BINDING OF OESTRADIOL BY THE NUCLEAR FRACTION OF RAT TESTIS INTERSTITIAL TISSUE

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1. Introduction

In most steroid target tissues the steroids are bound specifically by proteins from the cytosol and nuclear fraction. Available information indicates that the steroids are generally taken up by the target cell in a process which includes at least two steps: first binding to a receptor protein in the cytosol, and secondly the transfer of the steroid–cytoplasmic receptor complex to the nucleus [1].

In a previous publication [2] we have reported the presence of an oestradiol receptor in the cytoplasmic fraction of rat testis interstitial tissue. It was of interest to investigate if the observed binding of oestradiol in this tissue is limited to only cytoplasmic sites. Furthermore the possibility was considered that in testicular interstitial cells a comparable situation exists as in uterine tissue where in addition to cytoplasmic binding a large part of the oestradiol is associated with the nuclear fraction [3, 4].

In the present paper we report that after *in vivo* or *in vitro* administration of oestradiol, this steroid can be bound by the nuclear fraction of rat testis interstitial cells and that the cytoplasmic fraction is required for specific retention of oestradiol by the nuclear fraction from a cell-free system.

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 (U.K.). The radiochemical purity was verified by paper and thin-layer chromatography.

Thin-layer chromatography on silica gel plates in the solvent system toluene–ethyl acetate (2:1, v/v) showed that after the *in vivo* and *in vitro* studies more than 95% of the radioactivity in the nuclear extracts was still present as oestradiol.

2.2. In vivo labelling of testes and incubation procedures

Immature (26 days old) and mature (3 month old) Wistar rats were used. For the *in vivo* studies 0.6 μCi (6 pmoles) per g body weight oestradiol was injected subcutaneously. In some experiments as indicated in the Results section unlabelled oestradiol was injected together with the radioactive oestradiol. The time interval between injection and decapitation of the animals was varied in the different experiments. After decapitation of the rats the testes were isolated and the tunica albuginea were removed.

For *in vitro* studies one decapsulated testis of a mature rat, or 3 testes of immature rats were incubated in 1.5 ml Eagle's tissue culture medium, containing 6×10^{-9} M radioactive oestradiol (0.6 μCi/ml). Incubations were carried out at 32° for various periods of time in an atmosphere of 95% O_2 –5% CO_2.

2.3. Preparation of subcellular fractions

Interstitial tissue and seminiferous tubules were obtained from mature testis by wet dissection [5, 6] under

