Enhanced Ovarian Gonadotropin Receptors in the Testosterone-Induced Polycystic Ovary in Rats

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OTA, H., WAKIZAKA, A., FUKUSHIMA, M. and MAKI, M. Enhanced Ovarian Gonadotropin Receptors in the Testosterone-Induced Polycystic Ovary in Rats. Tohoku J. exp. Med., 1986, 148 (3), 313-325 — To establish the role of hormone receptors in patients with polycystic ovary (PCO), PCO rats were prepared by treating with testosterone propionate (TP). Five-day old immature female rats were subcutaneously injected with 1.25 mg TP in sesame oil. They were then killed at the age of 12 weeks. The ovarian receptors for LH and FSH as well as serum hormone levels were investigated in PCO rats and also in control rats at the various stages of the estrous cycle. The LH receptor binding in the TP-treated ovaries was elevated almost as high as that of proestrus control, and was observed to be higher than the other control. The FSH receptor binding of PCO rats was elevated to 173% of that of diestrus control, which showed the highest value throughout the cycle. Thus, the gonadotropin receptors in PCO rats appeared to be in an activated state. High levels of the receptor binding were due to an increase in receptor binding sites. Serum LH level was significantly higher than that of diestrus control but still remained lower than that of proestrus control. In contrast, FSH level was as low as that of diestrus control. Prolactin level was markedly elevated and 17- and 2-fold higher than that of diestrus and proestrus control, respectively. Estradiol level was higher than that of diestrus control, increasing to almost the same level during proestrus control. While progesterone level was largely depressed to 23 and 13% of that of diestrus and proestrus control, respectively, testosterone level was almost the same as that of diestrus control. From these results, it was suggested that tonic secretion of LH, low level of FSH, and markedly high levels of prolactin would increase the gonadotropin receptors and result in extremely low production of progesterone in rat ovaries. Clinically, elevated levels of the LH and FSH receptors may be a relevant occurrence in PCO patients.

In PCO, it is well recognized that half of the patients are refractory to clomiphene citrate, and ovarian hyperstimulation is likely to occur during hormone treatment (Schenker and Weinstein 1978; Holtz et al. 1982). PCO patients

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with the complication of hyperprolactinemia (Thorner 1977; Ota et al. 1979; Alger et al. 1980; Falaschi et al. 1980; Wortsman and Hirschowitz 1980) are even more refractory to hormone treatment. Bromocriptine (CB-154), a dopamine receptor agonist (Corrodi et al. 1973), is quite effective in inducing ovulation in such cases. However, it is poorly understood how the ovarian receptors interact with serum hormones in these patients. First, Rajaniemi et al. (1980) reported the reduction of ovarian LH receptors in PCO patients, then Copmann and Adams (1981) experimentally produced polycystic ovaries in hypothyroid-hCG stimulated rats and found that the FSH receptor binding was well maintained at higher levels than that of the control (euthyroid rats). In order to better understand ovarian function in PCO patients, it is very important to investigate the mode of regulation of gonadotropin receptors. In this study, gonadotropin receptors in polycystic ovaries produced by administration of testosterone propionate (already reported preliminarily [Ota et al. 1982b]) are investigated by comparing the serum hormone levels with the microscopic appearances in rat ovaries.

