Transformation of Mammary Cytoplasmic Glucocorticoid Receptor under Cell-Free Conditions†

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ABSTRACT: The transformation of glucocorticoid–receptor complex in the cytosol from lactating mouse mammary tissue was studied by using elevated temperature and KCl as promoters of the transformation reaction. The transformed receptor was identified from the nontransformed receptor by the following criteria: (a) increased binding to DNA–cellulose, (b) increased binding to ATP–Sepharose, (c) higher affinity for the steroid as determined by steroid dissociation kinetics, and (d) different sedimentation profiles on sucrose gradients containing KCl and sodium molybdate. A greater percentage of the nontransformed receptor was converted to the transformed state by an increased KCl concentration as opposed to increased temperature. Pretreatment of cytosol with 10 mM sodium molybdate prevented both the temperature- and salt-mediated transformation of the receptor.

The cytoplasmic glucocorticoid–receptor complex from several target tissues undergoes a transformation or activation[1] process which is believed to facilitate its nuclear translocation (Rousseau et al., 1973; Wira & Munck, 1974; Aronow, 1978; Munck & Foley, 1979). The in vivo transformation may be triggered by hormone binding and can be modelled, in vitro, by treatment of cytosolic steroid–receptor complex with such conditions as high ionic strength, elevated temperature, dilution, or increased pH (Higgins et al., 1973; Milgrom et al., 1973; Kalimi et al., 1975; Goidl et al., 1977; Bailly et al., 1978). The transformed complex exhibits an increased binding to isolated nuclei or polyanions such as DNA–cellulose, phosphocellulose, or ATP–Sepharose while the untreated, nontransformed complex binds minimally to these materials (Higgins et al., 1973; Milgrom et al., 1973; Kalimi et al., 1975; Goidl et al., 1977; Bailly et al., 1978; Atger & Milgrom, 1976; Colman & Feigelson, 1976; Simons et al., 1976; Climent et al., 1977; Cidlowski & Munck, 1978; LeFevre et al., 1979; Moudgil & John, 1980). For the uterine estrogen receptor, nontransformed and transformed estrogen–receptor complexes can also be identified on the basis of differing steroid-dissociation kinetics (Weichman & Notides, 1977, 1979) and sedimentation rates on sucrose gradients containing 0.4 M KCl (DeSombre et al., 1972; Jensen & DeSombre, 1973; Notides & Nielsen, 1974).

Extensive studies from several laboratories have revealed that in mammary tissues glucocorticoids enhance several tissue-specific biological responses such as increased production of casein, α-lactalbumin, and mammary tumor virus (Juergens et al., 1965; Stockdale & Topper, 1966; Turkington et al., 1968; McGrath, 1971; Ringold et al., 1975). To this end, cytoplasmic glucocorticoid receptors from both normal and neoplastic mammary tissues have been characterized in detail (Shyamala, 1973, 1974; Goral & Wittliff, 1975; McBlain & Shyamala, 1980; Costello, 1980). However, a detailed study on the transformation of the mammary cytoplasmic glucocorticoid receptor is still lacking, even though it has been shown that the response of the mammary tissues to a particular glucocorticoid is related to the level of the receptor complexed with that glucocorticoid in the nucleus (Shyamala, 1975; Shyamala & Dickson, 1976; Young et al., 1975). The present study on the transformation of mammary cytoplasmic glucocorticoid receptors was therefore undertaken so that it may facilitate our understanding of the precise role of glucocorticoid receptor in mediating biological responses in mammary tissues.

Materials and Methods

[6,7-^{3}H]Dexamethasone[2] (36.0–41.4 Ci/mmol) was obtained from New England Nuclear Corp. Unlabeled dexamethasone, monothioglycerol, and dithiothreitol (DTT) were purchased from Sigma Chemical Co., sodium molybdate was from British Drug Houses, and Sephadex G-50 was from Pharmacia. All other chemicals were from Fisher Scientific Co. Distilled deionized water was used to prepare all reagents.

