Two High-Affinity Ligand Binding States of Uterine Estrogen Receptor Distinguished by Modulation of Hydrophobic Environment

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ABSTRACT: The steroid binding function of soluble (cytosolic) estrogen receptors from calf uteri was evaluated under conditions known to modify the extent of hydrophobic interaction with receptor-associated proteins. Receptor preparations were equilibrated into 6 M urea (±0.4 M KCl) buffers and control buffers (±0.4 M KCl) by chromatography through small columns of Sephadex G-25 or by dialysis at 0–6 °C. Equilibrium dissociation constants (K_d) and binding capacities (n) of experimental and control receptor preparations were determined by 13-point Scatchard analyses using concentrations of 17β-[^{3}H]estradiol from 0.05 to 10 nM. Nonspecific binding was determined at each concentration by parallel incubations with a 200-fold molar excess of the receptor-specific competitor diethylstilbestrol. The control receptor population was consistently found to be a single class of binding sites with a high affinity for estradiol (K_d = 0.36 ± 0.09 nM, n = 14) which was unaffected by G-25 chromatography, by dialysis, by dilution, or by the presence of 0.4 M KCl. However, equilibration into 6 M urea induced a discrete (10-fold) reduction in receptor affinity (K_d = 3.45 ± 0.86 nM, n = 6) to reveal a second, thermodynamically stable, high-affinity binding state. The presence of 0.4 M KCl did not significantly influence the discrete change in receptor affinity induced by urea. However, KCl did help prevent the reduction in binding capacity induced by urea. The effects of urea on both receptor affinity and binding capacity were reversible, suggesting a lack of covalent modification. These results demonstrate nonenzymatic means by which not only the binding capacity but also the affinity of receptor for estradiol can be reversibly controlled, suggesting that high concentrations of urea might be more effectively utilized during the physicochemical characterization and purification of steroid receptor proteins.

That portion of the estrogen receptor which binds steroid has been characterized under structure-dissociating or denaturing conditions as a polypeptide of approximately 55–70 kilodaltons [e.g., see Erdos & Fries (1974), Sica et al. (1976), Auricchio et al. (1978), Redeuilh et al., (1981), Eckert & Katzenellenbogen (1982), Monsma et al. (1984), Sakai & Gorski (1984), and Green et al. (1986)]. In contrast, there is still no consensus on the native structure or subunit organization of the estrogen receptor (Greene & Press, 1986) or any other steroid receptor protein(s). Several possible quaternary receptor structures have been discussed [e.g., see Grody et al. (1982), Sherman & Stevens (1984), and Joab et al. (1984)]. These models emphasize the possibility of both homologous and heterologous component or subunit compositions and are experimentally based, to a large extent, on observed variations in size of the steroid–receptor complex associated with altered ionic strengths. Relative to the extent to which electrostatic interactions have been manipulated to characterize and purify steroid hormone receptors, hydrophobic interactions have been virtually ignored. This is despite some direct (Notides & Nielson, 1974; Kamur et al., 1978; Pavlik & Rutledge, 1980; Sica et al., 1980; Sato et al., 1981; de Boer & Notides, 1981; Eckert & Katzenellenbogen, 1982; Dunaway et al., 1985; Bell et al., 1986) but, more often, anecdotal evidence suggesting that hydrophobic interactions may be significant in vitro and, therefore, important for an accurate description of native receptor structure and steroid-induced changes in subcellular interactions or function.

