Dissociation Kinetics of the Nuclear Estrogen Receptor†

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ABSTRACT: A method was developed for measuring the [^{3}H]estradiol dissociation of the nuclear estrogen receptor from calf uterine nuclei that were isolated from tissue slices preincubated with [^{3}H]estradiol. The [^{3}H]estradiol dissociation from the nuclear estrogen receptor was a single, slowly dissociating component at 29 °C, $k = (6.02 \pm 0.26) \times 10^{-3}$ min^{-1}. This nuclear-bound estrogen receptor was similar to the salt-extracted nuclear estrogen receptor, $k = 8.8 \times 10^{-3}$ min^{-1}, and to the second phase of the biphasic [^{3}H]estradiol dissociation process of the cytoplasmic estrogen receptor, $k = 8.68 \times 10^{-3}$ min^{-1}. The second phase of the [^{3}H]estradiol dissociation was previously shown to be the activated form of the cytoplasmic estrogen receptor. The nuclear [^{3}H]estradiol–receptor dissociation rate was reduced by one-half to $2.83 \pm 0.21 \times 10^{-3}$ min^{-1} after the addition of uterine cytosol to the uterine nuclei in place of the buffer, indicating the presence of a factor in the uterine cytosol that interacts with the receptor only when it is chromatin bound or interacts with the chromatin and indirectly influences the chromatin–receptor interaction. The cytosol factor is a macromolecule found in uterus, but not in diaphragm; it is stable at 40 °C but unstable at 100 °C for 10 min and is precipitated by ammonium sulfate at 60–90% saturation. The fraction of the estrogen receptor that bound to isolated nuclei was 5-fold greater (9% vs. 43%) when the uterine cytosol was preincubated at 29 °C than at 0 °C. The dissociation of the [^{3}H]estradiol receptor adsorbed to the isolated nuclei whether preincubated at 0 °C or at 29 °C showed the same, slow [^{3}H]estradiol dissociation kinetics at 29 °C. The presence of 0.01% Triton X-100 or excessively high unlabeled estradiol concentrations (10–100 μM) resulted in anomalous [^{3}H]estradiol dissociation kinetics of the nuclear estrogen receptor, suggesting that high concentrations of steroids can interact with hydrophobic sites on the receptor to produce a nonspecific detergent-like action on the receptor. These data indicate that the nuclear estrogen receptor has estradiol dissociation kinetics similar to those of the activated form of the cytoplasmic estrogen receptor and there is present a protein factor in the uterine cytosol that affects the estrogen receptor–nuclear interaction.

The activation of the cytoplasmic, nonactive form of the estrogen receptor by an estrogen- and temperature-dependent reaction results in the translocation of the receptor to the nucleus (Jensen et al., 1968; Shyamala & Gorski, 1969). We have recently demonstrated that the nonactive and active conformational states of the estrogen receptor are reflected in two affinity states of the receptor. The [^{3}H]estradiol dissociation from the cytoplasmic estrogen receptor occurs as two exponential components. The first or fast component (k_{-1}) results from the [^{3}H]estradiol dissociating from the nonactive state of the receptor. The activated form of the cytoplasmic estrogen receptor produces the second or slower [^{3}H]estradiol dissociating component (k_{-2}). Estradiol binding modulates the receptor activation process by driving the equilibrium between the two forms of the receptor from the nonactive, low-affinity receptor state toward the higher affinity, activated state of the receptor (Weichman & Notides, 1977, 1979, 1980). The kinetics of [^{3}H]estradiol dissociation provides a sensitive indicator of the two states of the receptor. In this paper we investigate the [^{3}H]estradiol dissociation kinetics of the nuclear estrogen receptor, assess its relationship to the activated cytoplasmic estrogen receptor, and observe nuclear estrogen receptor–chromatin interactions previously not detected by other methods.

Experimental Procedures

Materials. The 17β-[2,4,6,7-^{3}H]estradiol (90 and 108 Ci/mmol) and Triton X-100 were purchased from Amersham/Searle. The radiochemical purity of the tritiated estradiol was evaluated by thin-layer chromatography and found to be greater than 95%. Nonradioactive estradiol was obtained from Steraloids. The Ultrapure grade Tris was from Schwarz/Mann. All other reagents were analytical grade.

