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DETERMINATION OF THE BINDING PROPERTIES OF ESTRADIOL-17β WITHIN THE CYTOPLASMIC AND NUCLEAR FRACTIONS OF RAT VENTRAL PROSTATE

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SUMMARY

A specific binding protein for estradiol-17β ($E_2$17β) has been detected in the cytosol of the ventral prostate of retired breeder CD strain rats. The binding protein was determined to have a sedimentation coefficient of 3.6 *S* by ultracentrifugation of [^{3}H]-estradiol-17β labeled cytosol on high salt sucrose density gradients. The estrogen binding protein appears to have a greater affinity for diethylstilbestrol and estradiol-17β than for estrone or estriol. Also, the estrogen binding protein appears to be inactivated by sulfhydryl group blocking agents.

The uptake of radioactivity by ventral prostate nuclei was investigated following the injection of [^{3}H]-E_2-17β *in vivo*. A radiolabeled protein with a sedimentation coefficient similar to the cytoplasmic estrogen binding protein was detected in the 0.6 M KCl extract of the purified nuclei. However, *in vitro* studies with purified ventral prostate nuclei failed to detect a temperature dependent nuclear translocation of cytoplasmic protein–steroid complex. These studies indicate that an estrogen binding protein with some of the properties of a receptor is present in rat ventral prostate. However, the physiological significance of this protein has not been determined.

INTRODUCTION

Recent reports have indicated the presence of specific estrogen binding proteins in the prostates of rat [1, 2], calf [3] and human [4]. These studies have been primarily concerned with the investigations of the binding of estradiol-17β to proteins within the high speed supernatant fraction (cytosol) of prostate. The estrogen binding proteins which have been identified within prostate cytosols appears to be distinct from those that bind androgen and have properties which are characteristic of steroid hormone receptors. A specific estrogen receptor could conceivably play a role in the regressive action of estrogens on the prostate.

Little information is available concerning the nuclear binding of estrogens within the prostate. Nuclear uptake and the binding of steroid hormone–receptor complexes to chromatin appear to be essential steps in the mechanism of action of steroid hormones in their target tissues. Rennie and Bruchovsky [5] have extracted macromolecular bound radioactivity from prostatic nuclei following the injection of [^{3}H]-Estradiol-17β into castrated adult rats. Mangan *et al.* [6] have detected binding of [^{3}H]-diethylstilbestrol ([^{3}H]-DES) to the protein fraction of nuclear homogenates prepared from rat ventral prostates which were directly injected with [^{3}H]-DES. Also, Stumpf *et al.* [7] have reported autoradiographic studies which indicated nuclear uptake of radioactivity by epithelial cells of prostates from rats injected with [^{3}H]-Estradiol-17β.

A previous report from this laboratory has described a specific estradiol-17β binding protein(s) in the cytosol of ventral prostate from intact retired breeder CD strain rats [1]. The protein(s) had a sedimentation coefficient of approximately 3.5 *S* on 5–20% sucrose density gradients containing 0.4 M KCl. It was found that the protein(s) had a greater affinity for estrogens than for androgens or progestogens and was distinct from steroid hormone binding proteins in rat plasma. Van Beurden-Lamess *et al.* [2] have quantitated specific E_2-17β binding in cytosols of the prostate and a variety of other tissues in the adult, intact rat. They reported the presence of macromolecules within ventral prostate cytosol which specifically bind E_2-17β and sediment at 4 and 8 *S* on sucrose density gradients, but they did not characterize the macromolecule any further.

In this study we wish to report the identification of a 17β-estradiol binding protein in ventral prostates of retired breeder rats on the basis of steroid specificity, tissue specificity, heat lability, inactivation by sulfhydryl blocking agents and temperature dependent nuclear uptake.

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EXPERIMENTAL

1. *Materials.* Radioactive steroids were purchased from New England Nuclear. All chemicals used were of reagent grade or better.

2. *Animals.* Male retired breeder rats of the CD strain (500–700 g, 10–12 months old) were purchased from Charles River Breeding Laboratories and were maintained as previously described [1].