Our own investigations of steroid receptor structure have been directed toward understanding the influence of steroid binding on alterations in native receptor structure associated with the transition to a form with increased affinity for nuclear components (Milgrom, 1981; Dahmer et al., 1984). Very little is known regarding the subunit structure or possible function of steroid receptor proteins prior to stimulation with ligand. During attempts to develop improved methodologies for the physicochemical characterization of unliganded receptor or aporeceptor (Hutchens et al., 1985a,b; Dunaway et al., 1985), we observed an increased recovery of steroid binding activity when unliganded receptor preparations were analyzed in the presence of 6 M urea additionally containing 0.4 M KCl (Dunaway et al. 1985). These results revealed the need to better evaluate physical means by which receptor binding capacity for steroid might be controlled. We selected calf uterus as a well-characterized model system (Notides, 1978; Sica et al., 1976, 1980: Hutchens et al., 1985a,b; Dunaway et al., 1985; Auricchio et al., 1986) to closely examine the influence of high urea concentrations on receptor binding capacity and affinity for 17β-estradiol. We report that modulation of the receptor's hydrophobic environment reversibly alters the receptor between two discrete high-affinity estrogen binding states in vitro. The possible relationship between these two forms of receptor and similar high-affinity forms described by others (Taylor & Smith, 1982; Raymoure et al., 1985) is discussed in terms of receptor structure, purification, and the regulation of steroid binding status.

Experimental Procedures

Materials. Uteri from small calves were obtained from a local slaughterhouse. The uterine horns (10–20 g) were rinsed

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in ice-cold saline immediately after removal, cut into 1–2-g pieces, frozen in liquid nitrogen, and stored frozen at −85 °C. 17β-[^{3}H]Estradiol (90–110 Ci/mmol) was purchased from New England Nuclear. Dextran (T-70) and prepacked Sephadex G-25 (PD-10) columns (6 cm × 1.5 cm ID) were obtained from Pharmacia. Spectra/Por 6 (1000-dalton molecular exclusion limit) dialysis membrane tubing, charcoal (alkaline Norit A), and disodium ethylenediaminetetraacetic acid (Na_2-EDTA) were from Fisher Scientific. Diethylstilbestrol (DES), ovalbumin, glycerol, and potassium chloride were from Sigma. Trizma base and dithiothreitol (DTT) were from Research Organics Inc. Ultrapure urea (enzyme grade) was purchased from Bethesda Research Laboratories. Protein assay dye reagent was from Bio-Rad and used with ovalbumin as standard. All chemicals were of reagent grade or better and used without further purification.

Buffers. All cytosols were prepared by using 10 mM tris(hydroxymethyl)aminomethane hydrochloride (Tris-HCl) (pH 7.4 at 0 °C) containing 1 mM dithiothreitol, 1.5 mM Na_2-EDTA, and 20% (w/v) glycerol.

The effects of 6 M urea and/or 0.4 M KCl on receptor interaction with estradiol were evaluated by Scatchard analyses using the following buffers: control buffer, 25 mM Tris-HCl (pH 7.4 at 0 °C), 1 mM dithiothreitol, 1.5 mM Na_2-EDTA, and 10% (w/v) glycerol; KCl buffer, control buffer containing 0.4 M KCl; urea buffer, control buffer containing 6 M urea; urea–KCl buffer, control buffer containing both 6 M urea and 0.4 M KCl.

All buffers containing urea were freshly prepared (at 0–6 °C) for each experiment by using precautions known to prevent cyanate formation (Stark et al., 1960).

Preparation of Cytosol in Various Buffers for Scatchard Analyses. Cytosol was prepared by homogenization and ultracentrifugation exactly as described previously (Hutchens et al., 1985a). To affect changes in cytosol buffer composition, freshly prepared cytosol (2–10 mL) was either chromatographed on a prepacked G-25 Sephadex column (PD-10) or dialyzed using Spectra/Por 6 membranes (four changes over 6–24 h; 250 mL each change). Cytosol aliquots were further diluted as indicated into appropriate buffers for Scatchard analyses. The cytosol protein concentrations before and after buffer exchange were determined by the method of Bradford (1976) using the dye reagent supplied by Bio-Rad.

