AN OESTRADIOL RECEPTOR IN RAT TESTIS INTERSTITIAL TISSUE

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1. Introduction

Investigations concerning a biochemical explanation for the mechanism of action of steroids have revealed the presence of specific receptors for steroids in target tissues [1]. Isolation of specific steroid binding receptors from testis tissue has not been reported, although Stumpf [2] has presented autoradiographic evidence for a nuclear localization of radioactivity in interstitial cells of immature rat testis after administration of radioactive oestradiol. In the course of a study on steroid receptors we observed a specific binding of oestradiol by the cytoplasm of whole rat testis tissue [3]. In the present paper, data will be given on the specific localisation of this receptor in interstitial tissue obtained after wet dissection of mature rat testis and on the characteristics of oestradiol binding by a cytoplasmic fraction obtained from whole testis tissue.

2. Materials and methods

2.1. *Steroid*

[2,4,6,7-$^3H_4$]Oestradiol (specific activity: 100 Ci/mmole); was obtained from the Radiochemical Centre, Amersham (Great Britain). The radiochemical purity was verified by paper- and thin-layer chromatography.

2.2. *Preparation of subcellular fractions*

Immature (26 days old) and mature (3 months old) Wistar rats were used. After decapitation of the rats the testes were isolated and the tunical albuginea was removed. Whole testis tissue was homogenized in 2 vol 10 mM Tris buffer, pH 7.4, containing 1.5 mM EDTA and 2 mM mercaptoethanol (TEM-buffer) using a motor driven Potter-Elvehjem homogenizer. After homogenisation glycerol was added to make a final glycerol concentration of 10%. The homogenate was centrifuged at 105,000 *g* for 60 min at 0°. For *in vitro* labelling the 105,000 *g* supernatant (cytosol) was incubated for 2 hr at 0° with steroid.

Interstitial tissue and seminiferous tubules were obtained by wet dissection of the testis [4, 5]. The tubules were washed in Krebs-Ringer bicarbonate buffer pH 7.4, containing glucose (10^{-2} M). In the isolated fractions no impurities could be detected as ascertained by determination of esterase activity [6]. The 105,000 *g* supernatants of interstitial tissue and tubules were prepared and incubated *in vitro* with steroid in the same way as described for whole testis tissue. For *in vivo* studies with immature rats 5 μCi [^{3}H]oestradiol was injected subcutaneously 5 min before decapitation. In mature rats the testis was perfused in situ for 2 min with 10 μCi [^{3}H]oestradiol dissolved in 0.5 ml Krebs-Ringer solution, subsequently the normal blood flow was restored for 1 min and then the testis was removed from the animal. The testis was dissected at 0–4° as described above.

2.3. *Sucrose gradient centrifugation*

Cytosols (200 μl, approx. 3 mg of protein), which had previously been incubated with steroid, were layered on linear 5–15% (w/v) sucrose gradients, prepared in TEM-buffer containing 10% glycerol and were centrifuged in a Beckman L2-65B centrifuge at 0° for 18 hr at 49,000 rpm in a SW 65 rotor. Yeast alcohol dehydrogenase ($s_{20,w}$: 7.4 S) and bovine serum albumin ($s_{20,w}$: 4.6 S) were used as markers for calculation of

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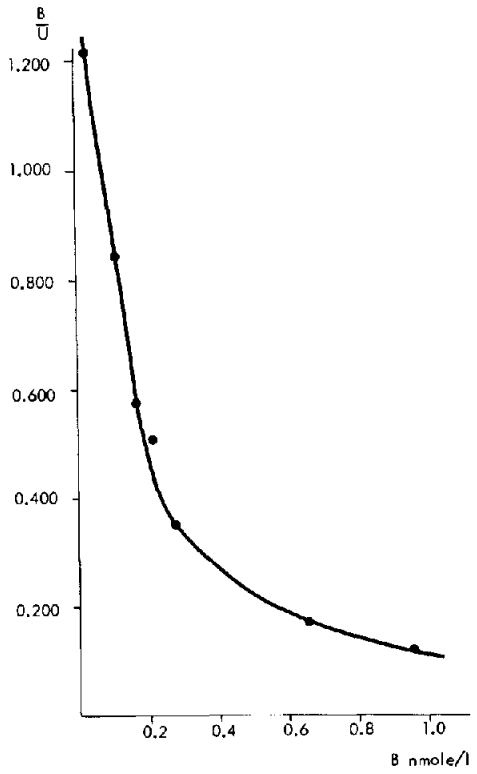