Incubation of Uterine Tissue and Isolation of Nuclei. Fresh calf uteri in Eagle's minimum essential medium (Gibco) were cut open, and the endometrium was scraped free with a scalpel. Four–five grams of tissue per 10 mL of medium was incubated at 37 °C for 1 h under an atmosphere of O_2–CO_2 (95:5) in the presence of 20 nM [^{3}H]estradiol or 20 nM [^{3}H]estradiol plus 5 μM unlabeled estradiol. The tissue was then washed with 10 volumes of cold medium, followed by a wash with 40 mM Tris, 1 mM dithiothreitol, 25 mM KCl, 3 mM $MgCl_2$, and 0.32 M sucrose, pH 7.5 (TKMS buffer). The tissue was homogenized at 0 °C with a Polytron PT-10 with four 15-s bursts at 50-s intervals at a power setting of 4. Nuclei were isolated by the method of Widnell et al. (1967). Where indicated, the nuclear pellet was washed once with 5 volumes of TKMS buffer containing 0.01% or 0.1% Triton X-100, followed by three washes with TKMS buffer without Triton X-100. The nuclear pellet was then used for measuring the [^{3}H]estradiol dissociation from the nuclear estrogen receptor.

In Vitro Incubation of Nuclei with Activated and Nonactivated Estrogen Receptor. Cytosol of calf uterus or diaphragm was prepared from fresh or frozen tissue (−80 °C) as described in the preceding paper (de Boer & Notides, 1981) by means of either 3–5 volumes of TKMS buffer or 40 mM Tris–1 mM dithiothreitol, pH 7.5 (TD buffer). Cytosol prepared with TD buffer was incubated with 10 nM [^{3}H]estradiol or 10 nM [^{3}H]estradiol plus 1 μM unlabeled estradiol at 0 °C for 60 min. Aliquots were incubated either at 0 °C (nonactivated receptor) or 29 °C (activated receptor) for 30 min. Unbound estradiol was removed by adsorption to 0.3%

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charcoal (Norit A)–0.03% dextran 500 at 0 °C for 5 min. The charcoal was removed by centrifugation for 5 min at 1500*g*. When necessary, the cytosol was adjusted to a final concentration of 0.32 M sucrose by the addition of 2 M sucrose in TD buffer.

The nuclear pellet was obtained as described above (see Incubation of Uterine Tissue and Isolation of Nuclei) with the exception that the uterine tissue was not preincubated with [^{3}H]estradiol. With the aid of a Teflon–glass homogenizer, 3 mL of cytosol containing either the activated or the nonactivated estrogen receptor was used to resuspend the nuclear pellet obtained from one uterine horn. After incubation of the cytosol–nuclear mixture at 0 °C for 1 h, the mixture was centrifuged for 5 min at 800*g* and the pellet washed three times with TKMS buffer. The final nuclear pellet was used for measuring the [^{3}H]estradiol dissociation from the estrogen receptor.

Assay of [^{3}H]Estradiol Dissociation from the Nuclear and Cytoplasmic Estrogen Receptors. The assay of the dissociation of the [^{3}H]estradiol–receptor complex in cytosol at 29 °C, the receptor stability, and the nonspecific binding have been described (Weichman & Notides, 1977; de Boer & Notides, 1981). The nuclear pellet (see Incubation of Uterine Tissue and Isolation of Nuclei, above) containing the [^{3}H]estradiol–receptor complex was suspended in 7 mL of buffer or cytosol (each preincubated with unlabeled estradiol) by use of a few strokes of a Teflon–glass homogenizer. The mixture of nuclear estrogen receptor with buffer or with cytosol was transferred to a 25-mL polycarbonate Erlenmeyer flask. The concentration of the unlabeled estradiol and preparations or additions to the cytosol or buffers are noted in the legends to the figures. The dissociation of [^{3}H]estradiol from the receptor was initiated by transferring the flasks to a 29 °C water bath, while the nuclei were maintained in suspension with a magnetic stirrer. At the times cited, 0.2 mL of the nuclear suspension was added to 2 mL of TKMS buffer at 0 °C, mixed, and centrifuged for 5 min at 5000*g*. The nuclear pellet was washed once with 2 mL of TKMS buffer and then extracted with 2 mL of ethanol. The [^{3}H]estradiol was measured by using 10 mL of Liquiscint (National Diagnostics), with an efficiency of 36%. During the dissociation assay an adhesion of nuclei to the Erlenmeyer flask was observed (adsorption of nuclei to polycarbonate is less than to glass). This loss of nuclei was corrected by measuring the radioactivity present in 0.2-mL aliquots of a [^{3}H]estradiol–receptor nuclear suspension. The [^{3}H]estradiol dissociation data were then corrected for the loss of nuclei, which did not exceed 15% during a 2-h incubation at 29 °C. The details of the analysis of the [^{3}H]estradiol dissociation data have been published (Weichman & Notides, 1977, 1979).