Scatchard Analyses. Cytosols were previously equilibrated and diluted with appropriate buffer before aliquots (200 μL) were incubated (0 °C) in triplicate or duplicate with 13 concentrations of 17β-[^{3}H]estradiol ranging from 0.05 to 10 nM. Parallel incubations were performed in the presence of a 200-fold molar excess of radioinert diethylstilbestrol to determine nonspecific binding. The protein-bound and free steroid fractions were separated after 2–18 h (see text) of incubation by addition of 0.4 mL of dextran-coated charcoal (DCC). After 15 min with DCC (intermittent shaking), the suspensions were centrifuged at 10000*g* for 10 min. Radioactivity in 0.4 mL of the supernatant fractions was determined by using a Beckman Model LS-250 liquid scintillation spectrophotometer at 33–38% counting efficiency. Control experiments measuring adsorption of steroid by DCC in the presence of urea and/or KCl buffers indicated little or no changes in DCC efficiency. Receptor-bound 17β-[^{3}H]estradiol (total binding less nonspecific binding) was calculated relative to free hormone (total added hormone less specifically bound hormone) at equilibrium for each concentration and analyzed according to Scatchard (1949).

Results

These results represent an extension of our original investigations aimed toward a better understanding of the mechanism by which steroid binding influences the degree of hydrophobic interaction(s) between other steroid binding subunits and/or non-steroid-binding, receptor-associated proteins. Recently, we reported evidence for steroid-induced alterations in receptor structure (size) which were amplified in buffers containing 6 M urea (Dunaway et al., 1985). Fractions of unliganded receptor analyzed by high-performance size-exclusion chromatography and postlabeled in buffers containing 6 M urea and 0.4 M KCl showed higher binding capacities than receptor analyzed in parallel using 6 M urea alone. Because of the importance of understanding receptor structure/function relationships and due to the effectiveness of including 6 M urea during receptor purification schemes, we have investigated the effects of 6 M urea (±0.4 M KCl) on both the binding capacity and affinity of the estrogen receptor for 17β-[^{3}H]estradiol.

It was important in these studies to accurately and unequivocally establish both the affinity (K_d) and binding capacity (n) of control (no urea) receptor preparations from several different calf uteri using more than one experimental approach to buffer exchange. Equal aliquots of receptor samples were equilibrated into control buffer, KCl buffer, urea buffer, and urea–KCl buffer by two means: (1) rapid chromatography through PD-10 columns of Sephadex G-25; (2) dialysis. After further dilution (1:5 to 1:20) with appropriate (homologous) buffers, the affinity (K_d) and specific binding capacity (n) of each receptor preparation were determined by 13-point Scatchard analyses. In agreement with previously published results [e.g., see Giannopoulos & Gorski (1971) and Sica et al. (1976)], we found calf uteri to contain a single class of high-affinity receptors when evaluated in hypotonic or hypertonic buffer. Figure 1 illustrates the Scatchard profiles obtained by using control and KCl buffers. There was consistently no evidence for the presence of a second class of lower affinity binding sites. There was no significant change in receptor affinity (undiluted control, $K_d = 0.39 \pm 0.08$ nM, $n = 4$) induced by G-25 chromatography ($K_d = 0.33 \pm 0.11$ nM, $n = 10$) or dialysis ($K_d = 0.42 \pm 0.06$ nM, $n = 4$) with control buffers. In separate experiments, a 10-fold dilution of cytosol with control buffer (or KCl buffer) resulted in no change of affinity (K_d). The receptor content varied (approximately 80–500 fmol/mg of cytosol protein) with different uteri; however, the estrogen binding capacity of receptors in a given preparation was not influenced by either method of buffer equilibration. In addition, Scatchard results obtained with 10 mM Tris-HCl or 50 mM potassium phosphate as control buffers (each containing 1 mM DTT, 1.5 mM Na_2-EDTA, and 20% glycerol at pH 7.4) were the same as those described by using the 25 mM Tris-HCl control buffer as shown in Figure 1A. Final receptor concentrations were at levels below those which typically result in curvilinear Scatchard plots (Notides et al., 1981).

