Nuclear Translocation of the Estrogen Receptor in Autonomous C3H Mouse Mammary Tumors

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ABSTRACT

The presence of ER has been demonstrated in all estrogen-dependent tumors. Estradiol and prolactin have been shown to be the most important hormones involved in the induction and growth of the mouse or rat mammary tumor. The reason for estrogen independence of C3H mouse mammary tumors has been sought in the initial steps of estradiol action. The characteristics of the estrogen receptor complexes were estimated by more specific methods such as protamine sulfate or hydroxylapatite precipitations, the estrogen receptor translocation into the nucleus was clearly shown. We therefore conclude that the estrogen independence of C3H mammary tumors cannot be explained by a defect in the two initial steps of the mechanism of action of estradiol, namely, cytosol binding and nuclear translocation of receptors.

INTRODUCTION

Estradiol and prolactin have been shown to be the most important hormones involved in the induction and growth of the mouse or rat mammary tumor. The presence of ER has been demonstrated in all estrogen-dependent tumors. However, ER's have also been found in estrogen-unresponsive breast cancer, leading to the proposal that the concentration of ER is not a sufficient criterion indicative of hormone dependence and that the quality of ER should also be evaluated. In the C3H mouse, the induction of mammary tumor is hormone dependent, while the growth of the constituted tumors is totally estrogen independent since the tumors continue to grow even though the hosts had been ovariectomized and adrenalectomized.

The purpose of these studies was to analyze the initial steps of the mode of action of estradiol in the C3H mammary tumors in order to explain the mechanism underlying the hormone independence of these tumors. We have therefore measured the concentration and characterized the estrogen cytosol receptor that had previously been detected in C3H mouse mammary tumors. The functional integrity of RC has then been assessed in evaluating its nuclear translocation after in vivo injection of estradiol.

MATERIALS AND METHODS

Reagents and Buffers. 17β-6,7-[3H]Estradiol (45 to 58 Ci/m mole) was obtained from CEA, Gif-sur-Yvette, France. Purity of the isotope was checked periodically by thin-layer chromatography. The following steroids (98% pure) were a gift from Roussel-Uclaf, Romainville, France: 17β-estradiol, progesterone, testosterone, 5α-dihydrotestosterone, and cortisol. [3H]R 5020 (51 Ci/mmol) and nonradioactive R 5020 were kindly provided by Dr. J. P. Raynaud, Roussel-Uclaf. The antiestrogen nafoxidine [U-11100A; 1-{2-[p-(3,4-dihydro-6-methoxy-2-phenyl)naphthal]phenyl)ethyl]pyrrolidine hydrochloride] was a gift from Dr. O. Kaduka, The Upjohn Company, Kalamazoo, Mich. Calf thymus DNA I, monothioglycerol, and Tris were obtained from Sigma Chemical Company, St. Louis, Mo. Pronase, free of nucleases, was from Calbiochem, Lucerne, Switzerland. Prostate androgens and DES were from Merck AG, Darmstadt, Germany. The 2 buffers used were TET buffer and TETK buffer.

C3H Mouse Mammary Tumors. C3H mice were kindly provided by Dr. G. Rudali (Institut du Radium, Paris, France) and by Dr. R. Van Nie (A. Van Leuwenhoekhuis, Cancer Institute, Amsterdam, The Netherlands). The animals were maintained in a temperature (22°)- and light (6 a.m. to 8 p.m.)-controlled room and were fed a diet of UAR A 04 (UAR S. A., Villemoisson, France) and tap water ad libitum. They developed mammary cancer within 8 months (forced breeding). Bilateral ovariectomy was routinely performed under light ether anesthesia when the tumor size reached 2 sq cm. Tumor size was measured with a caliper before and after ovariectomy in order to assess the ovarian independence of the tumors. The animals were sacrificed by decapitation following chloroform anesthesia. The tumors were immediately used or frozen in liquid nitrogen and stored at -20°. As a study of the in vivo effect of [3H]estradiol on the nuclear translocation of the ER complexes, a tumor biopsy (200 to 300 mg) was primarily obtained under light ether anesthesia; then the mouse was given a s.c. in-
jection of \(^{3}H\)estradiol (0.25 to 4 \(\mu\)g in 0.9\% NaCl solution containing 5\% absolute ethanol) in the middorsal area. One to 6 hr after \(^{3}H\)estradiol injection, the remaining labeled tumor was excised at sacrifice and assayed for ER.

