Modulation of Ovarian LH Receptor and Serum Hormone Levels in Rats with Hyperprolactinemia Induced by Administration of Ovine Prolactin or Sulpiride

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OTA, H., WAKIZAKA, A. and FUKUSHIMA, M. Modulation of Ovarian LH Receptor and Serum Hormone Levels in Rats with Hyperprolactinemia Induced by Administration of Ovine Prolactin or Sulpiride. Tohoku J. exp. Med., 1986, 148 (2), 213–227 — Hyperprolactinemia was experimentally produced in rats by administration of ovine prolactin (oPRL) and sulpiride, and tried to evaluate the effect of hyperprolactinemia on ovarian receptor for luteinizing hormone (LH) as well as that on serum gonadotropin and steroid hormone levels. Wistar-Imamichi strain mature female rats showing 4-day estrous cycles were treated with various doses of oPRL or sulpiride twice a day for 4 days from diestrus. They were killed on the fifth day. Binding of ovarian LH receptors was reduced by a small dose of oPRL (0.1 IU) or sulpiride (0.25 mg) and restored to normal by larger doses of oPRL. However, larger doses of sulpiride (50 or 100 mg) increased the receptor bindings beyond the control level (4.39±0.40 ng/mg homogenate protein). Serum prolactin levels decreased in rats treated with larger doses of oPRL, but increased with larger doses of sulpiride. Serum LH levels increased with both agents. Although the ovaries treated with either oPRL or sulpiride suggested the lack of ovulation, there were no significant changes of steroid hormones in oPRL groups. In contrast, sulpiride treatment resulted in a reduction of estradiol and an increase of progesterone secretion, suggesting the prolonged effect of the drug. Thus, prolactin appeared to act on the rat ovarian LH receptors in two different manners in hyperprolactinemia, depending on the amount of this hormone or a ratio of prolactin to LH. —— LH receptors; rats; ovary; hyperprolactinemia; receptor regulation

It is known that patients with hyperprolactinemia are often associated with luteal phase defect (Corenblum et al. 1976), polycystic ovary syndrome (Thorner 1977; Ota et al. 1979) or amenorrheas (Bohnet et al. 1975b; Healy et al. 1977).
These patients are more refractory to clomiphene citrate or gonadotropin therapy. 2-Bromo-α-ergocryptine (CB-154), a dopamine receptor agonist (Corrodi et al. 1973), is quite effective in restoring prolactin level to normal. However, it is not clearly understood how prolactin acts on the ovary of the human or animal. To date, there is increasing evidence that prolactin directly acts on the ovary as well as on the hypothalamic-pituitary level. That is, prolactin directly inhibits ovarian estrogen biosynthesis (Wang et al. 1980; Dorrington and Gore-Langton 1981; Wang and Chan 1982) or production of cyclic AMP (Kraiem 1981). Furthermore, we have previously reported some aspects of the hormonal regulation of ovarian receptors for prolactin, LH and FSH (Ota et al. 1982a, b, c). The number of LH receptors in the rat ovary was found to decrease after the administration of 0.1–0.5 IU ovine prolactin (oPRL) at 12 hr intervals for 4 days from diestrus as well as 5 IU hCG (human chorionic gonadotropin; Ota et al. 1982a, b). Recently, a similar decrease in LH receptors by prolactin was observed in the rat granulosa cells (Darbon and Ranta 1984). In contrast, there are several observations that prolactin enhances ovarian LH receptors in rats (Holt et al. 1976; Richards and Williams 1976). In order to reconcile these differences, especially in LH receptors, we report dose-dependent effects of exogenously administered prolactin and of prolactin secretion stimulated by sulpiride, a dopamine receptor antagonist (Debeljuk et al. 1974; Iwasaki et al. 1976; Puech et al. 1976), on the ovarian LH receptor as well as on the coincidently occurring changes in serum estradiol, progesterone and gonadotropin levels using mature female rats.