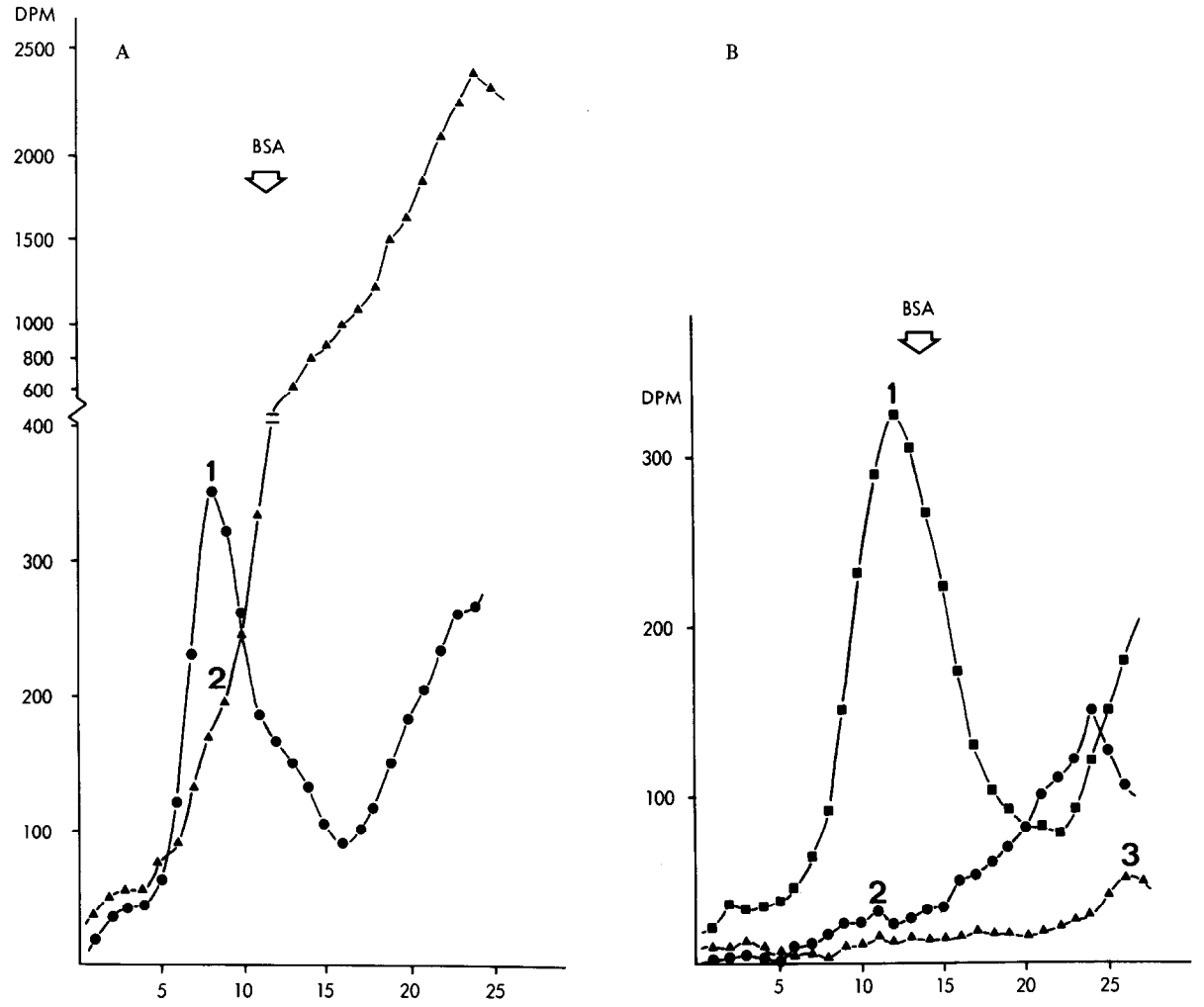

Fig. 1. *In vivo* labelling of testis tissue. A) Sedimentation profile of [^{3}H] oestradiol in the cytosol (curve 2; 3.9 mg protein) and nuclear extract (curve 1; 0.45 mg protein) after *in vivo* labelling of the testis from 26 day old rats. [^{3}H] Oestradiol (6 pmole per g body weight) was injected 90 min before decapitation. An excess of unlabelled oestradiol was added before homogenization as described in the method section. The sucrose gradient contained 0.4 M KCl, and bovine serum albumin (BSA) was used as sedimentation marker. B) Sedimentation profile of oestradiol in the nuclear extract after *in vivo* labelling of testis interstitial tissue (curve 1) and seminiferous tubules (curve 2) and of muscle (curve 3) from a mature rat, injected with 6 pmole [^{3}H] oestradiol per g body weight 90 min before decapitation.

Eagle's tissue culture medium at 4°. 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 an all-glass Potter–Elvehjem homogenizer. The homogenate was centrifuged at 700 *g* for 10 min at 0°. The 700 *g* pellet was resuspended in TEM-buffer, filtered through a nylon gauze (60 μm) and the filtrate was centrifuged for 5 min at 700 *g*. The pellet was called the nuclear fraction. Since less than 200 mg wet weight of rat testis interstitial tissue was available for each experiment no further purification of the small amounts of partially purified nuclear fraction was undertaken. A nuclear extract was prepared by extraction of the nuclear fraction with 10 mM Tris buffer pH 8.5, containing 1.5 mM EDTA and 0.4 M KCl (TEK-buffer) [3] for 90 min at 0° followed by centrifugation at 105,000 *g* for 30 min at 0°.

