A SPECIFIC BINDING PROTEIN FOR 17β-ESTRADIOL IN RETIRED BREEDER RAT VENTRAL PROSTATE

E. G. Armstrong and N. Bashirelahi

Department of Biochemistry
University of Maryland School of Dentistry
Baltimore, Maryland 21201

Received September 30,1974

A specific binding protein for 17β-estradiol has been detected in ventral prostate of normal retired breeder rats using sucrose density gradient techniques. The protein has an approximate sedimentation coefficient of 3.5S. It is distinguishable from serum proteins which bind 17β-estradiol on the basis of binding specificity and sedimentation coefficient. It is also distinct from the cytoplasmic androgen binding protein known to be present in rat ventral prostate.

The binding of estradiol and other steroid hormones to a cytoplasmic receptor protein has been indicated as a prerequisite for their biological activity. The binding of 17β-estradiol to uterine cytoplasmic receptors has been shown to precede the transport of the hormone into the nucleus and the subsequent stimulation of RNA synthesis (1).

The regressive action of estrogen on prostatic carcinoma was discovered by Huggins and Hodges (2) over three decades ago. However, the mechanism by which estrogens effect this regression is still unknown. Autoradiographic studies of the uptake of ^{3}H-17β-estradiol by human prostate carcinoma *in vitro* have revealed a localization of the radioactive hormone in the nucleus (3). The formation of estradiol-receptor complexes in prostatic cytoplasm as a prerequisite step in the regressive action of the hormone is an intriguing possibility. The present communication demonstrates that there is a specific binding protein for 17β-estradiol in the rat ventral prostate which is distinct from those which bind androgens and is not due to contamination by serum proteins.

EXPERIMENTAL PROCEDURE

Materials. 1,2,6,7-^{3}H-17β-Estradiol (^{3}H-E_2, 100 Ci/mMole) and 1,2-^{3}H

Dihydrotestosterone (^{3}H-DHT, 40 Ci/mMole) were purchased from New England Nuclear. Unlabelled steroids, DNase (Type I), RNase A, Trizma base, and Myoglobin (MGB, equine skeletal muscle) were purchased from Sigma. Ultrapure sucrose was purchased from Schwarz-Mann and Bovine Serum Albumin (BSA) from Pentex Incorporated.

Animals. Male retired breeder rats of the CD strain (500-700 g) were purchased from Charles River Breeding Laboratories. They were maintained on a diet of Purina Laboratory Chow and water ad libitum and were housed in individual cages in a temperature controlled room with a 12-hour light, 12-hour dark lighting cycle. The rats were from 10-12 months of age at the time of experimentation.

Methods. The animals were sacrificed by decapitation and the ventral prostate removed, dissected free of its capsule and weighed immediately. The tissue was minced with surgical scissors and homogenized in three volumes Tris-EDTA buffer (0.01 M Tris-HCl buffer, pH 7.4, containing 1.5 mM EDTA) using a Pyrex glass on glass homogenizer. Homogenates of diaphragm used as a control tissue were prepared in the same manner. Serum was prepared from blood collected after decapitation by allowing the blood to clot at 4°C and centrifuging at 3,000 rpm for 20 minutes in an IEC clinical centrifuge. The serum was diluted 1:3 (v/v) with Tris-EDTA buffer.

Homogenates were centrifuged at 123,000 x g for one hour in a Beckman Type 50.1 rotor. The supernatant (cytosol) was allowed to stand in the presence of 1,2,6,7-^{3}H-estradiol or 1,2-^{3}H-dihydrotestosterone for one hour at 4°C. Two-tenths ml of the labelled supernatant was layered onto linear 5-20% sucrose density gradients (5 ml volume) prepared in 50 mM Tris-HCl, 1.5 mM EDTA, 0.4 M KCl, pH 7.4 buffer. An external marker tube was prepared by layering 0.2 ml of a solution containing 15.0 mg/ml of Bovine Serum Albumin ($4.6S_{20,w}$) and 5.0 mg/ml of myoglobin ($2.0S_{20,w}$) in Tris-EDTA buffer onto a separate sucrose density gradient.