Preparation of Cytosol. Female Balb/c mice from our colony were used and, at sacrifice, had been lactating for 7–10 days. Cytosol preparation was done at 0–4 °C; mammary tissues were excised, weighed, rinsed twice in buffer, minced, and homogenized in 1 volume (w/v) of buffer (5 mM sodium phosphate, 12 mM monothioglycerol, and 10% glycerol (v/v), pH 7.6, at 23 °C) by using a Polytron PT-10 (Brinkmann) homogenizer for two 15-s intervals. The homogenate was centrifuged at 105000*g* for 1 h, and the resulting supernatant (cytosol) was drawn from beneath the lipid layer with a cold Pasteur pipet. Cytosol was used immediately or frozen in liquid nitrogen and stored at −70 °C; DTT (10–20 mM) was added to fresh or thawed cytosol.

Steroid Binding Assay. Samples of cytosol were incubated for 3–4 h in ice with 50 nM [^{3}H]dexamethasone alone or in the presence of a 100-fold excess of unlabeled dexamethasone. The steroids were added to the incubation in ethanol at concentrations which limited the addition of ethanol to 4%; bound steroid was determined by a dextran-coated charcoal (DCC) procedure based on that of Korenman (1968). Specific binding was defined as the amount of binding in the presence of [^{3}H]dexamethasone alone minus the amount of binding when the 100-fold excess of unlabeled dexamethasone was also

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[1] Transformation or activation refers to the process by which steroid-bound cytoplasmic receptor is transformed to its nuclear form. Inactivation refers to the process by which steroid-free receptor is converted to a form incapable of binding steroid.

[2] Abbreviations used: dexamethasone, 9α-fluoro-16β-methyl-11α,17β,21-trihydroxypregna-1,4-diene-3,20-dione; DTT, dithiothreitol.

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present. Nonspecific binding generally represented about 10% of the total dexamethasone binding.

DNA–Cellulose Binding Assay. DNA–cellulose was prepared essentially by the method of Alberts & Herrick (1971). All column procedures were carried out at 4 °C. Columns containing 2-mL of packed DNA–cellulose were washed with 10 mL of homogenization buffer containing 1 M KCl followed by 30 mL of homogenization buffer alone. Samples of [^{3}H]dexamethasone-labeled cytosol treated as described in the figure legends were applied to the packed columns, eluted at about 1 drop/s, and washed stepwise with 30 mL each of homogenization buffer containing 0, 0.1, 0.3, and then 1 M KCl. Fractions of the washes were sampled for the presence of radioactivity, and for later experiments only the buffer and the 0.3 M KCl washes were used. For selected samples, the [^{3}H]dexamethasone eluted from the DNA–cellulose by 0.3 M KCl was applied to columns of Sephadex G-50 to verify that the steroid was bound to material excluded from the gel. Nonspecifically bound [^{3}H]dexamethasone was not retained by DNA–cellulose.

ATP–Sepharose Assay. ATP–Sepharose was prepared as described previously (Moudgil & Toft, 1975), and the preparations used contained 3–8 μmol of ATP/mL of packed Sepharose. Columns of 3 mL of packed ATP–Sepharose were washed as described for the DNA–cellulose above. Samples of [^{3}H]dexamethasone-labeled cytosol treated as described in the figure legends were applied to the packed columns, eluted at about 1 drop/s, and washed stepwise with 30 mL each of homogenization buffer containing 0, 0.15, and then 1.0 M KCl. Fractions of the 0.15 M and 1.0 M KCl washes were sampled for the presence of radioactivity.