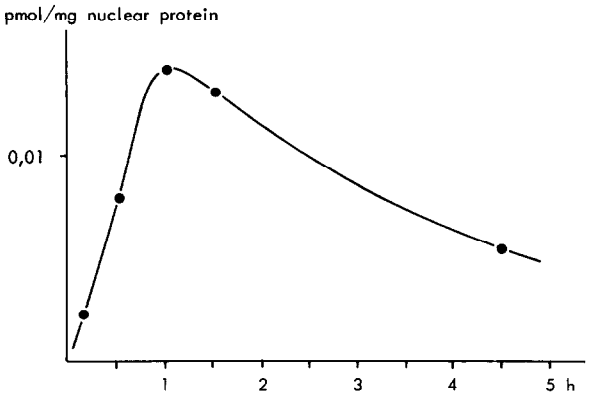

Fig. 2. Effect of time on *in vivo* labelling with oestradiol of nuclear extracts of testis tissue of 26 days old rats. The nuclear extract of total testis tissue was subjected to density gradient centrifugation as described in the legend of fig. 1A. The pmole amount of steroid per mg protein in the nuclear extract was calculated from the area under the 5 S peak in the sedimentation profile.

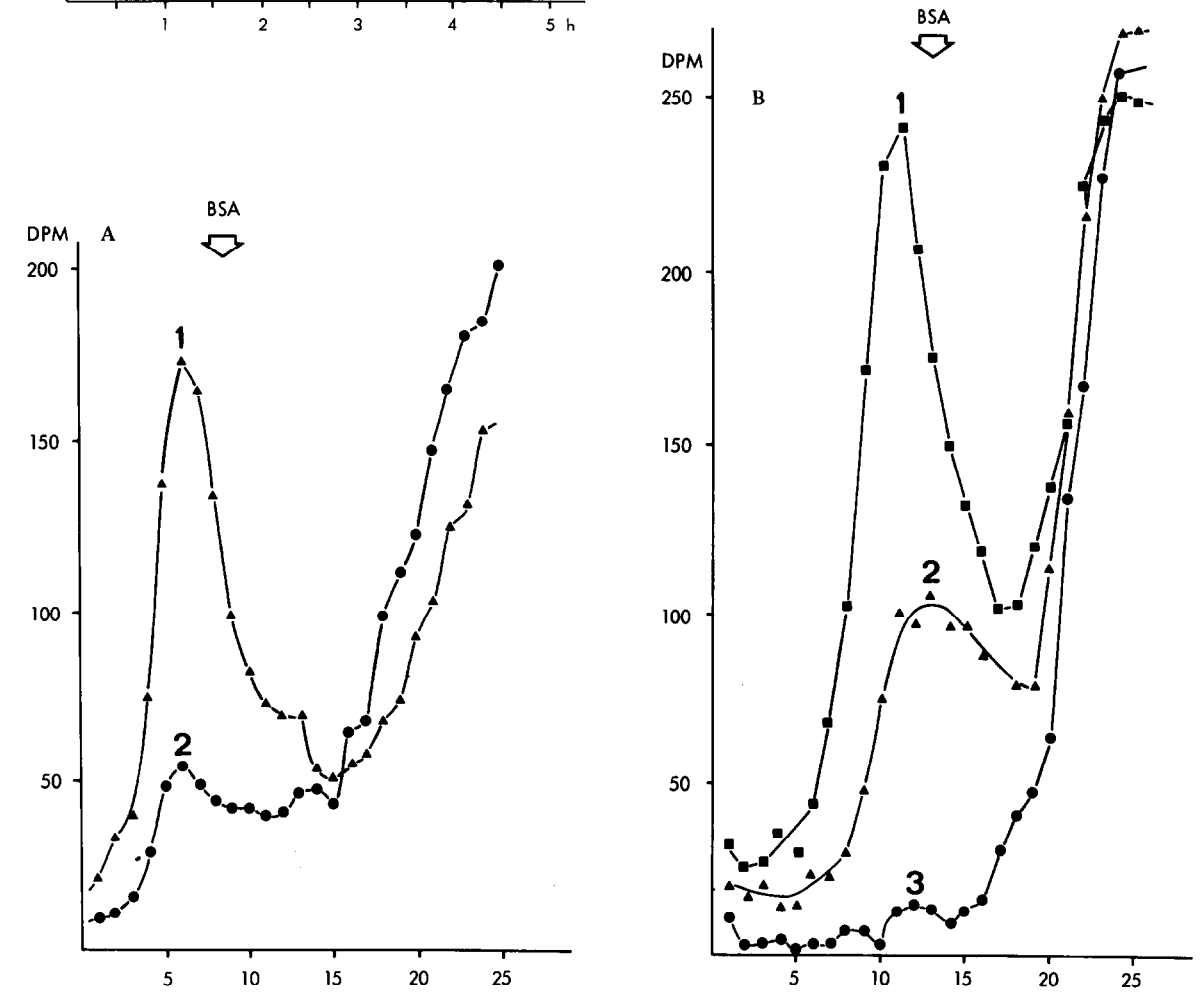

Fig. 3. *In vitro* labelling of testis tissue. A) Sedimentation profile of oestradiol in nuclear extracts of isolated interstitial tissue (curve 1) and tubules (curve 2) of rat testis. Total testis tissue was incubated with 6×10^{-9}M oestradiol at 32° before dissection as described in the Methods section. B) Sedimentation profile of oestradiol in nuclear extracts of interstitial tissue after incubation of total testis for 30 min at 32° (curve 1), for 10 min at 32° (curve 2) and for 30 min at 0° (curve 3) with 6×10^{-9}M oestradiol.