In contrast to results obtained at low and high ionic strengths, by modification of the hydrophobic milieu with urea, a discrete (10-fold) reduction in the affinity of receptor for estradiol is induced. Figure 2A is typical of Scatchard results obtained by using buffers containing 6 M urea, and Figure 2B demonstrates that the decreased affinity of receptor for estradiol in urea buffer is not significantly influenced by the presence of 0.4 M KCl. Table I summarizes our collective findings and indicates the reproducibility and magnitude of the results. It was interesting to find, however, that the specific

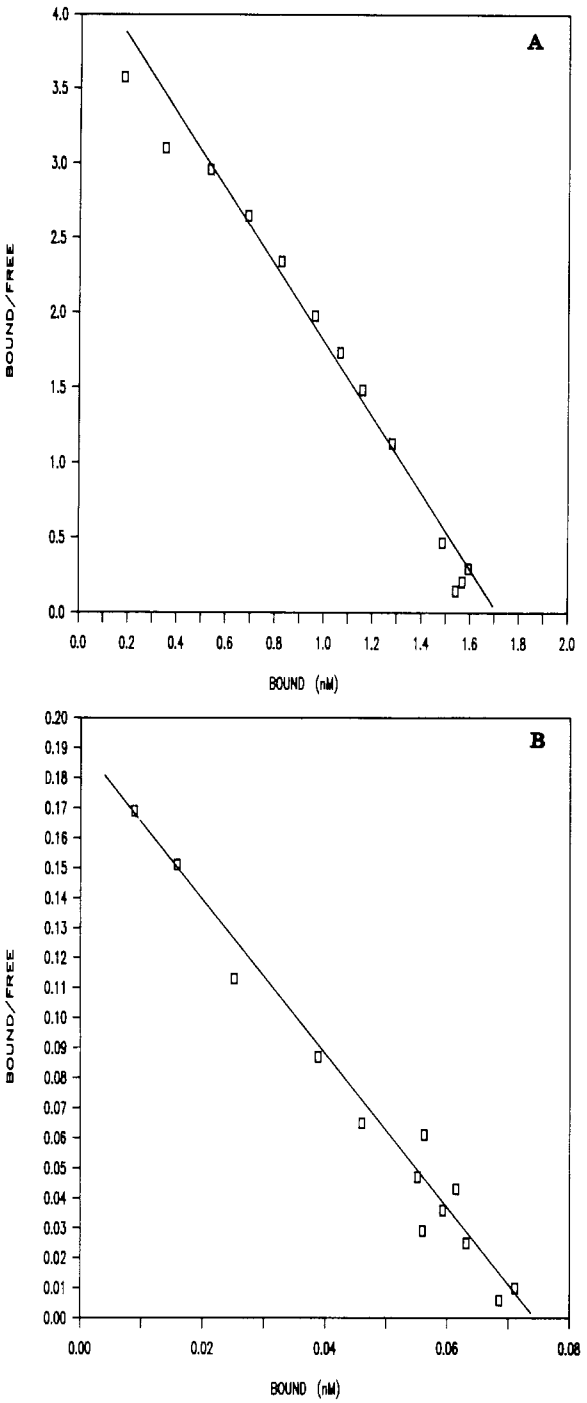

FIGURE 1: Scatchard analysis of receptor equilibrated into control buffer (A) or KCl buffer (B) as described under Experimental Procedures. A single class of high-affinity binding sites is typically observed in both buffer systems. Separate experiments are shown. The equilibrium dissociation constant (K_d) was 0.39 nM (r = 0.98), and the binding capacity (n) was 570 fmol/mg of protein for the data shown in the absence of KCl (A). In the presence of KCl (B), the results shown indicate a K_d of 0.40 nM (r = 0.97) and a binding capacity of 280 fmol/mg of protein.

