Journal of Steroid Biochemistry, 1977, Vol. 8, pp. 859–865. Pergamon Press. Printed in Great Britain

KINETICS OF *IN VITRO* BINDING OF OESTRADIOL IN SUBCELLULAR FRACTIONS OF TESTICULAR AND UTERINE TISSUE; CHARACTERIZATION OF OESTRADIOL BINDING IN TESTICULAR NUCLEI

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(*Received* 6 *December* 1976)

SUMMARY

1. Oestradiol in rat testicular and uterine tissue is specifically bound to nuclear receptor sites, which can be separated in KCl-extractable nuclear and nuclear residual (a nuclear fraction which resists KCl extraction) receptor sites.
2. The amount of "extractable" nuclear binding sites for oestradiol in testis could be increased by mild trypsin treatment. Treatment of testicular nuclei with deoxycholate or DNAse resulted in a decrease of residual receptor sites and a concomitant increase of unbound oestradiol in the "extractable" nuclear fraction.
3. The presence of KCN *in vitro* resulted in a relative increase in the number of oestradiol binding sites in the nuclear residual fraction in both uterine and testicular tissue; the number of binding sites in the KCl-extractable fraction was not affected by KCN.
4. During *in vitro* incubations of testicular tissue the number of oestradiol binding sites in the KCl-extractable nuclear fraction reached a maximum and remained constant after 30 min of incubation; the number of binding sites in the nuclear residual fraction decreased after incubation periods longer than 30 min.
5. During *in vitro* incubations of uterine tissue the number of oestradiol binding sites in the KCl-extractable nuclear fraction and the nuclear residual fraction after an initial increase decreased to 50% of the maximal value between 30 and 60 min of incubation.
6. It is concluded, that the testicular oestradiol receptor shows certain characteristics comparable with those of the uterine receptor. However, regarding the differences in retention time of steroids in the nucleus, it seems very unlikely that the oestradiol effect in uterus and the oestradiol effect in testis, if present, are mediated by identical receptor mechanisms.

INTRODUCTION

It is now well accepted that the cytoplasm of target cells for steroid hormones contains a specific binding protein called a receptor. The complete sequence of events in the response of a tissue to a steroid hormone is still unknown, but a postulated primary step is the interaction of the steroid with its receptor in the cytoplasm of the target tissue. The formed steroid–receptor complex migrates into the nucleus and binds to acceptor sites on the chromatin which ultimately results in a response of the tissue to the steroid *via* changes in RNA and protein synthesis [1–4].

The rat testicular Leydig cell contains a cytoplasm receptor for oestradiol which can be transported into the nucleus and which binds to the chromatin under the influence of endogenously produced oestradiol [5–8]. Whether the binding of oestradiol–receptor complexes to nuclear acceptor sites results in a physiological effect in the Leydig cell is still unclear [9]. In order to gain further insight in a possible function of oestradiol-receptors in the Leydig cell the processes of translocation and nuclear binding of receptor-oestradiol complexes *in vitro* in testicular tissue with similar processes in uterine tissue, a tissue which responds well to oestrogen administration, were compared [10,11].

In the present study we have also investigated the effects of trypsin, deoxycholate and DNAse treatment on the nature of nuclear oestradiol binding sites in testicular tissue. For uterine tissue it has been postulated that the number of nuclear residual oestradiol receptor sites (the fraction which resists KCl-extraction) determines the tissue response to oestradiol [12]. Therefore the distribution of KCl-extractable and nuclear residual binding sites was investigated after incubation of uterine and testicular tissue with oestradiol.

It has been reported that energy might be required for the action of glucocorticoids and progesterone [13–16]. In this respect we have also studied the effect of energy deprivation on the distribution of oestradiol binding sites in KCl-extractable and nuclear residual fractions of uterine and testicular tissue.

MATERIALS AND METHODS

Materials. Unlabelled oestradiol and diethylstilboestrol (DES) were purchased from Steraloids Inc. Pawling, NY, U.S.A. [^{3}H]-oestradiol (S.A. 96 Ci/mmol) was purchased from Radiochemical Centre, Amersham, U.K. The purity of the steroids was determined by t.l.c. Trypsin (analytical grade) was obtained from Boehringer Mannheim, West Germany, sodium deoxycholate from Merck, Darmstadt, West-Germany and DNAse from Sigma, St. Louis, U.S.A.