**Preparation of Soluble Extracts.** All procedures were performed at 0–4\°C. The tumors and uterus were homogenized in TET buffer. Cytosol was obtained by ultracentrifugation of the homogenate at 164,000 \(\times\) g for 70 min (24). The particulate pellet was then extracted by TETK buffer; the high-speed supernatant obtained was called "nuclear extract." Control experiments performed with purified nuclei had previously established that ER's were mainly localized in nuclei and not in other particles (8).

**ER Assays.** The E\(_2\)-R complexes were determined after labeling of the cytosol and KCl nuclear extract in vitro (Chart 1) or in vivo. In the latter case, the soluble extracts were either directly analyzed or subsequently resaturated up to 5 or 10 \(nM\) \(^{3}H\)estradiol in order to assess the in vivo saturation of the ER sites.

The bound estradiol was assayed by 3 different techniques. The DCC assay was performed as previously described (24). The nonsaturable estradiol binding was evaluated in vitro by a parallel incubation with 1 \(\mu\)M nonradioactive DES. It was systematically subtracted from the total binding assayed by DCC after the in vivo and in vitro experiments. Protamine sulfate precipitation was performed according to the method of Chamness et al. (3) on labeled extracts, the bound radioactivity being then extracted 3 times with 1 ml absolute ethanol. The hydroxylapatite assay was performed batchwise and adapted for microassay (P. P. Baskevitch and F. Capony, unpublished observations). Briefly, 200 \(\mu\)l of a suspension of 10\% hydroxylapatite (HTP; Bio-Rad Laboratories, Richmond, Calif.) in TET or TETK buffer were incubated with 50 \(\mu\)l of labeled cytosol or KCl nuclear extract for 30 min with vigorous horizontal shaking in disposable polypropylene tubes. The E\(_2\)-R complexes were adsorbed onto the hydroxylapatite that was washed 3 times with 3 ml TET buffer. The \(^{3}H\)estradiol was then extracted from hydroxylapatite in 15 ml Bray scintillation mixture and subsequently counted. Both protamine sulfate and hydroxylapatite assays were systematically done on extracts previously treated with a pellet of DCC for 1 hr at 2\°C. In these 2 latter procedures, the nonspecific binding was evaluated after pronase digestion (1 mg/ml for 1 hr at 25\°C) and after heating at 60\°C for 1 hr, or by isotopic dilution with 1 \(\mu\)M DES in vitro or 1 mg estradiol in vivo.

**Sucrose Density Gradients.** After treatment with a pellet of DCC to adsorb the excess of unbound estradiol, the cytosol or nuclear extract was layered onto a 5 to 20\% sucrose gradient and centrifuged at 150,000 \(\times\) g for 16 hr in an SW 50 rotor. Then 2-drop fractions were collected, and the sedimentation values were calculated (10) according to internal markers \([^{14}C]\)ovalbumin and \([^{14}C]\)–\(\gamma\)-globulin or endogenous hemoglobin.

**Miscellaneous Techniques.** Protein concentration was assayed by absorption measurements at 260 and 280 nm (9). The radioactivity was counted in a 0.3\% PPO-0.01\% POPOP-toluene scintillation mixture in a \(\beta\) SL30 scintillation counter (Inter Technique S. A., Plaisir, France), with 25\% efficiency as evaluated by external standard.