3. *Preparation of prostate cytosols and serum.* 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 TE buffer (0.01 M Tris–HCl buffer, pH 7.4, containing 1.5 mM EDTA) using a Pyrex glass on glass homogenizer. Homogenates were centrifuged at 123,000 *g* for one h in a Beckman Type 50.1 rotor. Serum was prepared from blood collected after decapitation by allowing the blood to clot at 4°C and centrifuging at 2000 *g* for 20 min in an IEC clinical centrifuge. The serum was diluted 1:3 (v/v) with TE buffer. The supernatant (cytosols) and serum were incubated with labeled steroids for one or two h at 4°C. In competitive inhibition studies a 1000 fold concentration of unlabeled steroid was included in the incubation mixture. In experiments in which the effects of sulfhydryl blocking reagents were tested, 1×10^{-2} M N-ethyl maleimide (NEM) or 1×10^{-3} M *p*-chloromercuribenzoic acid (PCMB) were incubated simultaneously with 1×10^{-9} M [^{3}H]-E_2-17β.

4. *Sucrose density gradient centrifugation.* The conditions for the assay of [^{3}H]-E_2-17β binding in prostate cytosol has been previously described [1]. The sedimentation coefficients of [^{3}H]-steroid labeled binding proteins were estimated by comparison to the rate of migration of [^{14}C]-protein markers added to the same/or a separate gradient (~ 9000 d.p.m. in 5 μl TE). [^{14}C]-BSA and [^{14}C]-ovalbumin were prepared by acetylation of the unlabeled proteins with [^{14}C]-acetic anhydride according to the method of Siiteri *et al.* [8].

Gradient fractions were dissolved in four ml of a scintillation fluid containing Toluene–Triton X-100 (2:1, v/v) and 0.4% (w/v) Omnifluor. Radioactivity was determined using a Nuclear Chicago Mark I scintillation spectrometer at 25% efficiency for tritium.

5. *Dextran-coated charcoal adsorption.* Adsorption of free steroids onto dextran-coated charcoal was performed according to the method of Chamness and McGuire [9]. The procedure used a suspension of 2.5 g/l of Norit A and 25 mg/l of dextran in TE buffer. An aliquot of charcoal suspension was centrifuged at 1000 *g* for 5 min in the IEC clinical centrifuge and the supernatant discarded. The pellet was resuspended directly into the cytosol and allowed to stand for 10 min. The charcoal was then pelleted by centrifugation at 1000 *g* for 10 min in the IEC clinical centrifuge, and an aliquot of the cytosol was removed for determination of bound radioactivity. All operations were carried out at 4°C.

The charcoal dextran assay was used to determine the heat sensitivity of the [^{3}H]-E_2-17β binding proteins in the ventral prostate cytosol of retired breeder rats. Aliquots of the same cytosol were kept at 0°C, 25°C, 40°C, and 60°C for 30 min and then cooled on ice for 10 min. Duplicate 0.25 ml aliquots of the four different cytosol preparations were incubated with 5×10^{-9} M [^{3}H]-E_2-17β (85 Ci/mmol) in the presence or absence of 5×10^{-6} M unlabeled E_2-17β for one h at 4°C. Each sample was then treated with the pellet from 2.0 ml of charcoal-dextran suspension for 15 min at 4°C. One hundred microliters of each sample was added to 4 ml of scintillation fluid and radioactivity determined.