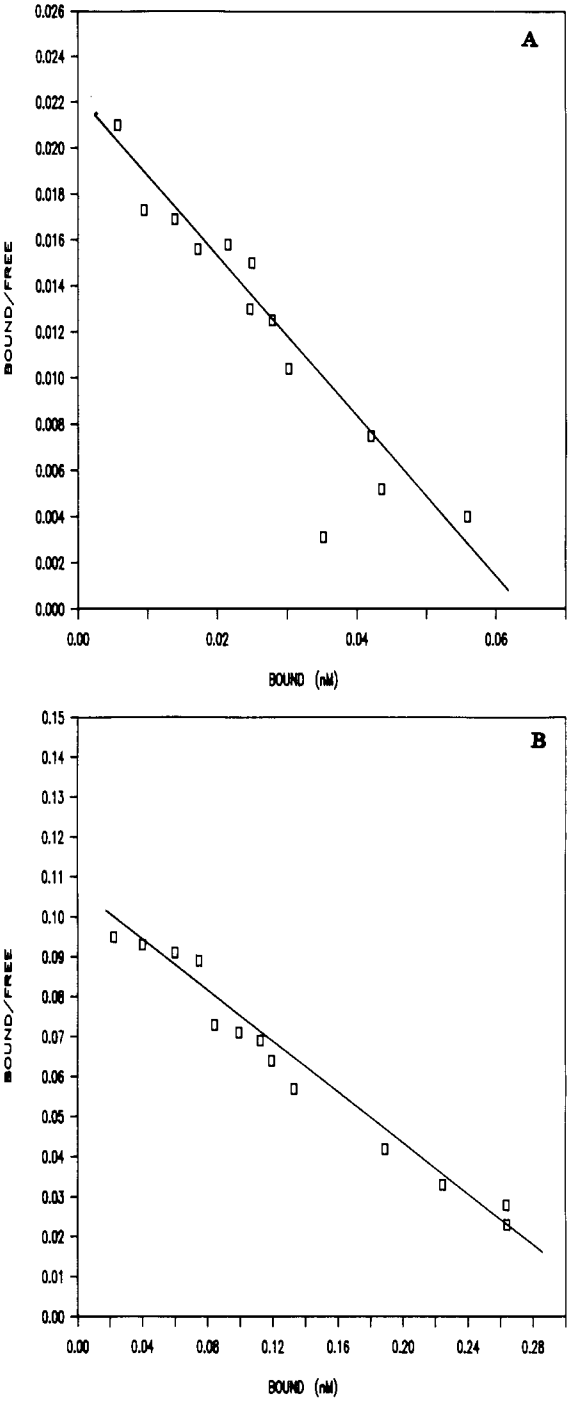

FIGURE 2: Scatchard analysis of receptor equilibrated into urea buffer (A) or urea–KCl buffer (B) as described under Experimental Procedures. A single class of high-affinity binding sites is typically observed in both urea buffer systems. Separate experiments are shown. The equilibrium dissociation constant (K_d) was 2.7 nM (r = 0.90), and the binding capacity was 26 fmol/mg of protein for the data shown in urea buffer alone (A). In urea–KCl buffer (B), the results shown indicate a K_d of 3.6 nM (r = 0.91) and a binding capacity of 300 fmol/mg of protein.

binding capacity of receptor in urea–KCl buffers was significantly increased relative to that observed in urea buffers alone. By Scatchard analyses, the binding capacities (n) of receptor preparations equilibrated into urea–KCl buffer were 60–80% (n = 6) of control values while receptor equilibrated into urea buffer alone typically revealed binding capacities which were 15–40% (n = 5) of control.

The influence of 6 M urea on the steroid binding site (K_d) was limited but nonetheless significant. Therefore, competitive binding analyses were performed in urea buffer to determine whether the absolute steroid specificity or the relative binding affinity for other estrogenic steroids was also altered. Complete inhibition (100%) of 17β-[^{3}H]estradiol binding is typically observed in the presence of either a 100-fold or a 200-fold molar excess of DES. These same concentrations of DES were also found to be as effective (100%) in urea buffer. Furthermore, progestin, androgen, and glucocorticoid steroids remained entirely ineffective as competitors when present at

Table I: High-Affinity Estrogen Binding Receptor Forms Distinguished by Modulation of Hydrophobic Environment

cytosol buffer[a]	affinity (K_d)[b] (nM)	cytosol buffer[a]	affinity (K_d)[b] (nM)
control (n = 14)	0.36 ± 0.09	urea (n = 4)	2.25 ± 0.83
KCl (n = 3)	0.44 ± 0.10	urea–KCl (n = 6)	3.45 ± 0.86

[a] Exact buffer compositions defined under Experimental Procedures. Buffer exchange accomplished by G-25 chromatography as described under Experimental Procedures. [b] Determined by linear regression of data analyzed according to Scatchard as described under Experimental Procedures. Correlation coefficients (r) ranged from r = 0.92 to r = 0.99.

a 200-fold molar excess. The partial inhibition of [^{3}H]estradiol binding observed in the presence of a 200-fold molar excess of estrone (80% inhibition) or estriol (87–88% inhibition) was also unchanged in the presence of urea.