Fig. 1. Scatchard plot of oestradiol binding *in vitro* by testis cytosol (105,000 *g* supernatant) from immature rats as determined by the dextran-coated charcoal adsorption method (for details see Methods section). B/U represents the amount of bound (B) over unbound (U) steroid at equilibrium. Protein concentration: 14.8 mg/ml.

sedimentation values [7]. After centrifugation, approx. 30 fractions were collected after piercing the bottom of the tube.

2.4. *Competition of oestradiol binding between cytosol and dextran coated charcoal*

[^{3}H]oestradiol (4 pg, 0.0015 μCi) dissolved in 20 μl ethanol was added to various amounts of unlabelled oestradiol and dried under nitrogen. Testis cytosol (200 μl) was added and allowed to equilibrate with the steroid at 30° for 1 hr. No appreciable metabolism of oestradiol occurred at 30° and thin-layer chromatography on silica gel plates in the solvent system toluene–ethyl acetate (2:1, v/v) showed that over 95% of the radioactivity in the cytosol was still present as oestradiol. After the equilibration period a 50 μl aliquot was taken for counting. To separate bound from unbound steroid a 100 μl aliquot was added to 200 μl of a 0.25% charcoal suspension containing 0.025% of dextran. After mixing for 5 sec the solution was kept at 4° for 15 min. Subsequently the solution was centrifuged at 1200 *g* at 4° for 10 min and 200 μl aliquots of supernatant were removed for counting of the receptor-bound steroid.

2.5. *Measurement of radioactive samples*

Radioactivity in the samples was measured in a Nuclear Chicago Mark I or a Packard model 3375 liquid scintillation spectrometer. Fractions obtained from sucrose gradients were diluted with 1 ml of water. These fractions and the supernatants prepared as described in sect. 2.4 were mixed with 15 ml of a dioxane solution containing 100 g naphthalene, 7 g 2,5-diphenyloxazole (PPO) and 0.3 g 1,4-bis-(5-phenyloxazole-2)-benzene (POPOP) per litre dioxane. Samples were counted until a standard error of 1–3% was reached. Quench corrections were calculated from external standard ratios.

3. Results

3.1. *Binding of oestradiol by cytosol of immature rat testis*

The binding of oestradiol in testis cytosol was studied by adsorption of the free steroid on dextran coated charcoal at 4°. Analysis of these results using a Scatchard type plot (fig. 1) gave an association constant of 3×10^9 M^{-1}. The concentration of binding sites in the cytosol was calculated to be in the order of 2×10^{-14} mole/mg protein.

3.2. *Characteristics of the receptor*

After labelling the cytosol of immature rat testis during a 5 min period with [^{3}H]oestradiol *in vivo* the radioactivity in the cytosol was associated with a fraction which sedimented at approx. 8 S (fig. 2A). If a 1000-fold excess of non-radioactive oestradiol was injected together with the tritiated oestradiol no detectable radioactivity was observed in the 8 S region, indicating that only a limited amount of high affinity binding sites is available. Essentially the same results were obtained *in vitro* (fig. 2B). The binding of [^{3}H]oestradiol by the 8 S macromolecules *in vitro*