In enzyme studies, the supernatant was incubated in the presence of

5 x 10^{-10} M ^{3}H-E_2 for 1 hr at 4°C. Then 1.0 ml aliquots were incubated in the presence of 1.0 mg of DNase, RNase, and Pronase 0.5 hr at 37°C. The DNase incubation mixture also contained 10 mM $MgCl_2$. The incubation mixtures were then cooled quickly in ice and 0.2 ml aliquots layered onto 5-20% sucrose density gradients. Gradients were centrifuged at 149,000 x g for 16 or 17 hr using a Beckman SW 50.1 swinging bucket rotor in a Sorvall OTD-2 ultracentrifuge.

Fractions were collected by inserting a thin steel tube from the top to the bottom of the gradient and removing the sucrose with a Technicon peristaltic pump. Three drop (≈0.2 ml) fractions were collected directly into liquid scintillation vials using an LKB Ultrorac Fraction Collector. Radioactivity was determined by the addition of 4.0 ml of a scintillation fluid containing Toluene-Triton X 100 (2:1) and 0.4% (W/V) Omnifluor (New England Nuclear) and counting in a Nuclear Chicago Mark I scintillation spectrometer. The percent efficiency, determined by the external standard ratios method, was 30%.

RESULTS AND DISCUSSION

The sucrose density gradient centrifugation pattern of rat ventral prostate

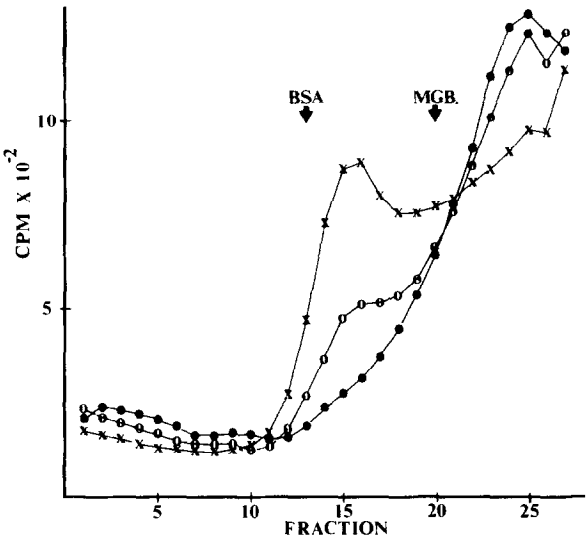

Fig. 1. Sucrose density gradient ultracentrifugal patterns of rat ventral prostate cytosol incubated alone with 5 x 10^{-10} M ^{3}H-17β-estradiol (x—x) or in the presence of 5 x 10^{-6} M unlabelled 17β-estradiol (o—o) or DES (●—●). Ultracentrifugation was for 17 hours. Experimental details are in Materials and Methods.

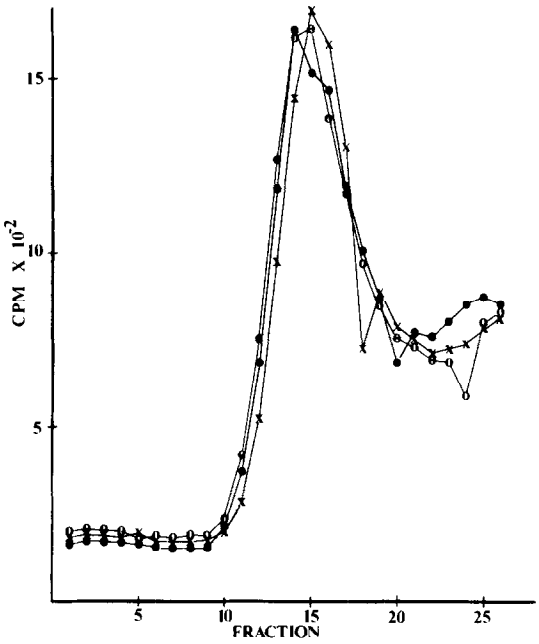

Fig. 2. Sucrose density gradient ultracentrifugal patterns of rat serum incubated alone with 5 x 10^{-10} M ^{3}H-17β-estradiol (x—x) or in the presence of 5 x 10^{-6} M unlabelled 17β-estradiol (o—o) or DES (●—●). Ultracentrifugation was for 16 hours. Experimental details are in Materials and Methods.

