Changes in lipid peroxide and antioxidant enzyme activities in corpora lutea during pseudopregnancy in rats

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This study investigated the involvement of lipid peroxidation and antioxidant enzymes in the regulation of luteal function in pseudopregnant rats. The activities of superoxide dismutase (SOD), a specific scavenger of superoxide radicals, and glutathione peroxidase, a scavenger of hydrogen peroxide, and lipid peroxide concentrations were measured in the corpus luteum on days 1, 3, 5, 7, 9, 11 and 13 of pseudopregnancy. The activity of SOD in the corpus luteum gradually increased until day 9 of pseudopregnancy and decreased thereafter, in a similar manner to serum progesterone concentration. Glutathione peroxidase activity significantly increased from day 1 to day 3 and remained high until day 11 of pseudopregnancy. The concentrations of lipid peroxides in the corpus luteum increased from day 3 to day 13 of pseudopregnancy. The involvement of prostaglandin F₂α (PGF₂α) in the production of lipid peroxides in regression of the corpus luteum was investigated by administering PGF₂α (3 mg kg⁻¹, s.c.) or saline on days 7, 9 and 12 of pseudopregnancy. Each group of rats was autopsied 2 h later, and SOD activity, glutathione peroxidase activity and the concentration of lipid peroxides in the corpus luteum were determined. PGF₂α significantly increased lipid peroxide concentrations in the corpus luteum on days 7, 9 and 12 of pseudopregnancy (approximately twofold increases on days 7 and 9, and a fivefold increase on day 12, compared with the control that received saline). The activity of SOD in the corpus luteum was significantly increased by PGF₂α on days 7 and 9, but not on day 12, of pseudopregnancy. PGF₂α did not cause any significant changes in glutathione peroxidase activity in the corpus luteum on days 7, 9 and 12 of pseudopregnancy. It is concluded that lipid peroxides play an important role in regulating luteal function in pseudopregnant rats.

Introduction

Reactive oxygen species are increased in the corpus luteum during the regression phase (Sawada and Carlson, 1989, 1991, 1994a; Riley and Behrman, 1991; Aten et al., 1992; Sugino et al., 1993a) and inhibit progesterone production in rats (Behrman and Preston, 1989; Behrman and Aten, 1991; Gatzuli et al., 1991; Sugino et al., 1993b; Kodaman et al., 1994; Musicki et al., 1994). Corpora lutea also contain antioxidant enzymes such as superoxide dismutase (SOD) and glutathione peroxidase (GP₅). Superoxide radicals are scavenged to hydrogen peroxide by a specific inhibitor, SOD, and further scavenged to H₂O by GP₅ or catalase. Sugino et al. (1993a) and Sugino and Kato (1994) reported that SOD and GP₅ activities in the corpus luteum change in a similar manner to serum progesterone concentrations during pregnancy in rats, and suggested an important role for these scavenging enzymes in the regulation of luteal function.

Prostaglandin F₂α (PGF₂α) is involved in corpus luteum regression (Rothchild, 1981; Olofsson and Selsam, 1988), at least in part, through the increase of reactive oxygen species in rats (Sawada and Carlson, 1989, 1991, 1994a; Riley and Behrman, 1991; Aten et al., 1992). However, the effects of PGF₂α on scavenging systems such as SOD and GP₅ in the corpus luteum are unknown. In addition, the luteolytic effects of PGF₂α depend on the age of the corpus luteum in rats (Khan et al., 1979; Lahav et al., 1988, 1989).

The present study was designed to clarify the changes in lipid peroxide concentrations and the activities of antioxidant enzymes in the corpus luteum during pseudopregnancy in rats, and to study the effects of PGF₂α on lipid peroxides and antioxidant enzymes at different ages of the corpus luteum.

Materials and Methods

Animals

Sprague–Dawley rats, 220–270 g, were housed under controlled conditions (lights on from 05:00 to 19:00 h) with free access to food and water from 06:00 to 18:00 h.
access to standard rat chow and water. Vaginal smears were obtained daily, and only those rats showing two consecutive 4 day oestrous cycles were used. Pseudopregnancy was induced by mechanical stimulation of the uterine cervix for about 1 min with a glass rod at 18:00 h at pro-oestrus and at 9:00 h at oestrus. The last day on which the rat exhibited an oestrous smear was designated as day 1 of pseudopregnancy. Vaginal smears from pseudopregnancy were checked every day.