6. *Purification of prostate nuclei.* Nuclei were prepared by a modification of the method of Busch [10]. Retired breeder rat ventral prostates were finely minced with surgical scissors and homogenized in three vol. of 50 mM Tris–HCl, 0.25 M sucrose, 3.3 mM $MgCl_2$ pH 7.4 buffer (TSM buffer) using a Teflon on glass homogenizer. The homogenate was filtered through one thickness of nylon gauze and centrifuged at 250 *g* for 10 min in the IEC clinical centrifuge. The supernatant was centrifuged again at 123,000 *g* for one h in the Beckman T50.1 rotor for preparation of the cytosol fraction. The crude nuclear pellet was resuspended in 63% sucrose containing 3.3 mM $MgCl_2$ (13 ml per gram of original tissue weight). The percent of sucrose in the suspension was measured with a Bausch and Lomb refractometer and adjusted to 60.5 to 61.6% when necessary. The suspension was centrifuged at 45,000 *g* for one h in a Beckman SW40 rotor. The purified nuclear pellet was washed twice with 1.0 ml of TSM buffer. Nuclei prepared by this method were found to be whole and essentially free of cytoplasmic debris by phase contrast microscopy.

7. *Nuclear uptake studies.* In *in vivo* nuclear uptake studies three retired breeder rats were anesthetized with ether and their prostates exposed by a ventral incision. Ten μCi of [^{3}H]-E_2-17β (85 Ci/mmol) in 50 μl of 0.9% NaCl–20% ethanol solution was injected directly into the ventral lobes. The incision was closed with suture clips and after one h the animals were sacrificed and the ventral prostates were removed and rinsed in sucrose buffer. The cytosol and nuclear fractions were prepared as described above.

The nuclei were extracted with one ml of 50 mM Tris–HCl, 1.5 mM EDTA, pH 8.5 buffer containing 0.6 M KCl for 1 h at 0°C. The nuclear suspension was then digested with 2.0 mg of DNase in the presence of 20 mM $MgCl_2$ for 0.5 h at 20°C and 2 h at 0°C. The insoluble material was removed by centrifugation at 1000 *g* for 10 min in the IEC clinical centrifuge. Four tenths ml of the nuclear extracts and cytosol preparations (diluted 1:2 with distilled water) were analyzed by ultracentrifugation on 5–20% sucrose density gradients containing 0.4 M KCl.

In vitro nuclear uptake experiments were conducted with purified nuclei and cytosol prepared as described

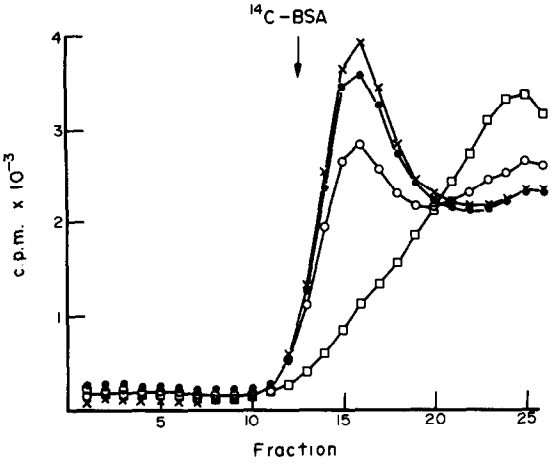

Fig. 1. The cytosol was incubated with 5×10^{-9} M [^{3}H]-E_2-17β (85 Ci/mmol) in the absence (×——×) or presence of 5×10^{-6} M unlabeled E_2-17α (●——●), E_2-17β (○——○), or DES (□——□). Two tenths ml aliquots containing 4.3 mg of protein were analyzed by sucrose density gradient centrifugation at 4°C for 16 h. The abbreviation [^{14}C]-BSA refers to the migratory position of ^{14}C labeled bovine serum albumin within a separate sucrose density gradient.

above. The cytosol preparations were incubated with 1×10^{-8} M [^{3}H]-E_2-17β in the presence or absence of 1×10^{-6} M unlabeled E_2-17β for two h at 4°C. Equal amounts of nuclei suspended in sucrose buffer were added to six different tubes and the buffer removed following centrifugation in the IEC clinical centrifuge at 250 *g* for 10 min. The nuclei were resuspended in 1 ml aliquots of cytosol previously incubated with 1×10^{-8} M [^{3}H]-E_3-17β with or without 1×10^{-6} M E_2-17β. Control tubes and tubes containing competitor 17β-E_2 were incubated at 0°C, 23°C, and 45°C. After various time periods (5–60 min), the nuclear suspensions were vortexed and 0.2 ml aliquots removed and brought to 4°C. The incubation medium was removed following centrifugation at 250 *g* in the IEC clinical centrifuge. The nuclear pellet was washed three times with one ml of TSM buffer. The pellet was resuspended in 0.2 ml of TSM buffer by vortexing and 0.1 ml of the suspension was placed in 4 ml of scintillation fluid for measurement of radioactivity. The remaining 0.1 ml of suspension was used for determination of the DNA concentration.