Results

[^{3}H]Estradiol Dissociation from the Nuclear Estrogen Receptor after in Vitro Incubation of Uterine Tissue with [^{3}H]Estradiol. The monophasic kinetics of [^{3}H]estradiol dissociation from the nuclear estrogen receptor, assayed in nuclei at 29 °C, showed slower dissociation ($k = 6.02 \pm 0.26 \times 10^{-3}$ min^{-1}) than the 0.4 M KCl extracted nuclear estrogen receptor ($k = 8.8 \times 10^{-3}$ min^{-1}) and slower dissociation than the activated cytoplasmic estrogen receptor ($k = 8.68 \times 10^{-3}$ min^{-1}) when measured in TKMS buffer containing 0.32 M sucrose (Figure 1). The slower rate of [^{3}H]estradiol dissociation from the activated nuclear estrogen receptor results from nuclear binding and the presence of sucrose and could be mimicked by immobilizing the activated estrogen receptor to hydroxylapatite (de Boer & Notides, 1981). The cytoplasmic estrogen receptor when activated and bound to hydroxylapatite showed a dissociation rate constant of 6.75×10^{-3} min, which decreased further in TKMS buffer to 4.51×10^{-3} min^{-1}.

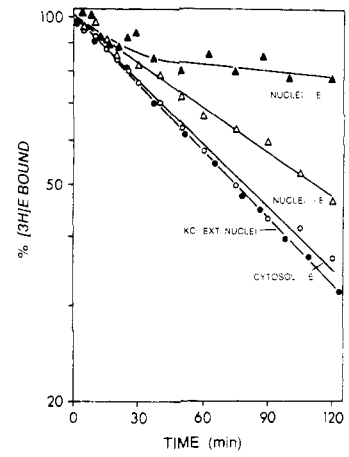

FIGURE 1: Dissociation of [^{3}H]estradiol from the nuclear estrogen receptor. Uterine tissue was incubated with 20 nM [^{3}H]estradiol in the absence or presence of 5 μM unlabeled estradiol at 37 °C for 1 h. The nuclear fractions were isolated and resuspended in TKMS buffer (▲) and in TKMS buffer containing 1 μM unlabeled estradiol (△) or made 0.4 M with respect to KCl at 0 °C for 1 h and the salt-soluble fraction was made 1 μM with respect to estradiol (●). Cytosol in TD buffer was equilibrated with 10 nM [^{3}H]estradiol or with 10 nM [^{3}H]estradiol and 1 μM estradiol at 0 °C for 1 h. After incubation at 29 °C for 30 min and addition of sucrose to 0.32 M, unlabeled estradiol (1 μM) was added and the dissociation assayed at 29 °C (○). The data shown are the specific [^{3}H]estradiol binding, the difference between the values with and without unlabeled estradiol. Receptor concentrations and dissociation rate constants: (●) 0.4 nM, 8.8×10^{-3} min^{-1}; (○) 2.2 nM, 8.68×10^{-3} min^{-1}; (△) 0.6 nM, 6.07×10^{-3} min^{-1}.