Experimental protocol

Experiment 1. Operations were carried out between 16:00 and 18:00 h on days 1, 3, 5, 7, 9, 11 or 13 of pseudopregnancy. Rats underwent laparotomy under ether anaesthesia. After sampling blood from the portal vein, the ovaries were perfused with saline via the portal vein during draining of the inferior vena cava, to remove the blood, as reported by Sugino et al. (1993a). The ovaries were removed, and the corpus luteum was dissected, cleaned of adhering tissue in a watch glass and weighed. For SOD or GPx assay, the corpus luteum was homogenized with Tris–HCl buffer (0.01 mol L⁻¹, pH 7.4) and centrifuged at 800 × g for 10 min at 4°C, and the supernatant was stored at −70°C. For the lipid peroxide assay, the corpus luteum was homogenized with 1.15% (w/v) KCl and the homogenate was stored at −70°C. The serum samples were stored at −20°C for progesterone assay.

Experiment 2. The effects of PGF₂α on the production of lipid peroxides and antioxidant enzymes in the corpus luteum were studied by injecting rats with PGF₂α (3 mg kg⁻¹ body mass, s.c., dissolved in 0.01 mol PB¹⁻¹, pH 7.4), at 18:00 h on day 7, 9 or 12 of pseudopregnancy. PGF₂α was the kind gift of Ono Pharmaceutical Co. Ltd (Osaka). Control rats received saline (s.c.). Each group of rats was autopsied under ether anesthesia before or 2 h after PGF₂α injection as described in Expt 1. Blood samples were taken and the ovaries were removed and used to determine serum progesterone concentrations, enzyme activities and lipid peroxide concentrations in the corpus luteum.

SOD assay

Total SOD activity and Mn-SOD activity in the corpus luteum were measured using the method described by Sugino et al. (1993a), based on the nitrite method of Oyanagi (1984). The amount of protein required for 50% inhibition in the absorbance at 550 nm was defined as one unit (Nitrite Unit; NU) of SOD activity. All data were expressed in NU of SOD activity mg⁻¹ protein. Protein concentration was determined using the method described by Lowry et al. (1951). Cu,Zn-SOD activity was determined by subtracting Mn-SOD activity from the total SOD activity. The intra- and interassay coefficients of variation were 3.8% and 9.6%, for the total SOD assay, and 4.7% and 6.4% for the Mn-SOD assay, respectively.

GPx assay

GPx activity in the corpus luteum was measured using the method described by Sugino et al. (1993b), based on the coupling of the enzyme to NADPH via glutathione reductase (Paglia and Valentine, 1967). The activity of GPx was defined as μmol NADPH oxidized min⁻¹ mg⁻¹ protein.

Lipid peroxide assay

Concentrations of lipid peroxides in the corpus luteum were measured by the thiobarbituric acid method of Ohkawa et al. (1979). The results were expressed as nmol of malondialdehyde (MDA) g⁻¹ wet mass of tissue.

Progesterone assay

Serum progesterone concentrations were determined by the specific radioimmunoassay described by Kato et al. (1982). The sensitivity of the assay was 100 pg per tube, and the intra- and interassay coefficients of variation were 7% and 14.4%, respectively.

Statistical analyses

Data were examined by analysis of variance and Duncan’s new multiple range test. Differences were considered to be significant if P < 0.05.

Results

Cu,Zn-SOD and Mn-SOD activities in the corpus luteum changed in a similar manner to total SOD activity, which significantly (P < 0.01) increased from day 1 to day 9 and decreased thereafter (Table 1). GPx significantly (P < 0.01) increased from day 1 to day 3, remained high until day 11, and slightly decreased on day 13 of pseudopregnancy. Total SOD activity in the corpus luteum and serum progesterone concentrations changed in parallel during pseudopregnancy, gradually increased until day 9 of pseudopregnancy and decreased thereafter (Fig. 1). A positive correlation was found between serum progesterone concentration and total SOD activity (r = 0.71, P < 0.01). The mass of the corpus luteum gradually increased from day 1 to day 13 of pseudopregnancy. Lipid peroxide concentrations in the corpus luteum also gradually increased from day 3 to day 13 of pseudopregnancy (Fig. 1).

