Bull. Tokyo Med. Dent. Univ., 28: 87–90, 1981

UTERINE ESTROGEN RECEPTOR ASSAYED BY THE CONTROLLED PORE GLASS (CPG) METHOD IN ADULT RAT AFTER STEROID ADMINISTRATION

BY

Yoshihisa IMAMURA, Shuji SASSA and Ryohei OKAMOTO*[1]

ABSTRACT

The interaction of estrogen and androgen was studied at the estrogen receptor level of adult rat uterus by the in vivo experiment. Both the free and total estrogen receptor in the cytosol and nucleus were assayed by the controlled pore glass beads method [2]. Testosterone was given by daily injection for 5 days to the rats treated with estradiol dipropionate (long-acting estradiol) 3 or 5 days before. In the estradiol dipropionate-treated rats the plasma estradiol concentration remained at an extremely high level for 7 days and then decreased to 5-fold of that of proestrus control by the 14th day. There was no significant change in the uterine estrogen receptor of the adult rat with a high estradiol concentration induced by testosterone.

INTRODUCTION

In the medical clinics, administration of androgen is frequently effective in the patients with estrogen-dependent diseases or symptoms. Investigations [1, 3, 4, 5] at the estrogen receptor (ER) level have been carried out to study the anti-estrogenic mechanism of androgen. In these experiments, estradiol (E_2) and testosterone (T) were given simultaneously to the immature rats; cytosolic and nuclear ER were assayed by the methods of dextran-coated charcoal (DCC) and pellet exchange, respectively. In the present experiment, cytosolic and nuclear ER were assayed by the same method, CPG, and the adult rats were pretreated with estradiol dipropionate (ED) to elevate the blood E_2 concentration, because the patients with estrogen-dependent diseases or symptoms are thought to be under the estrogenic influence for a fairly long period.

MATERIALS AND METHODS

I. Experimental animals

Fifty nine female Sprague-Dawley rats, 10 weeks old, were used. The rats were divided into 4 groups. Group I (n=6) was not treated in any way and was sacrificed at proestrus. Group II (n=33) was injected im (intramuscularly) with 1 mg/0.2 ml of estradiol dipropionate (ED: Teikoku Hormone Mfg. Co., Ltd.) and was sacrificed at days 1 (n=5), 3 (n=9), 5 (n=4), 7 (n=5), 11 (n=5) and 14 (n=5) after the ED injection. Group III (n=10) received daily intramuscular injections 1 mg of testosterone (T: Merck) dissolved in 0.2 ml of dimethylsulfoxide (Wako Pure Chemical Industries, LTD.) (n=5) or 0.2 ml of vehicle (V: n=5) for 5 days, 3 days after the ED injection, and was sacrificed 3 hours after the last injection. Group IV (n=10) was treated in the same way as Group III, except that the testosterone injection was

*[1] 今村好久・左雨秀治・岡本良平: Department of Endocrinology (Chief: Prof. R. OKAMOTO), Medical Research Institute, Tokyo Medical and Dental University (Tokyo Ika Shika Daigaku).
Received for publication, June 8, 1981.

started 7 days after the ED injection.

II. Plasma E_2 assay

Blood was drawn by cardiac puncture under urethane ethylcarbamate anaesthesia immediately before sacrifice and heparin was added. After centrifugation the plasma was stored at −20°C for E_2 determination by radioimmunoassay (RIA).

III. Uterine ER assay

Uterus was dissected and weighed after blood collection, and stored at −70°C for ER assay by the controlled pore glass (CPG) method. Cytosol and nuclear fractions were prepared as follows: (1) Pulverization of the tissue. (2) Homogenization in 4 ml of T buffer (10 mM Tris neutralized to pH 7.4 with HNO_3). (3) Centrifugation at 25,000×g for 15 minutes. The supernatant was defined as cytosol fraction and designated as S_1. (4) Washing of the sediment with 4 ml of T buffer. (5) Centrifugation at 25,000×g for 15 minutes. (6) (4) and (5) procedures were repeated. (7) Homogenization of the second sediment in 4 ml of TK buffer (10 mM Tris and 400 mM KNO_3 neutralized to pH 8.4 with HNO_3. (8) Centrifugation at 25,000×g for 15 minutes. The supernatant was defined as the nuclear fraction and designated as S_2. CPG method: (1′) Controlled pore glass beads (Electro Nucleonics, Inc.: Mean Pore diameter 5150 nm, mesh size 200/400) were added to an equal volume of TK buffer. (2′) A glass fiber disc was pushed to the bottom of the glass column. 400 μl of the CPG suspension was pipetted onto the disc. (3′) The column was washed with 1 ml of TK buffer. (4′) Added 500 μl of S_1 or S_2. (5′) Washed with 1 ml of TK buffer. (6′) Added 500 μl of 1.25 mM $AgNO_3$ in TK buffer. (7′) Washed with 5 ml of TK buffer. (8′) Added 200 μl of TK buffer containing 2 nM E_2^* (6, 7-tritiated estradiol obtained from New England Nuclear Co.)+10 mM dithiothreitol (DTT) or 2 nM E_2^*+200 nM E_2+10 mM DTT and incubated for 3 hours. (9′) Washed with 10 ml of TK