The slower dissociation rate of the nuclear [^{3}H]estradiol receptor complex assayed in nuclei, in comparison with the activated estrogen receptor assayed in cytosol, was not a consequence of an insufficient availability of unlabeled estradiol for exchange with the bound nuclear [^{3}H]estradiol–receptor complex, since at estradiol concentrations of 0.15–1 μM (a 200- to 1200-fold excess) the [^{3}H]estradiol dissociation rates were identical. Additions to the [^{3}H]estradiol dissociation assay of 10 and 50 μM estradiol resulted in 39% and 177% increases, respectively, in the nuclear [^{3}H]estradiol dissociation rates; 100 μM unlabeled estradiol produced biphasic dissociation kinetics with rate constants of 0.079 min^{-1} and 5.96×10^{-3} min^{-1} (data not shown). Thus, estradiol at an extremely high concentration that exceeds its solubility and forms micelle produces an anomalous effect on the [^{3}H]estradiol dissociation kinetics of the receptor that was observed when the receptor was in cytosol or bound to hydroxylapatite (de Boer & Notides, 1981).

When uterine nuclei containing the [^{3}H]estradiol–receptor complex were suspended in 25 mL of TKMS buffer at 29 °C, without unlabeled estradiol, an initial decrease in the specific bound [^{3}H]estradiol was induced by dilution and continued until a plateau was reached at approximately 60 min (75–80% of the initial nuclear [^{3}H]estradiol receptor concentration; Figure 1). Equation 1 [see Experimental Procedures in de Boer & Notides (1981)] was used to estimate the new equilibrium reached following the dilution-induced dissociation of the nuclear [^{3}H]estradiol–receptor complex, a process similar to that observed for the estrogen receptor immobilized by hydroxylapatite (de Boer & Notides, 1981). We calculated that 24% of the initial [^{3}H]estradiol would dissociate, which was in good agreement with the observed results.

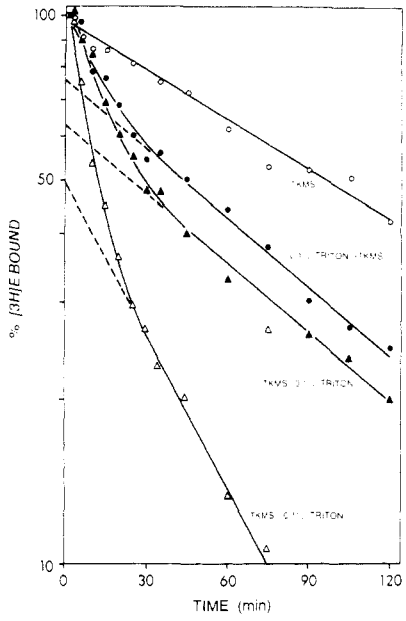

FIGURE 2: Effect of Triton X-100 on the [^{3}H]estradiol dissociation kinetics of the nuclear estrogen receptor. Uterine tissue was incubated with 20 nM [^{3}H]estradiol in the absence or presence of 5 μM unlabeled estradiol at 37 °C for 1 h. The nuclear fractions were isolated in TKMS buffer and resuspended in TKMS buffer (○), TKMS buffer containing 0.01% Triton X-100 (▲), or TKMS buffer containing 0.1% Triton X-100 (△). Nuclei isolated in TKMS buffer containing 0.1% Triton X-100 were washed five times and resuspended in TKMS buffer (●). Estradiol (1 μM) was added to the nuclei suspensions, and the dissociation of [^{3}H]estradiol from the nuclear estrogen receptor was assayed at 29 °C. Receptor concentrations and dissociation rate constants: (○) 0.62 nM, 6.52 × 10^{-3} min^{-1}; (●) 0.84 nM, 0.094 min^{-1} and 9.54 × 10^{-3} min^{-1}; (▲) 0.64 nM, 0.098 min^{-1} and 9.60 × 10^{-3} min^{-1}; (△) 0.59 nM, 0.151 min^{-1} and 20.81 × 10^{-3} min^{-1}.