**RESULTS**

**In Vitro Characterization of the Estradiol Receptor.** The estradiol receptor that had been previously detected in the cytosol of C3H mammary tumors (15, 16) was further characterized in castrated mice. Saturation analysis indicated a high-affinity binding of estradiol to cytosol proteins \(K_D = 0.17 \pm 0.02 \text{ nM (S.E.)}\). (Chart 1, a and b). Competitive binding experiments performed at equilibrium showed the high specificity of the binding, since only estradiol, estrone, estriol, and U-11100A, a synthetic antiestrogen, competed with estradiol for binding sites while 1 \(\mu\)M testosterone, 5 \(\alpha\)-dihydrotestosterone, progesterone, and cortisol had no competitive effect. On the 5 to 20\% sucrose gradient, an 8S peak was detected in all experiments (Chart 2); a saturable 4S peak could also be shown in some tumors. The 8S estradiol receptor of the C3H tumor was shown to interact with soluble DNA (Chart 2) as is the case for uterine estradiol receptor (1, 22). However, the degree of this interaction varied according to the tumor and was sometimes less than that represented in Chart 2. This finding was confirmed by adsorption onto DNA cellulose (C. Bousquet, unpublished results). The ER binding sites measured by DCC assay were destroyed when the cytosol had been heated at 60\°C for 1 hr or incubated with pronase (1 mg/ml) at 25\°C for 1 hr (Table 1). In addition, the E\(_2\)-R complexes were assayed by precipitation on protamine sulfate and retention on hydroxylapatite. When the in vitro \(^{3}H\)estradiol binding was assayed, a similar number of estradiol-binding

![Chart 1](image-url)
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20% sucrose gradient at 150,000 x g for 15 hr in an SW 50 rotor. Endogenous and the control level of receptor was determined in vitro on Therefore, in the C3H mouse mammary tumors, the ER's d). supernatant were further incubated without (•) or with (O) 170 µg of calf thymus DNA for 1 hr at 2°. The incubates were then ultracentrifuged in a 5 to concentration of the ER sites did not vary from Day 1 to Day 18 after castration (Chart 3) contrary to what was observed in the ovarian-dependent rat mammary tumor induced by DMBA (24). The mean value of receptors concentration in the cytosol was 18.5 ± 3.5 fmol/mg of protein (mean of 20 determinations ± S.E.). In the KCl nuclear extract some estradiol-accessible binding sites (3.5 ± 1.0 fmol/mg of cytosol protein) were also observed (Table 1) that displayed a high affinity and limited binding capacity (Chart 1, c and d).

Therefore, in the C3H mouse mammary tumors, the ER's displayed the same physical characteristics as did those observed in estrogen target tissues. We thus investigated the following step in the mechanism of action of estradiol which is currently assumed to be the nuclear translocation of the E₃R complexes.

Attempts to Demonstrate an in Vivo Nuclear Translocation of the ER by DCC Assay. In order to avoid the experimental artifacts observed with the in vitro and cell-free systems such as a lack of hormone dependence for ER nuclear translocation (18), we decided to analyze the nuclear translocation induced in vivo by estradiol. The concentrations of the ER complexes were compared in the cytosol and KCl nuclear extract before and after estradiol injection. In most of the cases, the same mouse was used and the control level of receptor was determined in vitro on a tumor biopsy collected immediately before estradiol injection (see "Materials and Methods"). For comparison, the ER concentration was also determined in the uterus of the same animals. The repartition of ³H-binding sites evaluated by DCC assay was similar in uterus and in mammary tumor before in vivo injection of estradiol since in both tissues 70 to 80% of the soluble sites were found in the cytosol (Chart 4). In contrast, 1 hr following the in vivo injection of estradiol, the repartition of ER sites was modified in utero in favor of the nuclear extract (70% of the total soluble binding) as normally found in an estrogen-responsive target tissue, demonstrating in the uterus the occurrence of an estradiol-dependent nuclear translocation of ER. Conversely, the subcellular localization of ER sites did not appear to be modified by estradiol injection in the mammary tumor, where most of the ³H binding remained in the cytosol. The constancy of the ratio between cytosol and nuclear ³H-binding sites was verified under different conditions of treatment by [³H]estradiol. No marked difference could be shown when the time of injection (0.5 to 6 hr) or the dose of [³H]estradiol injected (0.1 to 4 µg) was increased (Chart 5). In control mice given injections of 2 µg [³H]estradiol plus 1 mg nonradioactive estradiol, both the cytosol and the nuclear binding were depleted when analyzed by DCC or by sucrose gradient, thus suggesting a saturable binding (not shown). It was, however, surprising with the DCC assay to find a constant 10-fold increase of the ³H binding in the [³H]estradiol-treated tumor as compared to the untreated biopsy (Chart 4; Table 1, Section 1). This increase occurred rapidly and could not be due to a de novo protein synthesis. We thus further investigated the nature of the DCC-resistant binding observed in the cytosol after estradiol injection. When the ether-ethanol extract of the DCC-treated cytosol was analyzed by thin-layer chromatography (1-butanol equilibrated with 10% NH₄OH or chloroform-acetone), most of the radioactive ligand was not estradiol but rather polar metabolites that did not migrate in the second system. The characterization of the metabolites that are similar to estrogen sulfates and their biological significance will be described elsewhere. The small proportion of estrogen metabolites also observed in the uterus could be responsible for the remaining binding observed in the uterine cytosol after estradiol in vivo injection.