Source of tissues and treatments. Immature (25–30 day old) and mature (3 months old) rats of the R-Amsterdam strain were used in this study. Interstitial tissue of mature rat testis was obtained by wet dissection after incubation *in vitro* [17]. One decapsulated testis or one uterus stripped of adhering fat and mesentery, from immature rats, was incubated in 2.0 ml Krebs–Ringer bicarbonate buffer, pH 7.4, containing 0.2% glucose. For mature rat testis 4.0 ml of incubation medium was used per testis. Incubations were carried out for different time periods in an atmosphere of 95% O_2:5% Co_2. In studies where the effect of KCN was investigated tissues were incubated in Krebs–Ringer bicarbonate buffer, pH 7.4, containing 5×10^{-4} M KCN. Testicular tissue was incubated at 32°; uterine tissue at 37°.

Tissues were incubated either with 2×10^{-8} M [^{3}H]-oestradiol (total binding) or with 2×10^{-8} M [^{3}H]-oestradiol in the presence of 4×10^{-6} M DES (non specific binding). Specific binding of [^{3}H]-oestradiol is defined as the difference between the total binding and the non specific binding.

Preparation of subcellular fractions. After incubation total testicular tissue and interstitial tissue were homogenized in 10 vol. 10 mM Tris–HCl buffer, pH 7.4, containing 1.5 mM EDTA and 0.02% NaN_3 (TEN-buffer) with 6 strokes of a Potter–Elvehjem homogenizer at 1100 rev./min. The homogenate was rehomogenized in all-glass Potter–Elvehjem homogenizer and was subsequently centrifuged at 500 *g* for 10 min at 0°. The 500 *g* pellet was washed once with TEN buffer, twice with TEN-buffer containing 0.2% Triton X-100 and another time with TEN buffer. Uterine tissue of immature rats was homogenized in an Ultraturrax homogenizer for 10 s, rehomogenized in an Potter–Elvehjem homogenizer and centrifuged at 800 *g* for 10 min at 0°. The 800 *g* pellet was further washed and centrifuged at 800 *g* as described for testicular tissue. The nuclear preparations thus obtained appeared pure by phase contrast microscopy and had a protein/DNA ratio in the range of 1.7–1.9. The isolated pellets were used immediately after isolation for estimation of nuclear binding. Where indicated the 500 *g* and 800 *g* supernatants of the testicular and uterine tissue respectively were centrifuged at 105,000 *g* for 60 min to obtain the cytosol fraction.

Incubation of nuclei. In experiments concerning the nature of the oestradiol binding sites in testicular nuclei the following procedure was used. After incubation of testis tissue from mature rats in the presence of steroids for 60 min at 32° interstitial tissue nuclei were isolated. Nuclei obtained from the interstitial tissue of one testis were suspended in 10 mM Tris buffer pH 7.4 and incubated with DNAse (150 μg/ml; 30 min at 20°), trypsin (150 μg/ml; 30 min at 10°) or deoxycholate (1% DOC, 30 min at 0°) in a vol. of 1.0 ml. After incubation the nuclear suspensions were centrifuged for 30 min at 105,000 *g* and specific binding of [^{3}H]-oestradiol was measured in the supernatant ("soluble") and pellet ("nuclear residual") fraction as described below. A KCl extractable nuclear fraction and a nuclear residual fraction obtained after extraction of untreated nuclei with 0.4 M KCl served as a control.

Estimation of specific [^{3}H]-oestradiol binding. It was attempted to extract the [^{3}H]-oestradiol in the nuclear pellet with 0.4 M KCl in TEN buffer, pH 8.5. The nuclear pellet was mixed with an equal vol. of 0.8 M KCl in TEN buffer, pH 8.5. The final vol. of the extraction buffer was adjusted to 800 μl with 0.4 M KCl in TEN buffer, pH 8·5. After incubation for 60 min at 0° the extract was centrifuged for 30 min at 105,000 *g* and an aliquot of the supernatant fraction was analyzed on sucrose density gradients as described below (KCl-extractable nuclear binding). The residual nuclear pellet, from which no further [^{3}H]-oestradiol could be extracted with KCl–TEN buffer, was solubilized in 1 N NaOH and an aliquot was counted for radioactivity after addition of an equal vol. of 3 N perchloric acid (residual nuclear binding). Specific binding in both fractions was estimated by substracting the non specific binding from the total binding of [^{3}H]-oestradiol and was expressed as fmol/mg protein, present in each fraction. In the 105,000 *g* supernatants specific cytosol binding was estimated by sucrose density gradient centrifugation as described below.

