Effects of Intra-ovarian Bursal Injection of Eicosanoid Synthesis Inhibitors on Luteal Function in Immature Pseudopregnant Rats

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Abstract. To investigate the role of ovarian endogenous eicosanoids in regulation of luteal function, we examined effects of local and chronic administration of eicosanoid synthesis inhibitors on plasma progesterone levels in immature pseudopregnant rats. Injections of drugs in 20 μ l oil were performed into the ovarian bursal cavity and repeated three times every 36 h. As compared with the control (oil-injected) group, the phospholipase A₂ inhibitor dexamethasone (DEX) at the dose of 62.5 μ g/ovary delayed functional luteolysis for 3 days by treatments from day 6 of pseudopregnancy (D6). Indomethacin (IND) also extended luteal activity for 5 days after treatment from D6, while nordihydroguaiaretic acid (NDGA) extended it for only 1 day. The extension of pseudopregnancy by each drug was not so evident with treatments from D2 or D4 as compared to that from D6. From D6, imidazole also delayed luteolysis, while a doubled dose of NDGA did not. Systemic administration of DEX or IND increased plasma progesterone levels more rapidly and significantly than local treatments but were less effective in delaying luteolysis. These results suggest that ovarian endogenous eicosanoids, mainly prostanoids, in the late luteal phase are involved in functional luteolysis in the present model.

Key words: Luteolysis, Dexamethasone, Indomethacin, Nordihydroguaiaretic acid, Rat.

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Eicosanoids exert a variety of biological activities including regulation of mammalian luteal function. In rats, there are two possible sources of eicosanoids relevant to luteal function; one is the uterus and the other is the corpus luteum itself [1, 2]. Previous studies show that *in vivo* levels of several prostanoids in the luteal tissue increased or maintained high levels during luteal regression in pseudopregnant rats [3–6], suggesting a role of luteal endogenous eicosanoids in luteolysis. However, one of those studies demonstrated a

concomitant increase in prostaglandin F_{2 α} (PGF_{2 α}) in the uterus [5]. The relative importance of PGF_{2 α} originating from these tissues in regulation of luteal function and lifespan has not been well clarified.

The effects of systemic administration of eicosanoid synthesis inhibitors on rat luteal function were investigated previously [7, 8]. Furthermore, the effect of intraluteal infusion of a prostaglandin synthesis inhibitor on the luteal function and lifespan was tested using rhesus monkeys [9]; however, such an examination has not been done in rats. In the present study, we examined effects of eicosanoid synthesis inhibitors on plas-

ma levels of progesterone in immature pseudopregnant rats by local administration of four kinds of inhibitors.

Materials and Methods

Reagents and animals

PMSG was purchased from Shionogi Co. (Osaka, Japan). Ovine prolactin and inhibitors of eicosanoid biosynthesis, dexamethasone (DEX), indomethacin (IND), imidazole (IMZ) and nordihydroguaiaretic acid (NDGA) were from Sigma (St. Louis, MO, USA). Immature female Wistar rats were kept in an air-conditioned room with controlled light regimens (lights on 0500 h–1900 h). Immature, 26 day-old rats were given 5 IU PMSG to induce ovulation and pseudopregnancy. By vaginal smear examinations, the animals exhibited proestrus on day 28; estrus and ovulation occurred on the 29th day. Thereafter, diestrus continued for 13.5 ± 0.5 (mean $\pm$ SEM, $n=12$) days. Therefore, the 30th day in age was defined as D1 (day 1 of pseudopregnancy) in this study. To ensure a functional corpus luteum, animals in the present model were given subcutaneously 10 IU ovine prolactin twice daily from D1 to D5.