**Materials and Methods**

*Hormones and chemicals*

Testosterone propionate (TP) was kindly provided by Prof. A. Kanbegawa, Teikyo University School of Medicine (Tokyo). Ovine prolactin (oPRL; 32 IU/mg), hCG (human chorionic gonadotropin; 9,600 IU/mg) and human FSH (hFSH; NIAMDD-hFSH-2; FSH biopotency, 3.925 IU/mg; LH biopotency, 523 IU/mg) were obtained from Sigma (St. Louis, Mo, USA), Teikoku Hormone MFG (Tokyo, Japan) and NIH, NIAMDD (Bethesda, Md, USA), respectively, and used for [125I] labeled ligands. The purity of oPRL was confirmed by gel electrophoresis (Ota 1983). Iodogen was purchased from Pierce (Rockford, Il, USA). Ultrogel AcA44 was obtained from LKB (Sweden). Carrier free Na [125I] (NEZ-033H) was purchased from New England Nuclear (Boston, Ma, USA). Ovine FSH (oFSH; NIH FSH-S14; FSH potency, equal to 9 NIH FSH-S1 units/mg; LH activity, 0.02 NIH LH-S1 units/mg; PRL activity, <0.1% by weight) and radioimmunoassay (RIA) kits for LH, FSH and prolactin in rat serum were generous gifts from NIH (NIAMDD). The second antibody used for the RIAs was goat anti-rabbit Ig G antiserum (MBL, Nagoya, Japan). RIA kits for 17β-estradiol and progesterone were obtained from CIS (Midori Juji, Inc., Tokyo) and Daichi Radioisotope Laboratory (Tokyo), respectively. RIA kits for testosterone were supplied by Eiken Co., Inc. (Tokyo). The tracers of the kits were [125I] 17β-estradiol (approx. 14,000 cpm/100 µl), [125I]-progesterone (approx. 20,000 cpm/100 µl) and [125I]-testosterone (approx. 20,000 cpm/100 µl).

*Animals in experiments*

Three day old female Wistar-Imamichi rats, obtained from Imamichi Institute for Animal Reproduction (Omiya), were kept in an air-conditioned room as described previously (Ota et al. 1983). At 5 days of age, the rats were injected subcutaneously with 1.25 mg testosterone propionate in 0.2 ml sesame oil per rat Following the previous report (Ota et al. 1983). They were kept together with their mothers until weaning. After vaginas opened, the vaginal smear of rats were daily examined to inspect persistent estrus. At the age of 12 weeks, the rats that showed persistent estrus in vaginal smears were killed between 17:00 and 18:00 and blood samples were collected from bilateral cervical vessels. The ovaries were quickly removed, weighed, and stored at −80°C until assay. The blood samples were centrifuged at 2,500×g for 5 min and sera were stored under −20°C until
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For the control, 0.2 ml of sesame oil was injected into immature animals. Only the rats which showed at least two consecutive four day-estrous cycles were used in the experiment. At each stage of diestrus, proestrus, estrus and metaestrus according to the vaginal smears, the rats were sacrificed, and blood and ovaries were collected as described above.

**Iodination of hormones**

The hormones were labeled with Na $^{125}$I by the iodogen method (Fraker and Speck 1978). Ten $\mu$g hCG, 10 $\mu$g oPRL or 5 $\mu$g hFSH was dissolved in 50 $\mu$l of 50 mM phosphate buffer (pH 7.2). The hormones were added to a glass tube containing 1 $\mu$g adhered iodogen, which was first dissolved in chloroform and then dried under N$_2$. One mCi carrier free Na $^{125}$I was added to the vessel. The tube was allowed to stand at room temperature for 12 to 15 minutes with occasional mixing. The reaction was terminated by transferring the content to another glass tube, followed by an addition of 60 $\mu$l 0.5 M sodium iodide. Radioiodinated hormones were separated chromatographically from free iodide by gel filtration with an ultragel AcA44 column (2.0 x 25 cm). The iodinated peak fractions of the hormone were pooled and aliquots adequate for a single day’s assay were stored at $-20^\circ$C. The specific activities of the labeled hormone were estimated to be 45-70 $\mu$C/$\mu$g from the proportional distribution to the counts under each peak.

**Receptor binding assays for LH, FSH and prolactin**

The frozen ovaries were homogenized with 9 volumes of 10 mM Tris-HCl buffer (pH 7.2 ; homogenate buffer) containing 1 mM MgCl$_2$, 0.1 mM phenylmethylsulfonyl fluoride (Sigma, USA), 0.25 M sucrose, 0.01% NaN$_3$ and 10,000 KIE/l aprotinin (Trasyrol® ; Bayer, Leverkusen, GFR). One hundred $\mu$l of the homogenate (approx. 1 mg protein) was incubated with 40,000 cpm (5-10 ng) of either $^{125}$I iodo-hCG, $^{125}$I iodo-hFSH or $^{125}$I iodo-oPRL in 10 mM Tris-HCl buffer (pH 7.2 ; RRA buffer) containing 1 mM MgCl$_2$, 0.1% bovine serum albumin (BSA) and 0.01% NaN$_3$ in a shaking metabolic incubator for 30 min at 37°C with or without an excess amount of unlabeled hCG (1 $\mu$g), oFSH (1 $\mu$g) or oPRL (1 $\mu$g). The reaction was terminated by adding 10 mM Tris-HCl buffer (RRA buffer), followed by centrifugation of the tubes at 4,000 x g for 30 min at 4°C. After aspirating the supernatant, the radioactivity in the resulting pellets were counted by an automatic gamma counter.

