A RECEPTOR FOR TESTOSTERONE IN MATURE RAT TESTES

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1. Introduction

In most steroid target tissues the steroids can be bound by specific receptors in the cytosol and in the nuclear fraction. Androgens are known to affect spermatogenesis [1], it was therefore of interest to investigate if specific androgen receptors are present in testis tissue.

In this paper we discuss results obtained for the binding of testosterone in nuclear fractions of seminiferous tubules of mature hypophysectomized rats.

2. Materials and methods

2.1. *Preparation of subcellular fractions and incubation procedures*

[1,2,6,7-$^3H_4$]Testosterone (84 Ci/mmol) and [1,2-$^3H_2$]-5α-dihydrotestosterone (47 Ci/mmol) were obtained from the Radiochemical Centre, Amersham, England. Mature (3 months old) hypophysectomized male rats of the R-Amsterdam strain were used. 100 μCi of [^{3}H]testosterone was injected 45 min before sacrificing the animals. Interstitial tissue and seminiferous tubules were obtained by wet dissection at 0°C [2]. The isolated tissues were homogenized in ice-cold 10 mM Tris buffer, pH 7.4, containing 1.5 mM EDTA, with 3 strokes of a Potter–Elvehjem homogenizer at 1100 rpm. The homogenate was centrifuged at 700 *g* for 10 min at 0°C. The 700 *g* pellet was resuspended in buffer and filtered through a 60 μm nylon gause. The filtrate *(nuclear fraction)* was washed 3 times with ice-cold 0.25 M sucrose, containing 10 mM Tris–HCl and 0.2% Triton X-100. A *nuclear extract* was prepared by extraction of the nuclear fraction with 0.4 M KCl in 20 mM Tris buffer, pH 8.5, for 60 min at 0°C, followed by centrifugation at 105 000 *g* for 30 min at 0°C [3]. The cytosol fraction was prepared by centrifugation of the 700 *g* supernatant at 105 000 *g* at 0°C [4].

For in vitro studies one decapsulated testicle was incubated in 3 ml Eagle's Medium, containing 2×10^{-8} M radioactive steroids, for 45 min at 32°C in an atmosphere of 95% O_2 – 5% CO_2. Dihydrotestosterone labelled androgen binding protein (ABP) was prepared according to Ritzen [5], by treatment of normal rat testis cytosol with charcoal and incubation with [^{3}H]dihydrotestosterone.

2.2. *Estimation of steroid binding and identification of radioactive steroids*

Free and bound steroid fractions were separated with either agar electrophoresis or gradient centrifugation. Agar gel electrophoresis was performed essentially as described by Wagner [6]. For counting of radioactivity after electrophoresis 2 mm slices of the agar gel were solubilized in a Triton-containing scintillation fluid [7]. Sucrose gradient analysis of the nuclear extracts [3], protein estimation, and measurement of radioactivity in the samples were performed as described previously [4]. For estimation of radioactivity samples were counted until a standard error of 3% or less was obtained.

Testosterone, 5α-dihydrotestosterone, 5α-androstane-3β, 17β-diol and 5α-androstane-3α, 17β-diol were identified by thin-layer chromatography on silica gel plates in the solvent system toluene–ethyl acetate (5:2, v/v) both as the free steroids and as their acetylated derivatives [8].

3. Results

Testis tissue was obtained from rats 8 days after hypophysectomy and injected with [^{3}H] testosterone 45 min before sacrificing the animal. After gradient centrifugation of the samples obtained from seminiferous tubules a distinct peak of radioactivity was observed in the 4–5 S region on gradients with 0.4 M KCl (fig. 1). The nuclear extract of interstitial cells showed only a small elevation in the same region of the gradient. Dihydrotestosterone labelled androgen binding protein (ABP) obtained from the cytosol of intact rat testis also sedimented in the 4–5 S region. In order to distinguish a possible receptor protein in the nuclear fraction from a cytoplasmic contamination of the nuclei with ABP, agar gel electrophoresis was used. As shown in fig. 2 ABP remained close to the application site in the middle of the plate and in the nuclear extract (0.4 M KCl) a peak at the anodic side