8. *Protein and DNA determination.* Estimation of the concentration of protein in cytosol preparations was performed according to the method of Lowry *et al.* [11] using BSA as a standard. The DNA content of prostatic nuclei was determined by the diphenylamine reaction described by Burton [12] using calf thymus DNA as a standard.

9. *Statistical analysis.* Tests of statistical significance were done by a two sample *t*-test.

RESULTS

Characterization of estrogen binding in ventral prostate cytosol sucrose density gradient centrifugation

The relative abilities of various unlabeled estrogens to competitively inhibit the binding of [^{3}H]-E_2-17β in ventral prostate cytosol was examined using the technique of sucrose density gradient centrifugation. In the absence of competitor steroid, [^{3}H]-E_2-17β is bound to a macromolecule which sediments at 3.6 *S* ± 0.1 standard error (S.E.) (n = 28) on 5–20% sucrose density gradients containing 0.4 M KCl (Fig. 1). The addition of a 1000 fold concentration of unlabeled estradiol-17β to the incubation mixture consistently results in 20–25% reduction in the amount of bound radioactivity. The same concentration of estradiol-17α (E_2-17α) has little or no effect on the binding of [^{3}H]-estradiol-17β indicating that the binding reaction is stereospecific. A 1000 fold concentration of DES is a better inhibitor of [^{3}H]-estradiol-17β binding than are equivalent amounts of estradiol-17β itself.

The amounts of radioactivity bound in cytosols incubated with unlabeled estrogens relative to the control value were estimated from the total amounts of bound radioactivity on the sucrose density gradients (fractions 10–20). The results of three separate experiments are shown in Table 1. The results of these competitive inhibition studies indicate that the relative binding affinities of the estrogen binding protein for the various estrogens examined are: DES > E_2-17β > E_1 > E_2 17α or E_3. The ability of E_1 but not E_3 to competitively inhibit [^{3}H]-E_2-17β binding in ven-

Table 1. Effects of unlabeled estrogens on [^{3}H]-estradiol-17β binding in ventral prostate cytosol

Unlabeled hormone	Experiment 1		Experiment 2		Experiment 3	
	d.p.m./mg protein	% Of control	d.p.m./mg protein	% Of control	d.p.m./mg protein	% Of control
None	21,600	100	23,000	100	18,900	100
Estradiol-17β	17,100	79	18,300	80	14,400	76
Estradiol-17α	20,600	95	22,800	99	—	—
DES	9,200	43	9,500	41	—	—
Estrone	—	—	19,200	83	17,100	90
Estriol	—	—	21,900	95	21,100	112

The ventral prostate cytosol was incubated with 5×10^{-9} M [^{3}H]-estradiol-17β (85 Ci/mmol) in the presence or absence of 5×10^{-6} M unlabeled estrogen. Two-tenths ml aliquots of cytosol were analyzed by sucrose density gradient centrifugation. The data were calculated by summation of the ratioactivity present in eleven fractions corresponding to the 3.6 *S* peak of bound radioactivity. The protein concentrations of the cytosol preparations used in Experiments 1, 2 and 3 were 21.7, 20.6 and 13.2 mg/ml respectively.

tral prostate cytosol is consistent with the observation that [^{3}H]-E_1 but not [^{3}H]-E_3 forms complexes with macromolecules which sediment within the 3–4 *S* region of 5–20% sucrose density gradients (Data not shown).

