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# HIGH AFFINITY BINDING OF OESTRADIOL BY RAT TESTIS INTERSTITIAL TISSUE AND BY SEVERAL OTHER TISSUES OF THE MALE RAT

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## SUMMARY

Specific testicular oestradiol receptors were present in cytoplasmic and nuclear fractions of interstitial tissue, but not in seminiferous tubules of rat testis. A specific saturable binding protein for oestradiol could be demonstrated also in liver, adrenal, pituitary, prostate and epididymis.

## INTRODUCTION

For several steroid target tissues it has been shown, that the steroid molecule after entering its target cell, becomes immediately bound by a cytoplasmic receptor, which transfers the steroid into the nucleus. In the course of a study on steroid receptors in the testis we observed a specific binding of oestradiol by the cytoplasm of whole rat testis tissue[1]. A nuclear form of the receptor has also been found and it appears that the cytoplasmic receptor can be transferred to the nuclei of testis interstitial tissue[2]. In addition, the distribution of high affinity oestradiol binding components in the different tissues of the male rat was studied.

## METHODS

The subcellular fractions were prepared from 3 month old Wistar rats. The isolated tissues were homogenized in 1 volume of 10 mM Tris buffer pH 7·4 at 0°C with 3 strokes of a Potter–Elvehjem homogenizer at 1100 rev./min.

The homogenate was centrifuged at 105,000 *g* for 60 min at 0°C. The 105,000 *g* supernatant (cytosol) was incubated with steroid for 2 h at 0°C.

Isolated interstitial tissue and seminiferous tubules were obtained by wet dissection of decapsulated whole testis tissue. Binding of steroids to specific binding proteins was estimated by gradient centrifugation, agar gel electrophoresis, Sephadex chromatography and charcoal adsorption as described before[1, 3]. Nuclear fractions of testis interstitial tissue were prepared as described in Table 3 (see also [2]).

## RESULTS

The oestradiol binding protein was only present in interstitial tissue and not in seminiferous tubules. The sedimentation coefficient of the cytoplasmic complex was approximately 8*s* on a gradient containing 0·01 M Tris–buffer. The receptor showed the characteristics of a protein with respect to pronase sensitivity and was relatively thermolabile. Testosterone and 5α-dihydrotestosterone did not compete with oestradiol for the oestradiol binding sites (Table 1).

Cytosols of 13 different tissues of the male rat have been studied in order to investigate if the presence of an oestradiol receptor was restricted to testis interstitial tissue. Cytosols of all tissues were incubated with $4 \times 10^{-9}$ M labelled oestradiol and specific binding was determined by agar gel electrophoresis. The presence of an 8*s* binding protein was checked by sucrose gradient centrifugation of each cytosol. The uterus of the immature female rat was used as a control tissue. The results (Table 2) show that a specific binding protein with a sedimentation coefficient of 8*s* could be

Table 1. Characteristics of testicular cytoplasmic oestradiol receptor

| |
|---|
| Sedimentation coefficient $s_{20,w}$: 8*s* |
| Association constant $K_a$: $10^{10}$ $M^{-1}$ |
| Concentration in total testis: $2 \times 10^{-14}$ moles/mg cytosol protein |
| Localization: interstitial tissue |
| Heat-treatment (30′ at 37°): disappearance 8*s* macromolecules |
| Pronase treatment: disappearance 8*s* macromolecules |
| DNA-ase treatment: no disappearance 8*s* macromolecules |
| RNA-ase treatment: no disappearance 8*s* macromolecules |

Table 2. Specific binding of oestradiol by different tissues of the male rat

| Tissue | Specific binding ($10^{-15}$ mole/mg protein) | 8s-protein |
|---|---|---|
| Total testis tissue | 9·8 | + |
| Seminiferous tubules | 0·1 | − |
| Testis interstitial tissue | 140 | + |
| Epididymis | 8·7 | + |
| Prostate | 11·4 | + |
| Seminal vesicle | 0·1 | − |
| Pituitary | 75·6 | + |
| Hypothalamus | 2·6 | − |
| Adrenal | 22·2 | + |
| Liver | 2·3 | + |
| Kidney | 1·4 | − |
| Skeletal muscle | 0 | − |
| Plasma | 0 | − |

