Formation of Polycystic Ovary in Mature Rats by the Long-Term Administration of Human Chorionic Gonadotropin

HIROTAKA OTA, MINEKO FUKUSHIMA and MASAHIRO MAKI

Department of Obstetrics and Gynecology, Akita University School of Medicine, Akita 010

OTA, H., FUKUSHIMA, M. and MAKI, M. Formation of Polycystic Ovary in Mature Rats by the Long-Term Administration of Human Chorionic Gonadotropin. Tohoku J. exp. Med., 1987, 151 (1), 33-40 —— The critical role of high level of serum LH in polycystic ovary syndrome was evaluated in rats by the long-term administration of hCG. Wistar-Imamichi strain mature female rats (age: 12 weeks) which showed at least two consecutive estrous cycles in vaginal smears were daily injected 10 IU hCG subcutaneously from diestrus for 80 days. Control rats were received saline solutions. On the next day after the last administration the rats were killed, and serum hormone levels and histological changes in the ovaries were examined. In 8 of 11 control rats the vaginal smears showed the regular estrous cycles (group 1) during the experimental period. None of the controls exhibited polycystic ovaries. In 19 of 25 experimented rats there were old corpora lutea and degenerated follicles (group 2). The remained animals (n=6; group 3) showed polycystic ovaries and no corpora lutea except one. HCG treatment elevated the serum prolactin and estradiol levels in group 3, but reduced the progesterone level. Thus, it was suggested that the hCG-induced formation of PCO in rats might be able to refer to the pathogenesis in polycystic ovary syndrome. —— polycystic ovary; human; rat; hCG; hyperprolactinemia

In polycystic ovary syndrome, there are several endocrine features such as high levels of serum LH and testosterone, an elevated estrone/estradiol ratio and an excessive response of LH to LH-RH stimulation (Yen et al. 1970; Duignan 1976; Quigley et al. 1981). Moreover, the disease is frequently associated with hyperprolactinemia (Thorner 1977; Ota et al. 1979; Alger et al. 1980; Wortsman and Hirschowitz 1980). However, the mechanisms by which polycystic ovaries are caused are not clearly understood. One hypothesis for the pathogenesis of the disease is that inappropriate gonadotropin secretion with a high LH/FSH ratio would be raised. The high level of LH maintains the elevated estrogen level, which would turn to stimulate the hypothalamic-pituitary system. In addition,
Yen et al. (1970) or Futterweit (1984) suggested that this syndrome originates as an adrenal disorder during the early phase of sexual maturation. The elevated level of adrenal androgens could result in an increased extraglandular estrogen production, which in turn induces an elevated LH/FSH ratio and is associated with ovarian androgen secretion. In the present study, the authors attempted to establish the critical role of the high level of serum LH in the formation of polycystic ovary by the daily injection of HCG for 80 days.

**MATERIALS AND METHODS**

*Animals in experiments*

Wistar-Imamichi strain female rats at 12 weeks old (mean body weight 170 g) having 4 day regular estrous cycles were used in the present experiment. The rats were daily examined in vaginal smears and those which showed consecutive estrous cycles were only employed in the experiment. Ten IU hCG (Teikoku Hormone MFG, Tokyo) dissolved in 0.5 ml saline solutions was daily injected into rats subcutaneously from diestrus for 80 days. Control rats received saline solutions. On the next day after the last injection between 14:00 and 15:00, all of the animals were anesthetized with ethylether. Then, blood was obtained from cervical vessels and the bilateral ovaries were removed. Sera were kept in a freezer at $-20^\circ$C until the assay. The ovaries were fixed in 10% neutral formalin, serially sectioned at 2 $\mu$m and stained with hematoxylin and eosin.

*Radioimmunoassays of serum LH, FSH and prolactin*

The levels of serum LH, FSH and prolactin were measured using double antibody RIA kits supplied from NIH (NIAMDD; Bethesda, MD, USA). The details in the assays were described previously (Ota et al. 1986a).

