Steroid Hormone Receptors in the Uterus and Ovary of Immature Rats Treated with Gonadotropins

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Abstract

We administered either saline (group A) or 10 IU of pregnant mare serum gonadotropin (PMS; groups B and C) to female immature rats. Fifty-three hours later, the rats were injected with saline (groups A and B) or 30 IU of human chorionic gonadotropin (hCG; group C). The rats were decapitated 17 h after the last treatment, and the serum levels of progesterone (P_4) and estradiol (E_2) were measured by specific radioimmunoassays (RIA). The receptor levels of progesterone (PR) and estrogen (ER) in the uterus and ovaries were measured and the dissociation constant (K_d) of PR was obtained.

The highest serum level of P_4 was found in group C and that of E_2 in group B. Cytosol levels of PR and ER in the uterus and ovary of the group B were the highest. It was indicated that the PMS treated-group (B), which had developing follicles in the ovary and the high serum level of E_2 , showed the highest concentration of ER and PR in both the ovary and the uterus. In the PMS and hCG-treated group (C), the uterine and ovarian steroid receptors decreased probably because of the luteinization and the high serum level of P_4 . The K_d uterine PR value was less than that of ovarian PR.

It is well known that progestins and estrogens have stimulatory and inhibitory effects on the ovarian steroidogenesis (Wada *et al.*, 1984; Welsh Jr. *et al.*, 1983; Schreiber *et al.*, 1980; Fortune and Hansel, 1979). These effects are partly exerted through ovarian steroid receptors (Schreiber *et al.*, 1980). Many published data deal with rat ovarian steroid receptors (Schreiber and Hsueh, 1979; Schreiber and Erickson, 1979; Sakabe *et al.*, 1983; Fujii-Hanamoto *et al.*, 1985; Hamilton *et al.*, 1977; Saiduddin and Zassenhaus, 1977; Saiduddin and Zas-

senhaus, 1978), but the effect of gonadotropins on immature rat ovarian vs. uterine receptors of steroid hormones still remains to be clarified.

Materials and Methods

Chemicals

All chemicals and reagents used were of analytical grade. [17α -methyl- 3H]-R5020 (86.3 Ci/mmol), and [2,4,6,7- 3H (N)]-estradiol-17 β (E_2) (96.4 Ci/mmol) were purchased from New England Nuclear Corp. (Boston, MA, USA). Other non radioactive steroids were obtained from Sigma Chemical Company (St. Louis, MO, USA).

Pregnant mare serum gonadotropin (PMS) and human chorionic gonadotropin (hCG) were the product of Teikoku Hormone Co. (Tokyo, Japan). Trasylol was obtained from Bayer Co. (Leverkusen, Germany).

Animals

Immature female rats (24 days old) of the Wistar-Imamichi strain were used. The rats were divided into three groups. At 0900 h, saline (group A) or 10 IU of PMS (groups B and C) was injected subcutaneously (s.c.). Fifty-three hours later, saline (groups A and B) and 30 IU of hCG (group C) was injected s.c. Seventeen hours after the last treatment, the rats were decapitated and blood samples were collected. The ovaries and the uterus were dissected out, weighed and kept frozen at -80°C until steroid receptors were assayed.

Radioimmunoassay (RIA) of P_4 and E_2

The blood sample was centrifuged at 3000 rpm for 20 min and serum was separated. For the RIA of progesterone (P_4), the serum was extracted twice with three volumes of ether. For the RIA of E_2 , the serum was extracted first with hexane to remove lipid, and then extracted with ether in a similar way to P_4 . The antibodies to E_2 and P_4 were prepared in our laboratory; Japanese albino rabbits were immunized by injecting E_2 -6-carboxymethyloxime BSA or P_4 -11 α -hemisuccinate to produce specific antisera. The values for cross reaction of the P_4 antibody (diluted at 1:200,000) with 20 α -hydroxy P_4 and 17 α -hydroxy P_4 were 6.73 and 0.89%, respectively (Yoshida *et al.*, 1973). Those for the E_2 antibody (diluted at 1:30,000) to estrone and estriol were 3.2 and 1.8%, respectively (Makino, 1973).