The specific radioactivity of the hormone was calculated by subtracting the nonspecific binding from the total binding and expressed as pg of the specific binding of the added hormones to the receptors per mg homogenate protein. Nonspecific bindings of the assays were less than 2.2% of total bindings. Preliminary experiments revealed that the bindings of LH and FSH receptors reached equilibrium between 30 and 180 min of incubation at 37°C. Prolactin receptor showed the highest binding when incubated 30 min which was reduced after that time (Ota 1983). Protein concentration was determined by the described method (Lowry et al. 1951).

For the kinetic study of the receptors, the ovaries from 3 to 4 rats were collected. The mixed homogenates prepared as described above were incubated with increasing amount of the unlabeled hormones (hCG, oFSH or oPRL) and constant amount (40,000 cpm) of the labeled hormones to measure LH, FSH or prolactin receptors. The binding data were analyzed by Scatchard plots (Scatchard 1949) to determine the association constants (Ka) and the number of binding sites (maximal binding capacity) of the receptors.

**Radioimmunoassays for rPRL, rLH and rFSH in serum**

Aliquots (100 $\mu$l) of sera were added to the tubes which contained either 100 $\mu$l $^{125}$I iodo-rPRL, $^{125}$I iodo-rLH or $^{125}$I iodo-rFSH with the rabbit antisera in 0.01 M phosphate buffer (pH 7.6). This buffer contained 3% normal rabbit serum, 0.05 M EDTA (ethylene-diamine-tetracetic acid), 0.15 M NaCl and 0.1% NaN$_3$. Anti-rPRL, rLH and
rFSH were diluted to 1:5,000, 1:10,000 and 1:2,500. NIAMDD reference hormones were diluted in a 0.01 M phosphate buffer (pH 7.6) containing 0.5% BSA and 0.15 M NaCl. 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 was measured by a gamma counter. The concentrations of the hormones were expressed in terms of NIAMDD reference hormones. The intra- and interassay coefficients of variation for rPRL, rLH and rFSH assays were: rPRL 10.4% and 10.5%; rLH, 9.7% and 8.8%; rFSH, 5.4% and 12.7%, respectively. The lower limits of sensitivity for rPRL, rLH and rFSH assays were 5.2, 1.3 and 30 ng/tube, respectively.

Radioimmunoassays of serum estradiol, progesterone and testosterone

Concentrations of serum estradiol were measured by double antibody RIA kits. Iodination of 17β-estradiol was performed by chloramine-T method. Serum (300 μl) was extracted with 3 ml ethylether. The ether layer was evaporated under N₂ gas and the extract was resuspended in 300 μl 0.04 M phosphate buffer. After addition of 100 μl [¹²⁵I] 17β-estradiol (14,000 cpm) each tube was incubated with 100 μl antiserum raised in rabbits against estradiol-6-carboxymethoxyethyl-BSA for 18 hrs at room temperature. Goat antirabbit gamma-globulin (1 ml) was then added and the mixture was incubated for 15 min at room temperature. After centrifugation for 30 min at 2,000 × g, radioactivity in the resulting pellet was counted. The cross reactivity of this antiserum with estrone was 0.7%. The intra- and interassay coefficients of variation for the assay were 3.3% and 8.3%, respectively. The sensitivity was 1.8 pg/tube.