The estrogen binding protein described in these studies appears to be distinct from the androgen receptors of ventral prostate cytosol since 5×10^{-6} M concentrations of 5α-dihydrotestosterone (17β-hydroxy-5α-androstane-3-one, 5α-DHT) or testosterone do not compete with 5×10^{-9} M ^{3}H-$E_2$17β for binding sites (data not shown). Also we have been unable to demonstrate any specific binding of [^{3}H]-5α-DHT in ventral prostate cytosol of these aging, intact animals (data not shown). This finding may be due to saturation of the 5α-DHT receptors with endogenous androgen. Alternatively, it may be due to a decreased concentration of 5α-DHT receptors in the ventral prostate of the aging rat as reported by Shain and Axelrod [13].

Steroid hormone receptors have generally been found to be inhibited by sulfhydryl blocking agents [14]. We have examined the effects of N-ethylmaleimide (NEM) and *p*-chloromercuribenzoate (PCMB) on the binding of [^{3}H]-E_2-17β in ventral prostate cytosol (Fig. 2). It was found that 1×10^{-3} M PCMB or 1×10^{-2} M NEM markedly reduced the amount of bound radioactivity as measured by sucrose density gradient centrifugation. These results indicate that the estradiol-17β binding protein(s) of ventral prostate cytosol requires free sulfhydryl group activity.

The effects of sulfhydryl blocking reagents of [^{3}H]-E_2-17β binding in rat serum was also investigated. In contrast to the studies with ventral prostate cytosol, 1×10^{-3} M PCMB or 1×10^{-2} M NEM

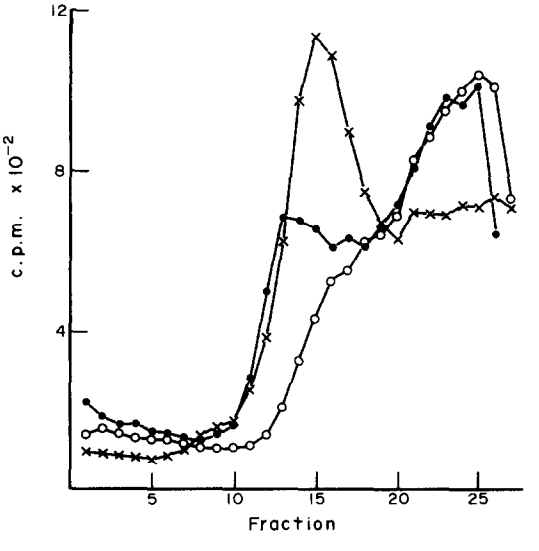

Fig. 2. The cytosol was incubated with 1×10^{-9} M ^{3}H-E_2-17β (100 Ci/mmol) in the absence (×——×) or presence of 1×10^{-2} M NEM (O——O) or 1×10^{-3} M PCMB (●——●). Two-tenths ml aliquots containing 5.8 mg of protein were analyzed by sucrose density gradient centrifugation at 4°C for 16 h.

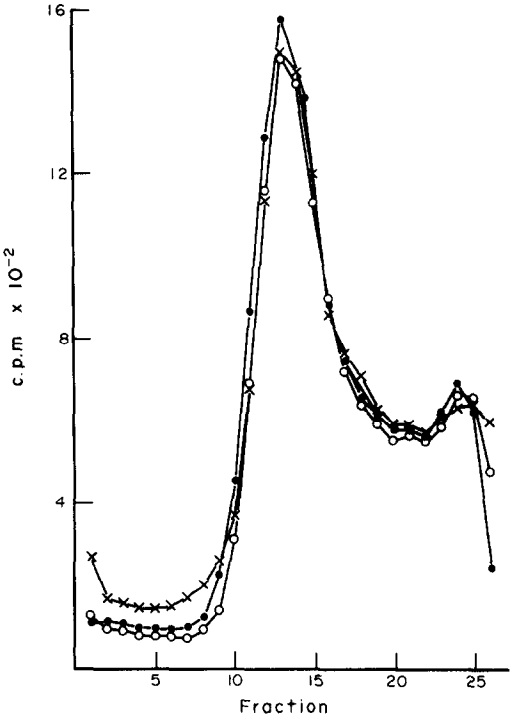

Fig. 3. The serum was incubated with 1×10^{-9} M [^{3}H]-E_2-17β (100 Ci/mmol) in the absence (×——×) or presence of 1×10^{-2} M NEM (O——O) or 1×10^{-3} M PCMB (●——●). Two tenths ml aliquots containing 7.0 mg of protein were analyzed by sucrose density gradient centrifugation at 4°C for 16 h.