Assay of [^{3}H]Dexamethasone Dissociation from the Glucocorticoid Receptor. The dissociation of [^{3}H]dexamethasone from the receptor was measured in the presence of an excess of unlabeled dexamethasone, and the data were analyzed according to the procedures of Weichman & Notides (1977, 1979). Mammary cytosol was incubated at 0 °C with 50 nM of [^{3}H]dexamethasone for 1 h. Aliquots were then incubated at either 0 (nontransformed receptor) or 25 °C (transformed receptor) for 30 min. The unbound steroid was removed from the cytosol by adsorption to a pellet of dextran-coated charcoal for 15 min at 0 °C. Then 50 μM unlabeled dexamethasone was added to the samples and incubated at 15 °C to initiate the dissociation of bound [^{3}H]dexamethasone. At the times indicated, aliquots were withdrawn and assayed for bound radioactivity by using the DCC assay. In all cases, the degradation of the [^{3}H]dexamethasone–receptor complex was measured by incubating parallel samples without the addition of unlabeled dexamethasone. The first-order dissociation rate constants were determined from the slopes of a plot of log (% [^{3}H]dexamethasone bound) vs. time of incubation at 15 °C. Each value was corrected for nonspecific binding of [^{3}H]-dexamethasone.

Sucrose Density Gradient Analysis. The samples to be analyzed were exposed to a DCC pellet (15 min) and centrifuged at 800*g* for 5 min, and the resulting supernatant (0.2–0.3 mL) was centrifuged on 4.5-mL gradients of 10–30% sucrose in specified buffers. Approximate sedimentation coefficients were determined by the method of Martin & Ames (1961) with ^{14}C-labeled bovine serum albumin (4.4 S) and ovalbumin (3.5 S) as internal standards. The *s* values for these standards were obtained from Castellino & Barker (1968).

Results

Temperature-Mediated Transformation of the Dexamethasone–Receptor Complex. Binding to DNA–Cellulose.

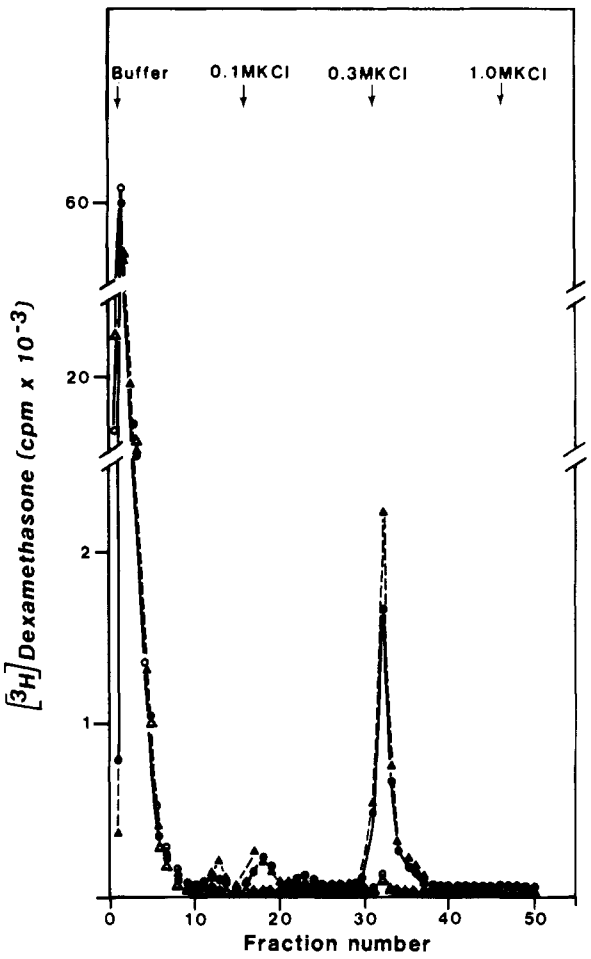

FIGURE 1: Profile of [^{3}H]dexamethasone–receptor complex eluted from DNA–cellulose columns by KCl. Cytosol was prepared and incubated with 50 nM [^{3}H]dexmethasone as described under Materials and Methods. Samples of this labeled cytosol were exposed to 25 °C for 30 min either in the presence (▲) or in the absence (●) of 10 mM Na_2MoO_4. Control samples were left untreated at 0–4 °C (○). Also, 10 mM Na_2MoO_4 was added to samples of cytosol previously exposed to 25 °C (▲). Columns were eluted as described under Materials and Methods and the text. The [^{3}H]dexamethasone–receptor complex bound by DNA–cellulose, expressed as a percent of receptor present in the cytosol, was about 22% for the 25 °C transformation in the experiment shown.