Sucrose gradient centrifugation. A 200 μl portion of the KCl-extractable nuclear fraction was layered on top of a linear 5–20% (w/v) sucrose density gradient prepared in TEN buffer, pH 8.5, containing 0.4 M KCl. Gradients were centrifuged at 260,000 g_{av} for 18 h at 0° in a Beckman SW65 rotor. Cytosol fractions (200–400 μl) were layered on top of linear 5–20% (w/v) sucrose gradients prepared in TEN buffer and gradients were centrifuged at 150,000 g_{av} for 16 h at 0°. On separate gradients 200 μg of bovine serum albumin (BSA) or alcohol dehydrogenase (ADH) were run as sedimentation markers (4.6 *s* and 7.6 *s* respectively). After centrifugation each gradient was fractionated in 27 fractions of 0.2 ml each and each fraction was assayed for radioactivity.

General procedures. Protein was determined by the procedure of Lowry *et al.* [18] with bovine serum albumin as a standard. Radioactivity was measured in a Packard model 3375 liquid scintillation spectrometer. The scintillation fluid consisted of a mixture of Triton X-100 (Rohm and Haas, Philadelphia, U.S.A.) and toluene (1:2, v/v) containing 0.1 g POPOP

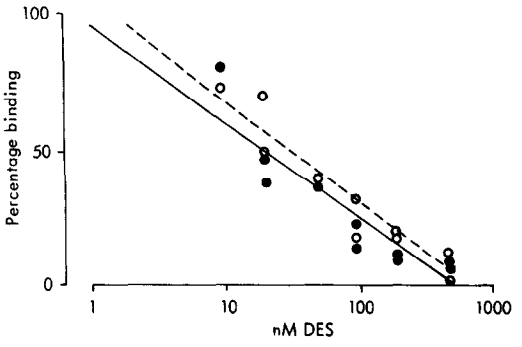

Fig. 1. Determination of two different nuclear binding sites for oestradiol. Testicular tissue from immature rats was incubated for 60 min at 32° either with 10^{-8} M [^{3}H]-oestradiol or with 10^{-8} M [^{3}H]-oestradiol in the presence of increasing amounts of nonradioactive diethylstilboestrol. Nuclei were isolated and extracted with 0.4 m KCl. Binding of oestradiol in the KCl-extractable fraction and the nuclear residual fraction was estimated as described in the methods section. The binding obtained in these two nuclear fractions in the absence of nonradioactive competitor is defined as the 100% value. ●——● nuclear extract; O-----O residual nuclear fraction.

(1,4-bis-(5-phenyloxazol-2-yl) benzene)/l and 4.8 g PPO (2,5-diphenyloxazol)/l (Packard Instrument S.A., Benelux, Brussels, Belgium).

RESULTS

Determination of two different types of specific oestradiol binding sites in testicular nuclei

Immature rat testicular tissue was incubated either with 10^{-8} M [^{3}H]-oestradiol or with 10^{-8} M [^{3}H]-oestradiol in the presence of increasing amounts nonradioactive DES (Fig. 1). Nuclei were isolated, extracted with 0.4 M KCl and oestradiol binding sites present in the KCl-extractable fraction and the nuclear residual fraction (the fraction which resists extraction) was estimated. In Fig. 1 it is shown that increasing amounts of nonradioactive DES decreased the binding of [^{3}H]-oestradiol in both the KCl-extractable and the nuclear residual fraction. A two and three fold excess nonradioactive DES reduced the initial amount of binding by a factor of two in the KCl-extractable fraction and nuclear residual fraction respectively.

In the order of 40% of the specifically bound steroid in testicular nuclei was generally recovered in the KCl-extractable fraction; 60% resisted KCl extraction. The distribution of specific binding sites in the two nuclear fractions did not change if nuclei were extracted several times with 0.4 M KCl. At 0.5 M and higher salt concentrations the nuclear material became quite viscous and could not be sedimented by centrifugation.

Nature of the oestradiol binding sites in testicular nuclei

In order to investigate the nature of the oestradiol binding sites in testicular nuclei, testes from mature rats were incubated with oestradiol and the obtained nuclear suspension from interstitial tissue nuclei was incubated, either with trypsin, DNAse or deoxycholate.