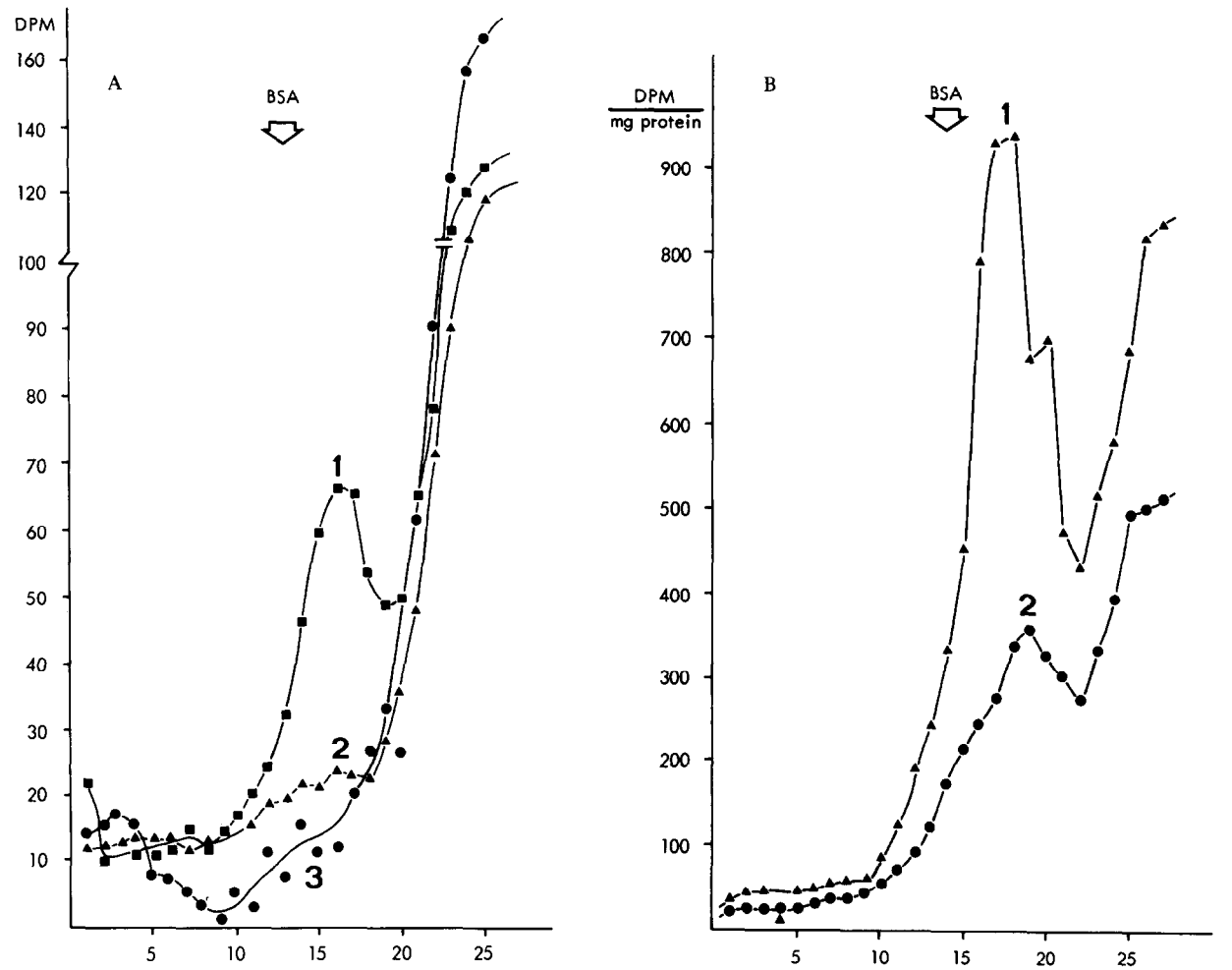

Fig. 4. Incubation of isolated nuclear fractions with cytosol. A) Sedimentation profiles of oestradiol in the nuclear extracts after incubation of isolated nuclear fractions from interstitial cells, in different media containing 0.06 μCi [^{3}H] oestradiol/ml and respectively cytosol (curve 1), heat denaturated cytosol (curve 2) or 10 mM Tris-EDTA buffer, pH 7.4 (curve 3). Before incubation with nuclear fractions the cytosol or heat denaturated cytosol (20 min 60°) obtained from interstitial tissue was incubated with [^{3}H] oestradiol for 90 min at 0°. Subsequently nuclear fraction from interstitial tissue obtained from 2 testes was mixed with the labelled cytosols or buffer and incubated for 45 min at 0° followed by 15 min at 25°. After incubation the nuclei were washed with 10 mM Tris buffer. B) Comparison of the sedimentation profile of [^{3}H] oestradiol in the cytosol (curve 2) and in the nuclear fraction after incubation as described under A (curve 1).

The cytosol fraction was prepared by centrifuging the 700 *g* supernatant at 105,000 *g* for 60 min at 0°.

In most experiments with *in vivo* or *in vitro* labelled testis an excess of unlabelled oestradiol (10^{-6}M) was added to the medium used for the dissection and to the TEM-buffer used for homogenization [4].

2.4. Sucrose gradient centrifugation and measurement of radioactive samples