cytosol incubated with 5 x 10^{-10} M ^{3}H-17β-estradiol for 1 hr indicates the presence of a binding protein(s) with a sedimentation coefficient of approximately 3.5S (Fig. 1). The addition of 5 x 10^{-6} M 17β-estradiol as a competitor to the incubation medium significantly reduced the amount of bound radioactivity (~70%). Diethylstilbesterol (DES) at a concentration of 5 x 10^{-6} M completely inhibited the binding of ^{3}H-17β-estradiol. Conversely, dihydrotestosterone, testosterone, or progesterone at 5 x 10^{-6} M did not compete with the labelled steroid for binding sites. These results indicate the presence of a high affinity, low capacity protein within the peak of bound labelled steroid which is specific for 17β-estradiol.

The binding of ^{3}H-17β-estradiol in rat serum was examined in order to determine if the binding in prostate supernatant could be due to contamination by serum proteins which bind estrogen. It was found that, in contrast to the case in prostate supernatant, 5 x 10^{-6} M concentrations of estradiol and DES did not

reduce the binding of tritiated 17β-estradiol in serum (Fig. 2). Also, the peak of bound radioactivity in serum consistently migrates ahead of the peak in prostate supernatant with an approximate sedimentation coefficient of 4.6S (Fig. 3). Based on this evidence, it appears that the 17β-estradiol binding proteins in rat prostate supernatant and serum are distinct entities with different specificities and sedimentation coefficients.

High speed supernatants of diaphragm used as a control tissue were incubated in the presence of 5×10^{-10} M ^{3}H-17β-estradiol at 4°C for 1 hr. The sedimentation pattern following sucrose density gradient centrifugation does not indicate the presence of any bound labelled estrogen (Fig. 3).

The sedimentation pattern of rat prostate cytosol incubated with 1×10^{-9} M

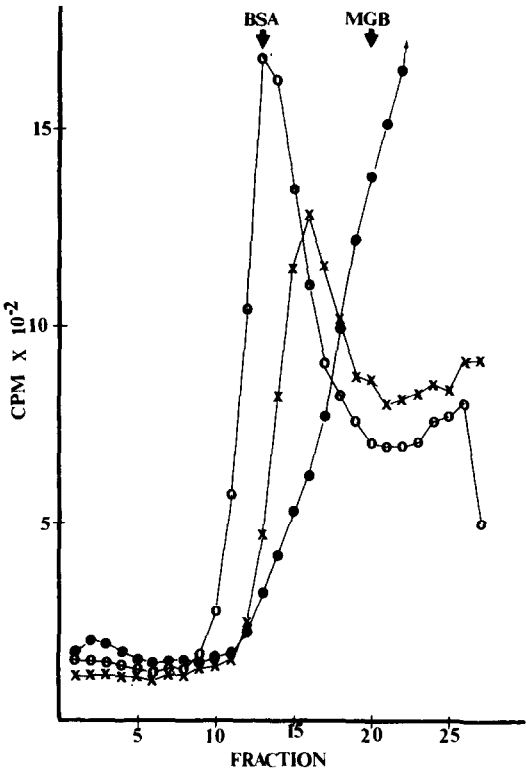

Fig. 3. Sucrose density gradient ultracentrifugation patterns of rat ventral prostate cytosol (x—x), diaphragm cytosol (•—•), and serum (o—o) incubated in the presence of 5×10^{-10} M ^{3}H-17β-estradiol. Ultracentrifugation was for 17 hours. Experimental details are in Materials and Methods.