The results in Fig. 2 show that treatment of nuclei with trypsin resulted in an increased amount of specifically bound [^{3}H]-oestradiol in the soluble fraction. Further analysis of this soluble fraction on sucrose density gradients revealed that [^{3}H]-oestradiol was specifically bound to a 4 *s* sedimenting macromolecule. If nuclei were treated with DNAse or deoxycholate an oestradiol binding macromolecule with a sedimentation value of 5 *s* was observed in sucrose density gradients. This value is similar to the value obtained after extraction of nuclei with 0.4 M KCl. Treatment of nuclei with either DNAse or deoxycholate did not result in a further change of specifically

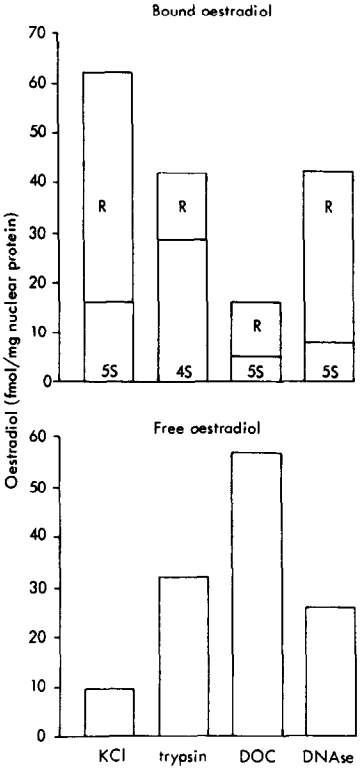

Fig. 2. Effect of different treatments on the binding of oestradiol in testicular nuclei. Testicular tissue from mature rats was incubated for 60 min at 32° in the presence of steroids and after dissection of the interstitial tissue, nuclei were isolated. Aliquots of the nuclear suspension in 10 mM Tris-buffer, pH 7.4, were treated either with trypsin (150 μg/ml; 30 min at 10°), with deoxycholate (1% DOC; 30 min at 0°) or with DNAse (150 μg/ml; 30 min at 20°). After incubation the suspensions were centrifuged at 105,000 *g* for 30 min and specific binding in the supernatant ("nuclear extractable") and pellet fraction ("residual nuclear fraction") were measured as described in Materials and Methods. Nuclei extracted with 0.4 M KCl were used as a control. Radioactive steroid in the nuclei is subdivided in radioactivity bound by the nuclear extractable fraction (sedimentation value of 5 *s* or 4 *s*), free radioactive oestradiol in the extractable fraction and specifically bound residual nuclear radioactivity (R).

bound radioactivity in the soluble fraction. But with all three different methods a shift of bound radioactive oestradiol from the nuclear residual fraction to free oestradiol recovered from the soluble fraction was observed.

Distribution of specifically bound [^3H]-oestradiol during in vitro *incubation*

Testis. The effect of incubation time on the distribution of oestradiol binding sites was studied in testicular tissue of immature rats preincubated in the presence of steroids for 60 min at 0° and subsequently incubated at 32° for various time periods. During the first 30 min of incubation specific oestradiol binding in the KCl-extractable and residual nuclear fraction gradually increased (Fig. 3). After longer incubation the amount of bound oestradiol in the KCl-extractable fraction did not change, but a considerable decrease of the binding of oestradiol in the nuclear residual fraction was observed. The major part of the oestradiol binding sites in the cytosol fraction, after the incubation of testicular tissue with radioactive steroid, consisted of nonspecific binding sites. Therefore, it was impossible to obtain an accurate measurement of the amount of specific binding of oestradiol in the cytosol.

Uterus. Uterine tissue was incubated at 37° for different time periods after an initial incubation of 60 min at 0° in the presence of steroids. Nuclei were isolated and extracted with 0.4 M KCl in order to obtain a KCl-extractable and nuclear residual fraction. Specifically bound oestradiol in both nuclear fractions increased during the time of incubation