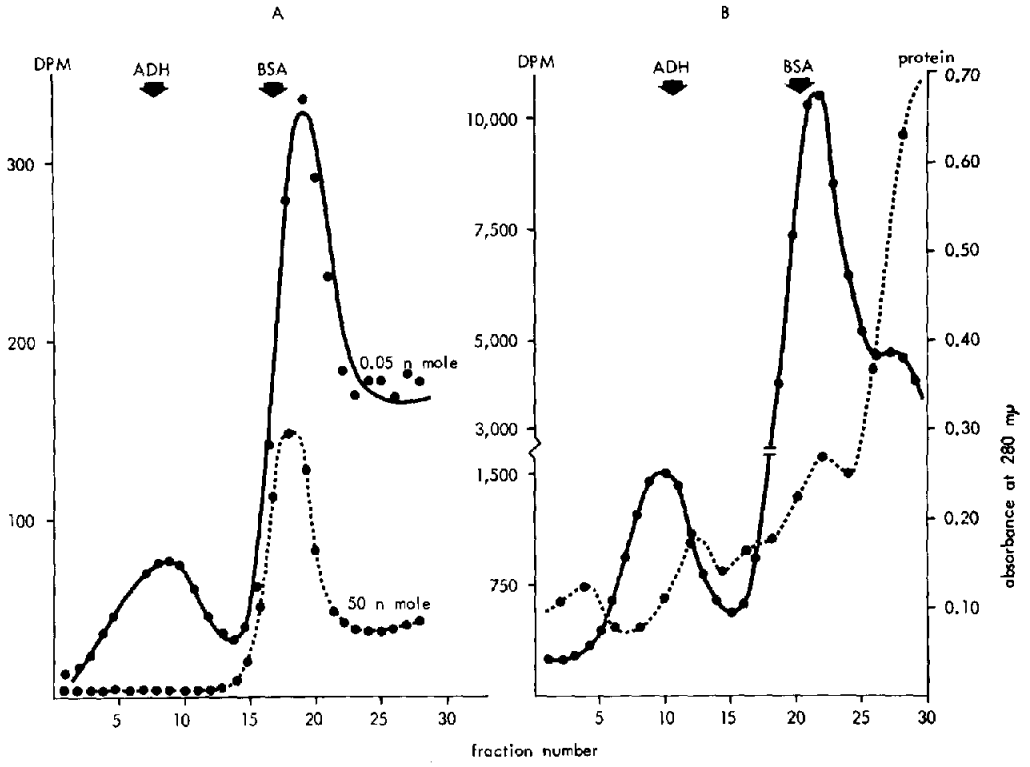

Fig. 2. A) Sedimentation of oestradiol after *in vivo* labelling of testis cytosol from immature rats. Either 0.5×10^{-10} mole of [^{3}H]oestradiol (solid line) or 0.5×10^{-10} mole of [^{3}H]oestradiol + 500×10^{-10} mole of oestradiol (dashed line) was injected into the rats 5 min before decapitation and isolation of the testis. B) Sedimentation of oestradiol after *in vitro* labelling of testis cytosol from immature rats with 45×10^{-10}M [^{3}H]oestradiol (solid line). The dashed line represents the protein sedimentation profile of testis cytosol. Yeast alcohol dehydrogenase (ADH) and bovine serum albumin (BSA) were used as sedimentation markers.

was almost completely abolished by the addition of a 1000-fold excess of non-radioactive oestradiol (fig. 3). When a 1000-fold higher amount of non-radioactive dihydrotestosterone was added only a small decrease in binding of oestradiol in the 8 S region was observed (fig. 3). Addition of excess testosterone and corticosterone also did not influence the binding of oestradiol by the 8 S macromolecules (fig. 4). Analysis of oestradiol binding by cytosol from interstitial tissue and seminiferous tubules is shown in figs. 5A and 5B. A sharp peak of radioactivity was observed in the 8 S region when the cytosol obtained from interstitial tissue was either labelled during in situ perfusion of the testis (fig. 5A) or by *in vitro* incubation (fig. 5B) with [^{3}H]oestradiol. The sedimentation profile of the tubular preparation showed a very small elevation in the 8 S region, possibly due to a contamination by interstitial tissue.