**Materials and Methods**

*Hormones and chemicals*

oPRL (32 IU/mg) purchased from Sigma Chemical Company (St. Louis, MO, USA) was dissolved in sterile 0.1 M veronal buffer (pH 8.4) containing 0.5% bovine serum albumin (BSA) for injection. The homogeneity of this preparation was previously confirmed by gel electrophoresis (Ota 1983). Sulpiride (Dogmatyl®) supplied from Fujisawa Pharmaceutical Company (Osaka) was dissolved in sterile 0.9% saline solution just before injection. hCG (9,600 IU/mg) used for iodination was supplied from Teikoku Hormone MFG (Tokyo). Iodogen for the iodination was purchased from Pierce (Rockford, IL, USA). Ultrogel AcA44 was from LKB (France). Carrier free Na[^125]I (NEZ-033H) was from New England Nuclear (Boston, MA, USA). Radioimmunoassay (RIA) kits for prolactin, LH and FSH were provided by NIH (NIAMDD). The second antibody used for the RIAs was goat anti-rabbit IgG (MBL, Nagoya). RIA kits for 17β-estradiol and progesterone were purchased from CIS (Midori Juji, Inc., Tokyo) and Daiichi Radioisotope Laboratory (Tokyo), respectively.

*Experimental animals and administration methods*

Ninety day-old Wistar-Imamichi strain female rats weighing 160 to 185 g (mean 175 g) and having a regular 4-day estrous cycle were used throughout the experiment. The animals were kept under the same conditions as described previously (Ota et al. 1983). Various concentrations of oPRL (0.1, 0.5, 1 and 2.5 IU) or sulpiride (0.25, 5, 50 and 100 mg) were administered to 6 animals in each group by subcutaneous injection at 12 hr intervals (8:00–9:00, 20:00–21:00) for 4 days beginning on diestrus. The vaginal smear during
the treatment showed continuous diestrus in all of the groups except 0.25 mg sulpiride group, in which some cornified or nucleated cells were observed in the vaginal smears. On the fifth day, blood was obtained by cutting off the cervical vessels and the ovaries were removed between 13:00 and 14:00. The resected ovaries were weighed and immediately stored at -80°C until the assay.

To examine the histological changes, some of the ovaries were fixed in 10% neutral formalin, serially sectioned at 2 μm and stained with hematoxylin and eosin.

**Iodination of hormones**

The hormones were labeled with Na[¹²⁵I] by the iodogen method (Fraker and Speck 1978). In brief, 10 μg hCG was reacted with 1 mCi Na[¹²⁵I] under 1 μg iodogen in a glass tube. The tube was allowed to stand at room temperature for 12 to 15 minutes with occasional mixing. The reaction was terminated by removing the content to another glass tube, followed by an addition of 60 μl 0.5 M sodium iodide. Radioiodinated hormones were separated chromatographically from free iodide by gel filtration. The specific activity of the labeled hormone was estimated to be 40-65 μCi/μg from the proportional distribution of the counts under each peak.

**LH receptor binding assay**

The frozen ovaries were homogenized in 9 volumes of 10 mM tris-HCl buffer (homogenate buffer) containing 1 mM MgCl₂, 0.1 mM phenylmethylsulfonyl fluoride (Sigma), 0.25 M sucrose, 0.01% NaN₃ and 10,000 KIE/liter aprotinin (Trasylol®; Bayer, Leverkusen, GFR). Duplicate aliquots (100 μl) of the homogenates (approx. 1 mg protein) were mixed with 40,000 cpm (2-4 ng) of the labeled hormone in 200 μl of 10 mM tris-HCl buffer (pH 7.2) containing 1 mM MgCl₂ and BSA (RRA buffer) with or without an excess amount of the unlabeled hormone (hCG). After incubation for 30 min at 37°C with constant shaking, the reaction was terminated by addition of 1 ml of ice-cold RRA buffer and the tubes were centrifuged at 4,600 x g for 30 min at 4°C. After aspirating the supernatant, radioactivity in the resulting pellet was measured in an automatic gamma counter. Nonspecific binding was measured in the presence of an excess of hCG (1 μg) and was less than 2.2% of the added radioactivity. The specific radioactivity of the hormone was calculated by subtracting the nonspecific binding from the total binding and expressed as the specific binding of the added hormone to the receptor (ng) per mg homogenate protein. Protein concentration was determined by the method reported by Lowry et al. (1951).