For determination of the effect of elevated temperature on the cytoplasmic dexamethasone–receptor complex, cytosol labeled with dexamethasone was either left untreated or exposed to 25 °C for 30 min and then applied to columns of DNA–cellulose. The columns were initially washed with buffer to remove the free [^{3}H]dexamethasone and then with varying concentrations of KCl as shown in Figure 1. As may be seen, the majority of the radioactivity in the unheated cytosol eluted with the buffer wash while a portion of the radioactivity in the heated cytosol was retained by the column during both the buffer and 0.1 M KCl washes but was released with an increase of the ionic strength to 0.3 M KCl. Thus, only the receptor-bound [^{3}H]dexamethasone in the heat-treated cytosol was bound by DNA–cellulose while that in the untreated control was not bound (see Table I also.) Therefore, as with other glucocorticoid receptors (Milgrom et al., 1973; Kalimi et al., 1975; Goidl et al., 1977), mammary cytoplasmic glucocorticoid receptors could be transformed by heat to a physical state capable of binding to DNA. Figure 1 and Table I also show that the addition of 10 mM molybdate to cytosol prior to heating almost completely inhibited the binding of the receptor to DNA but did not impair the DNA binding if cytosol was heated prior to the addition of molybdate.

Table I: Binding of Glucocorticoid Receptor to DNA-Cellulose or ATP-Sepharose[a]

treatment	cytosolic receptor bound (%) (mean ± SE, n = 3) to DNA-cellulose	to ATP-Sepharose
4 °C control	2.2 ± 0.7	4.9 ± 1.8
25 °C for 30 min	28.5 ± 2.1	19.9 ± 0.3
10 mM MoO_4, then 25 °C for 30 min	1.7 ± 0.2	4.4 ± 1.2
25 °C for 30 min, then 10 mM MoO_4	29.9 ± 2.1	21.2 ± 0.8
4 °C buffer control	3.0 ± 0.7	3.6 ± 1.4
0.4 M KCl for 60 min at 4 °C	65.7 ± 8.4	58.1 ± 16.9
10 mM MoO_4, then 0.4 M KCl for 60 min at 4 °C	2.8 ± 0.4	6.1 ± 1.8
0.4 M KCl for 60 min at 4 °C, then 10 mM MoO_4	66.4 ± 12.1	63.9 ± 18.3

[a] Labeled cytosol was prepared as described under Materials and Methods and treated as indicated above. Heated samples and KCl-treated samples (diluted 5-fold) were applied to columns of DNA-cellulose or ATP-Sepharose, washed, and eluted as indicated under Materials and Methods. Radioactivity was determined in the column eluates, and the percent of cytosolic receptor bound by the resin was calculated as a percentage of the receptor revealed by DCC analysis of the 4 °C control cytosol (mean of 250 fmol/mg of cytosol protein).