4. Discussion

The results in this paper demonstrate the presence of a fraction in interstitial tissue of rat testis which specifically binds oestradiol with a high affinity. These macromolecules are present in both immature and mature rat testis cytosol and have a sedimentation coefficient of approx. 8 S. The affinity constant of 4×10^9 M^{-1} at 4° for this oestrogen–macromolecular complex is of the same order of magnitude as the affinity constants reported for cytoplasmic receptors for oestradiol and dihydrotestosterone in uterus

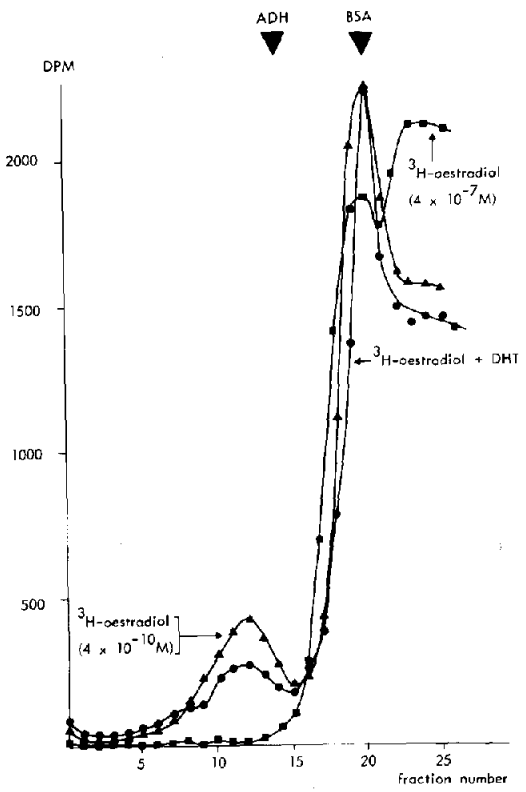

Fig. 3. Sucrose gradient analysis of oestradiol binding by cytosol of testis from immature (26 days old) rats. Cytosols were incubated respectively with 3 × 10^{-10}M [^{3}H]oestradiol; 3 × 10^{-10}M [^{3}H]oestradiol + 3 × 10^{-7}M oestradiol; 3 × 10^{-10}M [^{3}H]oestradiol + 3 × 10^{-7}M 5α-dihydrotestosterone (DHT) and layered on 5–15% sucrose gradients.

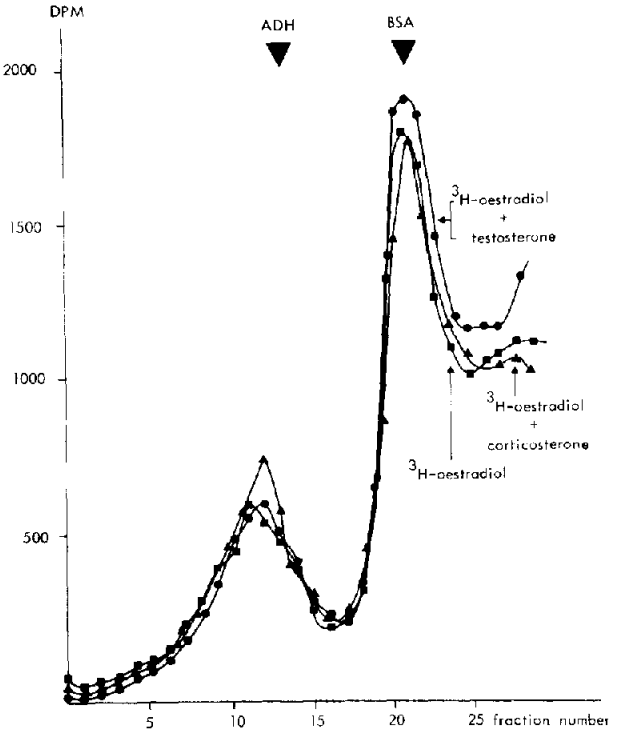

Fig. 4. Sucrose gradient analysis of oestradiol binding by cytosol of testis from immature rats. Cytosols were incubated respectively with 3 × 10^{-10}M [^{3}H]oestradiol; 3 × 10^{-10}M [^{3}H]oestradiol + 3 × 10^{-7}M testosterone; 3 × 10^{-10}M [^{3}H]oestradiol + 3 × 10^{-7}M corticosterone and layered on 5–15% sucrose gradients.

and prostate tissue [8]. Other steroids did not compete with oestradiol binding to the testis cytoplasmic receptor, indicating a high specificity of the binding sites for oestradiol.