had no effect on the binding of [^{3}H]-E_2-17β by serum proteins (Fig. 3). These findings indicate that the presence of the sulfhydryl blocking agent sensitive binding protein(s) in ventral prostate cytosol is not due to contamination by serum proteins. It was also found that the proteins which bind E_2-17β in rat serum migrate at a faster rate (4.3 *S* ± 0.1 S.E., $n = 15$) than those in ventral prostate cytosol. These results and the previous finding [1, 2] that the serum of the adult rat does not contain a saturable estrogen binding protein indicate that the specific estrogen binding protein(s) of ventral prostate cytosol is distinct from the estrogen binding proteins of serum.

Temperature sensitivity of estrogen binding protein

The effects of pretreatment of ventral prostate cytosol at various temperature on E_2-17β binding are shown in Fig. 4. The data indicate that pretreatment of ventral prostate cytosol at a temperature up to 40°C does not decrease the specific binding of E_2-17β. However, pretreatment of the cytosol at 60°C for 30 min completely eliminates the specific estrogen binding sites.

Interestingly, pretreatment of the cytosol at 40°C or 60°C appeared to increase nonspecific binding so that the total amount of binding was greater than (40°C) or approximately equal to (60°C) the total amounts in 0°C and 25°C cytosols. The results show

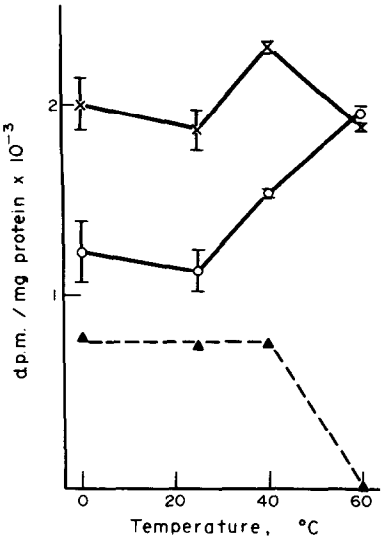

Fig. 4. Ventral prostate cytosol containing 22.5 mg of protein per ml was preincubated at 0°C, 25°C, 40°C, and 60°C for 30 min. The cytosol preparations were then incubated with 5×10^{-9} M [^{3}H]-E_2-17β (85 Ci/mmol) in the absence (×——×) or presence of 5×10^{-6} M unlabeled E_2-17β (O——O) at 4°C for one h. Free steroid was removed by charcoal-dextran adsorption. The amount of specifically bound radioactivity (▲---▲) was calculated from the difference between the amounts bound in the presence and absence of unlabeled E_2-17β. The data are expressed as d.p.m. bound per mg of cytosol protein and represent the means of duplicate determinations. The vertical bars represent the standard error.

that although heat treatment at 60°C does not reduce the total amount of [^{3}H]-E_2-17β binding, it completely eliminates specific binding. Therefore, it appears that specific binding sites for E_2-17β in ventral prostate are degraded by elevated temperature (60°C) whereas nonspecific binding is actually promoted.

Nuclear uptake of 17β-E_2 *by prostatic nuclei*; in vivo *nuclear studies*

Following the injection of [^{3}H]-E_2-17β directly into the ventral prostate *in vivo* macromolecular bound radioactivity was extracted from purified nuclei with 0.6 M KCl (Fig. 5). Bound radioactivity within the nuclear extract sediments in the same region of 5-20% sucrose density gradients containing 0.4 M KCl as the cytosol binding proteins (3.6 *S*). The radioactivity bound within the nuclear extract represents approximately 20% of the total amount of bound radioactivity recovered from the nuclear and cytosol fractions. These findings are in agreement with those of other investigators [5, 6] who have reported a greater retention of radioactivity within the cytosol fraction than the nuclear fraction of rat ventral prostate following *in vivo* administration of [^{3}H]-estrogen.