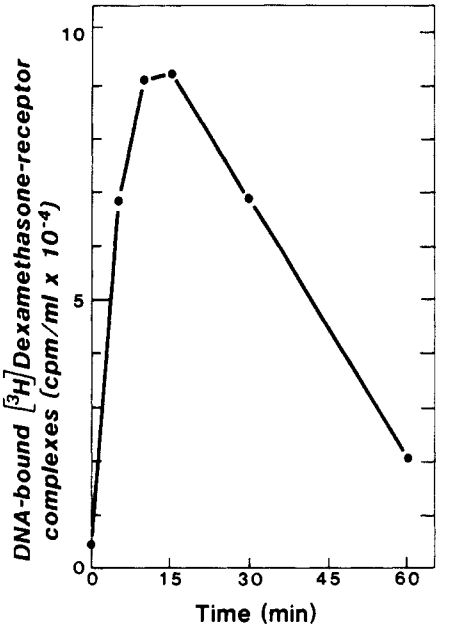

FIGURE 2: Time course for the transformation of [^{3}H]dexamethasone-labeled receptor exposed to 25 °C. For transformation, [^{3}H]dexamethasone-labeled cytosol was exposed to 25 °C, and at the times indicated 0.3-mL samples were cooled to 0–4 °C and applied to DNA–cellulose columns as described under Materials and Methods. At 15 min, the amount of receptor complex bound to DNA–cellulose was 33% of the total (as measured by charcoal adsorption).

Therefore, molybdate inhibits the temperature-mediated transformation reaction but does not affect the DNA-binding reaction. The effect of molybdate on the temperature-mediated transformation was reversible; if receptor heated in the presence of molybdate was dialyzed to remove molybdate and subjected to a second heat treatment, the receptor was able to bind to DNA (data not shown).

The time course for the transformation of receptor at 25 °C is shown in Figure 2. Maximal DNA binding of the dexamethasone–receptor complex (33% of total receptor complex) was observed after about a 15-min exposure of the cytosol to 25 °C, and by 30 min the DNA binding had begun a progressive decline; this result is explained by our finding that the 25 °C treatment of dexamethasone-labeled cytosol not only transforms the steroid-bound receptor to the DNA-binding form but also increases the rate of irreversible dissociation of the steroid from the receptor (degradation) as revealed by steroid-binding assays. This loss of bound steroid accounts for the decrease in detectable DNA binding of the receptor after longer times of exposure to 25 °C (Figure 2) or after exposure to temperatures above 25 °C (not shown). A 20 °C treatment requires about 90 min to transform the receptor to the level seen for 15 min at 25 °C, and while temperatures of 20 °C or lower transform the receptor more slowly, they cause less concomitant steroid dissociation (data not shown).

Binding to ATP–Sepharose. It has been reported that ATP–Sepharose binds transformed avian progesterone receptor but does not bind the nontransformed receptor (Miller & Toft, 1978), and similar results have been shown for glucocorticoid receptor from rat liver (Moudgil & John, 1980). Therefore, to further ascertain that mammary glucocorticoid receptor was transformed by heat, we tested the ability of cytosol labeled with dexamethasone to bind to ATP–Sepharose before and after exposure to elevated temperature. As shown in Figure 3 and Table I, [^{3}H]dexamethasone-labeled receptor in untreated cytosol was not bound by ATP–Sepharose, whereas

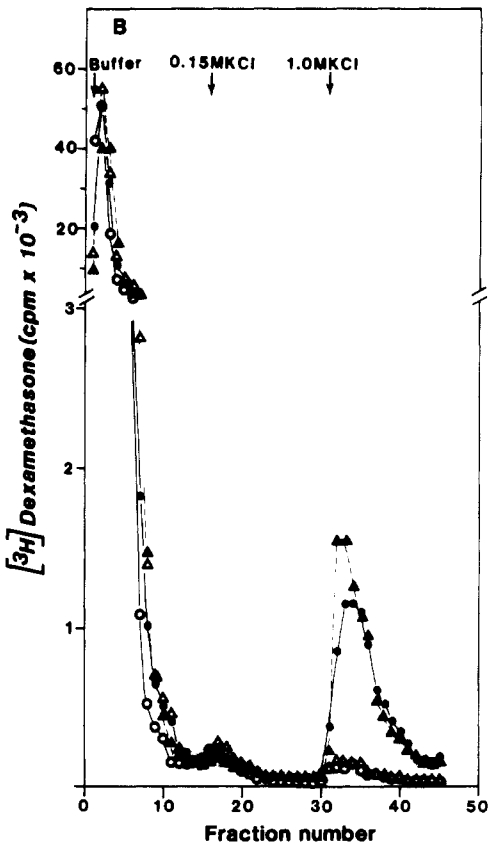

FIGURE 3: Profile of [^{3}H]dexamethasone–receptor complex eluted from ATP–Sepharose columns by KCl. Legend and symbols identical with Figure 1. The receptor bound by the ATP represented about 21% of the receptor present in the cytosol for the experiment shown.