Effect of Triton X-100 on the [^{3}H]Estradiol Dissociation Kinetics of the Nuclear Estrogen Receptor. The presence of 0.01% Triton X-100 in the TKMS buffer altered the dissociation kinetics, assayed in nuclei, of the nuclear [^{3}H]estradiol–receptor complex from the slow, monophasic (k_{-2} = 6.02 ± 0.26 × 10^{-3} min^{-1}) dissociation of the activated estrogen receptor to the biphasic dissociation process, composed of an initial, fast component with a rate constant of 0.098 min^{-1} and a second, slower component with a rate constant of 9.6 × 10^{-3} min^{-1} (Figure 2). A higher concentration of Triton X-100 (0.1%) increased the [^{3}H]estradiol dissociation rates further, showing rate constants of 0.151 min^{-1} and 20.81 × 10^{-3} min^{-1}, respectively. The magnitude of the fast component was increased from 37% in 0.01% to 50% in 0.1% Triton X-100 (Figure 2). The effect of Triton X-100 upon the nuclear estrogen receptor was not reversed by washing the Triton X-100 isolated nuclei with detergent-free TKMS buffer; the fast component showed a dissociation rate constant of 0.094 min^{-1} with a magnitude equal to 24%; the slow component, k_{-2} = 9.54 × 10^{-3} min^{-1} (Figure 2). Sucrose gradient analysis indicated that incubation of the activated cytoplasmic estrogen receptor in 0.01% Triton X-100 at 29 °C converted the 5S receptor to a 4S sedimenting form; however, a significant loss of [^{3}H]estradiol binding was noted (data not shown).

Dissociation of [^{3}H]Estradiol after in Vitro Binding of the Activated Cytoplasmic Estrogen Receptor to Isolated Nuclei. Seventeen percent of the estrogen receptor in uterine cytosol, preincubated at 0 °C for 30 min, bound to isolated nuclei after incubating the cytosol with nuclei at 0 °C for 60 min, whereas 63% of the estrogen receptor in the uterine cytosol bound to isolated nuclei when the estrogen receptor was activated by preincubating the cytosol at 29 °C for 30 min. After the isolated nuclei were washed with TKMS buffer, only 9% of the estrogen receptor from the nonactivated cytosol remained bound to the nuclei whereas 43% of the estrogen receptor of the 29 °C preincubated cytosol remained bound to the nuclei.

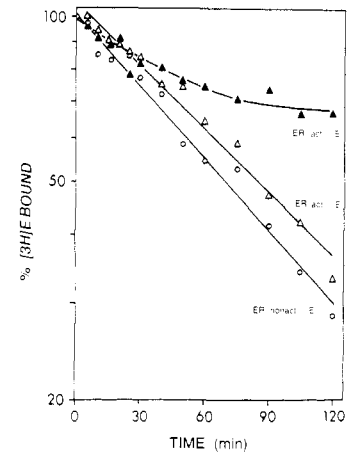

FIGURE 3: Dissociation of [^{3}H]estradiol after an in vitro reconstitution of the cytoplasmic estrogen receptor and isolated nuclei. Cytosol in TD buffer was equilibrated with 10 nM [^{3}H]estradiol in the absence or presence of 1 μM unlabeled estradiol at 0 °C for 1 h. Aliquots were then incubated at 0 °C for 30 min (○) (the nonactivated estrogen receptor) or at 29 °C for 30 min (△, ▲) to obtain the activated estrogen receptor. The free [^{3}H]estradiol was removed by adsorption to charcoal–dextran. After the sucrose concentration was adjusted to 0.32 M, the cytosol receptor preparation was incubated with isolated nuclei at 0 °C for 1 h. The nuclei were washed and resuspended in TKMS buffer (▲) or in TKMS buffer containing 1 μM unlabeled estradiol (△, ○). Receptor concentrations and dissociation rate constants: (○) 0.05 nM, 10.10 × 10^{-3} min^{-1}; (△) 0.25 nM, 8.96 × 10^{-3} min^{-1}.

The [^{3}H]estradiol dissociation kinetics of the nuclear estrogen–receptor complex showed a slow monophasic dissociation with a rate constant of 9.89 ± 0.20 × 10^{-3} min^{-1} for the 0 °C preincubated cytosol and 8.17 ± 0.59 × 10^{-3} min^{-1} for the 29 °C preincubated cytosol (Figure 3). These values were similar to those of the estrogen receptor from the salt-extracted nuclei and of the activated cytoplasmic estrogen receptor, but higher than the rate of [^{3}H]estradiol dissociation from the nuclear estrogen receptor of calf uterine tissue incubated in vitro with [^{3}H]estradiol (Figure 1). In the absence of unlabeled estradiol, the [^{3}H]estradiol dissociation of the activated form of the cytoplasmic estrogen receptor that had been bound to nuclei reached a new equilibrium within 60 to 90 min. The new equilibrium, defined by eq 1 of de Boer & Notides (1981), indicated that the estrogen receptor would dissociate to 64% of the initial [^{3}H]estradiol bound, in good agreement with the 65–70% observed (Figure 3).