Evidence for the in Vivo Translocation of the ER Obtained from Hydroxylapatite and Protamine Sulfate Assays. Since the cytosol binding determined by DCC after in vivo injection of [³H]estradiol was resistant to heating, to pronase digestion, and to precipitation on protamine sulfate and hydroxylapatite (Table 1, Section 2) it could not be due to the interaction of estradiol with its receptor. The apparent saturability observed with DCC assay in the in vivo experiments was therefore not a good criterion in favor of ER sites. It could be due, in this case, to the in vivo saturation of the enzymes that conjugate estradiol. We therefore corrected the DCC assay according to temperature and pronase criteria and used the hydroxylapatite and protamine sulfate assays described in “Materials and Methods” to quantify the E₃R complexes (Table 1; Chart 6). The repartition of the E₃R complexes in the cytosol and nuclear extract before (in vitro biopsy) and 1 hr after [³H]estradiol treatment clearly demonstrated an estradiol-dependent receptor translocation in the nucleus (Chart 6). The same

* F. Vignon and H. Rochefort, manuscript in preparation.
Table 1

Subcellular localization of [3H]estradiol "binding" in C3H mammary tumor

A. The [3H]estradiol receptor was evaluated in the tumors of castrated mice that were not treated by estradiol, with the use of the indicated assays following the in vitro incubation of extracts with saturating concentrations of [3H]estradiol.

B. The [3H]estradiol receptor was evaluated in the tumors of castrated mice that had been given in vivo injections of 4 μg [3H]estradiol. The extracts were further incubated in vitro with saturating concentrations of [3H]estradiol as in Chart 4 and assayed for binding complexes as in A.

1. Number of binding sites as determined by DCC assay after correction for in vitro nonsaturable [3H]estradiol binding.

2. In 1 typical experiment, the binding was estimated in the 2 extracts before and after estradiol injection by different techniques, described in "Materials and Methods," in order to assess the specificity of the DCC assay. These results are given without correction for nonsaturable binding.

3. This experiment shows the specificity of the hydroxylapatite and protamine sulfate techniques. Two procedures, pronase digestion (1 mg/ml for 1 hr at 25°) and heating for 1 hr at 60°, have been used to evaluate the nonspecific binding obtained before and after estradiol injection. The specific binding calculated by difference is represented in Chart 6.