To evaluate the possibility that urea was modifying receptor susceptibility to enzymatic modification, we investigated the reversibility of urea-induced changes in both the affinity (K_d) and binding capacity (n) of receptor for estradiol. Scatchard analyses were performed on samples dialyzed against starting (control) buffer as well as parallel samples which were dialyzed into urea buffer (equilibrated 6.5–24 h) and then redialyzed back into control buffer. The effects of urea on receptor affinity were completely reversible. Yet, urea effects on binding capacity were only partially (up to 80%) reversible. Table II shows representative data for three individual experiments where the time of equilibration in 6 M urea buffer varied from 6.5 to 24 h. Experiments 1 and 2 were performed with 50 mM phosphate and 25 mM Tris-HCl control buffers, respectively. The finding that urea-induced changes in receptor affinity (K_d) for estradiol were completely reversible in both phosphate and Tris buffers argues that direct carbamylation is not an issue. While Tris (primary amine) can act as a carbamylation trap, phosphate cannot.

In both the presence and absence of urea (±0.4 M KCl), we have not observed any evidence for the simultaneous presence of a second class of reduced-affinity binding sites regardless of experimental design. The urea-induced reduction in receptor affinity for estradiol is complete within 3–4 h at 0–6 °C with no further significant change for at least 24 h (see Table II). However, the exact time dependence for the reversible shift in receptor equilibrium between the two high-affinity binding states has not yet been evaluated.

Discussion

The results presented here demonstrate that the mammalian estrogen receptor may reversibly exist in two functionally distinct, thermodynamically favored states, each with a significantly different (7–10 X) but nonetheless high affinity for estradiol. It is revealing that the consistent reduction in receptor affinity induced by the presence of 6 M urea was discrete and of limited magnitude regardless of incubation time. We believe these results would be less significant if the urea-induced decrease in affinity had been more pronounced (i.e., 100- or 1000-fold) or more variable and not completely reversible. The two high-affinity receptor forms demonstrated here (K_d = 0.33 nM and K_d = 2.3–3.4 nM) are remarkably similar in affinity to those reported for the designated X (K_d = 0.1 nM) and Y (K_d = 1 nM) components of the estrogen receptor from chick oviduct (Taylor & Smith, 1982). It is tempting to speculate upon a structural basis for the parallel affinities between these two sets of receptor forms. However, Giannopoulos and Gorski (1971) reported no apparent difference in K_d for the 4S, 5S, 6S, and 8S forms of estrogen receptor from rat uteri.

There are no previous studies which examine the influence of urea on the equilibrium binding constant (K_d) and binding capacity (n) of estrogen receptor for estradiol. There are, however, several reports of using chaotropic salts to perturb the dissociation rate of estrogen from receptor. Kumar et al. (1978) found increased estrogen dissociation rates in the presence of various chaotropic anions. Weichman and Notides (1979) used calf uterine estrogen receptor to demonstrate an increase in the estradiol dissociation rate with increasing concentrations of NaSCN (up to 0.4 M). The reversibility of these phenomena was not evaluated. Sica et al. (1980) reported that calf uterine estrogen receptor showed an increase in both the steroid association rate (k_1) and dissociation rate (k_{-1}) in the presence of 0.5 M NaSCN. Interestingly, Sato et al. (1981) reported the formation of a second high-affinity estrogen binder by exposure of rat liver cytosol to 0.4 M NaSCN. Receptor forms displaying different estrogen dissociation rates have been referred to as high- and low-affinity forms in other studies using different experimental conditions without confirming whether the corresponding association rate constants have changed. It is an overassumption to state that a change in only one rate constant (kinetic parameter) affects the equilibrium (thermodynamic) binding constant since the latter is also influenced by association rates which are more difficult to accurately assess.