*Radioimmunoassays of serum estradiol, progesterone and testosterone*

The levels in serum estradiol, progesterone and testosterone in rats were measured using the kits obtained from CIS (Midori Juji Co., Tokyo), Daiichi Radioisotope Lab. (Tokyo) and Eiken Co. (Tokyo), respectively. The assay procedures and accuracies were already described elsewhere (Ota et al. 1986a, b).

*Statistics*

All values were expressed as the mean±s.e. Statistical significances were calculated by Duncan's new multiple range test. A value of $p<0.05$ was chosen as the limit of statistical significance.

**RESULTS**

*Incidence of polycystic ovary rats after administration of hCG for 80 days*

Among the controls $(n=11)$ 8 rats showed the regular estrous cycles throughout the experimented period and were at diestrus in vaginal smears on the 81st day. The estrous cycles in the remained rats $(n=3)$ became irregular and retarded, but none of them showed persistent estrus during the last two estrous cycles. Histologically, the ovaries in all rats did not have PCO patterns. Therefore, 8 rats which showed the regular estrous cycles, were employed as the control (group 1).

In the hCG-treated group, 19 of 25 animals had old corpora lutea and
degenerated follicles in various sizes (group 2). In 5 of the remained animals (group 3; \( n = 6 \)) there were no corpora lutea and polycystic ovaries. The last animal had a hyalinized, very small corpus luteum and was combined into group 3. In all of the animals the vaginal smears showed persistent estrus during the last three consecutive cycles just before the completion of the experiment.

**Changes of serum LH, FSH and prolactin levels**

In group 2, serum LH level tended to increase insignificantly in contrast with the control (2.2 ± 0.3 ng/ml) as shown in Fig. 1. Serum FSH levels did not vary among the three groups. Serum prolactin level was 14.7 ± 1.8 ng/ml in the control group, and showed 3- and 8-fold increases in groups 2 and 3, respectively, as compared with the control group, which were associated with the disappearance of corpora lutea and the formation of cysts.

**Changes of serum estradiol, progesterone and testosterone levels**

Serum estradiol level was 13.6 ± 0.7 pg/ml in the control as shown in Fig. 2. HCG treatment significantly \( (p < 0.01) \) raised the estradiol level in group 3 over the control. On the contrary, serum progesterone levels in the treated groups were lower than the control (37.8 ± 7.1 ng/ml). HCG treatment reduced the level of progesterone significantly in group 3 \( (p < 0.01) \). Testosterone levels did not vary among the groups.

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**Fig. 1.** Changes in serum LH, FSH and prolactin levels after treatment with 10 IU hCG for 80 days. **\( **p < 0.01 \)** compared to the control (group 1).  
- group 1 (control);  
- group 2;  
- group 3.

**Fig. 2.** Changes in serum estradiol, progesterone and testosterone levels after treatment with 10 IU hCG for 80 days. **\( **p < 0.01 \)** compared to the control (group 1).  
- group 1 (control);  
- group 2;  
- group 3.
Fig. 3. An ovarian section from the rat at diestrus before hCG treatment. ×10.4

Fig. 4. An ovarian section from the control rat (group 1) on the next day after hCG treatment for 80 days. ×10.4
Fig. 5. An ovarian section from the hCG-treated rat (group 2) showing the old corpora lutea, degenerated follicles and wide stroma. ×10.4
Fig. 6. An ovarian section from the hCG-treated polycystic ovary rats (group 3) showing no corpora lutea, multiple cysts and wide stroma. ×10.4
Histological changes after the treatment with 10 IU hCG for 80 days

The ovarian weight in group 2 was not different from the control (99.3 ± 10.2 g). However, the weight in group 3 was significantly reduced to 52% of the control.