Studies with dissected testis tissue from mature rats showed that this specific binding protein is mainly located in the interstitial tissue. This observation is in agreement with autoradiographic studies of Stumpf [2]. Since De Jong and Van Der Molen [9] have demonstrated testicular oestradiol secretion, it might be expected that some endogenous oestradiol is present in testis tissue. The binding of exogenously added oestradiol to the cytoplasm of interstitial tissue might, however, indicate that not all receptor sites for oestradiol are occupied in testis tissue of mature rats.

Testosterone is synthesized in the interstitial tissue of the testis and this steroid influences spermatogenesis in the seminiferous tubules. It is of interest, to investigate if the occurrence of oestradiol receptors in interstitial tissue are relevant in this respect.

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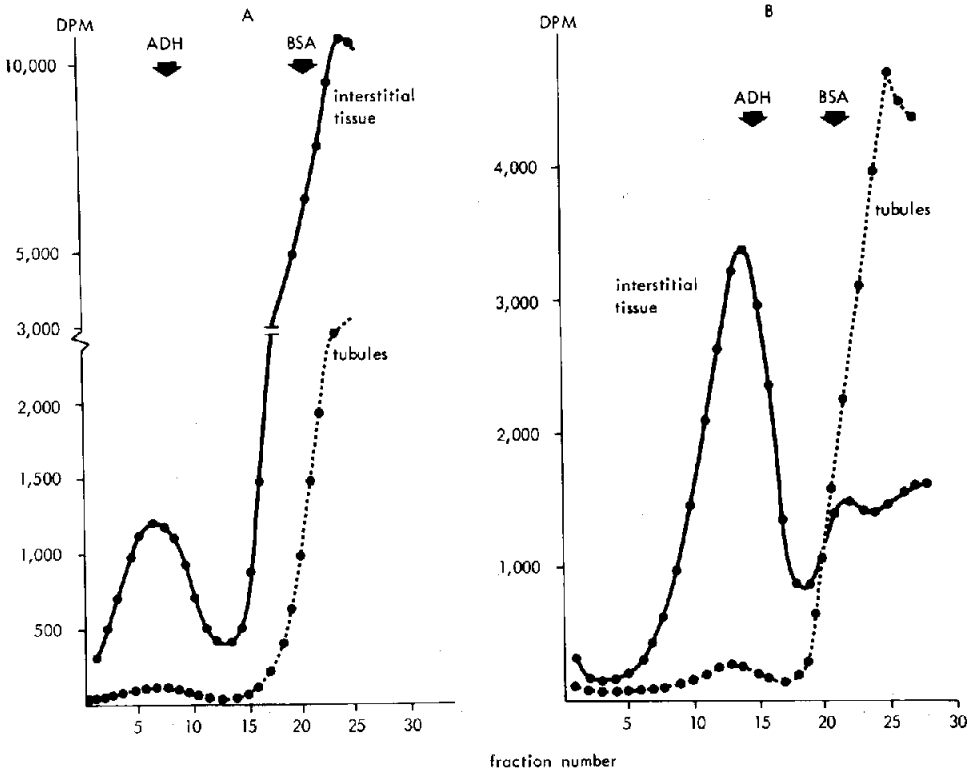

Fig. 5. Sucrose gradient analysis of oestradiol binding by cytosols of interstitial tissue (solid lines) and seminiferous tubules (dashed lines). Cytosols were labelled either A) *in vivo* during in situ perfusion of the testis with 10^{-10} mole of [^{3}H]oestradiol or B) by *in vitro* incubation with 0.7×10^{-10}M [^{3}H]oestradiol.

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