Nuclear extracts (200 μl) containing approx. 1.5 mg of protein (whole testis) or 0.5 mg of protein (isolated interstitial tissue) were layered on linear 5–20% (w/v)

sucrose gradients, prepared in TEK-buffer and were centrifuged in a Beckman L2-65B centrifuge at 0° for 18 hr at 60,000 rpm in a SW 65 rotor. Bovine serum albumin (BSA, $s_{20,w}$: 4.65) was used as sedimentation marker. After centrifugation, approx. 25 fractions were collected after piercing the bottom of the tube.

The radioactivity of the samples collected from the gradients was determined by liquid scintillation counting as described previously [2]. Samples were counted until a standard error of 3% or less was reached.

3. Results

3.1. Testis tissue labelled in vivo or in vitro

After incubation of isolated cytosol of mature and immature rat testis with [^{3}H] oestradiol, the radioactivity in the cytosol was associated with a fraction, which sedimented at a value of approx. 4 S on a gradient prepared in TEK-buffer (curve 2 in fig.4B). Similar results were obtained both after *in vitro* or *in vivo* labelling of testis tissue. Williams and Gorski [4] have described for uterine tissue, that addition of excess unlabelled oestradiol to the buffer used for homogenization, will prevent labelling of unoccupied cytosol receptor sites during the homogenization procedure. When excess unlabelled oestradiol was added during homogenization of labelled testis tissue a similar effect was observed.