the steroid–receptor complex in cytosol exposed to 25 °C for 30 min was bound. As seen for DNA binding, the addition of molybdate to cytosol prior to heating blocks the ability of receptor to bind to ATP, but molybdate does not interfere with

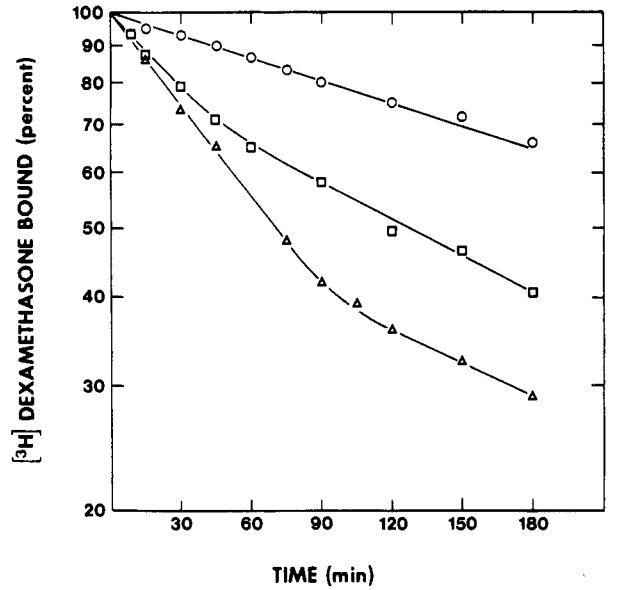

FIGURE 4: Dissociation of [^{3}H]dexamethasone from mammary cytoplasmic glucocorticoid receptor. Mammary cytosol was equilibrated at 0 °C with 50 nM [^{3}H]dexamethasone for 1 h, and then aliquots were either kept at 0 °C (□) or heated at 25 °C for 30 min with (△) or without (○) 10 mM molybdate. Subsequently, free dexamethasone in all samples was removed by charcoal/dextran adsorption, and the dissociation assay was initiated at 15 °C, as described under Materials and Methods. Each dissociation measurement has been corrected for nonspecific binding. The 100% value represents the amount of specifically bound [^{3}H]dexamethasone at the beginning of the dissociation assay and is equivalent to 197 fmol/mg of protein for the experiment shown. The magnitude (amplitude) of the fast component equals the difference between the extrapolated slow component at zero time and 100%, divided by 100%.

the ATP binding if the receptor has been heated prior to the molybdate addition (Figure 3 and Table I).

Effect of Heat and Molybdate on [^{3}H]Dexamethasone Dissociation from the Receptor. On the basis of their studies on uterine estrogen receptor transformation, Weichman & Notides (1977, 1979) propose that steroid bound to the nontransformed state of the receptor has a faster dissociation rate than that bound to the transformed receptor. Furthermore, when the steroid dissociation kinetics of the receptor are used as an index of transformation, it has been demonstrated that sodium molybdate is an inhibitor of uterine estrogen receptor transformation (Mauck & Notides, 1980; Shyamala & Leonard, 1980). At present there is not evidence that the transformed glucocorticoid receptor can be distinguished from the nontransformed receptor by its kinetics of dissociation. So that this possibility could be tested, the rates of dissociation of receptor-bound [^{3}H]dexamethasone from the unheated and heated cytosol were determined.