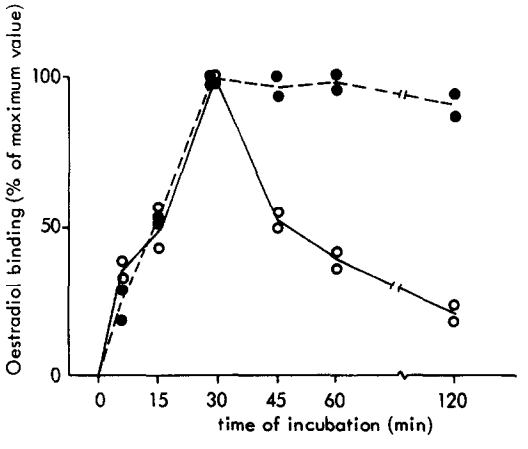

Fig. 3. Time course of oestradiol binding in testicular nuclei during incubation of testis *in vitro*. Testicular tissue from immature rats was pre-incubated at 0° for 60 min either with 2×10^{-8} M [^{3}H]-oestradiol or with 2×10^{-8} M [^{3}H]-oestradiol plus 4×10^{-6} M DES. After this period the temperature was elevated to 32° and incubation was continued for different time periods. Tissues were rinsed with buffer, homogenized and nuclei were isolated. After extraction of nuclei with 0.4 M KCl specific oestradiol binding in the KCl-extractable and nuclear residual fraction was estimated. Each point reflects an individual estimation. ●——● nuclear extract; O----O residual nuclear fraction.

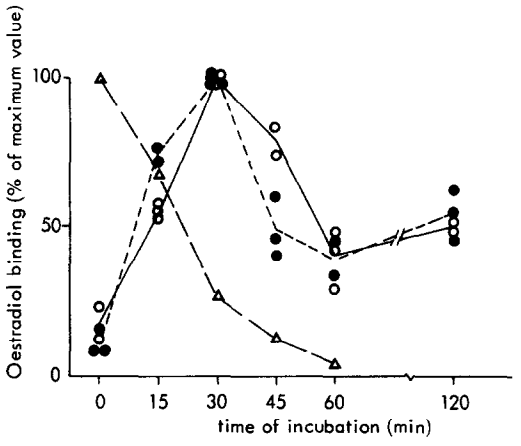

Fig. 4. Time course of oestradiol binding in subcellular fractions after incubation of uterine tissue *in vitro*. Uteri from immature rats were preincubated at 0° for 60 min either with 2×10^{-8} M [^{3}H]-oestradiol or with 2×10^{-8} M [^{3}H]-oestradiol plus 4×10^{-6} M DES. After this incubation period the temperature was elevated to 37° and incubation was continued for different time periods. Uteri were rinsed with buffer, homogenized and nuclei were isolated. After extraction of nuclei with 0.4 M KCl specific oestradiol binding was estimated in the KCl-extractable fraction and the nuclear residual fraction. Specific binding in the cytosol fraction, obtained as described in the method section, was also estimated. Each point reflects an individual estimation. ●——● nuclear extract; O----O nuclear residual fraction; △----△ cytosol fraction.

reaching a maximum after 30 min (Fig. 4). After continued incubation the specific binding of oestradiol in the KCl-extractable and nuclear residual fraction declined reaching a plateau after 60 min. During the first period of incubation the initial increase in nuclear oestradiol binding was accompanied by a decrease in the amount of specifically bound oestradiol in the cytosol fraction. After 60 min specific binding sites for oestradiol in the cytosol fraction became undetectable.

Effect of energy deprivation on the distribution of specifically bound [^3H]-oestradiol in testicular and uterine nuclei

To determine the effect of energy deprivation on the distribution of oestradiol binding sites, testicular and uterine tissue were incubated in the presence of steroid and 5×10^{-4} M KCN. Tissues incubated in the presence of steroids but with the omission of KCN from the incubation medium served as control tissues. One hour after the addition of KCN the number of specific oestradiol binding sites in the nuclear residual fractions of both testicular (Fig. 5A) and uterine (Fig. 5B) tissue were enhanced significantly compared to controls. For testicular tissue a twofold increase was measured, for uterine tissue the increase was slightly lower (1.6 fold). The addition of KCN had no specific effect on the specific binding of oestradiol in the KCl-extractable nuclear fraction of both tissues.