In order to know the kinetics of the receptor bindings, ovaries from 3 to 4 rats were collected. Mixed homogenates prepared as described above were incubated with increasing amount of the unlabeled hormone (hCG) and constant amount (40,000 cpm) of the labeled hormone to measure LH receptors. The binding data were analyzed by Scatchard plots (Scatchard 1949) to determine the association constant (Ka) and the number of binding site (maximal binding capacity) of the receptor.

**Radioimmunoassays for rat PRL, LH and FSH in serum**

Aliquots (100 μl) of sera were added to the tubes which contained 100 μl labeled hormones with the rabbit antisera in 0.01 M phosphate buffer (pH 7.6). Anti-rat PRL, LH and FSH were diluted to 1 : 5,000, 1 : 10,000 and 1 : 2,500, respectively. The mixture was incubated for 48 hrs at 4°C. Goat anti-rabbit IgG (MBL, Nagoya) diluted 1 : 10 (200 μl) was added to the mixture. After allowing it to stand for 18 hrs at 4°C, the mixture was centrifuged at 2,000 x g for 30 min and radioactivity in the pellets measured in a gamma counter. The lower limit of sensitivity for rat PRL, LH and FSH assays were 5.2, 1.3 and 30 ng/tube, respectively.

**Radioimmunoassays of serum estradiol and progesterone**

Concentrations of serum estradiol were measured using CIS kits according to the
methods given by the manufacturer. In brief, serum (300 μl) was extracted with 3 ml ethyl ether. The ether layer was evaporated under N₂ gas and the extract resuspended in 300 μl 0.04 M phosphate buffer. After the addition of 100 μl [¹²⁵I] 17β-estradiol (14,000 cpm), each tube was incubated with 100 μl antiserum raised in rabbits for 18 hrs at room temperature. Goat antirabbit γ-globulin (1 ml) was added then and the mixture incubated for 15 min at room temperature. After centrifugation, the radioactivity in the resulting pellet was counted. The sensitivity was 1.8 pg/tube.

The serum progesterone level was determined by the polyethylene glycol method using Daiichi radioisotope RIA kit (Tsushima et al. 1976). Briefly, serum 0.1 ml, 1 ml ethyl ether and 50 μl propylene glycol were mixed together. After the ether was evaporated under N₂ gas, 0.5 ml phosphate buffer and 0.1 ml (20,000 cpm) of [¹²⁵I] iodo-progesterone were added to the tube. The mixture was incubated with 0.1 ml antiserum raised in rabbits for 18 hrs at room temperature. Then, 0.1 ml bovine serum gamma globulin and polyethylene glycol were added to the mixture followed by centrifugation for 10 min at 2,000 × g. The radioactivity was measured in the resulting pellet. The lower limit of sensitivity was 0.6 ng/tube.

Statistics

All results were expressed as the mean ± s.e. of 6 animals. Experimental data were analyzed using analysis of variance and Duncan’s new multiple range test. A value of p < 0.05 was chosen as the limit of statistical significance.

Results

Changes in LH receptors in the rat ovary treated with ovine prolactin or sulpiride

As shown in Fig. 1 (left panel), a small dose (0.1 IU) of oPRL caused a marked decrease in LH receptor to 24% of the control. On the other hand, higher doses of this hormone restored the receptor to normal. A similar decrease in LH receptors was observed by a small dose (0.25 mg) of sulpiride (Fig. 1; right panel). However, increasing doses of the drug elevated the receptor binding in a dose-
Modulation of LH Receptor by Prolactin in Rats

Table 1. Kinetic study of ovarian LH receptor in the rats treated with saline, oPRL and sulpiride

<table>
<thead>
<tr>
<th>Treatment</th>
<th>LH receptor</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$K_a^*$ ($\times 10^{11}$ M$^{-1}$)</td>
</tr>
<tr>
<td>Saline (control)</td>
<td>7.8</td>
</tr>
<tr>
<td>oPRL 0.1 IU</td>
<td>6.5</td>
</tr>
<tr>
<td>0.5</td>
<td>8.7</td>
</tr>
<tr>
<td>2.5</td>
<td>7.3</td>
</tr>
<tr>
<td>Sulpiride 50 mg</td>
<td>5.1</td>
</tr>
</tbody>
</table>

*Association constant; †Maximal binding capacity (site).