Serum progesterone level was determined by the described method using Daiichi radioisotope RIA kit (Tsushima et al. 1976). Briefly, serum 0.1 ml, 1 ml ethylether and 50 μl propylene glycol were mixed together. After the ether was evaporated under N₂ gas, 0.5 ml phosphate buffer and 100 μl (20,000 cpm) of [¹²⁵I] iodo-progesterone were added to the tube. The mixture was incubated with 100 μl antiserum raised in rabbits against progesterone-11 α-hemisuccinyl-BSA for 18 hrs at room temperature. One hundred μl bovine serum gamma globulin and polyethylene glycol were then added to the mixture followed by centrifugation for 10 min at 2,000 × g. The radioactivity in the resulting pellet was measured.

Concentrations of serum testosterone were measured by double antibody RIA kits (Arai et al. 1979). In brief, serum (100 μl) was extracted with 2 ml n-hexane-ethylether (1:1) on ice. After centrifugation at 2,500 rpm for 5 min, 1 ml supernatant was transferred to the glass tube. The organic solvent was evaporated at 45°C under nitrogen gas. After addition of 100 μl [¹²⁵I] testosterone (20,000 cpm), each tube was incubated with 100 μl rabbit antiserum for 20 hrs at room temperature. One hundred μl goat antirabbit gamma-globulin was added, then the mixture was incubated for 30 min at room temperature. After centrifugation for 30 min at 2,000 × g at 4°C, the supernatant was aspirated. The radioactivity in the resulting pellet was counted by an automatic gamma counter.

Statistics

All results were expressed as the mean ± S.E. from 5 to 8 animals. Experimental data were analysed 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**

*Ovarian receptor levels for LH, FSH and prolactin during the estrous cycle and in the TP-treated rats.*

LH receptor binding during the estrous cycle was found to vary at different stages as seen in Table 1. LH receptor binding was the lowest at metaestrus, increased steeply at diestrus with follicular maturation, and reached the peak at proestrus. Then, the receptors decreased sharply at estrus. LH receptor binding in the TP-treated ovaries was significantly elevated to a level 32% higher than the diestrus level ($p < 0.01$). It showed an insignificantly higher level than that of proestrus level. The $K_a$ (association constant) of the LH receptor at diestrus was $9.5 \times 10^{11} \text{ M}^{-1}$ and no changes in binding affinity were observed during the estrous cycle as shown in Table 2.

**Table 1.** Receptor levels for LH, FSH and prolactin during the estrous cycle and in the TP-treated rats

<table>
<thead>
<tr>
<th>Group</th>
<th>LH receptor (pg/mg homogenate protein)</th>
<th>FSH receptor (pg/mg homogenate protein)</th>
<th>Prolactin (pg/mg homogenate protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diestrus</td>
<td>$882 \pm 36$</td>
<td>$661 \pm 41$</td>
<td>$350 \pm 51$</td>
</tr>
<tr>
<td>Proestrus</td>
<td>$951 \pm 88$</td>
<td>$657 \pm 64$</td>
<td>$379 \pm 54$</td>
</tr>
<tr>
<td>Estrus</td>
<td>$683 \pm 93^{**}$</td>
<td>$262 \pm 41^{**}$</td>
<td>$250 \pm 37^{**}$</td>
</tr>
<tr>
<td>Metaestrus</td>
<td>$649 \pm 88^{**}$</td>
<td>$391 \pm 52^{**}$</td>
<td>$474 \pm 88^*$</td>
</tr>
<tr>
<td>TP-treated</td>
<td>$1139 \pm 88^{**}$</td>
<td>$1148 \pm 152^{**}$</td>
<td>$-$</td>
</tr>
</tbody>
</table>

Receptor bindings are expressed as pg of the specific binding of the added hormone to the receptor per mg homogenate protein. Values given are means ± s.e. from 6 to 8 animals.

$^{*}p < 0.05$ compared to the diestrus control.

$^{**}p < 0.01$ compared to the diestrus control.