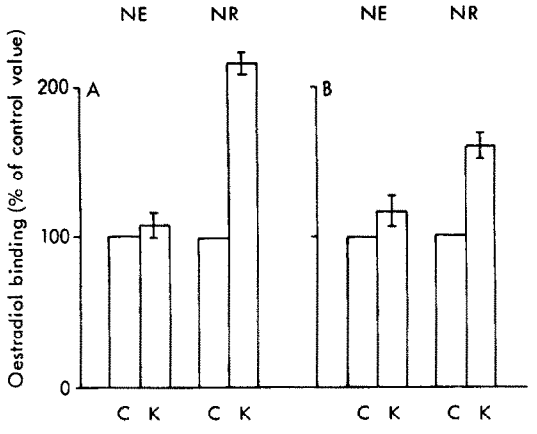

Fig. 5. *In vitro* effect of KCN on the nuclear binding of oestradiol in testicular (A) and uterine tissue (B). Tissues were preincubated in KRBG-buffer at 32° or 37° for 60 min either with 2×10^{-8} M [^{3}H]-oestradiol or with 2×10^{-8} M [^{3}H]-oestradiol plus 4×10^{-6} M DES. After this period the tissues were washed with KRB-buffer and further incubated in KRB-buffer for a period of 60 min at 32° or 37° in the presence of both steroids and 5×10^{-4} M KCN. Nuclei were isolated and specific oestradiol binding in the KCl-extractable and nuclear residual fractions was estimated. The specific binding obtained after incubation of testis and uterus in KRBG for a period of 120 min in the presence of steroids is defined as the 100% value. Results are expressed as the mean of 3 experiments ±S.E.M. C = control incubation. K = incubation in the presence of KCN.

DISCUSSION

The testicular Leydig cell contains a receptor protein which is specific for oestradiol [7, 19]. This oestradiol receptor has also been demonstrated in total testicular tissue from immature rats [20] and in dissected interstitial tissue of mature rats [5, 20]. From the present results it appears that two different fractions of specifically bound oestradiol can be demonstrated in testicular nuclei: one fraction (40%) is extractable with 0.4 M KCl, the other fraction resists KCl-extraction. The number of specific binding sites in the KCl-extractable fraction did not show any increase if nuclei were extracted repeatedly or if nuclei were extracted with concentrations of KCl above 0.4 M. The present observations are compatible with those for steroid-hormone receptors in other tissues where nuclear binding could also be distinguished in a KCl-extractable and a nuclear residual form [12, 13, 21, 22, 24–26].

The role of different types of nuclear binding sites for steroid hormones is not yet clear. Evidence has been presented, however, that the residual nuclear binding proteins for oestradiol and testosterone might be important in the mechanism of action of steroid hormones [12, 27, 28]. The demonstration of two different nuclear binding sites for oestradiol in Leydig cells raises the possibility that actions of oestradiol in these cells can also be mediated via a similar receptor mechanism. The *in vitro* incubations of testicular tissue with [^{3}H]-oestradiol showed that both types of nuclear oestradiol binding were similarly affected by increasing amounts of nonradioactive DES (Fig. 1). The addition of a 2–3 fold excess of nonradioactive DES caused a 50% reduction of the [^{3}H]-oestradiol binding in both nuclear fractions. This observation is in good agreement with those obtained with the cytoplasmic oestradiol receptor [19].

Different approaches were used to characterize the nature of the nuclear oestradiol binding sites in testicular tissue. Mild trypsin treatment of nuclei, which contained oestradiol receptor complexes, caused a release of binding sites for oestradiol with a sedimentation value of 4 *s*. This value is different from the sedimentation value of 5 *s* which is normally observed for KCl-extractable nuclear oestradiol receptors. Receptor complexes with a sedimentation value of 4 *s* were also solubilized after treatment of liver and uterine nuclei with trypsin [22, 23]. It has been reported previously that trypsin treatment of the oestradiol receptor from cytosols of calf uteri resulted in a loss of binding affinity of the receptor for DNA and it has been suggested [29] that trypsin treatment releases part of the receptor molecule, which contains the chromatin-binding unit, whereas the oestrogen binding unit remains unaffected and shows a sedimentation value of 4 *s* on sucrose density gradients.

Extraction of isolated liver nuclei with NaCl solutions in a concentration range of 0.14 M–2 M causes removal of DNA-histones and associated proteins; the residual protein fraction contains acidic proteins [30]. For other tissues it has been reported that nuclear steroid hormone receptors are associated with acidic nuclear proteins [27, 31]. It has been reported by Wang [30], that deoxycholate (DOC) could solubilize 95% of the nuclear residual proteins from rat liver, including the acidic protein fraction. In the present experiments deoxycholate treatment of testicular nuclei, containing oestradiol–receptor complexes, caused the release of a considerable amount of total nuclear radioactive oestradiol, but only a minor fraction was bound to macromolecules with a sedimentation value of 5 *s*. Addition of DOC to cytoplasmic oestradiol–receptor complexes did not result in a dissociation of the hormone from the receptor molecule. Therefore the observed release of unbound radioactivity from deoxycholate-treated nuclei might reflect a lowered affinity of the nuclear receptor molecule for the hormone subsequent to its binding by the chromatin. DNAse was used in another attempt to characterize the nature of the nuclear steroid binding. After treatment with DNAse of testicular nuclei, containing accumulated oestradiol–receptor complexes, a considerable increase of radioactive oestradiol in the soluble nuclear fraction was obtained, but again only a small part of the released oestradiol was bound to macromolecules.