Table 2. Serum prolactin, LH and FSH levels in the rats treated with saline, oPRL and sulpiride

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Serum level (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Prolactin</td>
</tr>
<tr>
<td>Saline (control)</td>
<td>15±2</td>
</tr>
<tr>
<td>oPRL 0.1 IU</td>
<td>12±2</td>
</tr>
<tr>
<td>0.5</td>
<td>14±1</td>
</tr>
<tr>
<td>1.0</td>
<td>8±1†</td>
</tr>
<tr>
<td>2.5</td>
<td>9±2*</td>
</tr>
<tr>
<td>Sulpiride 0.25 mg</td>
<td>8±1*</td>
</tr>
<tr>
<td>5</td>
<td>10±2</td>
</tr>
<tr>
<td>50</td>
<td>170±19§</td>
</tr>
<tr>
<td>100</td>
<td>92±6§</td>
</tr>
</tbody>
</table>

Values given are the mean±s.e. from 6 animals.

* $p<0.05$ compared to the saline controls; † $p<0.02$ compared to the saline controls; ‡ $p<0.01$ compared to the saline controls; § $p<0.001$ compared to the saline controls.

dependent manner. The rat ovaries treated with 50 and 100 mg sulpiride showed significantly higher bindings than that of the control ($p<0.01$, $p<0.001$).

Typical findings from Scatchard analyses of the ovarian LH receptors are shown in Table 1. The changes in the receptor binding with either oPRL or sulpiride treatment were mainly due to alteration of the maximal binding capacity (hormone binding site). A small dose (0.1 IU) of oPRL reduced the LH receptor binding site in the ovary to 14% of the control and 50 mg sulpiride administration increased the receptor site up to 170% of the control.
Changes in serum prolactin, LH and FSH levels

As shown in Table 2, treatment with 1 or 2.5 IU oPRL at 12 hr intervals caused a significant reduction in the prolactin level compared to the control. Sulpiride administration also resulted in a decrease of the prolactin level to 53% of the control in 0.25 mg-treated rats, but the level increased 11- or 6-fold higher in 50 or 100 mg-treated rats. Rats treated with 50 mg sulpiride showed the highest level of serum prolactin.

Interestingly, serum LH levels rose by an increase in the dose of both agents. With oPRL, every group except for one (1 IU) showed a significantly higher level in LH than the control. Though small doses of sulpiride (0.25 or 5 mg) did not show statistically significant increases in the level, large doses (50 or 100 mg) caused a significant rise in the level.

Serum FSH level was not altered in any of the treatment groups compared with the control.

Changes in serum estradiol and progesterone levels

No significant change was observed in the estradiol level among any of the oPRL-treated groups, whereas a large dose of sulpiride administration reduced the level approximately one half to one third of the control (Table 3).

Level of serum progesterone was also unchanged in all of the oPRL-treated groups. However, sulpiride administration increased progesterone levels 2- to 3-fold higher than the control level.
Fig. 2. An ovarian section (×10.4) from the control rat at diestrus. The figure shows multiple corpora lutea and growing follicles.

Fig. 3. An ovarian section (×65) from the control rat at diestrus. The figure shows active lutein cells and rich intercellular capillaries.
Fig. 4. An ovarian section (×10.4) from the rat treated with 0.5 IU oPRL at 12 hr intervals for 4 days from diestrus. The figure shows degenerated follicles, fibrotic corpora lutea and well-developed stroma.