PGF₂α significantly decreased serum progesterone concentrations on days 7 and 9, but not on day 12 of pseudopregnancy (Fig. 2). PGF₂α significantly increased lipid peroxide concentrations in the corpus luteum on days 7 and 9 of pseudopregnancy, and further increased them on day 12 of pseudopregnancy (Fig. 2). The lipid peroxide concentrations induced by PGF₂α on day 12 of pseudopregnancy were significantly higher than those on day 7 or 9 of pseudopregnancy. Total SOD activity in the corpus luteum was increased by PGF₂α on days 7 and 9 of pseudopregnancy, but not on day 12 of pseudopregnancy (Fig. 2). The total SOD activity in the corpus luteum on day 12 of pseudopregnancy was significantly lower than that on day 7 or 9 of pseudopregnancy in each group. PGF₂α did not cause any
Superoxide dismutase and lipid peroxide in corpus luteum

Table 1. Changes in total superoxide dismutase (SOD), Cu,Zn-SOD, Mn-SOD and glutathione peroxidase (GPX) activities in the corpus luteum during pseudopregnancy

<table>
<thead>
<tr>
<th>Day of pseudopregnancy</th>
<th>Total SOD activity (NU mg⁻¹ protein)</th>
<th>Cu,Zn-SOD activity (NU mg⁻¹ protein)</th>
<th>Mn-SOD activity (NU mg⁻¹ protein)</th>
<th>GPX activity (µmol NADPH × 10⁻³ min⁻¹ mg⁻¹ protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>6</td>
<td>57.4 ± 5.7a</td>
<td>52.8 ± 4.6a</td>
<td>4.6 ± 2.6b</td>
</tr>
<tr>
<td>3</td>
<td>5</td>
<td>115.2 ± 5.0</td>
<td>106.2 ± 5.0</td>
<td>9.0 ± 1.3</td>
</tr>
<tr>
<td>5</td>
<td>8</td>
<td>137.3 ± 4.8</td>
<td>123.0 ± 5.4</td>
<td>14.3 ± 1.3</td>
</tr>
<tr>
<td>7</td>
<td>6</td>
<td>158.0 ± 6.3</td>
<td>153.5 ± 8.2</td>
<td>22.7 ± 2.0</td>
</tr>
<tr>
<td>9</td>
<td>6</td>
<td>172.2 ± 3.3</td>
<td>152.7 ± 4.4</td>
<td>19.5 ± 3.0</td>
</tr>
<tr>
<td>11</td>
<td>8</td>
<td>150.1 ± 5.3</td>
<td>120.4 ± 5.2</td>
<td>29.7 ± 2.1</td>
</tr>
<tr>
<td>13</td>
<td>5</td>
<td>107.7 ± 14.0b</td>
<td>93.8 ± 12.1b</td>
<td>13.9 ± 4.2b</td>
</tr>
</tbody>
</table>

Values are means ± SEM for the number of animals given; NU: naiture unit.

*P < 0.01 compared with the other days; *P < 0.01 compared with days 5, 7, 9 and 11; *P < 0.05 compared with day 7 (Duncan's new multiple range test).

Fig. 1. Changes in serum progesterone concentration (●), total superoxide dismutase (SOD) activity (○), lipid peroxide (LPO) concentration (□) in the corpus luteum (CL) and CL masses (▷) during pseudopregnancy. MDA: malondialdehyde. Values are means ± SEM for the number of animals given in parentheses. *P < 0.01 compared with days 1, 3 and 5; *P < 0.05 compared with day 7; *P < 0.05 compared with days 7 and 9; *P < 0.01 compared with days 5, 7, 9 and 11; *P < 0.01 compared with the other days; and *P < 0.01 compared with days 3, 5, 7 and 9 (Duncan's new multiple range test).

