the CL and serum progesterone levels on day 15 of pregnancy in the control group, the hypophysectomy group and the aspiration group. The results are expressed as mean ± SEM, with the number of rats indicated in each bar.

*p<0.01, significantly lower than values of the control and hypophysectomy group.

**p<0.05, significantly lower than values of the control and hypophysectomy group.

Fig. 2. The luteal concentrations of cholesterol, pregnenolone and progesterone in the control group, the hypophysectomy group, and the aspiration group.

*p<0.05, significantly lower than values of the control and aspiration group.

Table 1. Total luteal contents of cholesterol, pregnenolone, and progesterone in the control group, the hypophysectomy group, and the aspiration group.

<table>
<thead>
<tr>
<th>Group</th>
<th>N</th>
<th>Cholesterol (µg/CL)</th>
<th>Pregnenolone (ng/CL)</th>
<th>Progesterone (ng/CL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>7</td>
<td>21.1±2.5</td>
<td>18.0±3.3</td>
<td>139±10</td>
</tr>
<tr>
<td>Hypophysectom</td>
<td>9</td>
<td>15.0±0.9*</td>
<td>16.3±1.9</td>
<td>149±13</td>
</tr>
<tr>
<td>Aspiration</td>
<td>5</td>
<td>13.8±0.7*</td>
<td>12.6±1.9</td>
<td>103±10**</td>
</tr>
</tbody>
</table>

*p<0.05, significantly lower than value of the control group.

**p<0.05, significantly lower than values of the control and hypophysectomy group.
Discussion

The present study showed that hypophysectomy significantly decreased the luteal concentration of cholesterol in pregnant rats, which confirmed previous findings in non-pregnant rats that cholesterol storage in the CL was reduced after hypophysectomy. Interestingly, hypophysectomy did not affect the growth of the CL or the production of progesterone in the present study. Armstrong noted that hypophysectomy in non-pregnant rats resulted in decreases in the circulating progesterone levels. Christie et al. also reported that treatment with 4-amino-pyrazolo-[3,4-d] pyrimidine, an inhibitor of hepatic secretion of lipoproteins, markedly decreased the plasma cholesterol levels, and resulted in a reduction of the luteal cholesterol concentration and serum progesterone levels in rats. The present results, i.e. that the hypophysectomy did not induce any decline in the circulating progesterone levels in pregnant rats, would indicate that although the cholesterol accumulation is regulated, at least in part, by the pituitary factor, the cholesterol in the CL appears to be further maintained by other factors than the pituitary, possibly the placental hormones. Several reports indicated that prolactin coluld maintain cholesterol concentration in the CL. The placenta secretes a large amount of rPL, which is a potent prolactin-like substance. Recently, Blank et al. reported that hypophysectomy increased the secretion of placental substances in the pregnant rat. It is likely that cholesterol in the CL is maintained by some placental hormones during the second half of pregnancy, particularly after hypophysectomy, resulting in the maintenance of cholesterol levels which are high enough to maintain the growth of the CL and the production of progesterone. The present study showed that hypophysectomy did not influence the concentrations of pregnenolone or progesterone. However, Taya et al. have shown that hypophysectomy causes significant decreases in the amounts of estradiol in the serum and in the CL after day 18 of pregnancy, but not on day 15. It is possible, therefore, that the declines in cholesterol on day 15 may be connected to some changes of steroidogenesis (e.g. the production of estradiol) after dar 18 of pregnancy.

The present study, which shows that the weights of the CL and the circulating progesterone levels are significantly lower in rats bearing a single conceptus than in those bearing a full complement of conceptuses confirms the findings of Kato et al. Surprisingly, the reduction of the placental hormones (e.g., the reduction of conceptus number in the present study) caused no changes in the concentrations of cholesterol, pregnenolone, and progesterone in the CL. The low progesterone levels in the circulation in the aspiration group would, therefore, seem to be mainly due to the small size of the CL in this group (Table 1).

On the other hand, we cannot exclude the possibility that the placental hormones may influence not only the growth of the CL but also the steroidogenesis in the CL. The rat's placenta secretes androgen into the general circulation, and androgen is readily converted to estrogen in the CL. A direct relationship has been demonstrated between the size of the CL and the circulating estrogen levels. Further, it is well documented that estrogen maintains 3β-HSD activity, but suppresses 20α-HSD activity, of the ovary in the pregnant rat. Estrogen also stimulates the progesterone production after day 12 of pregnancy in rats. These results suggest that the production rate of progesterone is also reduced in the aspiration group. It is not clear why there is no difference in the concentration of progesterone of the CL between the aspiration group and the control group.

One possible explanation may be that the greatest part of progesterone is released from the cells as soon as it is produced in the CL, so that we cannot find any significant decrease in the progesterone concentration of the CL in the aspiration group.

Acknowledgments

The author expresses deep appreciation to Prof. Tadashi Torigoe for his encouragement and to Dr. Hiroshi Kato for his appropriate advice. The author also thanks Prof. Masaharu Horino and Dr. Seikoh Nishida (Kawasaki Medical School) for the gift of the pregnenolone antiserum.

References


（Accepted: No. 5551, July 16, 1984）

概要 妊娠中期ラットの黄体機能は胎盤性ホルモンにより維持されている。一方妊娠中期内において下垂体は黄体機能を抑制するととの報告も認められる、そこで今回は、妊娠中期ラット黄体のステロイド産生に及ぼす胎盤性ホルモン及び下垂体の影響を検討した。

SD系ラットを用い、妊娠7日目にConception 1個を残し他を吸引除去したAspiration群、妊娠12日日に下垂体を摘出したHypophysectomy群、及びControl群を作成した。妊娠15日目に屠殺し、黄体重量、血中Progesterone値、及び黄体中Cholesterol、Pregnenolone、Progesterone量を測定した。Aspiration群では黄体重量、血中Progesterone値は有意に低下した。しかし黄体中のCholesterol、Pregnenolone、Progesterone量には有意差を認めなかった。一方Hypophysectomy群では黄体中のCholesterol量のみ有意に低下したが、黄体重量、血中Progesterone値、黄体中のPregnenolone、Progesterone量には有意差を認めなかった。

以上の結果より、妊娠中期ラットにおいて胎盤性ホルモンは主として黄体の発育に影響し、それに伴い血中Progesterone値を調節している事が示された。一方下垂体は黄体中のCholesterol量の調節に関与している事が明らかとなつたが、そのステロイド産生における意義については今後の検討が必要である。