^{3}H-dihydrotestosterone shows only a small amount of labelled steroid-receptor complex(es) under these experimental conditions in which intact animals were used. The addition of 1 x 10^{-5} M unlabelled DHT to the incubation medium reduced the amount of bound radioactivity. Unlabelled 17β-estradiol at the same concentration had little, if any, effect. These results are in contrast to those with ^{3}H-17β-estradiol which shows a distinct profile of bound radioactive steroid in the intact animal. It is apparent that the binding of 17β-estradiol in the ventral prostate of normal retired breeder rats is not due to the presence of free DHT receptor molecules.

The effects of various degradative enzymes on the sedimentation pattern of ^{3}H-17β-estradiol labelled prostate supernatant on sucrose density gradients were examined (Fig. 4). Pronase completely eliminated the peak of bound radioactivity whereas RNase and DNase were ineffective. These results strongly indicate that the macromolecule which binds 17β-estradiol in rat ventral prostate is a protein.

The presence of cytoplasmic and nuclear binding components in the immature

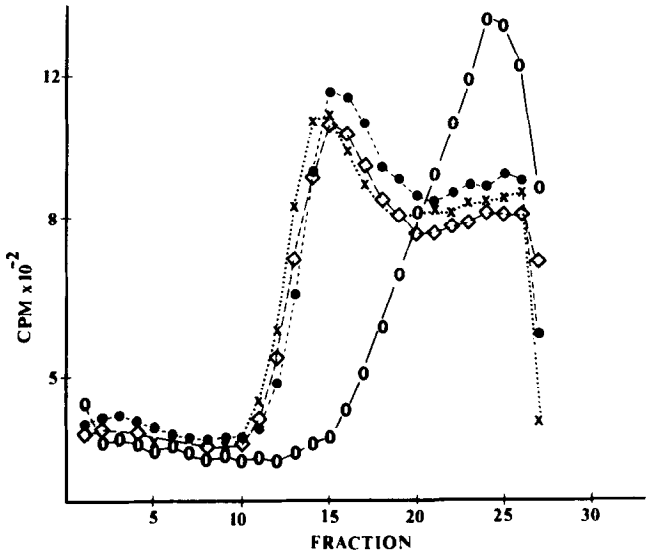

Fig. 4. Sucrose density gradient ultracentrifugal patterns of rat ventral prostate cytosol incubated alone with 5 x 10^{-10} M ^{3}H-17β-estradiol (x—x) or in the presence of Pronase (o—o), RNase (●—●), or DNase (◇—◇). Ultracentrifugation was for 17 hours. Experimental details are in Materials and Methods.

rat uterus with high affinity and specificity for testosterone has been demonstrated by Giannopolos (4). He proposed that the uterotropic effect of testosterone was due to a direct action by the hormone on the uterus. This proposal is supported by the fact that other workers (5) have demonstrated that androgen increases the RNA content of the uterus. It has also been shown that the uterine 17β-estradiol-protein receptor complex stimulates RNA synthesis in the isolated nuclei of calf uterus (6). Using competitive hybridization techniques of RNA with DNA, we have demonstrated the presence of some species of RNA in rat ventral prostate after estradiol treatment which were absent from the control (7).

The mechanism by which the regressive action of 17β-estradiol on prostate takes place is unknown, but the participation of an estradiol-receptor complex in a direct action of the hormone on the gland is an intriguing possibility. Such a direct action could conceivably take place by the stimulation of the synthesis of specific RNA(s) responsible for the regressive effect by an estradiol-receptor complex.

1. Jensen, E. V. and DeSombre, E. R., Science, 182, 126, 1973.

2. Huggins, C. and Hodges, C. V., Cancer Res., 1, 293, 1941.

3. Sinha, A. A., Blackard, C. E., Doe, R. P., Seal, U. S., Cancer, 31, 682, 1973.

4. Giannopoulos, G., J. Biol. Chem., 248, 1004, 1973.

5. Lerner, L. J., Hilf, R., Turkheimer, R., Michel, I., and Engel, S. L., Endocrinology, 78, 111, 1966.

6. DeSombre, E. R., Mohla, S., and Jensen, E. V., Biochem. Biophys. Acta, 48, 1601, 1972.

7. Bashirelahi, N., McRoberts, P., and Villee, C. A., Submitted for publication.