Experimental procedures

Methods for local administration of drugs were as follows. Bilateral abdominal incisions were made under ether anesthesia. The ovarian bursa was exposed and ligated with thread across the oviduct but not across the blood vessels. Injections of drugs dissolved in sesame oil (20 μ l per side) were performed into the ovarian bursal cavity using a microliter syringe. This treatment started at 0700 h on D2, D4 or D6 and was repeated three times at 36 h intervals. During and after the second injections, any remaining vehicle was removed before applying a new one. As we confirmed that the control (oil injection) animal showed a similar progesterone secretion pattern and luteal lifespan as intact animals, the present experimental procedures, such as repeated abdominal operations and oviductal ligation, had no serious effects on luteal activity. Eicosanoid synthesis inhibitors were prepared at the standard concentrations of 62.5 μ g/ovary or 125 μ g/rat, which was chosen with reference to previous reports using systemically administered DEX or IND in immature rats [8, 10].

As the drugs were most effective in extending luteal function with treatment from D6, experiments of systemic (subcutaneous) treatment with DEX or IND at the same dose were undertaken from D6. Furthermore, experiments of local treatment with variable doses of drugs (indicated in the Results) were also undertaken on D6.

Progesterone assay and statistics

Systemic blood (about 200 μ l) was collected from the tail vein at noon every other day. Mean plasma progesterone levels were determined daily in one of the duplicate experimental groups for each treatment; that is, responses were documented on days 2, 4, 6, 8, 10, 12 and 14 in one group and in another group on days 3, 5, 7, 9, 11, 13. Plasma progesterone levels were determined by radioimmunoassay as described earlier [11]. The inter- and intra-assay coefficients of variances were 6–8% and 12–14%, respectively. All data were displayed as mean $\pm$ SEM ($n=6$). The means of different treatment groups from the same collection time were analysed by one-way ANOVA and least significant difference test. The level of significance was established to be $P<0.05$.

Results

Effects of DEX on progesterone secretion

The effects of DEX, a phospholipase A_2 inhibitor, on luteal activity of immature pseudopregnant rats are shown in Fig. 1. Local injection of DEX (62.5 μ g/ovary, 125 μ g/rat) from D2 induced no effect on plasma progesterone levels (Fig. 1-A). However, treatment from D4 tended to increase plasma progesterone as compared to the control group (Fig. 1-B). When the onset of treatment was delayed to D6, prolonged secretion of progesterone was evident, and half of that dose of DEX was sufficient to induce prolonged luteal function (Fig. 1-C). After systemic treatment with DEX (125 μ g/rat) from D6, plasma progesterone levels became significantly higher on D6 and D7 than those of control or locally injected groups and tended to decrease to the basal level earlier (Fig. 1-D).

Effects of IND on progesterone secretion

When the rats were locally treated with a cyclooxygenase inhibitor IND (62.5 μ g/ovary) from D2, they showed a similar pattern in progesterone

Fig. 1. Changes in plasma progesterone levels in immature pseudopregnant rats following intra-ovarian bursal (ib) or subcutaneous (sc) injections of DEX. The ordinate and abscissa show plasma progesterone levels (ng/ml) and day of pseudopregnancy, respectively. The injections of drug or vehicle are shown by the open circle (○) in each experiment. The doses of DEX in a single injection were 62.5 µg/ovary (125 µg/rat) and 31.25 µg/ovary (62.5 µg/rat) in ib injected groups and 125 µg/rat in sc injected groups. *: significantly different from the control group ($P < 0.05$). #: significantly different from the DEX (ib, 62.5 µg/ovary) group ($P < 0.05$).

secretion to that of control groups (Fig. 2-A). Treatment from D4 raised plasma steroid levels compared to controls (significantly different on D5, 7, 9, 10) (Fig. 2-B). Treatment from D6 brought about more prominent effects (Fig. 2-C). The experimental groups exhibited higher progesterone levels on D6, 8, 10, 12 and 13, and vigorous progestational activity was prolonged about 5 days. A half dose (31.25 µg/ovary) also delayed the decline in plasma progesterone. In animals injected subcutaneously with IND from D6, plasma progesterone was instantly increased on D6 and D7 as compared to control or intrabursally injected groups (Fig. 2-D). This treatment showed significantly higher progesterone levels by D11 than controls, but the decrease to the basal level oc-

curred earlier than locally injected groups.