**Table 2.** Kinetic study of ovarian receptors for LH, FSH and prolactin in the rat during the estrous cycle and those treated with testosterone propionate

<table>
<thead>
<tr>
<th>Group</th>
<th>LH receptor $K_a$ (× $10^{11}$/M)</th>
<th>LH receptor $B_m$</th>
<th>FSH receptor $K_a$ (× $10^{11}$/M)</th>
<th>FSH receptor $B_m$</th>
<th>PRL receptor $K_a$ (× $10^{11}$/M)</th>
<th>PRL receptor $B_m$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diestrus</td>
<td>$9.5$</td>
<td>$70$</td>
<td>$4.3$</td>
<td>$14$</td>
<td>$6.4$</td>
<td>$25$</td>
</tr>
<tr>
<td>Proestrus</td>
<td>$10.1$</td>
<td>$79$</td>
<td>$3.9$</td>
<td>$14$</td>
<td>$-$</td>
<td>$-$</td>
</tr>
<tr>
<td>Estrus</td>
<td>$9.6$</td>
<td>$11$</td>
<td>$3.3$</td>
<td>$8$</td>
<td>$6.2$</td>
<td>$20$</td>
</tr>
<tr>
<td>Metaestrus</td>
<td>$11.0$</td>
<td>$6$</td>
<td>$-$</td>
<td>$-$</td>
<td>$6.0$</td>
<td>$59$</td>
</tr>
<tr>
<td>TP-treated</td>
<td>$8.2$</td>
<td>$83$</td>
<td>$3.7$</td>
<td>$43$</td>
<td>$-$</td>
<td>$-$</td>
</tr>
</tbody>
</table>

$K_a$, association constants; $B_m$, maximal binding capacity (site), expressed as fmole/mg homogenate protein.
FSH receptor binding was rather low at metaestrus, then increased sharply during diestrus to 169% of the metaestrus level. The mode of change of the receptor was similar to that observed for LH receptor. FSH receptor level at proestrus was almost as high as that at diestrus. After ovulation, the receptor binding decreased sharply to a minimum at estrus, about 40% of diestrus control ($p < 0.01$). FSH receptor binding in the TP-treated ovaries was observed to be much higher than the control levels in any other stages, and was 173% of that of diestrus control, which showed the highest FSH receptor binding through the cycle. The high level of the receptor was due to an increase of the binding sites as shown in Table 2. Thus, both gonadotropin receptors were in a much elevated state.

On the other hand, change of prolactin receptor binding showed a different mode through the cycle from those of the gonadotropin receptors. The receptor showed two peaks through the cycle. The highest one was found at metaestrus and the second at proestrus. The receptor level was lowest at estrus. The $K_a$ of the ovarian lactogenic receptor at diestrus was $6.4 \times 10^{11}$ M$^{-1}$ and did not change at the different stages of the estrous cycle. The prolactin receptor in the TP-treated ovaries was not determined due to the insufficient number of the samples.

Changes of serum hormone levels in the control rats during estrous cycle and in the TP-treated rats

Changes of serum LH levels during the estrous cycle were shown in Table 3. The serum LH levels were not changed at metaestrus and diestrus. The surge of the hormone was observed at proestrus, being 19-fold higher than the diestrus level. The LH level at estrus returned to a similar level at diestrus. In the TP-treated rats, LH level was significantly higher than the diestrus level ($p < 0.05$), but it was far lower than that of the proestrus level.

Serum FSH levels were relatively constant at metaestrus and diestrus, then significantly increased at proestrus ($p < 0.05$). The level was declined at estrus,

<table>
<thead>
<tr>
<th>Group</th>
<th>LH (ng/ml)</th>
<th>FSH (ng/ml)</th>
<th>prolactin (ng/ml)</th>
<th>Estradiol (pg/ml)</th>
<th>Progesterone (ng/ml)</th>
<th>Testosterone (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diestrus</td>
<td>29± 4</td>
<td>195±14</td>
<td>31± 9</td>
<td>12±1</td>
<td>44±6</td>
<td>81± 9</td>
</tr>
<tr>
<td>Proestrus</td>
<td>560±41**</td>
<td>356±52*</td>
<td>271±21**</td>
<td>27±3**</td>
<td>78±3**</td>
<td>173±16**</td>
</tr>
<tr>
<td>Estrus</td>
<td>33± 5</td>
<td>296±37*</td>
<td>108±13**</td>
<td>3±2**</td>
<td>24±4*</td>
<td>137±28</td>
</tr>
<tr>
<td>Metaestrus</td>
<td>29± 4</td>
<td>208±24</td>
<td>10± 5</td>
<td>5±1**</td>
<td>64±2*</td>
<td>66±15</td>
</tr>
<tr>
<td>TP-treated</td>
<td>41± 3*</td>
<td>207±17</td>
<td>532±71**</td>
<td>25±2**</td>
<td>10±4**</td>
<td>78± 7</td>
</tr>
</tbody>
</table>