Effect of a Uterine Cytosol Factor on the [^{3}H]Estradiol Dissociation Kinetics of the Nuclear Estrogen Receptor. The [^{3}H]estradiol dissociation rate from the nuclear estrogen receptor was reduced by one-half, from 6.02 ± 0.26 × 10^{-3} min^{-1} to 2.83 ± 0.20 × 10^{-3} min^{-1} after the addition of uterine cytosol (9 mg of protein mL^{-1}) to the uterine nuclei (Figure 4). A decreased rate of [^{3}H]estradiol dissociation was also observed when the uterine cytosol was preheated for 60 min at 40 °C or dialyzed overnight at 4 °C against 1000 volumes of TKMS buffer, or when the nuclear–cytosol mixture was made 150 mM with respect to KCl. The decrease of the nuclear [^{3}H]estradiol–receptor complex dissociation rate was produced by the uterine macromolecular fraction that was precipitated by a 60–90% saturation with ammonium sulfate. The 0–30% ammonium sulfate fraction, which contained the estrogen receptor, did not influence the [^{3}H]estradiol dissociation rate from the nuclear estrogen receptor. The dissociation of the

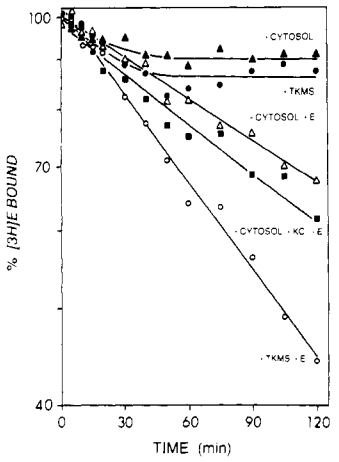

FIGURE 4: Effect of uterine cytosol on the dissociation of [^{3}H]estradiol from the nuclear estrogen receptor. Uterine tissue was incubated with 20 mM [^{3}H]estradiol in the absence or presence of 5 μM unlabeled estradiol at 37 °C for 1 h. The nuclei were isolated and resuspended in TKMS buffer (●); cytosol prepared in TKMS buffer (▲); cytosol prepared in TKMS buffer with 1 μM estradiol (△); cytosol prepared in TKMS buffer with 1 μM estradiol and 150 mM KCl (■); TKMS buffer with 1 μM estradiol (○). Receptor concentrations and dissociation rate constants: (○) 0.5 nM, 7.02×10^{-3} min^{-1}; (■) 0.5 nM, 3.76×10^{-3} min^{-1}; (△) 0.5 nM, 3.26×10^{-3} min^{-1}.

Table I: Effect of Cytosolic Fractions on the [^{3}H]Estradiol Dissociation from the Nuclear Estrogen Receptor[a]

addition to nuclear [^{3}H]estradiol receptor	dissociation rate constant, k_{-2} (10^3 min^{-1})	n
TKMS buffer	6.02 ± 0.25	16
+uterine cytosol	2.83 ± 0.21	10
dialyzed	3.18	1
preheated 1 h at 40 °C	3.61	1
preheated 10 min at 100 °C	7.14	1
+ 8 μM actinomycin D	7.19 ± 1.12	2
0–30% $(NH_4)_2SO_4$ fraction	7.50	1
30–60% $(NH_4)_2SO_4$ fraction	4.60 ± 0.41	2
60–90% $(NH_4)_2SO_4$ fraction	2.90 ± 0.80	2
+ diaphragm cytosol	7.00 ± 0.05	3

[a] Uterine and diaphragm cytosol were prepared with TKMS buffer. The [^{3}H]estradiol receptor nuclear fraction was prepared with TKMS buffer; the additions noted above and unlabeled estradiol (1 μM) were added, and then the dissociation of [^{3}H]estradiol at 29 °C was measured. Dialysis of the cytosol and ammonium sulfate fractions were against TKMS buffer at 0–4 °C for 20 h with a volume 1000-fold greater than the sample. The data obtained were corrected for nonspecific binding, and the dissociation rate constants shown are the mean ± SEM of n (number of determinations).