Cytosols of different tissues and plasma of the male rat were incubated either with $4 \times 10^{-9}$ M labelled oestradiol or with $4 \times 10^{-9}$ M labelled plus $4 \times 10^{-7}$ M unlabelled oestradiol. Specific binding was determined using agar gel electrophoresis and is expressed as $10^{-15}$ mole oestradiol bound/mg protein of the cytosol. The presence (+) or absence (−) of a binding protein with a sedimentation value of 8s after sucrose gradient centrifugation is indicated in the last column.

demonstrated in testis interstitial tissue, prostate, epididymis, pituitary, adrenal and liver. Such a protein, however, was absent in seminiferous tubules, seminal vesicle, hypothalamus, kidney, muscle and plasma. The amount of specific binding in the uterus was 240 fmole/mg protein. The highest amount of oestradiol binding macromolecules in male rats was found in testis interstitial tissue (140 fmole/mg protein) and in pituitary (75 fmole/mg protein).

In most steroid target tissues the steroids can be bound by specific receptors in the cytosol and in the nuclear fraction. Therefore, we have considered the possibility that a comparable situation exists in testis interstitial tissue and whether in addition to cytoplasmic binding, oestradiol can also be bound by the nuclear fraction. Testis was labelled either *in vivo* by subcutaneous injection of oestradiol, or by *in vitro* incubation of decapsulated testis tissue in a medium with labelled oestradiol. After dissection of the testicular tissue the nuclear fraction of the interstitial cells was prepared (Table 3). In some experiments this nuclear fraction was further purified on a discontinuous sucrose gradient with a bottom layer of 2·0 M sucrose. Through this purification procedure the crude nuclear fraction was divided in a precipitate $N_1$ and a residue $N_2$ on top of the 2·0 M sucrose (Fig. 1). After this purification the distribution of marker molecules in the different fractions was also measured (middle part of Fig. 1). DNA was mainly present in the $N_1$ fraction. There was little contamination of this fraction with mitochondrial cytochrome-C oxidase and cyto-

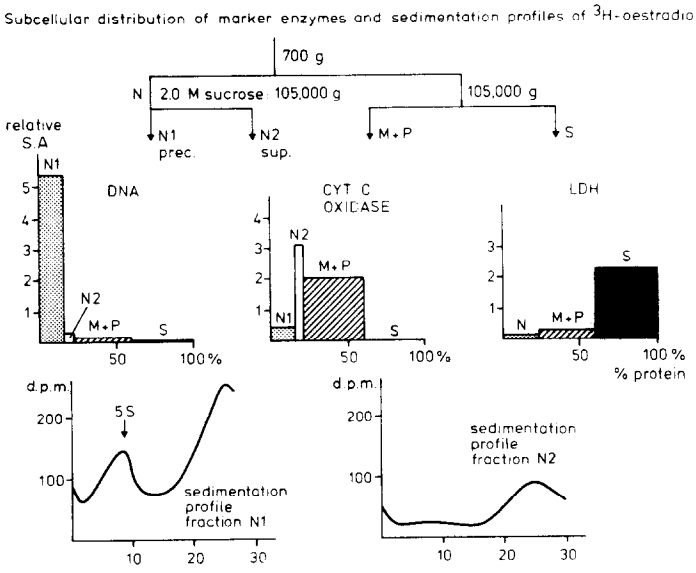


Fig. 1. Marker enzymes and sedimentation profiles of $^3$H-oestradiol in subcellular fractions of isolated interstitial tissue. Total testis tissue was incubated with $6 \times 10^{-9}$ M $^3$H-oestradiol for 30 min at 32° in Eagle's tissue culture medium before the dissection.

The block diagrams show relative specific (enzyme) activities of marker molecules. DNA was used as a marker for nuclei, cytochrome-C oxidase as a marker for mitochondria, lactate dehydrogenase (LDH) as a marker for cytoplasm. $N_1$ and $N_2$ : nuclear fractions; M + P: mitochondria and microsomes; s: cytosol. The lower part of the figure shows sedimentation profiles of oestradiol after centrifugation of nuclear extracts in sucrose gradients containing 0·4 M KCl.