Initially it was established that the optimum temperature for conducting the [^{3}H]dexamethasone dissociation assay was 15 °C; at lower temperatures, the dissociation was much slower, and at higher temperatures, especially after 30 min, there was a significant degradation of the receptor (data not shown). The dissociation of [^{3}H]dexamethasone from the glucocorticoid–receptor complex, measured by exchanging the labeled with unlabeled dexamethasone at 15 °C, is shown in Figure 4. The data for the unheated cytosol reveal a biphasic curve; the $t_{1/2}$ for dissociation of the more rapid component is 79.3 ± 4.7 min and, for the more slowly dissociating component, 188 ± 5.6 min (mean ± SE, n = 3). Preincubation of cytosol labeled with dexamethasone at 25 °C for 30 min (to produce transformation) resulted in the presence of only the second slowly dissociating component. If 10 mM molybdate is added to cytosol prior to heating to 15 °C, the data reveal both the first component and the second component. However, the proportion of the fast component was higher in the cytosol heated with molybdate compared to the unheated control cytosol which was heated without molybdate. The data on [^{3}H]dexamethasone dissociation kinetics are summarized in Table II. Thus, it appears that as the mammary glucocorticoid–receptor complex is heated, there is a loss of the lower affinity state of the receptor and a concomitant appearance of the higher affinity state. For estrogen receptor, the generation of the higher affinity state of the receptor is believed to be associated with the transformation reaction (Weichman & Notides, 1977, 1979). This may also be true for the glucocorticoid receptor since the 25 °C treatment, which resulted in the appearance of the high-affinity state only (Figure 4), also resulted in a receptor form which bound DNA or ATP (Figures 1 and 3 and Table I).

Table II: [^{3}H]Dexamethasone Dissociation Kinetics of Receptor from Cytosol Exposed to Varying Experimental Conditions

treatment[a]	$k_{-1} \times 10^3$ (min^{-1})	$k_{-2} \times 10^3$ (min^{-1})	amplitude[b]	receptor level[c] 100%
none	8.8 ± 0.6	3.7 ± 0.1	0.22 ± 0.02	8.24 ± 0.74
25 °C for 30 min	*d*	3.0 ± 0.4	*d*	8.62 ± 1.6
25 °C for 30 min with 10 mM molybdate	8.9 ± 0.6	2.9 ± 0.3	0.48 ± 0.02	12.7 ± 1.2

[a] Refers to treatment of cytosol after addition of [^{3}H]dexamethasone and prior to dissociaton assay. [b] Refers to the magnitude of the fast component and equals the difference between the extrapolated slow component at zero time and 100%, divided by 100%. [c] The 100% value represents the nanomolar amount of specifically bound [^{3}H]dexamethasone at the beginning of the dissociation assay. [d] Not applicable.

The observed effect of molybdate on inhibiting the appearance of the slowly dissociating component could be reversed insofar as that if the labeled cytosol was first treated with molybdate, then dialyzed (to remove molybdate), and then transformed by heating and subjected to the exchange assay, only the slowly dissociating component was detectable (data not shown).

Effect of Heat on the Sedimentation Behavior of the Receptor. Extensive studies on uterine estrogen receptor have documented that the transformed receptor can be distinguished from the nontransformed receptor by its sedimentation behavior; the nontransformed receptor sediments at 4 S on sucrose gradients containing 0.4 M KCl while the transformed receptor sediments at 5 S (DeSombre et al., 1972; Jensen & DeSombre, 1973; Notides & Nielsen, 1974). However, in contrast to estrogen receptors, both transformed and nontransformed hepatic glucocorticoid receptors sediment at 4 S on sucrose gradients containing 0.4 M KCl (Kalimi et al., 1975; Bailly et al., 1980). In the following experiments, we examined the effect of temperature-mediated transformation on the sedimentation behavior of mammary cytoplasmic glucocorticoid–receptor complex.