Steroid radio receptor assay (RRA)

1) Buffer

The buffer for the RRA consisted of 10 mM Tris, 1.5 mM EDTA, 1 mM dithiothreitol, 10% glycerol, 10 mM sodium molybdate, and 500 KIE/ml Trasylol. The PH of the buffer was 7.4 for the preparation of the cytosol fraction and 8.0 for the incubation.

2) Preparation of the cytosol fraction

The ovaries and uterus were homogenized in a Polytron homogenizer and centrifuged at 105,000 g for 70 min at 4°C . The supernatant was treated with dextran coated charcoal (DCC)

(0.25% Norit A and 0.025% dextran) at 0°C for 10 min. We used this cytosol fraction for the RRA. Protein in the cytosol fraction was measured by Bradford's method (Bradford, 1976).

3) RRA of progesterone receptor (PR)

^3H -R5020 was used as a radioactive ligand for the assay of PR. To measure the quantity and the dissociation constant (K_d) of PR, the cytosol fraction was incubated with 0.125-8 nM ^3H -R5020 overnight at 0°C . With this condition we can measure the B_{max} of total PR, not free PR, because the dissociation rate of uterine and ovarian PR is very high (Feil *et al.*, 1972, Milgrom *et al.*, 1972, Schreiber *et al.*, 1979). To rule out non specific binding, a 100 fold excess of cold R5020 was added to the control incubation tubes. After the incubation, unbound ^3H -R5020 was removed by DCC, and bound ^3H -R5020 was counted in a liquid scintillation spectrometer (Aloka Model LSC-703). The data were analysed by Scatchard plot (Scatchard, 1949), and the number of maximum binding sites (B_{max}) and K_d were calculated. For the competition assay, the cytosol fraction was incubated with 2 or 8 nM ^3H -R5020 and a 100 fold excess of various other steroids. This gives percent specific binding of ^3H -R5020 to the cytosol fraction.

4) Assay of estrogen binding capacity

^3H - E_2 was used as a radioactive ligand for the assay of estrogen binding capacity. The cytosol fraction was incubated for 30 min at 37°C with 2-8 nM ^3H - E_2 in the presence or absence of a 100 fold excess of cold E_2 . Counts of ^3H - E_2 specifically bound to the cytosol fraction were obtained. However, because there was insufficient material, Scatchard analysis could not be performed for E_2 .

Statistical analysis

Statistical analyses of the results were made by Student's *t*-test, and a P value of less than 0.05 was considered significant.

Results

Weight of ovaries and uterus

A pair of ovaries from groups A, B, and C weighed (mean $\pm$ SE) 21.0 ± 4.61 , 63.1 ± 12.11 , and 78.3 ± 12.6 mg, respectively. There were significant differences among the three groups ($p < 0.001$). The uteri

from groups A, B, and C weighed (mean $\pm$ SE) 61.1 ± 14.1 , 154.0 ± 24.9 , and 123.9 ± 17.6 mg, respectively. There were significant differences among the three groups ($p < 0.001$).

Serum P_4 and E_2

The serum P_4 level for group B was higher than that for group A, but the difference was not significant (Fig. 1a). The P_4 level for group C was significantly higher than that for groups A and B ($p < 0.001$, $p < 0.05$). The serum E_2 level for group B was higher than that for group A ($p < 0.05$; Fig. 1b).

RRA of uterine and ovarian PR

1) K_d

The average K_d value for uterine PR was 0.315 ± 0.080 nM, and that for ovarian PR was 3.37 ± 1.53 nM. No significant difference in the K_d of the PR was observed among the group. The K_d value for ovarian PR was significantly higher than that for uterine PR ($p < 0.001$). Fig. 2 shows the Scatchard plot of equilibrium binding at 0°C of ^3H -R5020 to the uterine and ovarian PR.

2) Competition assay

Fig. 3 shows specific binding of ^3H -R5020 to PR in the ovaries and uterus as various competing steroids were added.

Fig. 1. Title: The serum level of (a) P_4 and (b) E_2 in the immature rat.

Legend: A: without gonadotropin treatment; B: treated with PMS; C: treated with PMS and hCG. Vertical bars indicate standard errors.