Values given are means±S.E. from 5 to 7 animals.

* $p < 0.05$ compared to the control at diestrus.

** $p < 0.01$ compared to the control at diestrus.
but still higher than the diestrus level. The FSH level in the TP-treated rats was as low as that of diestrus control.

The mode of change in serum prolactin level was similar to that of FSH level. That is, the level was the lowest at metaestrus, increased slightly at diestrus and reached its maximum at proestrus. The prolactin level dropped at estrus, but it was still higher than the diestrus level ($p < 0.01$). It is noteworthy that serum prolactin level in the TP-treated rats was 17- and 2-fold higher compared with that of diestrus and proestrus controls, respectively.

Serum estradiol level was low at metaestrus, increased at diestrus according to the follicular maturation, reached its maximum at proestrus, and then dropped sharply to its minimum at estrus. In the TP-treated group, the level was as high as that of proestrus.

In contrast, serum progesterone level was the highest at proestrus and lowest at estrus, and a slight increase was observed at metaestrus. The progesterone level in the TP-rats was as low as 23% diestrus control ($p < 0.01$).

Serum testosterone level was the lowest at metaestrus, increased slowly at diestrus and sharply rose to a peak at proestrus, and then decreased at estrus near that of diestrus control. In the TP-treated rats, the level was the same as that of diestrus control.

Microscopic appearances of the control and TP-treated ovaries in rats

As for the control ovary (Fig. 1), large amounts of corpora lutea were visible and also growing small follicles existed in the lower side of the picture. On the contrary, the ovaries treated with TP showed distinct morphological changes (Figs. 2 and 3). That is, multiple cysts under tunica albuginea, atretic change of follicles, thickening and luteinization of thecal layer around the cysts, degeneration and luteinization in some parts of granulosa layers, and no corpora lutea were seen. In the stromal cells, the nuclei became round and the cytoplasm transparent, indicating luteinization of the cells.

DISCUSSION

Endocrinological changes in the testosterone-treated rats were studied by comparing ovarian gonadotropin receptors and serum hormone levels with their changes during the estrous cycle. LH receptor binding in the rat ovaries during the estrous cycle was shown to vary at each stage as already observed in rat (Cheng 1976; Uilenbroek and Richards 1979; Solano et al. 1980), sheep (Niswender and Diekman 1979; England et al. 1981), pig (Ziecik et al. 1980) and human (Wardlaw et al. 1975; Rajaniemi et al. 1981). The receptor binding at diestrus increased 136% of that of metaestrus, reached its maximum at proestrus before ovulation, and declined toward estrus. FSH receptor binding also showed cyclic change, similar to the results described by others (Cheng 1976; Solano et al. 1980). The receptor level was low at metaestrus since the follicle was still immature and
Fig. 1. An ovarian section (×10.4) from the control rat at diestrus.

Fig. 2. An ovarian section (×10.4) from the TP-treated rat, showing polycystic ovaries and absence of corpora lutea.
the number of granulosa cells were not sufficient. Along with follicular maturation, the number of FSH receptors at diestrus elevated in binding capacity 69% more than that of LH receptor. The FSH receptor reached a peak at proestrus and declined after ovulation. Prolactin receptor showed a characteristic pattern of change different from that of LH or FSH receptor during the estrous cycle. The receptor level showed two peaks, one at metaestrus and the other at proestrus. This observation is consistent with that by Solano et al. (1980).

