Estrogen Receptor in a Human Cell Line (MCF-7) from Breast Carcinoma*

(Received for publication, May 16, 1973)

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SUMMARY

A stable cell line (MCF-7) derived by pleural effusion from a breast cancer patient has been demonstrated to contain significant amounts of 17β-estradiol receptor. This binding protein is specific for 17β-estradiol and has a Kd equal to 2.5 nm, a sedimentation constant of 1.0 S (and 9.2 S), and a mechanism capable of transporting the 17β-estradiol into the nucleus.

Considerable work, carried out in recent years, has culminated in elucidation of the receptor mechanism for the stimulation of "target" tissues by steroid hormones (1–4). This knowledge recently has given investigators greater insight into the molecular events involved in hormonal control of normal and neoplastic tissue (5–7). Present understanding regarding the estrogen receptor has been acquired through in vivo studies and by short term in vitro incubations of tissue slices or cell fractions. We would like to report herein the first demonstration of 17β-estradiol binding protein in a stable cell line.

The primary culture of human breast carcinoma cells was obtained originally by pleural effusion from a female patient with metastatic disease. A stable epithelioid cell line, MCF-7, was derived from free-floating passages and had been maintained through 71 weekly subcultivations. The cells were cultured in Eagle's minimal essential medium supplemented with nonessential amino acids and 20 μg per ml of insulin prepared in Hanks' salt solution. All media contained 250 units of penicillin and 250 μg of streptomycin per ml and were made 10% with respect to calf serum. Details of culturing and cell morphology will be published elsewhere (8).

For the experiments described herein, cells were inoculated into closed plastic containers (Falcon T-75) and allowed to grow into a confluent monolayer (approximately 2.5 X 10⁶ cells per bottle, 15 to 21 days). Cells from passages 71 through 87 were used in these investigations. The microsome-free supernatant from these cells contained a significant number of binding protein. The absence of estrogen binding protein in these cells from a tumor of a nontarget tissue indicates that prolonged maintenance in cell culture did not generate receptor. This was true in spite of constant exposure to the plasma hormones found in calf serum (12).

The 17β-estradiol binding protein was somewhat labile to extended storage of cells at −70°C. For example, storage for 36 days reduced the number of binding sites in the experiment described in Fig. 2 to approximately one-third (2.7 X 10⁻² pmole of 17β-estradiol per mg of protein) that of an aliquot of the same cells stored for 14 days and utilized in the studies described in Fig. 1. Although this decrease in picomoles of 17β-estradiol bound per mg of protein occurred, the binding constant of the remaining receptor would be expected to be unchanged (5). The Scatchard plot which resulted from analysis of nine aliquots of the microsome-free supernatant fraction from frozen cells is shown in Fig. 2. These binding determinations were carried out according to a procedure previously reported from this laboratory which has taken into account the competitive receptor. The absence of estrogen binding protein in these cells from a tumor of a nontarget tissue indicates that prolonged maintenance in cell culture did not generate receptor. This was true in spite of constant exposure to the plasma hormones found in calf serum (12).

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binding of 17β-estradiol by Sephadex G-25 column material (9). The $K_a$ was found to be 2.5 nM which is comparable to the dissociation constants determined by others for estrogen receptor in human breast tumors (5).

The specificity of the binding for 17β-estradiol is shown in Table I. Preincubation of cytosol from cells with a 103-fold excess of progesterone did not depress the binding by tritiated 17β-estradiol (1.8 nM). However, prior to unlabeled 17β-estradiol at 10 and 100 times the concentration of tritiated estrogen significantly decreased radioactivity in the receptor complex. This inhibition was observed also in experiments with high concentration of a specific estrogen blocking agent, U11, 100A (13).

While sedimentation constants reported for the complex vary according to conditions of the experiment, most of the values obtained for tissues homogenized in Tris buffer with high or low KCl concentrations are near 4 and 9, respectively (14). The density gradient pattern of cytoplasmic 17β-estradiol binding protein derived from the incubation of MCF-7 cells in Kreb-Ringer bicarbonate buffer with 20 nM tritiated 17β-estradiol showed peaks at 4.0 and 9.2 S (Fig. 3). Although incubated in bicarbonate buffer salts, these cells were homogenized and centrifuged through a gradient in Tris-EDTA buffer containing no KCl. The two sedimentation constants found for the estrogen receptor complex are typical of those described for incubated tissues (15).

A recognized property of the cytoplasmic estrogen receptor is its migration into the nucleus of target tissue (16). Nuclear migration has been thought to be temperature dependent; how

### Table I

| Compound added          | Concentration (nM) | Per cent of 17β-estradiol bound |
|------------------------|-------------------|---------------------------------|
| 17β-[3H]estradiol      | 1.8               | 100                             |
| + 17β-Estradiol        | 180               | 41                              |
| + Progesterone         | 1000              | 12                              |
| + U11, 100A            | 210               | 14                              |

![Fig. 2. Scatchard plot derived from the 17β-estradiol receptor in frozen MCF-7 cells (20 x 10^6). Homogenization and incubations with tritiated 17β-estradiol were carried out as described in Fig. 1. As outlined in a previous publication (9), each point was determined from three analyses and represents the radioactivity of bound tritiated 17β-estradiol which has been corrected for competitive binding to column material.](image)

Inhibition of 17β-[3H]estradiol binding to cytoplasmic receptor

Homogenization (20 x 10^6 frozen cells) and incubations were carried out as described in Fig. 1 except that in these experiments the tritiated 17β-estradiol (in 10 µl of ethanol) was added 10 min after the unlabeled compound (in 5 µl of ethanol). The bound radioactivity was determined in the effluent from 3-g Sephadex columns.