Fig. 5. An ovarian section (×65) from the rat treated with 0.5 IU oPRL at 12 hr intervals for 4 days from diestrus. The figure shows the lutein cells having pyknotic nuclei and vacuoles in the cytoplasm and poor intercellular capillaries.
Fig. 6. An ovarian section (×10.4) from the rat treated with 5 mg sulpiride at 12 hr intervals for 4 days from diestrus. The figure shows cystic follicles, regressed corpora lutea and wide stroma.

Fig. 7. An ovarian section (×65) from the rat treated with 5 mg sulpiride at 12 hr intervals for 4 days from diestrus. The figure shows relatively active lutein cells in corpora lutea.
Histological changes of the treated ovaries

oPRL treatment caused a decreasing tendency in ovarian weight to 93% compared to the control (95 ± 3 mg). Furthermore, the ovaries treated with sulpiride were significantly decreased in weight to 79% (75 ± 2 mg) of the control in all of the groups except for 0.5 mg-treated one (87 ± 4 mg).

Histologically, the control ovary showed a number of corpora lutea and growing follicles (Fig. 2). In the corpora lutea active lutein cells and developed capillaries were observed between the lutein cells (Fig. 3). The ovaries treated with oPRL or sulpiride showed degenerated follicles, cystic formation and well-developed stroma (Figs. 4 and 5). Also, regression and a decrease in the number of corpora lutea were observed. In oPRL-treated corpora lutea the pyknotic nuclei and cytoplasmic vacuoles were clearly visible (Fig. 6). On the contrary, in the sulpiride-treated corpora lutea the nucleoli were apparent and cytopasms were similar to those of the controls rather than to those of oPRL-treated corpora lutea, suggesting the source of the increasing production of progesterone (Fig. 7).

Discussion

The present study indicates that both exogenous prolactin and sulpiride in the state of hyperprolactinemia influence the LH receptor in two different manners depending on the dose. A smaller amount of oPRL and sulpiride reduced the LH receptor, while larger doses of sulpiride increased it. Prolactin is considered to act luteotropically on the rat ovary and stimulate LH receptors. It is already shown (Holt et al. 1976) that administration of oPRL and ovine LH to FSH-pretreated immature female rats stimulated the ovarian LH receptor and raised serum progesterone level. Other investigators (Richards and Williams 1976) also found a time-dependent increase in LH receptors in granulosa cells obtained from estrogen-primed FSH-pretreated hypophysectomized rats. Prolactin is also thought to regulate the LH receptor upward (Bohnet et al. 1975a) or downward (Sharpe 1976; Zipf et al. 1978; Purvis et al. 1979; Chan et al. 1981) in the testis of a mature male rat. However, there are different observations (Hsueh et al. 1976; Morris and Saxena 1980) that the LH receptor in the rat testis is regulated upward or downward according to the dose of administered prolactin. These findings are similar to those obtained in the present study with the rat ovary. Moreover, additional factors such as a ratio of LH to prolactin or duration of effects of these hormones may influence the receptor binding.

In addition to the present study, several reports have recently indicated that prolactin may affect the gonadotropin receptors in the rat ovary. Darbon and Ranta (1984) observed that addition of oPRL to rat granulosa cell cultures reduced LH receptors in a dose-dependent manner as well as cyclic AMP level in the absence of androstenedione. Similar decreases in FSH receptor and cyclic...
AMP level were confirmed in the granulosa cells by the present authors (Brodie and Ota 1985). Therefore, it is likely that prolactin may have its ability to decrease the gonadotropin receptors in hyperprolactinemia.

The decreased serum prolactin level in the rats treated with higher doses of oPRL may be related to a negative feedback effect of this hormone on the pituitary. In the rats treated with small doses of sulpiride, a decrease in prolactin may be caused by a transient increase of dopamine due to dissociation of sulpiride from once-occupied dopamine receptors in the hypothalamus or pituitary (Debeljuk et al. 1974; Puech et al. 1976). High levels of serum prolactin in rats treated with higher amounts of sulpiride would be brought about by prolonged inhibition of dopamine receptors by accumulation of the drug or its metabolites (Koishi et al. 1969).