In vitro *nuclear studies*

The finding that radioactivity is bound to macromolecules with similar sedimentation coefficients within the nuclear and cytosol fractions of ventral prostate following the injection of [^{3}H]-E_2-17β *in vivo* suggests that cytoplasmic molecules might transport the estrogen into the nucleus. A temperature dependent activation of steroid hormone–receptor complexes within the cytoplasm appears to be a prerequisite for nuclear translocation of steroid hormones *in vitro* [15]. The uptake of radioactivity by prostatic nuclei with time in a reconstituted *in vitro* system was investigated in order to determine if the process was influenced by temperature (Table 2). There were no significant increases in nuclear bound radioactivity with time at 0°, 23°, or 45°C. The inclusion of a 100-fold concentration of unlabeled E_2-17β in the cytosol had no consistent effect on the amount of radioactivity associated with the washed nuclei. Also, there was little difference in the amounts of nuclear bound radioactivity following incubation at the three temperatures. Incubation at 45°C appears to result in a slight increase in nuclear binding, but the effect appears to be nonspecific since the presence of a 100-fold concentration of unlabeled E_2-17β did not reduce the amount of bound radioactivity. Therefore, the nuclear uptake of radioactivity measured under these experimental conditions does not appear to be dependent upon the prior formation of [^{3}H]-E_2-17β–receptor complex within prostatic cytosol which subsequently undergoes temperature activated nuclear translocation. However, it is possible that nuclei prepared under these experimental conditions have lost the temperature sensitivity property of receptor–steroid complex translocation.

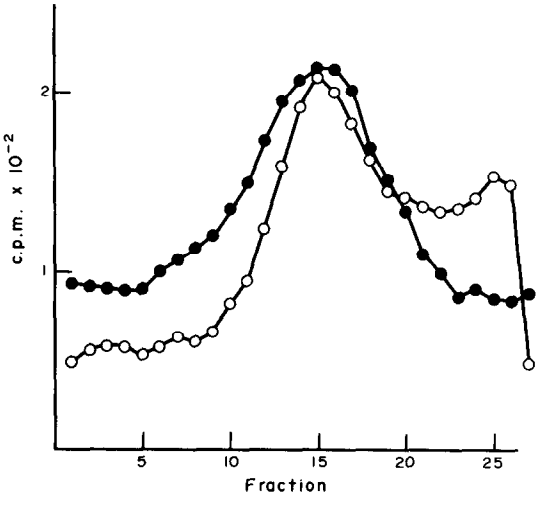

Fig. 5. Rats were injected with 10 μCi of [^{3}H]-estradiol-17β directly into their ventral prostates and one hour later nuclei and cytosol were prepared. The nuclei were extracted with 0.6 M KCl in the presence of DNase. Four-tenths ml aliquots of nuclear extracts (●——●) and cytosol (O——O), containing 3.3 and 3.7 mg of protein respectively, were analyzed by sucrose density gradient centrifugation at 4°C for 16 h.

Table 2. The effect of temperature on [^{3}H]-estradiol-17β uptake by ventral prostate nuclei *in vitro*

Time (min)		0°C		23°C		45°C	
		d.p.m./mg DNA	% Of control	d.p.m./mg DNA	% Of control	d.p.m./mg DNA	% Of control
5	Control +	234 ± 1*	100	276 ± 12*	100	334 ± 26*	100
	Estradiol-17-β	252 ± 12	108	218 ± 6	79	265 ± 85	79
10	Control +	287 ± 29	100	278 ± 17	100	376 ± 48	100
	Estradiol-17-β	319 ± 3	111	238 ± 20	86	342 ± 44	91
15	Control +	298 ± 74	100	294 ± 26	100	346 ± 14	100
	Estradiol-17-β	420 ± 82	141	232 ± 14	79	413 ± 27	119
30	Control +	234 ± 28	100	303 ± 21	100	350 ± 19	100
	Estradiol-17-β	230 ± 14	98	242 ± 6	80	300 ± 102	85
60	Control +	286 ± 32	100	338 ± 50	100	382 ± 84	100
	Estradiol-17-β	281 ± 35	98	280 ± 6	83	428 ± 30	112