From the observed effects of DNAse in the present experiments as well as in previous experiments by others [13, 32–39] it is not possible to derive unambi-

gous information about the nature of the nuclear acceptor sites which bind hormone–receptor complexes. From the available evidence [4, 27, 31, 40–42] it appears most likely that the nuclear acceptor sites are composed of both DNA and nonhistone proteins.

The observed increase in the amount of nuclear binding sites in the KCl-extractable and nuclear residual fraction of testicular tissue during the first 30 min of incubation (Fig. 3) might reflect translocation of cytoplasmic receptor molecules. Whereas the level of nuclear binding sites in the KCl-extractable fraction remained rather constant, the number of oestradiol binding sites in the nuclear residual fraction decreased, which might reflect either an inactivation of binding sites in this fraction or a redistribution of binding sites over nuclear and cytosol fractions. A possible return of nuclear binding sites into the cytoplasmic fraction could not be measured due to very high levels of nonspecific binding sites in this fraction. Different results were obtained for uterine tissue (Fig. 4). After an initial increase, the specific binding to KCl-extractable and residual nuclear fractions declined to levels about 50% of the maximum value, which was observed at 30 min after the start of the incubation. This pattern is in close agreement with observations of Mester *et al.* for uterine tissue after *in vivo* injection of immature rats with oestradiol [24]. The measured amount of specific oestradiol binding sites in the nuclear residual fraction 60 min after incubation (160 fmol/uterus) is comparable with the amount estimated by Clark *et al.* 1 and 6 h after *in vivo* injection of oestradiol [12]. These authors suggested that this rather constant amount of accumulated nuclear residual binding sites might be the important fraction for the response of the uterus to oestradiol [10, 11]. In testicular tissue the amount of nuclear residual binding sites, after a maximum at $t = 30$ min, dropped gradually, without reaching a plateau. From the results presented in Fig. 3 and Fig. 4 it can be concluded that the *in vitro* translocation of oestradiol–receptor complexes into the nuclear fractions of uterus and testis follow similar time courses. However, differences were observed for the retention times of the oestradiol–receptor complexes in the nuclear fractions of both tissues. The incubation temperatures used in the experiments were 37° and 32° for uterus and testis respectively. Therefore it cannot be excluded that the observed differences in the nuclear retention reflect a difference in the stability of the binding of hormone–receptor complexes to uterine and testicular chromatin under physiological conditions.

If hormone effects in cells are mediated via a receptor mechanism in the nuclear fraction, then the short retention time of tightly bound nuclear oestradiol receptor molecules (the binding sites in the residual nuclear fraction) could offer an explanation for the difficulties in the search for an oestrogen effect in the testis. In the literature some evidence is available that cellular ATP is involved in the mechanism of action of steroid hormones. It has been observed that the progesterone receptor in oviduct binds ATP [16] and that KCN could inhibit the glucocorticoid receptor release from fibroblast nuclei [13]. In our studies the presence of KCN increased the amounts of oestradiol binding in the residual nuclear fraction in testicular (2-fold increase) and uterine (1.6 fold) tissue, while the oestradiol binding in the KCl-extractable fraction remained unaffected. The increased residual nuclear binding in the presence of KCN might indicate that a continuous production of ATP is necessary for the release of receptor molecules, possibly in an inactive form [43], into the cytosol. Further investigations are needed to elucidate the energy requirements in the mechanism of action of steroid hormones.

From the present results it can be concluded that the testicular Leydig cell shares the large group of cells that contain receptors for steroid hormones. Under the influence of added oestradiol the cytoplasmic receptor was translocated into the nuclei where it could be recovered from two different nuclear binding sites which differed in extractability with KCl. The retention times of bound oestradiol in the nuclear fraction which resisted KCl extraction were different for uterine and testicular tissue. Therefore it seems very unlikely that the oestradiol effect in uterine tissue and in testicular tissue, if present, are mediated by identical nuclear receptor mechanisms.

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