Effects of NDGA on progesterone secretion

Similar to DEX or IND, local treatment with NDGA, a lipoxygenase inhibitor, from D2 made little difference in circulating progesterone levels (Fig. 3-A). Although its injection from D4 tended to increase the steroid level compared to control levels, a significant difference was observed only on D5 (Fig. 3-B). Treatment from D6 delayed the decrease in plasma progesterone to as late as D9, resulting in extension of the corpus luteum activity for only one day (Fig. 3-C). Doubling the dose of NDGA (125 µg/ovary) seemed to diminish such an effect.

Fig. 2. Changes in plasma progesterone levels in immature pseudopregnant rats following intra-ovarian bursal (ib) or subcutaneous (sc) injections of IND. Details are the same as in Fig. 1. The doses of IND in a single injection were 62.5 µg/ovary (125 µg/rat) and 31.25 µg/ovary (62.5 µg/rat) in ib injected groups and 125 µg/rat in sc injected groups.

*: significantly different from the control group ($P < 0.05$).

#: significantly different from the IND (ib, 62.5 µg/ovary) group ($P < 0.05$).

Effects of IMZ on progesterone secretion

The effect of administering IMZ (62.5 µg/ovary), a supposed inhibitor of thromboxane synthase, from D6 was further examined (Fig. 4). Rats in the experimental group showed a similar change in extended progesterone secretion as compared to the animals receiving the preceding inhibitors. Significant differences in the plasma steroid levels appeared on D8 and D10.

Discussion

The present study demonstrates that modulation of ovarian eicosanoid metabolism following local

and repeated treatments with their synthesis inhibitors resulted in the delay of luteolysis in immature pseudopregnant rats. These results suggest that ovarian endogenous eicosanoids are involved in regulation of rat luteal activity and lifespan.

First, DEX challenge at the late luteal phase (from D6 to D10) delayed luteolysis in the present animal model but not from D2 or D4. DEX is known to suppress arachidonate production through inhibition of phospholipase A₂ (PLA₂) activity [12, 13]. In a similar pseudopregnant model, microsomal PLA₂ activity, lipid peroxides level in the whole ovary [14, 15] and a 85 kDa cytosolic PLA₂ (cPLA₂) protein level in corpora lutea (Kurusu *et al.*, unpublished data) increased as the plasma

Fig. 3. Changes in plasma progesterone levels in immature pseudopregnant rats following intra-ovarian bursal (ib) injections of NDGA. Details are the same as Fig. 1. The doses of NDGA in a single injection were 62.5 μg/ovary (125 μg/rat) or 125 μg/ovary (250 μg/rat).

*: significantly different from the control group ($P < 0.05$).

Fig. 4. Changes in plasma progesterone levels in immature pseudopregnant rats following intra-ovarian bursal (ib) injection of IMZ on the day indicated by the open circle (). The dose of IMZ in a single injection was 62.5 μg/ovary (125 μg/rat).

*: significantly different from the control group ($P < 0.05$).

progesterone level fell. Consequently, DEX might have revealed these effects through inhibition of the increased PLA₂ activity and subsequent eicosanoids production. This was further supported by the results of experiments using IND or NDGA.

Both cyclooxygenase (IND) and lipoxygenase inhibitors (NDGA) delayed luteal regression by treatment from D6, and IND was more potent in delaying luteolysis than NDGA at the dose of 62.5 μg/ovary (125 μg/rat). Furthermore, the half dose of IND sustained the effect of extending active progestational activity, while the doubled dose of NDGA (125 μg/ovary) appeared to diminish the effect. In immature pseudopregnant rats without luteotropic stimulation by exogenous prolactin, luteal PGF_{2α} showed a temporal rise on D4–5 just before plasma progesterone began to fall on D6 [6]. In addition, luteal concentrations of PGF_{2α}, PGE₂ and 6-keto-PGF_{1α} (the stable metabolite of PGI₂) moderately increased or remained high during the luteal regression phase in adult pseudopregnant rats while declining in pregnant rats [3, 5]. Taken together, the increase or persistence in prostanoid biosynthesis in the late luteal phase is thought to be an important process in initiation of luteolysis. While both PGE₂ and PGI₂ are supposed to be luteotropic *in vitro* [16], the