nuclear [^{3}H]estradiol was not affected by diaphragm cytosol (9 mg of protein mL^{-1}) or by uterine cytosol that was heated for 10 min at 100 °C (Table I). The uterine cytosol diluted with diaphragm cytosol, to maintain a constant protein concentration, showed a concentration-dependent decrease in the rate of the nuclear [^{3}H]estradiol dissociation kinetics (data not shown). Actinomycin D, which has been reported to inhibit "receptor processing" (Horwitz & McGuire, 1978), did not affect the [^{3}H]estradiol dissociation properties of the receptor (Table I). The slower dissociation rate of the nuclear [^{3}H]-estradiol–receptor complex in the presence of cytosol was not due to an insufficient concentration of free estradiol for the exchange reaction, since unlabeled estradiol from 0.5 to 5 μM produced the same rate.

The addition of uterine cytosol to the activated cytoplasmic estrogen receptor that had been bound to hydroxylapatite did not affect the [^{3}H]estradiol dissociation kinetics, whereas the uterine cytosol added to the in vitro reconstituted nuclei containing activated estrogen receptor showed a decrease in the dissociation rate constant ($k = 3.94 \times 10^{-3}$ min^{-1}).

Discussion

The [^{3}H]estradiol dissociation kinetics of the estrogen receptor in isolated calf uterine nuclei showed a single, slow exponential component in comparison with the biphasic [^{3}H]estradiol dissociation kinetics of the cytoplasmic estrogen receptor. The slow [^{3}H]estradiol dissociation of the nuclear estrogen receptor ($t_{1/2}$ = 115 min) is very similar to the dissociation rate of the activated cytoplasmic estrogen receptor ($t_{1/2}$ = 80 min). We previously established that the second, slow component of the biphasic [^{3}H]estradiol dissociation was produced by the activated form of the cytoplasmic estrogen receptor while the first, rapid phase was produced by the nonactivated estrogen receptor (Weichman & Notides, 1977, 1979). In contrast, Sala-Trepat and Reti (1974) noted a biphasic [^{3}H]estradiol dissociation in the cytosol and nuclear fractions. The small difference between the rates of [^{3}H]estradiol dissociation from the nuclear estrogen receptor and from the activated cytoplasmic estrogen receptor was consistent, and may be a consequence of the activated estrogen receptor binding to nuclei. The less than 2-fold change in the dissociation rate is relatively small and may not reflect any significant change in the estradiol-binding mechanism or in receptor affinity. The slightly slower dissociation rate of the nuclear estrogen receptor may reflect an effect on the receptor's conformation, distal to the estrogen-binding site, provoked by nuclear binding or the nuclear estrogen receptor's microenvironment (diffusional limitations, pH, ionic strength, and/or the increased viscosity of the TKMS buffer).

The fraction of estrogen receptor that bound to isolated nuclei was 5-fold greater when the estrogen receptor was preincubated at 29 °C than at 0 °C. The kinetics of the estrogen receptor that had been adsorbed to the isolated nuclei, whether the cytosol was preincubated at 0 °C or at 29 °C, showed the same slow [^{3}H]estradiol dissociation from the receptor ($t_{1/2}$ = 70–80 min at 29 °C). These data are consistent with previous observations that (1) there is always present in the cytosol a small fraction of the estrogen receptor in the activated 5S form, even when maintained at 0 °C (Notides & Nielsen, 1974; Notides et al., 1975), (2) the 5S form of the receptor preferentially binds to nuclei (De Sombre et al., 1975), and (3) the slow [^{3}H]estradiol dissociation rate is characteristic of the activated 5S receptor (Weichman & Notides, 1977).

Although the [^{3}H]estradiol dissociation kinetics of the estrogen receptor is a sensitive indicator of the receptor's conformation, this parameter provides little compelling evidence that the interaction of the activated estrogen receptor with "specific nuclear acceptor sites" results in a change of the receptor's [^{3}H]estradiol dissociation properties. On the basis of differential salt extractability, the nuclear estrogen receptor binding to specific rather than nonspecific acceptor sites has been proposed (Clark & Peck, 1976; Baudendistel & Ruh, 1976); however, these data have been questioned (Juliano & Stancel, 1976; Traish et al., 1977). The single, slowly dissociating [^{3}H]estradiol component from the calf uterine nuclei is consistent with a single, high-affinity binding site, which is in contrast to reports based upon nuclear [^{3}H]estradiol exchange (Markaverich & Clark, 1979) of two binding sites in the rat uterine nucleus. Recently, high concentrations of actinomycin D have been shown to arrest "receptor processing" or the replenishment of cytoplasmic receptors from the nucleus (Horwitz & McGuire, 1978). Our data indicate that the action of actinomycin D does not perturb

the [^{3}H]estradiol dissociation kinetics of the nuclear estrogen receptor, thereby emphasizing the lack of a direct effect on the receptor's conformation.