The excess of oestradiol during homogenization did not, however, affect the amount of [^{3}H] oestradiol in the nuclear extract which sedimented on a TEK-sucrose gradient with a sedimentation value of approx. 5 S (fig.1A). These 5 S [^{3}H] oestradiol–macromolecule complexes were obtained both after *in vivo* and *in vitro* labelling of the testis (figs.1 and 3). Optimal labelling of this macromolecule was obtained *in vivo* 90 min after injection of 6 pmole oestradiol per g body weight (fig.2). Oestradiol radioactivity was observed only in the 5S region after centrifuging the nuclear fraction of interstitial tissue of the testis and this oestradiol binding macromolecule could not be extracted from the nuclei of either seminiferous tubules or muscle (fig. 1B).

Essentially similar results were obtained by *in vitro* incubation of whole testis tissue (fig.3). Incubation at 0° of whole testis did not yield a labelled 5 S binding protein (fig.3B).

3.2. Incubation of isolated cell fractions

After incubation of the isolated nuclear extract of interstitial cells for 2 hr at 0°, or for 1 hr at 25° with various amounts of [^{3}H] oestradiol, labelled macromolecules with a sedimentation value of 4 or 5 S could not be observed. Labelled macromolecules sedimenting at approx. 4 S could be extracted from the isolated nuclear fraction of interstitial cells only after incubation of the isolated nuclear fraction with [^{3}H] oestradiol labelled cytosol. [^{3}H] Oestradiol solubilized in denaturated cytosol or in buffer solution could not replace the labelled cytosol fraction (fig.4A). The specific radioactivity per mg of protein of the macromolecules extracted from the nuclear fraction was higher than the specific activity of the labelled cytosol fraction, used for the incubation (fig.4B).

4. Discussion

The results in this paper demonstrate that under *in vivo* conditions and after incubation of whole testis *in vitro* radioactive oestradiol is bound specifically by macromolecules that can be extracted from the nuclear fraction of rat interstitial tissue. In this respect the autoradiographic evidence presented by Stumpf [7] for a nuclear localization of radioactivity in interstitial cells of immature rat testis after administration of radioactive oestradiol, is also noteworthy.

For the present experiments it was not attempted to characterize carefully the nuclei using biochemical markers. However, in comparable studies on estrogen uptake by uteri [3], or by chick liver [8], and 5α-dihydrotestosterone uptake by prostate tissue [9] it was demonstrated that similar crude preparations (washed 700 *g* pellets) could be used for the study of nuclear uptake of steroids.

The incubation experiments with isolated subcellular fractions demonstrated that labelling of the nuclear fraction could be achieved only in the presence of the cytosol fraction. This suggests that for the uptake of oestradiol by nuclei of testis interstitial tissue a similar mechanism is operating as in most target tissues for steroid hormones where the steroid molecule after binding by cytoplasmic proteins is transferred to nuclear binding sites. A difference was observed for the sedimentation coefficients obtained for the macromolecules in the nuclear extracts after labelling of whole tissue (approx. 5 S; figs. 1 and 3) and after la-

belling in a reconstituted system (approx. 4 S; fig.4). From our results no explanation for this difference can be given, although it could reflect a comparable situation to uterine tissue [10], where presumably a 4 S subunit is transferred from the cytosol to the nucleus and subsequently becomes associated with a nuclear protein as an approx. 5 S macromolecule. It is conceivable that during incubation of the isolated nuclear fraction from testis tissue the conversion of a 4 S to a 5 S complex might not have occurred.

The physiological meaning of the uptake of oestradiol by the testis interstitial cells is not yet clear. The concentration of oestradiol used for *in vitro* incubation in this study (6×10^{-9}M) was in the order of the concentration normally present in uterine tissue, but higher than that present in total testis tissue (10^{-10}M [11]). However, preliminary evidence indicates that oestrogens are probably synthesized in testis interstitial tissue and consequently local concentrations in interstitial tissue might be higher than concentrations in total testis. Actions of oestradiol in the testis on DNA, RNA and protein synthesis have been reported [12]. It would therefore be of interest to investigate if these effects are mediated by the oestrogens bound to the macromolecules in the nuclear fraction of interstitial cells.

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