Table 2. Changes in SOD, Cu,Zn-SOD, Mn-SOD, GPX activities and lipid peroxide (LPO) concentration in serum and corpus luteum during pseudopregnancy

<table>
<thead>
<tr>
<th>Day of pseudopregnancy</th>
<th>Serum progesterone (ng ml⁻¹)</th>
<th>CL wet mass (mg)</th>
<th>Total SOD activity (NU mg⁻¹ protein)</th>
<th>Cu,Zn-SOD activity (NU mg⁻¹ protein)</th>
<th>Mn-SOD activity (NU mg⁻¹ protein)</th>
<th>GPX activity (µmol NADPH × 10⁻³ min⁻¹ mg⁻¹ protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>65.0 ± 7.5</td>
<td>5.0 ± 0.5</td>
<td>57.4 ± 5.7</td>
<td>52.8 ± 4.6</td>
<td>4.6 ± 2.6</td>
<td>1.80 ± 0.23</td>
</tr>
<tr>
<td>3</td>
<td>115.2 ± 5.0</td>
<td>5.0 ± 0.5</td>
<td>115.2 ± 5.0</td>
<td>106.2 ± 5.0</td>
<td>9.0 ± 1.3</td>
<td>2.63 ± 0.18</td>
</tr>
<tr>
<td>5</td>
<td>137.3 ± 4.8</td>
<td>5.0 ± 0.5</td>
<td>137.3 ± 4.8</td>
<td>123.0 ± 5.4</td>
<td>14.3 ± 1.3</td>
<td>2.68 ± 0.10</td>
</tr>
<tr>
<td>7</td>
<td>158.0 ± 6.3</td>
<td>5.0 ± 0.5</td>
<td>158.0 ± 6.3</td>
<td>153.5 ± 8.2</td>
<td>22.7 ± 2.0</td>
<td>2.15 ± 0.05</td>
</tr>
<tr>
<td>9</td>
<td>172.2 ± 3.3</td>
<td>5.0 ± 0.5</td>
<td>172.2 ± 3.3</td>
<td>152.7 ± 4.4</td>
<td>19.5 ± 3.0</td>
<td>3.28 ± 0.19</td>
</tr>
<tr>
<td>11</td>
<td>150.1 ± 5.3</td>
<td>5.0 ± 0.5</td>
<td>150.1 ± 5.3</td>
<td>120.4 ± 5.2</td>
<td>29.7 ± 2.1</td>
<td>3.31 ± 0.15</td>
</tr>
<tr>
<td>13</td>
<td>107.7 ± 14.0</td>
<td>5.0 ± 0.5</td>
<td>107.7 ± 14.0</td>
<td>93.8 ± 12.1</td>
<td>13.9 ± 4.2</td>
<td>2.78 ± 0.06</td>
</tr>
</tbody>
</table>

Values are means ± SEM for the number of animals given; NU: naiture unit.

*P < 0.01 compared with the other days; *P < 0.01 compared with days 5, 7, 9 and 11; *P < 0.05 compared with day 7 (Duncan's new multiple range test).

Discussion

The present study confirms that lipid peroxide concentrations in the corpus luteum gradually increase towards the end of pseudopregnancy. It is not fully understood, however, why serum progesterone values were high from day 3 to day 9 of pseudopregnancy in spite of the increasing production of lipid peroxides in the corpus luteum. The inhibitory effect of lipid peroxides on progesterone production may be blocked by some factors during the mid-luteal phase. This may be supported by the findings of Sawada and Carlson (1994b) that progesterone secretion was increased by LH in spite of the increase of superoxide radical generation in pseudopregnant rats. In addition, the amount of lipid peroxides may not be enough to suppress the luteal function during the mid-luteal phase. However, SOD scavenges superoxide radicals by catalysing them to hydrogen peroxide, which is further converted to H₂O by GPX. Although neither superoxide radical nor hydrogen peroxide concentrations were measured in the present study, the increasing activities of SOD and GPX would have reduced these reactive oxygen species and contributed to the maintenance of luteal function during the mid-luteal phase. Heslo et al. (1992) reported that ovarian progesterone production is significantly and positively correlated with Mn-SOD activity throughout pseudopregnancy in rabbits. Previous data from our laboratory also demonstrate that serum progesterone concentrations change in a similar manner to the luteal SOD and GPX activities in pregnant rats (Sugino et al., 1993a; Sugino and Kato, 1994), and that SOD, in the presence of catalase, apparently blocks the antisteroidogenic effect of reactive oxygen species induced by ischaemia–reperfusion injury of the
peroxides on day 12 of pseudopregnancy is unclear, but it may play a role in the accumulation of macrophages in the corpus luteum that precedes luteolysis by phagocytosis, as demonstrated in the pathogenesis of atherosclerosis (Steinberg et al., 1989).