More attention should be paid to the fact that the LH receptor in the TP-treated rats increased higher than the proestrus level. This result would be explained by the heterologous regulation mechanism of prolactin on LH receptor as described with granulosa cells (Casper and Erickson 1981), luteal cells (Holt et al. 1976; Richards and Williams 1976) or ovarian homogenates (Ota et al. 1982a) in the rat. High estradiol levels in the TP-rats would also potentiate the receptor to increase as observed in the granulosa cells (Richards et al. 1976; Ireland and Richards 1978). Tonic secretion of serum LH beyond the diestrus level but far lower than the proestrus level in the TP-rats does not seem to be sufficient to regulate the receptor downward in such circumstances. FSH receptor in the experimented animals was markedly higher than the control level. There are several reasons for this. First, prolactin may perform some role in inducing FSH receptor, since it was found to increase the receptor in the granulosa cell culture in the presence of androstenedione (Brodie and Ota 1985). Secondly, the low
level of serum FSH should be considered. Insufficient secretion of the gonadotropin might induce an increase in the FSH receptor level. This must be a reversed phenomenon of the so-called down-regulation of a receptor (Conti et al. 1977). Moreover, high levels of estradiol could raise FSH receptor as observed in the rat granulosa cells (Knecht et al. 1984).

The result of the present study as to gonadotropin receptor levels is partly inconsistent with that by Copmann and Adams (1981). They described the reduction in LH receptors and enhancement in FSH receptors in hypothyroid rats with daily injections of hCG. Such a difference between their study and the present result must be due to the distinct procedure employed. In human PCO, Rajaniemi et al. (1980) reported that the tonic secretion of LH would be a key stone of producing PCO and reducing LH receptors. However, Ota et al. (1984) have recently reported that LH receptor did not decrease to the late follicular level in human PCO, and that FSH receptor increased to nearly that of the late follicular level, and these results are compatible with those of the present study.

Serum LH level in TP-treated rats exceeded the diestrus control level. There are several reasons for this phenomenon. One is that the control mechanism of LH by dopamine in the pituitary might be disturbed in these animals as observed in human PCO cases (Quigley et al. 1981). Another is that abnormal ratio of hormone levels such as estrogen to progesterone may be involved. The serum prolactin level was 17-fold higher than that of diestrus control. The markedly high level of the hormone was observed since 40 days after TP injections in immature rats (Ota et al. 1983). Hyperprolactinemia in rats can also be induced by dehydroepiandrosterone (Knudsen et al. 1975; Ward et al. 1978) or hypophysial stalk transection (Nikitovitch-Winer 1965). This would be explained as the disturbance of the inhibitory control of prolactin by dopamine in the pituitary. This mechanism was already shown by Alger et al. (1980) using metoclopramide in PCO patients. Furthermore, elevated estradiol level near that during the proestrus level may be promoting prolactin secretion from the pituitary (Thorner 1977).

Level of serum estradiol was increased to nearly that of proestrus control, and is inconsistent with human cases (Ota et al. 1979; Alger et al. 1980; Rajaniemi et al. 1980). In human, high level of estrone seems to augment the sensitivity of pituitary to LH releasing hormone (LH-RH) and to inhibit the release of pituitary FSH leading to a derangement of follicular maturation (Rebar et al. 1976). Similar augmentation of estradiol on the pituitary might exist in the TP-treated rats. Estradiol would also stimulate prolactin secretion as mentioned above, which leads to anovulation and persistent estrous cycle. It is reasonable that progesterone level was lower in the TP-treated rats than that of any stage of the controls, since there were not corpora lutea in these animals.

Histological study revealed the multiple cysts in the TP-treated ovary. The granulosa cells were degenerated and partly luteinized. The theca cells were
thickened, which may explain estradiol level. Also, luteinization of the stroma and fewer primordial follicles were observed. These findings were consistent with those of human ovaries in PCO patient, although there was no thickening of tunica albuginea.

In conclusion, elevated levels of the gonadotropin receptors were clearly observed in polycystic ovary in rats, which seemed to be partly caused by insufficient secretion of gonadotropin and partly by much elevated levels of prolactin and estradiol. Clinically, the high levels of the receptors appear to be relevant to PCO patients.

Acknowledgments

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