overall effects of prostanoids during the late luteal phase would be luteolytic. The mechanism by which NDGA affects luteal activity seems to be complex. The reason for variations in the response to NDGA treatment is unclear. Because NDGA did not affect [17] or increased [18] arachidonate metabolites of the cyclooxygenase pathway, the high dose of NDGA, through inhibition of the lipoxygenase pathway, might induce a rise in the arachidonate pool available for prostanoid biosynthesis. Two kinds of eicosanoids of the lipoxygenase pathway are synthesized in human luteal cells and suppress its ability to secrete progesterone [19, 20]. Whether eicosanoids of the lipoxygenase pathway are involved in regulation of rat luteal function is obscure at present. But their luteolytic potencies, even if they have any, may be less than those of cyclooxygenase metabolites. In the present study, the thromboxane synthase inhibitor IMZ also induced a sustained secretion of progesterone but was less potent than DEX or IND at the dose of 62.5 μg /ovary as to the extent of the modulation of progesterone secretion. This suggests a possibility that luteal prostanoids, possibly other than thromboxanes, may be more potentially involved in mechanisms of luteolysis.

In accordance with previous reports [7, 8], systemic administration of DEX or IND prolonged luteal activity in the present study. According to Espey *et al.* [10], systemic injection of IND (0.1 mg/immature rat) decreased ovarian PGE and PGF contents to less than 10% in 5 h. While it is described that a single dose of IND can reduce PG levels in the entire body for at least 24 h [21], this drug disappears rapidly from circulation with a plasma half-life of about 6 h [22, 23]. Furthermore, in rabbits injected intravenously with 10 mg/kg IND (about 10 times higher than the present dose), ovarian prostaglandin production at 14–16 h after injection was similar to that of control rabbit ovaries [24]. Therefore, it is more likely that ovarian eicosanoids must have been suppressed in both locally animal and systemically administrated animals shortly after treatment, but the suppression must have persisted much longer in locally treated animals because of its high concentrations in the ovary. Moreover, the drugs

gradually leaked out of the ovarian bursa in locally injected animals, and dilution in the systemic plasma would render them ineffective for the other tissues.

Uterine involvement in regulation of luteal function may be possible because the uterus produces luteolytic $\text{PGF}_{2\alpha}$ [1, 5], and hysterectomy delays luteal regression in immature pseudopregnant rats [8]. However, Olofsson *et al.* showed that: 1) $\text{PGF}_{2\alpha}$ in luteal contents *in vivo* and produced by the isolated corpora lutea *in vitro* increased toward the end of the luteal phase in adult pseudopregnant rats, 2) the increase in luteal contents also persisted in hysterectomized rats, and 3) $\text{PGF}_{2\alpha}$ content in the remainder of the ovary was unaltered during the luteal phase [2–4]. Taken together with our present result that local treatment was more effective than systemic treatment in delaying luteolysis, it is suggested that the rat corpus luteum can intrinsically produce any amount of eicosanoids, which play an important role in the demise of luteal function. This appears to support the previous hypothesis of Rothchild [25]. The uterus may influence luteal eicosanoid production. It is noteworthy that exogenous cloprostenol ($\text{PGF}_{2\alpha}$ analogue) increased the luteal synthesis of $\text{PGF}_{2\alpha}$ *in vitro* [26, 27].

Unexpectedly, systemic injections with DEX or IND induced an instant and significant increase in circulating progesterone. This suggests a possible mechanism in extra-ovarian tissue(s) which appear to negatively regulate luteal function through prostanoids production. The effect of systemically injected inhibitors on luteal activity may be mediated by substances other than eicosanoids. There are some reports that the secretion of LH or prolactin, which is relevant to rat luteal function, is modulated by arachidonate or eicosanoids in the rat pituitary [28–31].

In conclusion, modulations in ovarian endogenous eicosanoids by local administration with several kinds of synthesis inhibitors prolonged luteal activity in immature pseudopregnant rats. The present findings suggest that ovarian endogenous eicosanoids in the late luteal phase may be involved in initiation of functional luteolysis in rats.

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