Injected PGF<sub>2α</sub> could increase SOD activity in the corpus luteum on days 7 and 9, but not on day 12 of pseudopregnancy. Since superoxide radicals by themselves stimulate SOD activity (Hassan and Fridovich, 1977; Dryer et al., 1980), the PGF<sub>2α</sub>-induced superoxide radicals may stimulate SOD production, i.e. the corpus luteum on day 7 or 9 may have a greater responsiveness against the increase of superoxide radicals to induce SOD. In addition, during the regression phase, the number of macrophages is increased in the corpus luteum (Brännström et al., 1994). Since PGF<sub>2α</sub> stimulated superoxide radical production by macrophages in our unpublished data, the production of hydrogen peroxide by macrophages (Fattone and Ward, 1982) should also be increased. Since hydrogen peroxide inhibits SOD activities (Hodgson and Fridovich, 1975), PGF<sub>2α</sub> may decrease SOD activity through macrophages. Also, during the regression phase, the number of macrophages is increased in the corpus luteum on day 12 of pseudopregnancy (Sugino et al., 1993b). These possibilities may explain why SOD activities in the corpus luteum were not increased by PGF<sub>2α</sub> on day 12 of pseudopregnancy.

There was a clear discrepancy in the present study between the changes in serum progesterone concentration and luteal mass after day 9 of pseudopregnancy. Wang et al. (1993) reported that functional luteolysis, defined as a decrease in serum progesterone and structural luteolysis, characterized as involution of the corpus luteum, are clearly separate events in rats. Niswender et al. (1994) also suggested that morphological luteolysis induced by PGF<sub>2α</sub> is manifested through apoptosis, which does not need the activation of the protein kinase C system, which is necessary to mediate the antisteroidogenic effects of PGF<sub>2α</sub> (Leung et al., 1986; Gibori et al., 1988). The present study indicates that the regulation of progesterone production may be independent of the regulation of luteal masses.

In summary, the present study indicated that lipid peroxides and antioxidant enzymes play important roles in regulating luteal function during pseudopregnancy, and that PGF<sub>2α</sub> in the corpus luteum may contribute to its regression through lipid peroxidation.

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Fig. 2. Effects of PGF<sub>2α</sub> (3 mg kg<sup>−1</sup>, s.c.) treatment on (a) serum progesterone concentrations, (b) lipid peroxide (LPO) concentrations and (c) total superoxide dismutase (SOD) activities in the corpus luteum on days 7, 9 and 12 of pseudopregnancy. (A) 0 h, (B) 2 h after saline injection, and (C) 2 h after PGF<sub>2α</sub> injection, (D) h: nitrite unit; MDA: malondialdehyde. Each bar represents the mean ± SD for the number of animals shown. *P < 0.01 compared with the saline group and #P < 0.01 compared with the same group on the other days of pseudopregnancy (Duncan's new multiple range test).

Ovary (Sugino et al., 1993b). These findings indicate the reciprocal relationship between reactive oxygen species and their scavengers in the regulation of uterine function.

PGF<sub>2α</sub> generates reactive oxygen species to induce luteolysis (Sawada and Carlson, 1989, 1991, 1994a; Riley and Behrman, 1991; Aten et al., 1992). The present study further demonstrated that these oxidative effects of PGF<sub>2α</sub> changed as the age of the corpus luteum advanced; PGF<sub>2α</sub> induced a fivefold increase in lipid peroxide concentrations in the corpus luteum on day 12, but only twofold increases on days 7 or 9 of pseudopregnancy. The findings that serum progesterone increased from day 3 to day 9 of pseudopregnancy in spite of the increased lipid peroxide concentrations may be controversial to the results that serum progesterone was decreased with the increased lipid peroxides induced by PGF<sub>2α</sub>. This may be because PGF<sub>2α</sub> induced much greater amounts of lipid peroxides, which were actually greater than those on day 13 of pseudopregnancy. The role of the greater induction of lipid peroxides on day 12 of pseudopregnancy is unclear, but it may play a role in the accumulation of macrophages in the corpus luteum that precedes luteolysis by phagocytosis, as demonstrated in the pathogenesis of atherosclerosis (Steinberg et al., 1989).
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