Because certain electrostatic interactions have come to be definitive during receptor characterization in vitro and are proposed to be important to receptor structure and possibly function in vivo, we felt that reagents used to probe the relative importance of hydrophobic interactions should be uncharged. Therefore, chaotropic ions of the Hofmeister series (Hofmeister, 1888) were not included in these studies because they are known to influence protein structure and intermolecular associations by disrupting not only hydrophobic interactions but also hydrogen bonding and, more importantly, electrostatic interactions. Reports of reversible receptor denaturation have

Table II: Reversibility of Urea-Induced Reduction in Receptor Affinity (K_d) and Binding Capacity (n) for Estradiol

expt	condition[a]	time in 6 M urea (h)	affinity (K_d) (nM)	correlation coefficient (r) of Scatchard plot	binding capacity (fmol/mg of protein)	% recovery
1	before urea		0.41	0.98	17.1	
	after removal of urea	24	0.40	0.99	5.1	30
2	before urea		0.46	0.98	14.7	
	after removal of urea	24	0.51	0.98	5.3	36
3	before urea		0.42	0.96	16.1	
	after removal of urea	6.5	0.37	0.99	12.5	78

[a] Buffer exchange accomplished by dialysis as described under Experimental Procedures. To monitor reversibility of the urea effects, receptor samples were dialyzed into urea buffer and maintained in urea buffer for the times indicated before being dialyzed back into control buffer (18–20 h). Control samples (i.e., before urea) were dialyzed in control buffer for the duration of the experiments (up to 44 h). Schatchard analyses were performed on control and experimental samples simultaneously.

been quite limited, and these did not include investigation of urea as a denaturant. Erdos and Fries (1974) used 6 M guanidine hydrochloride to denature estrogen receptor prepared from calf uteri and reported the ability to renature 20–40% of the receptor's steroid binding activity. Although no data are presented, no change in receptor affinity was observed upon renaturation. A decade later, Sakai and Gorski (1984) reported the reversible denaturation of estrogen receptor from rat uterus using guanidine hydrochloride as well as sodium dodecyl sulfate; however, recoveries of specific estradiol binding capacity were quite variable and ranged from 15% to over 100% depending upon the specific experimental conditions. No change in receptor affinity (K_d) for estradiol was reported after renaturation.

The use of 6 M urea in these studies (at 0–6 °C in the presence of glycerol) does not necessarily imply receptor denaturation. The reversible loss of binding activity may be due to denaturation; however, it is certainly not complete, and other possibilities seem worthy of consideration. These include dissociation of receptor-associated components [e.g., see Hutchens et al. (1982, 1984, 1985a,b) and Joab et al. (1984)] or perturbation of a hydrophobic surface binding site modulated by an as yet unidentified interaction with other subcellular structures or effector molecules (i.e., nuclear matrix, phospholipid, nucleotides, or nucleic acids). Auricchio et al. (1981), Fleming et al. (1983), and Raymoure et al. (1985) have reported an increased steroid binding capacity (augmentation) of estrogen receptors incubated with nucleotides. In these reports, both calcium-dependent and calcium-independent phosphorylation events are proposed. In other reports, it has been proposed that the steroid binding properties of glucocorticoid receptors are influenced by an ADP-mediated protection mechanism (Barnett et al., 1983) and by stabilizing of phosphate (molybdate binding) groups vicinal to essential sulfhydryl groups (Dahmer et al., 1984). However, our studies suggest that stabilization of a hydrophobic interaction site on steroid receptors might be involved in the maintenance of the estrogen receptor in its highest affinity steroid binding state. Of particular interest is our observation that the presence of 0.4 M KCl affords the receptor considerable protection from loss of binding capacity in 6 M urea buffer. The precise mechanisms by which as yet unspecified hydrophobic interactions, nucleotides, sulfhydryl reactions, etc. influence the steroid binding properties of one or more receptor forms remain unknown and may require the identification and purification of the putative effector components to ascertain with any degree of certainty. We have, in fact, observed changes in the apparent structure of the calf uterine estrogen receptor during analyses by high-performance size-exclusion chromatography and chromatofocusing in 6 M urea buffer. The effects of urea on receptor size and surface charge are varied depending upon ionic strength (manuscript in preparation). Notides and Nielsen (1974) have previously demonstrated that 3 M urea differentially affects both the sedimentation coefficient and Stokes radius of calf uterine estrogen receptor in an ionic strength dependent manner. We have also recently obtained evidence suggesting that access to the receptor DNA binding site is dramatically influenced upon exposure to 6 M urea (Hutchens et al., 1986). A more complete understanding of the relative influence of hydrophobic and electrostatic interactions toward the maintenance of both native and transformed receptor structure is clearly warranted but will require extensive investigations.