Table 3. Sucrose gradient analysis of oestradiol binding by macromolecule extracted from the nuclear fraction of rat testis interstitial cells

rat
↓
*testis* isolation
↓
dissection of *interstitial tissue*
↓
homogenization in 10 mM Tris, EDTA; pH = 7·4
↓
centrifugation $\xrightarrow[100g]{}$ supernatant $\xrightarrow[105{,}000\,g]{}$ *cytosol*
↓
*pellet*
filtration over nylon gauze
centrifugation 700 *g*
↓
*nuclear fraction*
extraction with 0·4 M KCl in Tris buffer pH = 8·8
60 min 0°
↓
*nuclear extract*
sucrose gradient analysis (gradient with 0·4 M KCl)
18 h, 400,000 *g*
↓
collection of fractions from *gradients*
measurement of radioactivity

plasmic lactate dehydrogenase. The sedimentation profile of $^3$H-oestradiol in extracts from the $N_1$ nuclear fraction, obtained by treatment with a 0·4 M KCl solution, showed radioactivity in the 5*s* region. This 5*s* $^3$H-oestradiol-macromolecule complex was not present in the extract from fraction $N_2$ (Fig. 1).

## DISCUSSION

The physiological meaning of the oestradiol-17$\beta$ receptors in the different tissues of the male rat is not yet clear. It has been suggested that the presence of specific steroid binding macromolecules in the cytoplasm is a prerequisite for steroid hormone action[4]. The concentration of oestradiol used for *in vitro* incubation of rat tissue in this study ($4 \times 10^{-9}$ M) was in the order of the concentration normally present in uterine tissue and rat testis interstitial tissue, but higher than that present in total testis tissue ($10^{-10}$ M) [5]. Actions of oestradiol in the testis on DNA, RNA and protein synthesis have been reported for Balb/c mice[6]. Steinberger *et al.*[7] and Danutra *et al.*[8] have observed an effect of oestradiol on testosterone concentrations in the rat, without a concomitant change in LH level, which might imply a direct effect of oestradiol on steroidogenesis in testicular tissue. In other experiments carried out in our laboratory, a simultaneous fall in both testosterone and LH levels in the rat was observed after oestradiol benzoate injections[9]. Further studies are clearly required to find out whether oestradiol has a direct regulatory effect on testis steroidogenesis or spermatogenesis.

## REFERENCES


1. Brinkmann A. O., Mulder E., Lamers-Stahlhofen G. J. M., Mechielsen M. J. and van der Molen H. J.: *FEBS Lett.* **26** (1972) 301–305.
2. Mulder E., Brinkmann A. O., Lamers-Stahlhofen G. J. M. and van der Molen H. J.: *FEBS Lett.* **31** (1973) 131–136.
3. van Beurden-Lamers W. M. O., Brinkmann A. O., Mulder E. and van der Molen H. J.: *Biochem. J.* **140** (1974) 495.
4. Jensen E. V. and deSombre E. R.: *A. Rev. Biochem.* **41** (1972) 203–230.
5. de Jong F. H., Hey A. H. and van der Molen H. J.: *J. Endocr.* **60** (1974) 409.
6. Samuels L. T., Uchikawa T. and Huseby R. A.: *Ciba Fdn. Colloq. Endrocr.* **16** (1967) 211–232.
7. Steinberger E.: communicated at the 9th Acta Endocrinologica Congress, Oslo, 1973.
8. Danutra V., Harper M. E., Boyns A. R., Cole E. N., Brownsey B. G. and Griffiths K.: *J. Endocr.* **57** (1973) 207–215.
9. Verjans H. L., de Jong F. H., Cooke B. A., van der Molen H. J. and Eik-Nes K. B.: *Acta endocr., Copenh.* (1974) in press.


## DISCUSSION

*Lindner*:

I found your discovery of an oestrogen receptor in the interstitial cell of the testis very intriguing. What function could one postulate for such a receptor? Is this a general phenomenon, or peculiar to one strain of rats? I ask this because of the observation by Dr. L. T. Samuels that in the BALB/c strain of mice and not in others, oestrogen would inhibit androgen formation by the testis *in vitro*; it was a direct effect on the interstitial cell not mediated by the pituitary. Later he found that in that strain of mice a single injection of stilbestrol will cause formation of interstitial cell tumours. This could be a very nice source for your receptor.

*Mulder*:

May I first comment on the species specificity. We have also studied monkeys and mice and in the testes of both species an estrogen receptor was present. With respect to your question on the function of estradiol. I have one Table to show.