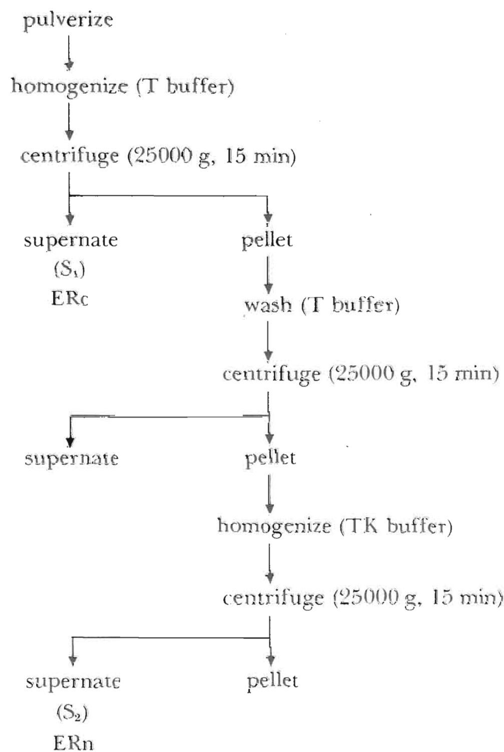

Fig. 1. Cytosol and nuclear extract preparation.

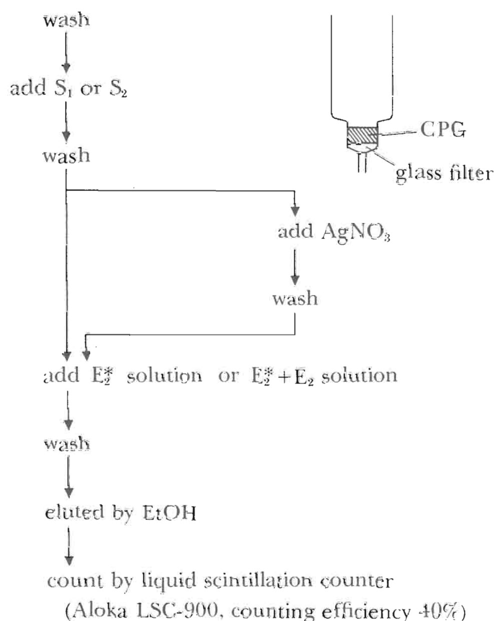

Fig. 2. CPG method.

buffer. (10′) Added with 1 ml of EtOH. (11′) The elute with EtOH was for counting. For free receptor assay, (6′) and (7′) were omitted. All steps except (10′) were carried out at 4°C.

IV. Statistical Analysis

The difference of statistical significance was analyzed using Student's t test.

Results and Discussion

As shown in Fig. 3, plasma E_2 increased 1 day after ED injection and remained at a high concentration, 1500 pg/ml, for 7 days; then decreased to 300 pg/ml by day 14, which was still higher than 60 pg/ml of the proestrus control. T was injected between day 3 and 7 (E_2 level was high) or day 7 and 11 (E_2 level was decreasing). As shown in Fig. 3, there was no significant change in the plasma E_2 concentration by T or V injections. For ER assay in cytosol (ERc) and nucleus (ERn), two methods of DCC and pellet exchange were used, respectively. With these methods, the material was incubated with E_2^* with or without E_2 at high temperature (30~37°C) to measure the total ER. When the CPG method was used, both the ERc and ERn were assayed by the same method and heating was omitted to measure the total ER. Figs. 4 and 5 show the total and free ER. The bound ER is calculated from the total ER minus the free ER. As seen in Fig. 4, the total ERc was slightly lower than the free ERc in the non-treated control rat. This contradiction might be attributed to the partial destruction of ER by the addition of Ag^+ by the CPG method. This result was seen specially in the rats with a low E_2 concentration. As shown in Fig. 4, both the total and free ERc were decreased 1 day after the ED injection and remained low for 7 days, then gradually increased to

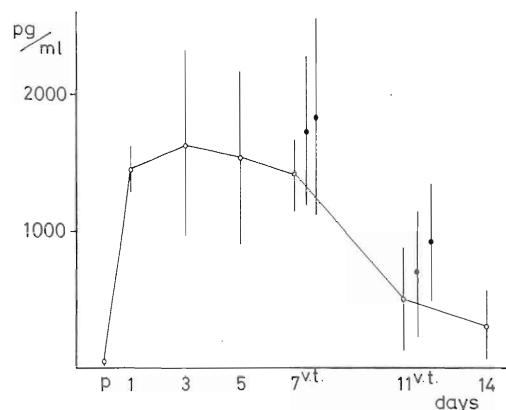

Fig. 3. Estradiol concentration in plasma.
○ Group I and Group II. p: proestrus
● Group III and Group IV. v: vehicle injected group, t: testosterone injected group
Each point is the mean S.D. from 4 to 9 rats.