The efficacy of urea use during protein characterization and purification is well documented. Continued use of urea as a probe of native receptor structure/function relationships offers an important experimental advantage in that it is compatible with the continued utilization of ion-exchange techniques, both electrophoretic and chromatographic focusing procedures, and ionic strength dependent affinity chromatography. The calf uterine estrogen receptor appears to be a good model system for further studies because, unlike the rat uterine (Clark et al., 1978) or chick oviduct (Taylor & Smith, 1982) model systems, results are not complicated by changing relative ratios of more than one estrogen binding species.

Registry No. Estradiol, 50-28-2; urea, 57-13-6.

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Regulation of 3-Hydroxy-3-methylglutaryl Coenzyme A Reductase mRNA Levels by L-Triiodothyronine†

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ABSTRACT: In hypophysectomized rats, hepatic 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase activity, immunoreactive 97-kilodalton (97-kDa) protein, and mRNA were all reduced to undetectable levels. Administration of triiodothyronine (T_3) resulted in large increases in all three after a 36-h lag period. HMG-CoA reductase activity, immunoreactive 97-kDa protein levels, and reductase mRNA levels were tightly correlated. Feeding hypophysectomized rats diets containing the bile acid sequesterant colestipol, together with the potent reductase inhibitor mevinolin, resulted in an increase in HMG-CoA reductase activity similar to that seen with T_3 but a lesser stimulation of reductase mRNA levels. These results suggest that agents which cause depletion of mevalonate-derived products may share in part with T_3 a common mechanism for increasing levels of HMG-CoA reductase activity in order to satisfy cellular needs for these products. Dexamethasone treatment, which is known to prevent the T_3-mediated stimulation of reductase activity, caused a marked decrease in 97-kDa immunoreactive material but had little effect on reductase mRNA levels.

Hepatic 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA)[1] reductase, the enzyme which catalyzes the rate-limiting reaction of cholesterol biosynthesis, plays a key role in maintaining cholesterol homeostasis (Spady et al., 1985). Both rapid and large changes in reductase activity occur in response to body needs for cholesterol, its products, and other mevalonate-derived metabolites. These changes are effected by both dietary and hormonal signals. Perhaps the best studied regulatory process is the LDL-mediated feedback system. It has been shown that the decrease in reductase activity caused by LDL is due to suppressed transcription of the reductase gene and accelerated degradation of the reductase protein (Faust et al., 1982; Edwards et al., 1983; Luskey et al., 1983; Gil et al., 1985; Chin et al., 1985).

In contrast with regulation by LDL, relatively little is known concerning the mechanisms by which various hormones regulate HMG-CoA reductase activity. With respect to pituitary hormones, it has been shown that hepatic reductase activity is reduced to very low levels in hypophysectomized rats (Ness et al., 1973). Administration of T_3 to such animals increases reductase activity to levels about 3-fold above normal. Administration of hydrocortisone inhibited the T_3-mediated stimulation. Actinomyocin D and cycloheximide also blocked the T_3-mediated increase in reductase activity. This suggested

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[1] Abbreviations: HMG-CoA, 3-hydroxy-3-methylglutaryl coenzyme A; T_3, triiodothyronine; kDa, kilodalton(s); LDL, low-density lipoprotein; Tris-HCl, tris(hydroxymethyl)aminomethane hydrochloride; SDS, sodium dodecyl sulfate; kb, kilobase(s); EDTA, ethylenediaminetetraacetic acid; BSA, bovine serum albumin.

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