In both treatment groups, serum LH levels rose by an increase of the dose of prolactin or sulpiride. LH secretion is stimulated by LH-RH, which is secreted from the hypothalamus and is considered to be regulated in part by dopamine (Quigley et al. 1981). This elevation in serum LH appears to be an abnormal phenomenon and may be resulted in by disturbance in dopamine turnover by high levels of prolactin (Judd et al. 1979; Alger et al. 1980). At higher levels than normal, LH acts downward on its ovarian receptors as we have previously described (Ota et al. 1982a). This down-regulation mechanism is resulted in by so-called internalization or endocytosis of the receptor (Ascoli and Puett 1977; Conn et al. 1978). In the present study, however, a higher level of LH induced by hyperprolactinemia did not inhibit its ovarian receptor bindings. This could be due to the markedly high level of serum prolactin or changes in the ratio of prolactin to LH.

Serum FSH level did not show the remarkable change in both treated groups, being coincident with that in rat hyperprolactinemia (Furuhashi and Fang 1981; Ota et al. 1983). Prolactin may not affect serum FSH level as much.

Levels of serum estradiol and progesterone did not vary in the oPRL treated rats compared to the controls. On the other hand, administration of sulpiride resulted in a decrease of estradiol level except for 0.25 mg group and a marked increase of progesterone level. This divergent action of prolactin by sulpiride has been observed elsewhere (Wang and Chan 1982). Several possibilities could account for mechanisms of those changes in the steroid levels in the two groups. First, the mechanisms of action of oPRL or sulpiride may be different, since oPRL influences the hypothalamus and pituitary gland or directly inhibits the ovary mediated by its own receptors located in the luteal, granulosa or interstitial tissue (Richards and Williams 1976; Magoffin and Erickson 1982; Navickis et al. 1982). Furthermore, half life of prolactin in peripheral blood is less than an hour, and this hormone would become undetectable within several hours (Kato 1980), which might lead to restoration of the steroid levels in the oPRL-group on the fifth day. On the other hand, sulpiride would first act on the central nervous system to
stimulate release of prolactin from the anterior pituitary (Debeljuk et al. 1974; Iwasaki et al. 1976; Puech et al. 1976). And it is likely that larger amounts of sulpiride or its metabolites accumulated in the area could cause the prolonged effect on the steroid levels as mentioned above (Koishi et al. 1969).

Prolactin is known to be one of the major stimuli to induce and maintain pseudopregnancy in rats (Nikitovitch-Winer and Everett 1958; Smith et al. 1975). In the case of pseudopregnancy, the corpora lutea are rescued, secreting progesterone markedly (Smith et al. 1975). However, in the present experiments the corpora lutea were decreased in number and showed regression as well as growth of stroma cells in the ovaries treated with the agents compared to the control. The decrease in corpora lutea was also accompanied with low level of progesterone in oPRL-treated group. On the contrary, the corpora lutea in sulpiride-treated ovaries did not regress as much as those in oPRL-treated ovaries, suggesting the prolonged effect of prolactin and the resultant accumulation of progesterone. The microscopic appearances in both treated groups seem to be similar to that described by Nikitovitch-Winer and Everett (1958), who observed an effect of prolactin on pseudopregnancy by transplanting the pituitary under the kidney capsule at various stages of estrous cycle. Pituitary graft on the afternoon of diestrus 3 or proestrus did not institute pseudopregnancy, while the operation on diestrus 2 did. Thus, short-term hyperprolactinemia produced in the present study did not seem to induce pseudopregnancy in view of the histologic appearances with or without low level of progesterone. On the contrary, it seemed to disturb cyclic changes of the steroids and impair follicular growth, leading to atresia.

Although we employed the ovarian homogenates to measure the LH receptor, the present experiments provide evidence of the divergent and dose-dependent action of prolactin on the receptors and steroid levels in the rats with hyperprolactinemia. The modulation of LH receptors and concomitant abnormal alterations in gonadotropin and/or steroid levels may be, in part, relevant to the pathophysiological state in human hyperprolactinemia.

Acknowledgments

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