![Fig. 3. Sucrose gradient sedimentation of cytoplasmic receptor prepared from MCF-7 cells. A total of 62 x 10^6 viable cells were incubated with 20 nM tritiated 17β-estradiol in 2 ml of Kreb-Ringer bicarbonate buffer, pH 7.4, for 60 min at 0°C. After incubation the cells were centrifuged at 600 x g for 10 min, the supernatant was discarded, and the cells were washed in 6 ml of Kreb-Ringer phosphate buffer, pH 7.4, followed by two washes with 2 ml of 10 mM Tris, pH 7.4, containing 1 mM Na_2EDTA (Tris EDTA). Then the cells were ruptured with 10 strokes of a Dounce homogenizer in 2 ml of Tris-EDTA containing 5 mM dithiothreitol. After centrifugation, 0.5 ml of detergent (0.05%)-coated charcoal (0.8%) was added to 0.6 ml of the 105,000 x g supernatant and the mixture was allowed to stand 30 min at 0°C. The charcoal was sedimented and 0.4 ml of the supernatant was layered on top of 4.5 ml of a sucrose gradient (5 to 20%) in Tris-EDTA. The proteins were sedimented in a 50.1 SW rotor at 41 x 10^3 rpm for 15 hours at 4°C. The tube was punctured and 3-drop fractions were collected for measurement of radioactivity. The marker protein, 10 mg per ml of bovine serum albumin (arrow), was treated similarly and the fractions were assayed at 260 nm.](image)
ever, many of the experiments carried out to demonstrate uptake of the receptor complex by nuclei are presently in doubt due to evidence of posthomogenization binding of extracellular tritiated 17β-estradiol by released cytoplasmic receptor (17). In Table II experiments are presented which utilized a wash with a 102-fold excess of nonlabeled 17β-estradiol prior to homogenization of the tissue, cells, or nuclei. The results clearly show migration of cytoplasmic 17β-estradiol receptor complex into the nuclei during incubation at 37°. Both porcine uterine nuclei and nuclei from MCF-7 cells exhibited appreciable nuclear uptake after 1 hour at 0°; a similar observation has been reported in the recent publication of Williams and Gorski (17).

These experiments demonstrated the presence of significant amounts of 17β-estradiol binding protein in a stable cell line derived from a human breast tumor. As previously reported for the receptor in human tumors, the estrogen binding protein from MCF-7 has a $K_D$ equal to 2.5 nm, a sedimentation constant of 4.0 S (and 9.2 S), and a mechanism capable of transporting the 17β-estradiol complex into the nucleus.

Utilizing in vivo experimentation and short term incubations, it previously has not been possible to investigate the induction of steroid receptor protein with strictly controlled environmental exposure to hormones and nutrients. With cell culture techniques these investigations are now feasible; and, in addition, a stable cell line will permit experiments to be carried out which will add to the present knowledge regarding intracellular binding constants, transport mechanisms, and the mode of nuclear uptake.

REFERENCES

1. JENSEN, E. V., AND JACOBSON, H. I. (1962) Recent Progr. Hormone Res. 18, 387
2. JENSEN, E. V., AND DESOMBRE, E. R. (1972) in Biochemical Actions of Hormones (Litwack, F., ed) p. 215, Academic Press, New York
3. FANG, S., AND LIAO, S. (1971) J. Biol. Chem. 246, 10
4. O'MALLEY, B. W., MILAND, A. R., AND SHIBAO, M. B. (1971) in The Sex Steroids (McKerns, K. W., ed) p. 315, Appleton-Century-Crofts, New York
5. HAYDN, R., AND TWADDLE, E. (1972) Cancer Res. 32, 550
6. McGUIRE, W. L., HUYT, K., JENNINGS, A., AND CHERNESS, G. C. (1972) Science 175, 335
7. McGUIRE, W. L., AND JULIAN, J. A. (1971) Cancer Res. 31, 1440
8. SOULE, H. D., VAZQUEZ, J., ALBERT, S., AND LONG, A. (1972) J. Nat. Cancer Inst., in press
9. GODFREY, R. R., AND BROOKS, S. C. (1973) Anal. Biochem. 51, 335
10. LOWRY, O. H., ROSEBROUGH, N. J., FARRELL, A. L., AND RANDALL, R. J. (1951) J. Biol. Chem. 193, 265
11. PETITBOURG, W. D., STUMPF, W. E., AND SIMPSON, W. F. (1971) Proc. Soc. Exp. Biol. Med. 136, 1187
12. ESSER, H. J., PAYNE, I. J., AND BOGDEN, A. E., J. Nat. Cancer Inst. 58, 559
13. JENSEN, E. V., JACOBSON, H. I., FLIESHER, J. W., SAI, N. N., GUPTA, G. N., SMITH, S., COULCI, J., SHIPLACOFF, D., NEUMANN, H. G., DESOMBRE, E. R., AND JUNGBLUT, P. W. (1966) in Steroid Dynamics (Pincus, G., NAKAO, T., AND TAIT, J., eds) p. 135, Academic Press, New York
14. CHAMNESS, G. C., AND MCGUIRE, W. L. (1972) Biochemistry 11, 2466
15. STALOULIS, A. W., AND KINO, R. J. B. (1970) Biochem. J. 116, 609
16. JENSEN, E. V., SUZUKI, T., KANASHIMA, T., STUMPF, W. E., JUNGBLUT, P. W., AND DESOMBRE, E. R. (1968) Proc. Nat. Acad. Sci. U. S. A. 59, 672
17. WILLIAMS, D., AND GORSKI, J. (1971) Biochem. Biophys. Res. Commun. 45, 258