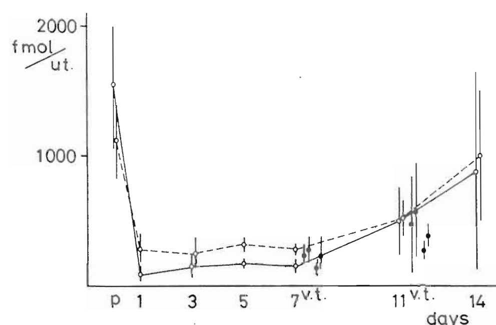

Fig. 4. Uterine cytosol estrogen receptor.
○—○ Group I and Group II. Free ER.
○--○ Group I and Group II. Total ER. p: proestrus
● Group III and Group IV. v: vehicle injected group, t: testosterone injected group
Each point is them mean S.D. from 4 to 9 rats.

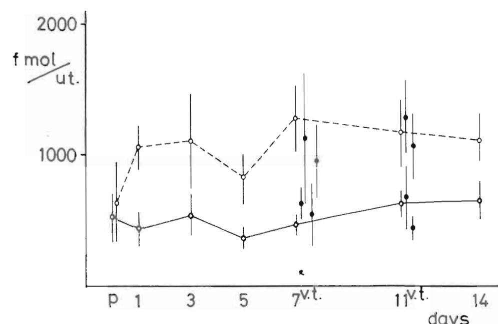

Fig. 5. Uterine nuclear estrogen receptor.
○—○ Group I and Group II. Free ER.
○--○ Group I and Group II. Total ER. p: proestrus
● Group III and Group IV. v: vehicle injected group, t: testosterone injected group
Each point is the mean S.D. from 4 to 9 rats.

the control level. This ERc movement is opposite to the plasma E_2 concentration, as shown in Fig. 3. T or V injections did not induce a significant change in the ERc of the rat with a high plasma E_2. As shown in Fig. 5, the total ERn increased 1 day after ED injection and remained high during the subsequent 14 days, whereas the free ERn did not change, remaining constant during the same period. It was shown clearly that the increased E_2 in the blood was bound to the uterine ERc first and then this bound ER was translocated to the nucleus. The bound ERn remained at a high level in accordance with the high concentration of plasma E_2. Many investigations have been carried out as to whether androgen has an influence on this mechanism of ER activity. Schmidt et al. [4] found that ERc was translocated to the nucleus of the immature rat uterus incubated with a large amount of T. Rochefort et al. [3] concluded that the free ERn was induced by T because the KCl extract from the centrifugal sediment of immature rat uterus, which was pretreated with a large amount of T, was bound to E_2^* easily. On the other hand, Toft & Gorski [5] and Anderson et al. [1] described that T had no influence on the binding of estrogen to ER. In these experiments, T was given with estrogen simultaneously to the immature rat and ER was assayed by the method other than CPG. In the present in vivo experiment by the CPG method using an adult rat, whose plasma E_2 was elevated, previously, T did not induce a significant change in both the ERc and ERn of the uterus.

REFERENCES

[1] Anderson, J., Clark, J. H., and Peck, J., Jr.: Oestrogen and nuclear binding sites. Biochem. J., 126: 561–567, 1972.

[2] Hospelhorn, V. D., Shida, T., DeSombre, E. R., and Jensen, E. V.: Nuclear estrophilin estimation by a novel exchange of bound estradiol. In the Endocrine Society 60th Annual Meeting Program and Abstracts, 1978, pp. 335.

[3] Rochefort, H., Lignon, F., and Capony, F.: Formation of estrogen nuclear receptor in uterus: Effects of androgens, estrone and nafoxidine. Bioch. Biophy. Res. Comm., 47: 662–670, 1972.

[4] Schmidt, W. N., Sadler, M. A., and Katzenellebogen, B. S.: Androgen-uterine interaction: Nuclear translocation of the estrogen receptor and induction of the synthesis of the uterine-induced protein (IP) by high concentration of androgens in vitro but not in vivo. Endocrinology, 98: 702–716, 1976.

[5] Toft, D., and Gorski, J.: A receptor molecule for estrogens: Isolation from the rat uterus and preliminary characterization. Proc. Natl. Acad. Sci., U.S.A., 55: 1574–1581, 1966.