<table>
<thead>
<tr>
<th>ER assay</th>
<th>A. In vitro [3H]estradiol binding (fmol/mg cytosol protein)</th>
<th>B. [3H]Estrogen &quot;binding&quot; following [3H]estradiol in vivo (fmol/mg cytosol protein)</th>
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<td></td>
<td>Cytosol</td>
<td>Nuclear extract</td>
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<td>1. DCC</td>
<td>18.5 ± 3.5a (20)b</td>
<td>3.5 ± 1.0 (17)</td>
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<tr>
<td>2. [3H]Estradiol + DCC</td>
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<td>21</td>
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<tr>
<td>[3H]Estradiol + DES + DCC</td>
<td>18</td>
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<td>60° + DCC</td>
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<tr>
<td>Pronase + DCC</td>
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<td>13</td>
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<tr>
<td>Protamine sulfate</td>
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<td>12</td>
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<tr>
<td>3. Hydroxylapatite</td>
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<td>60° + hydroxylapatite</td>
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* Mean ± S.E.

b Numbers in parentheses, number of tumors assayed.

c For the in vivo experiment, the nonsaturable DCC binding was evaluated in control mice receiving [3H]estradiol plus 1 mg nonradioactive estradiol. The mean value that has been calculated from 5 different experiments is represented.

e Estradiol-dependent decrease of cytosol ER sites and increase of nuclear extract ER sites were observed when the ER sites were assayed by the 4 different procedures (Chart 6). The estradiol-dependent increase of the nuclear extract ER sites was similar whether their concentrations were expressed per mg of cytosol protein (Table 1) or per mg of nuclear protein (not shown). These results confirmed the occurrence of the ER nuclear translocation when taking into account any possible differential nuclear losses during the preparation of the salt nuclear extract.

The recovery of the soluble ER sites after in vivo nuclear translocation could be evaluated when their concentrations in the cytosol and the nuclear extract were calculated per mg of cytosol protein. A similar concentration of total soluble ER sites was found before or after [3H]estradiol injection (Table 1, Section 3) which is in agreement with a simple translocation of the receptor to the nucleus without modification of the number of ER sites.

In addition, on the sucrose gradient, while the 8S peak of the cytosol labeled in vitro was depleted, a 4 to 5S peak appeared in the nuclear extract following in vivo labeling (Chart 7). This nuclear peak was shown to be thermolabile since it disappeared after 60° treatment. After estradiol injection, the radioactivity of the cytosol migrated with a broad polydisperse entity (Chart 7) that resisted DCC, temperature, and pronase treatments (not shown). In another control experiment, the nuclear binding peak totally disappeared when an excess (1 mg) of nonradioactive estradiol was injected with 4 μg of [3H]estradiol (not shown).

DISCUSSION

In the C3H mouse mammary tumor, the 2 initial steps of estrogen action, i.e., the binding to the cytosol receptor and the resulting nuclear translocation of the complex, have been investigated in order to find a possible cause or criterion for the estrogen resistance of these tumors. The quality of the cytosol ER's appeared to be normal, since its affinity, sedimentation constant, binding specificity, and DNA-binding ability were not altered. In addition, the cytosol-ER complexes were shown to be translocated to the nucleus 1 hr after estradiol injection. This is in agreement with the in vivo results obtained in the DMBA-induced ovarian-independent rat mammary tumors (2, 17, 23). Conversely, Shyamala (21) has found no in vitro nuclear translocation of the ER in the hormone-independent GR mouse...
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Chart 3. Concentration of the cytosol (Rc) and nuclear estrogen receptor (Rn) sites after ovariectomy. Tumor biopsy or whole tumors were excised from C3H mice at various days after castration (OVX). The cytosol and nuclear ER's were then assayed by DCC after in vitro labeling of the soluble extracts with saturating concentrations of [3H]estradiol.

Chart 4. Intracellular distribution of the charcoal-resistant 3H "binding" before and after [3H]estradiol (E2) injection. A biopsy of the mammary tumor (a) was taken immediately before injection of 2 µg [3H]estradiol, and the remaining tumor was excised 1 hr later (c). Uteri of control mice (b) were compared with those of [3H]estradiol-treated mice (d). The cytosol (cyt.) and nuclear extracts (nuc.) of tumor biopsies or control uteri were incubated in vitro with [3H]estradiol and the ER complexes were assayed by DCC (as described in "Materials and Methods"). The 3H binding in the cytosol and KCl nuclear extracts of estradiol-treated mice was assayed by DCC before in vivo and after [3H]estradiol in vitro resaturation up to the indicated final concentrations of [3H]estradiol (as in "Materials and Methods"). In all experiments the nonspecific binding estimated in vitro with nonradioactive DES was subtracted from the total binding. P, protein.