Prostatic nuclei which had been isolated by sedimentation through heavy sucrose were incubated with cytosol previously charged with 1×10^{-8} M [^{3}H]-estradiol-17β in the presence or absence of 1×10^{-6} M unlateled estradiol-17β. The nuclear suspensions were incubated at 0°C, 23°C and 45°C, and at various time periods 0.2 ml aliquots were removed and the amounts of radioactivity taken up by the nuclei was determined. The data are expressed as d.p.m. of nuclear DNA and represent the mean values from two replicate experiments.

* Standard error.

DISCUSSION

The specific estrogen binding protein(s) of rat ventral prostate has several characteristics of a steroid hormone receptor. The binding of [^{3}H]-E_2-17β in ventral prostate cytosol appears to be ligand specific in that estrogens (E_2-17β, DES, E_1), but not androgens (5α-DHT, testosterone) competitively inhibit the binding of [^{3}H]-E_2-17β. The binding protein is inactivated by sulfhydryl group blocking agents and proteolytic enzymes [1]. The specific estrogen binding protein(s) of rat ventral prostate is distinct from the nonspecific steroid binding proteins of rat serum based on several criteria. Specific E_2-17β binding sites in ventral prostate cytosol were inactivated at 60°C but not 40°C.

Following the *in vivo* injection of [^{3}H]-E_2-17β directly into rat ventral prostate, there is an indication of the presence of a macromolecule(s) binding to E_2-17β. The nuclear binding of E_2-17β needs further experimentation. We have been unable to demonstrate a temperature dependent activation of nuclear uptake of [^{3}H]-E_2-17β in the rat ventral prostate *in vitro*. Forsberg and Hoisaeter [16] recently demonstrated the *in vitro* uptake of estradiol-17β-cytostatic complexes by the nuclei of rat ventral prostate. In those studies, [^{3}H]-estradiol-17-3-(N,N-*bis*-2-chloroethyl)carbamate was recovered from purified nuclei following incubation of the complex with minced ventral prostate at 37°C. Also Bashirelahi *et al.* [17] have demonstrated a temperature dependent activation of [^{3}H]-E_2-17β uptake by nuclei isolated from human benign hyperplastic prostate which is dependent on the presence of a saturable cytoplasmic binding protein. Sinha *et al.* [18] have shown *in vitro* nuclear uptake of [^{3}H]-E_2-17β by human prostate using autoradiographic techniques.

In our studies we have not detected the presence of macromolecular bound radioactivity in the 8 *S* region of low salt (no KCl) sucrose density gradients following ultracentrifugation of [^{3}H]-E_2-17β labeled ventral prostate cytosol. It is possible that 8 *S* macromolecules which bind E_2-17β are present in rat ventral prostate cytosol but their presence is masked by endogenous steroids since intact animals were used in these studies. Van Beurden-Lamers *et al.* [2] have reported the presence of both 8 *S* and 4 *S* specific estrogen binding macromolecules in the ventral prostate cytosol of adult, intact Wistar rats. In their studies, unbound steroid was removed by charcoal-dextran adsorption from [^{3}H]-E_2-17β labeled steroid prior to ultracentrifugation on low salt 5–20% sucrose density gradients.

The physiological significance of the estrogen binding protein(s) of rat ventral prostate is as yet uncertain. Although the protein(s) has several properties which are typical of steroid hormone receptor we have not yet demonstrated that the protein has an 8 *S* form or undergoes a temperature activated nuclear translocation. Further research in these particular areas seems especially warranted.

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