Before the beginning of hCG injection, the ovaries showed a number of corpora lutea and limited space of stroma (Fig. 4). On the other hand, hCG treatment for 80 days resulted in a development of stroma having many vessels, degenerated follicles and decreased corpora lutea without fresh one (Fig. 5). In group 3, the ovaries contained multiple cystic follicles without corpora lutea except one (Fig. 6). The granulosa cell layers in the follicles became thin and inner surfaces of the layers irregular. Nuclei of the cells showed pyknosis and few mitosis. In some of the middle-sized follicles, the nuclei became round and the cytoplasm transparent, indicating the luteinization of the granulosa cells. Theca layers exhibited to be thick, but less prominent luteinization. Stroma developed the lymph-vessels. Nuclei of the stromal cells were round and cytoplasms transparent, indicating the luteinization.

DISCUSSION

The present study clearly indicated that the continuous treatment of hCG could make polycystic ovaries in mature rats, namely in 24% of the treated rats. Similar formation of PCO with hCG was previously reported by Greene and Burrill (1941). They suggested that the primary pathogenesis of PCO appeared to derive from the disturbance in hypothalamic-pituitary system. In the case of polycystic ovary syndrome, tonic secretion of serum LH is well maintained despite of lack of cyclic secretion, which would result in the stimulation of ovary to form multiple cysts and in testosterone secretion. Thus, endocrinological changes induced by the administration of hCG in rats appear to be related to those in polycystic ovary syndrome.

Serum prolactin levels increased in group 3, which showed the lack of corpora lutea and multiple cystic follicles. Hyperprolactinemia in rats can be produced experimentally by treatment of testosterone propionate (Ota et al. 1983) or dehydroepiandrosterone sulphate (Knudsen et al. 1975; Ward and Mahesh 1978). The mechanisms of inducing hyperprolactinemia in rats by androgen or hCG appear different. In the case of androgen, androgen appears to disturb the cyclic center located in the suprachiasmatic area of the hypothalamus (Barraclough and Gorski 1961), resulting in an acyclicity and hyperprolactinemia. However, the continuous stimulation with hCG would first promote estrogen secretion in the ovary. Then, this high level of estrogen would again modulate the hypothalamic-pituitary system and disturb the dopamine turnover (Falaschi et al. 1980), which would eventually stimulate prolactin secretion (Thorner 1977). Moreover, prolactin as a luteotropic hormone in rats might also be elevated compensatorily
due to the lack of corpora lutea.

In polycystic ovary syndrome, it is known that the dopaminergic control of prolactin and/or LH secretion is disturbed. That is, a drip infusion of dopamine hydrochloride typically inhibits LH secretion (Quigley et al. 1981), suggesting the decreased control of dopamine on LH. Also, a single injection of metoclopramide, a dopamine receptor antagonist (Vazquez-Matute et al. 1979), raised the prolactin level over the normal level in the women with regular menstrual cycles (Alger et al. 1980). Normalization of the high prolactin level and resultant induction of ovulation by bromocriptine (CB-154) in PCO patient support this possibility. Thus, in the present study, the high level of estrogen seems to modulate the dopamine neuron, and reduce endogenous dopamine inhibition of LH-RH secretion, resulting in an elevated level of LH secretion.

Histological study revealed that the ovaries in group 3 were lacking in corpora lutea and showed multiple cystic follicles and wide stroma, and that in some of the follicles the granulosa cells were luteinized. We have previously reported the formation of PCO associated with hyperprolactinemia in neonatal rats treated with testosterone propionate (Ota et al. 1983). In human, similar findings, such as thickened tunica albuginea, multiple cysts, atretic follicles and luteinization of theca cells, are observed (Stein and Leventhal 1935; Stein and Cohen 1939).

In conclusion, tonic secretion of LH would induce the formation of polycystic ovaries and stimulate estrogen secretion, which in turn would enhance prolactin secretion. The high level of estrogen would modulate dopamine turnover, reduce the dopaminergic control of LH-RH secretion in hypothalamus and result in an increase of pulsatile LH release, forming circulus vitiosus.

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References