The rate of [^{3}H]estradiol dissociation from the nuclear estrogen receptor was reduced by one-half when the uterine nuclei were suspended in uterine cytosol instead of TKMS buffer during the dissociation assay. These data indicate that a factor is present in the uterine cytosol which (1) interacts with the receptor directly, but only when the receptor is associated with the nucleus (presumably the chromatin), or (2) interacts with the chromatin and indirectly influences the receptor–chromatin interactions as indicated by the change in the receptor's [^{3}H]estradiol dissociation kinetics. The cytosol factor is a macromolecule, presumably a protein, that has molecular properties discretely different from those of the estrogen receptor; it is precipitated by ammonium sulfate at 60–90% saturation and is stable at 40 °C for 1 h in the absence of estradiol. The reduction in the rate of the [^{3}H]estradiol dissociation induced by the uterine factor occurs in the presence of cytosol containing 0.15 M KCl, which suggests that the action of the cytosol factor is not a nonspecific protein–protein interaction produced by the low ionic strength of the cytosol or buffer.

The reduction of the [^{3}H]estradiol dissociation by the uterine factor does not necessarily indicate that the function of this factor is to specifically change the estradiol-binding properties of the receptor. Rather, it indicates that the receptor interacts with a specific protein, and it is this interaction that is detected by the change in the receptor's [^{3}H]estradiol dissociation kinetics, a sensitive indicator of the receptor's conformation. These data indicate the complexity of the estrogen receptor interaction with the nucleus and the possibilities for other non-estrogen-binding proteins to be involved in estrogen receptor action in the nucleus.

A similar conclusion can be reached from the observation that a non-androgen-binding protein factor from the prostate inhibits binding of the androgen receptor to chromatin (Shyr & Liao, 1978).

Triton X-100 containing buffers have been used for the isolation of uterine nuclei free from cytoplasmic contamination so that an accurate estimation of the nuclear estrogen receptor content can be made (Edwards et al., 1980). We observed that nuclei isolated with TKMS buffer containing 0.1% Triton X-100 compared with TKMS buffer alone at 0–4 °C showed less cytoplasmic contamination and a slightly, but not significantly, decreased nuclear estrogen receptor content. However, the estrogen receptor is detrimentally affected by contact with the Triton X-100, as indicated by the anomalous behavior of the [^{3}H]estradiol dissociation rate at 29 °C. The Triton X-100 may be tightly bound by the receptor since repeated washings with TKMS buffer did not restore the normal [^{3}H]estradiol dissociation kinetics of the activated receptor. Characterization of the molecular, ligand-binding, or affinity properties of the nuclear estrogen receptor isolated from tissue with Triton X-100 containing buffers may lead to erroneous conclusions, particularly when the receptor is exposed to temperatures higher than 4 °C or to other biochemical perturbations, such as high salt concentrations or ion-exchange chromatography.

The anomalous increase in the [^{3}H]estradiol dissociation rate of the receptor produced by Triton X-100 or a high concentration of unlabeled estradiol suggests that the receptor may contain a hydrophobic domain with which these lipophilic substances nonspecifically interact (Clarke, 1975). Interestingly, some investigators of the glucocorticoid receptor (Suthers et al., 1976; Svec et al., 1980) have noted that high concentrations of progesterone in excess of 10 μM increased the rate of glucocorticoid dissociation. It remains to be established whether this action reflects a true allosteric site of pharmacological or physiological significance or a nonspecific action.

The present study shows that the nuclear estrogen receptor in calf uterine tissue shows estradiol dissociation kinetics essentially identical with the activated form of the cytoplasmic estrogen receptor. A protein factor in uterine cytosol that affects the estrogen receptor–nuclear chromatin interaction has been described and warrants further investigation.

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