Chart 5. Intracellular distribution of the charcoal-resistant 3H "binding." a, function of time following the in vivo injection of 1 µg [3H]estradiol (E2). b, function of doses 1 hr after [3H]estradiol treatment. The 3H "binding" was assayed by DCC in the tumor cytosol and nuclear (N) extract, either after in vitro incubation for the control (time 0) or following in vivo injections, as described in Chart 4. The results are expressed in percentage of the total bound radioactivity recovered in cytosol and nuclear extract. Numbers in parentheses, single determinations or mean ± S.E. of the different experiments.

Chart 6. Nuclear translocation of the ER following [3H]estradiol (E2) in vivo injection. The ER's were assayed in the cytosol (cyt.) and KCl nuclear extract (nuc.) of tumor before (a) or after (c) [3H]estradiol in vivo injection (1 hr, 0.25 to 4 µg). The detailed experimental procedures are described in "Materials and Methods." For hydroxylapatite and protamine sulfate precipitations, the nonspecific binding was evaluated after pronase digestion or heating to 60° as in Table 1 and systematically subtracted. The results are expressed in percentage of the concentration of the soluble ER sites (cytosol + nuclear extract) per mg cytosol protein.
ER's in Autonomous Tumors

effect of estradiol on the concentration of the progesterone receptor, which remained low (≤4 fmol/mg protein) before and after estradiol treatment when assayed with [3H]R5020 (unpublished results).

The reason for the inefficiency of the nuclear ER complexes is therefore unknown. The quality of the translocated receptor was not ascertained by our experiments. Since only the KCl-extracted nuclear receptor was evaluated, one cannot exclude a defect concerning the formation of a KCl-resistant nuclear receptor (4). The length of retention of the receptor in the nucleus, which has been considered to be critical to give a full response in the uterus (4) was not determined either. Another possibility to explain the inefficiency of these ER’s would be their relatively low concentration. Although the C3H mammary tumors could be considered as receptor-positive tumor, since they contained more than 10 fmol sites per mg of cytosol protein, their value before castration might be lower as suggested by the few experiments performed before castration (Chart 3). It is therefore possible, as in the case of androgen receptors in rat uterus (7), that the concentration of the E2R complex in the nucleus is too low to transfer efficiently the hormonal message to the specific sites involved in the tumor growth.

Methodologically, these results show also the advantages and the difficulty of the in vivo experimental approach to evaluate a steroid-induced nuclear translocation of the cytosolic receptor. By the in vivo method, artifacts of the in vitro systems can in fact be avoided; however, other difficulties may arise such as possible metabolism of the [3H]-steroid injected. In these studies, estrogen conjugates that were not bound to the receptor completely masked the E2R complexes when the DCC assay was used (23). The nature of the DCC- and temperature- and pronase-resistant binding found in the C3H tumors and its occurrence under other experimental conditions will be described separately. It is unlikely that this conversion of estradiol into polar metabolites would be responsible, however, for the estrogen independence of these tumors, since, even at the low dose of estradiol injected (0.25 µg), ER’s were occupied in vivo by estradiol and subsequently translocated to nuclei as shown with the protamine sulfate and hydroxyapatite receptor assays.

We conclude that in the C3H spontaneous tumors, as well as in the DMBA-induced rat mammary tumors, the estrogen independence for tumor growth cannot be explained by an absence of nuclear translocation of ER. Estrogen independence may be due to low concentration of nuclear ER or to abnormalities beyond the nuclear translocation step. In this respect, the quality of the translocated receptors and their efficiency to interact with specific nuclear sites to trigger the synthesis of specific mRNA merit evaluation (17).

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REFERENCES

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