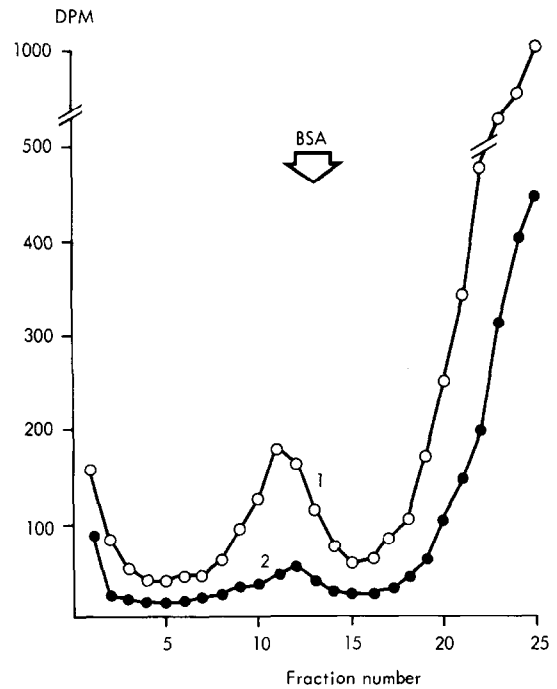

Fig. 1. Sucrose gradient analysis of testosterone binding by the nuclear extract of seminiferous tubules (curve 1, 0.5 mg protein) and interstitial tissue (curve 2, 0.2 mg protein). Bovine serum albumin (BSA, $S_{20,w}$: 4.6S) was used as a sedimentation marker.

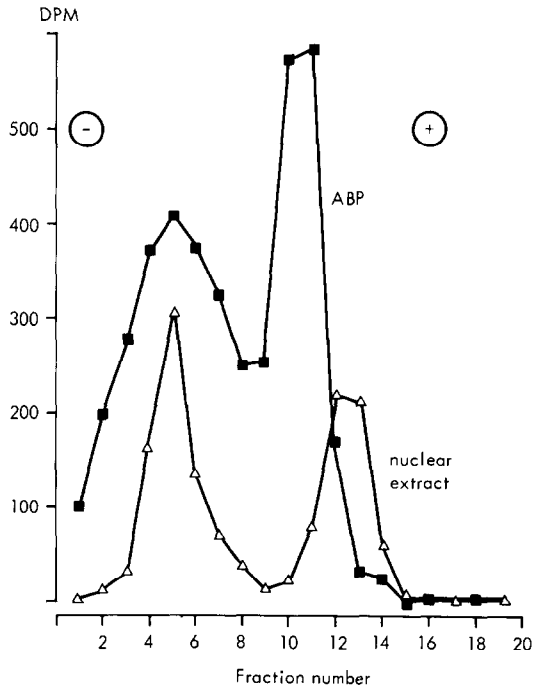

Fig. 2. Agar gel electrophoresis of androgen binding protein obtained from rat testis tissue. ABP: Electrophoretic pattern of dihydrotestosterone labelled androgen binding protein. Nuclear extract: The nuclear extract of testicular tissue of rats 8 days after hypophysectomy injected with [^{3}H] testosterone (100 μCi; 1.2 nmoles).

of the gel was present in fractions 12–15. The 0.4 M KCl in the extract did not interfere with the electrophoretic process.

The amount of testosterone that could be bound per mg of testis tissue increased gradually during the time period of 3 to 20 days after hypophysectomy. Metabolism of the radioactive steroid might have occurred between the time of injection in vivo and the recovery of the steroid receptor complex from the nuclei. Thin-layer chromatography of the radioactivity present in the anodic peak on the agar gels of the nuclear extract of testis of adult rats revealed, however, that less than 5% of the radioactive testosterone was converted into the metabolites: 5α-dihydrotestosterone, 3α- and 3β-androstanediol. In competition experiments with unlabelled steroid for [^{3}H] testosterone binding sites in vitro, the highest competition was achieved with dihydrotesterone. Other physiologically occurring steroids did not significantly

compete. Cyproterone acetate inhibited testosterone binding by the nuclear extract in vitro by more than 50% at a concentration of 2×10^{-6} M, a concentration known to inhibit dihydrotestosterone binding in prostate nuclei [9]. The binding of [^{3}H] dihydrotestosterone to androgen binding protein (ABP) obtained from the cytosol of intact rat testis decreased only 20% in the presence of 2×10^{-6} M cyproterone acetate.

4. Discussion

The results in this paper demonstrate the presence of a receptor-like testosterone binding protein in the nuclear fraction of the testis of mature rats. Testis tissue consists for the major part of seminiferous tubules and the receptor is mainly present in this fraction.

The presence of an androgen binding component different from ABP was also recently observed by Hansson et al. [10] for the testis of immature hypophysectomized rats. Hansson et al. reported that after in vivo administration of radioactive testosterone the steroid was recovered from acrylamide gels partly as testosterone and partly as 5α-dihydrotestosterone. In contrast, in our experiments with mature rats the steroid recovered from the binding protein in testis nuclei after in vivo injection of testosterone was mainly unmetabolized testosterone. In this respect the situation in the mature testis is different from most androgen dependent accessory sexual glands, where dihydrotestosterone is the predominant androgen bound to receptor proteins. The increasing binding capacity during the 20 days after hypophysectomy might imply that this protein is predominantly localized in Sertoli cells or in other cell types remaining in the testis during the degeneration process after hypophysectomy. The localization of the receptor in the different cell types in testis tubules will be the subject of further studies.

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