Table 1. Action of estradiol in testis (Mulder).

| Effect of oestradiol on | Animal species | |
|---|---|---|
| DNA, RNA, steroid converting enzymes | mice (BALB/c) | Samuels *et al.* (1967) |
| Induction of interstitial-cell tumours | mice | Bonsor and Robson (1940)<br>Huseby (1958) |
| | rat | Noble (1972)<br>Jull *et al.* (1973) |
| Steroidogenesis | rat | Steinberger *et al.* (1973)<br>Danutra *et al.* (1973) |

This is a summary of what is known about the possible function of estradiol. In the first place, the data of Samuels on the effect of estradiol in the BALB/c mice. Little is known about the function of the estradiol receptor in rats. Only very recent data from the group of Griffiths in Cardiff indicate that after 10 days administration of a large amount of estradiol (100 $\mu$g a day) the 17$\beta$-hydroxysteroid dehydrogenase activity is lower. That might indicate a direct action of estradiol on testosterone production, but there is only preliminary evidence for this.

*Jensen*:

I, too, find your observations on receptor in the interstitial cells both interesting and important. We are particularly interested in this, because a few years ago we also looked for estrogen receptors in many tissues. As we reported in 1969, we found all rat tissues to contain small amounts of estrogen receptor. We just looked at the whole testis, not being as clever as you were to look at the interstitial cells alone and see this marked concentration. There are two points of difference, though, between our findings and yours. One is that you had some tissues where you found no receptor whereas we found that all the tissues we examined contained small amounts of the 8S estrogen receptor. I think the major difference is in the relation of the pituitary content to that of other tissues. Your pituitary level seems much lower than ours, something like 8 times the whole testis, whereas ours was more like 50 times. I wonder if the difference might be in the rats used. We used the immature Sprague Dawley rat, both male and female. Were you using immature rats?

*Mulder*:

We use mature Wistar rats.

*Jensen*:

That may be the reason your pituitary level tends to be lower.

*Mulder*:

We only used one strain of rats so I don't have an answer on other species or other situations. The amount of *specific* binding in the hypothalamus of the female rat, as observed by King, was relatively small (40 counts/min above background in the 8S region of a sucrose gradient). We have used the normal rat and it might be—as was mentioned before this morning—that in the male rat, in the hypothalamus, testosterone is converted to estradiol. The receptor might already be occupied with unlabelled estradiol and therefore we don't see *specific* binding of labelled estradiol.

*Ungar*:

I'd like to make one remark about the adrenal. I noticed that you had a fairly high receptor level in the adrenal. I have one slide to show just to indicate something that is fairly well known, that is the increase in the adrenal weight of the female rat. We are doing some studies on the male-female differences in terms of the corticosterone production and the cyclic nucleotides. I think it's interesting that the female on a *weight basis* does not respond as well to ACTH as the male. (Production of Corticosterone *per adrenal* is the same). These sexual differences have never been explained adequately either at the local or at the hypothalamic level.

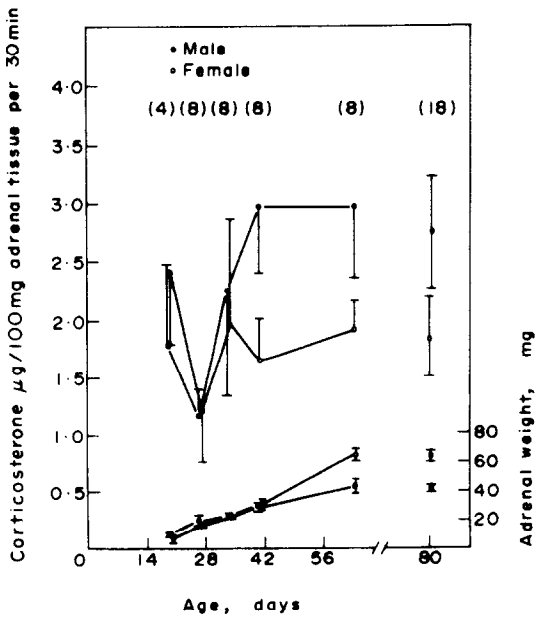


Fig. 1. (Ungar)

Effect of ACTH on corticosterone secretion and weight of adrenal.

I know other groups have looked for an estrogen receptor in the adrenal but have not been able to find one. I find it very interesting that you have.

*Mulder*:

We have only studied the receptor in the male rat. We have compared a series of receptors in male rats.

*Ungar*:

Have you looked at the female and the male to contrast the two of them?

*Mulder*:

No, we did not look at the female.