E₂, dexamethasone (DEX), and testosterone (T) slightly interfered with the binding of ³H-R5020 to PRs. On the other hand, ³H-R5020 was effectively replaced with R5020 and P₄ in ovarian and uterine PRs.

3) *Number of maximum binding sites (B_{max}) of PR*

Fig. 4 shows the B_{max} of cytosol PR in the uterus and ovary. The B_{max} of PR in the uterus, as expressed per mg protein, of group A was significantly higher than

Fig. 2. Title: Scatchard plot of the uterine and ovarian PR.

Legend: (a) Uterine PR of the immature rat treated with PMS; (b) ovarian PR of the immature rat without gonadotropin treatment.

Fig. 3. Title: The competition assay of the (a) uterine and (b) ovarian PR.

Legend: The cytosol fraction was incubated with ³H-R5020 and 100 fold excess of other steroids. The Figures indicate percent specific binding of ³H-R5020 to the cytosol fraction compared to the control incubation with ³H-R5020 only.

Fig. 4a Uterine PR **Fig. 4b** Ovarian PR

Fig. 4. Title: The Bmax of the (a) uterine and (b) the ovarian cytosol PR per unit weight of protein or per organ of the immature rat.

Legend: A: No gonadotropin treatment; B: Treated with PMS; C: Treated with PMS and hCG. Vertical bars indicate standard errors.

Fig. 5a E₂ bound to the uterus **Fig. 5b** E₂ bound to the ovaries **Fig. 5.** Title: The bound ³H-E₂ to the (a) uterine and (b) ovarian cytosol fraction per unit weight of protein or per organ of the immature rat.

Legend: A: No gonadotropin treatment; B: Treated with PMS; C: Treated with PMS and hCG. Vertical bars indicate standard errors.

that of group C. When Bmax was expressed per uterus, group B showed the highest value. In the ovary, the Bmax of cytosol PR both per protein and per two ovaries of group B was significantly higher than that of group A ($p < 0.05$, $p < 0.001$). The Bmax of PR per protein of group C was significantly lower than that of group B ($p < 0.05$).

Assay of uterine and ovarian estrogen binding capacity

Fig. 5 shows specific binding of E_2 to the cytosol fraction of the rat uterus and ovary. The total amount of $^3H-E_2$ bound to the uterus of group B was significantly higher than that of the groups A and C ($p < 0.001$, $p < 0.01$). In the ovary, there was no significant difference among three groups. However, the least binding of $^3H-E_2$ to the ovarian preparation was observed in group C.

Discussion

The present study showed that the administration of PMS increased ovarian weight and the serum level of E_2 . This indicates that the FSH component of PMS stimulates the development of the ovarian follicles, which secrete elevated levels of E_2 . The hCG administration after PMS-treatment increased ovarian weight and the serum level of P_4 , but the serum level of E_2 decreased. This result indicates that hCG induces ovulation and the ovary is luteinized. The corpus luteum has high 3β -HSD activity and produces P_4 in quantities. But the corpus luteum has less 17α -hydroxylase and $17-20$ lyase than the follicles, and consequently E_2 secretion decreases after luteinization (group C) (Suzuki and Tamaoki, 1979).

It is well known that E_2 increases uterine weight and P_4 antagonizes this effect of E_2 (Jordan *et al.*, 1978; Koseki *et al.*, 1977).

In the present experiment, the heaviest uterine weight was found in group B, which also showed the highest level of serum E_2 . As the level of serum P_4 reached the peak (group C), the serum level of E_2 dropped and uterine weight decreased.

In our experiment, the Kd value for ovarian cytosol PR was greater than that of uterine PR. According to the reported data, the Kd value for the rat uterus is 1.1 nM (Vu Hai and Milgrom, 1978), 0.29 nM (MacLusky and McEwen, 1980), 0.66 nM (Kirchhoff *et al.*, 1983) or 2.22 nM (Nagasaka, 1981) and that for the ovary is 14 nM (Schreiber and Hsueh, 1979) or 22-4.8 nM (Fujii-Hanamoto, 1985). The values vary, but the Kd for ovarian PR is usually smaller than that for uterine PR. Further study will be needed to clarify the difference between the Kd values.

The number of maximum binding sites of uterine cytosol PR in group B was significantly larger than that in group A or C, suggesting that E_2 increased uterine PR, and P_4 antagonized this effect of E_2 (Koseki *et al.*, 1977; Milgrom *et al.*, 1973). There is no report confirming that E_2 increases ovarian PR. The present results revealed that group B, having the biggest number of mature follicles with granulosa cells, had the largest number for PR; this agrees with previous findings that PR is mainly located in granulosa cells (Schreiber and Erickson, 1979; Sakabe *et al.*, 1983). On the other hand, the Bmax of PR in group C, which showed the highest level of serum P_4 , was lower than that in group B both in the uterus and ovary. The finding that ovarian PR is higher on the proestrous and diestrous days, and is lower on the metestrous and estrous days in cycling rats, suggests that follicular development increases ovarian PR and luteinization decreases it (Sakabe *et al.*, 1983; Fujii-Hanamoto *et al.*, 1985).

If the Kd value for ER is constant, E_2 binding capacity is parallel to the number of ERs. No evidence could be presented

showing that the K_d value was constant in our experiments. Morris (Morris, 1976) reported that the K_d value for uterine ER of ovariectomized rat shortly after treatment with E_2 was not constant, but this might be a special case because the E_2 administered would interrupt the RRA and increase the K_d value. Ginsburg (Ginsburg *et al.*, 1975) reported that the K_d value for rat uterine ER was constant in the estrous cycle. The K_d value for the ER of pregnant rat corpus luteum was reported to be 0.22 nM (Saiduddin *et al.*, 1977) and that of immature rat was 0.413 nM (Richards, 1974). These values are fairly close. It may be assumed that the K_d value was constant in our series of experiments and that largest number of ER were found in the group B both in the uterus and ovary.

In the present series of experiments the maximal binding of E_2 to uterine and ovarian cytosol was seen in the group B among the three groups studied (Fig. 5). Although intrinsic E_2 bound to ER could not completely be exchanged under the present experimental conditions, our results indicated that the E_2 binding capacity of group B was highest because the serum concentration of E_2 was highest (Fig. 1); binding sites are also abundant.

It is reported that E_2 increases uterine ER, and P_4 antagonizes this effect as in the case of the PR (Jordan *et al.*, 1978; Okulicz *et al.*, 1981; Bhakoo and Katzenellenbogen, 1977; West *et al.*, 1978). In our experiments the number of uterine cytosol ER in group B was higher than that in groups A and C (as expressed per organ). Few reports compare the ovarian and uterine ER; Saiduddin's experiments showed that E_2 increases, whereas P_4 decreases ER in the uterus but not in the ovary (Saiduddin and Zassenhaus, 1978). Since the ovarian ER is mainly located in the granulosa cells (Stumpf, 1969; Richards, 1975), it is reasonable that group B, in the present experiment, had more ER than the other 2

groups as expressed per organ. Because the level of serum E_2 was high in group B, the "translocation" process might have been enhanced, and the number of cytosol ER in this group was maximum. It may be due to an increase in ER synthesis, the stability of ER or a change in the velocity of reactivation in the recycling system. Further study will be needed to clarify these points. In the present series of experiments, group C had less ER than group B in both the ovary and the uterus. The decrease in uterine ER may be due to the high level of serum P_4 (Okulicz *et al.*, 1981; Bhakoo and Katzenellenbogen *et al.*, 1977; West *et al.*, 1978), but in the case of the ovary it is also possible that the granulosa cells lost ER after luteinization; Richards (1975) reported that luteinization induced by LH decreased ovarian ER in the granulosa cells.

The number of steroid binding sites of the ovary may vary according to the serum concentration of the steroid. However, since the ovary is subjected to cyclic changes, such morphological variables may also have to be considered together with the serum concentration of steroids, when the binding capacity is discussed.

It may be concluded that PMS increased PR and ER in the uterus and ovary, and hCG decreased them in both tissues, suggesting that changes in PR and ER occurred concomitantly in the uterus and ovary under various endocrinological conditions.

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