As shown in Figure 5, if the cytosol labeled with dexamethasone is centrifuged on gradients containing 0.4 M KCl, the receptor in both the unheated and heated cytosol sediments at 4 S (4.4 ± 0.02 S; mean ± SE, n = 4); i.e., both the nontransformed and transformed receptors appear to have a similar rate of sedimentation. However, it is known that salt alone can induce transformation of certain steroid–receptor complexes (Kalimi et al., 1975; Bailly et al., 1978; Leach et al., 1979). Thus it raised the possibility that the 4S receptor of the native cytosol might in itself represent transformed receptor insofar as that it was exposed to 0.4 M KCl during

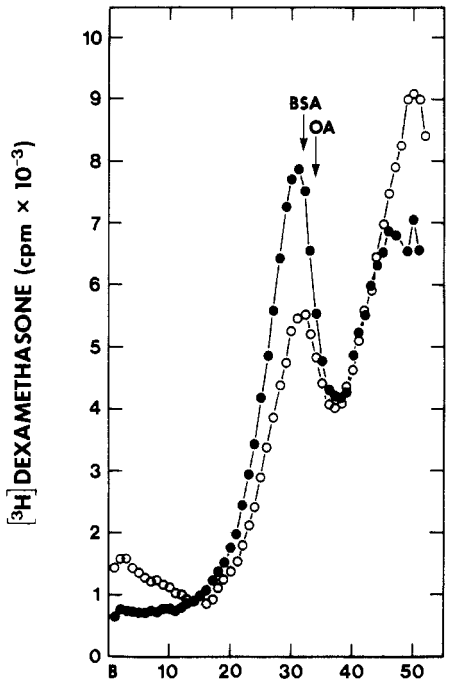

FIGURE 5: Sucrose gradient profiles of dexamethasone–receptor complex of mammary cytosol. Cytosol was equilibrated for 2 h at 0 °C with 50 nM [^{3}H]dexamethasone subsequent to which it was either left untreated (●) or heated at 25 °C (○) for 15 min. Next aliquots of these samples were treated with charcoal/dextran to remove free steroid and then centrifuged at 216000*g* for 22 h on sucrose gradients containing 0.4 M KCl. The bottom of the gradient in each panel is indicated by B. The arrow indicates the sedimentation of the standards, bovine serum albumin (BSA), and ovalbumin (OVA).

prolonged centrifugation. To this end, we decided to examine whether KCl alone can cause transformation of mammary cytoplasmic glucocorticoid receptor.

KCl-Mediated Transformation of Dexamethasone–Receptor Complex. Binding to DNA–Cellulose. As may be seen in Table I, receptor in the buffer-treated cytosol exhibits very little affinity for DNA–cellulose while the receptor in the cytosol treated with 0.4 M KCl for 60 min and then diluted 5-fold is bound by the DNA. Thus, the receptor could be transformed to a DNA-binding form by KCl in a manner similar to that previously seen for the exposure of the receptor to heat (Figure 1 and Table I). As with heat-induced transformation, sodium molybdate inhibits the KCl-mediated transformation of the receptor since the receptor pretreated with molybdate prior to addition of KCl does not bind to DNA.

Binding to ATP–Sepharose. The data in Table I also show that [^{3}H]dexamethasone–receptor complex in buffer-treated cytosol is not bound by ATP–Sepharose while that in the cytosol treated with 0.4 M KCl is bound. Also, molybdate added to cytosol prior to the addition of KCl can inhibit the KCl-mediated transformation of the receptor to the form capable of binding to ATP; molybdate again does not interfere with the binding of the transformed receptor to ATP if added after exposure of the cytosol to KCl. Thus, high ionic strength is capable of transforming mammary glucocorticoid receptor to either the DNA- or ATP-binding form.

Molybdate Concentration Requirement for Inhibition of KCl-Mediated Receptor Transformation. We observed that 10 mM sodium molybdate inhibited the transformation of mammary glucocorticoid–receptor complex to its DNA- or ATP-binding form (Figures 1 and 3 and Table I); to determine the minimal effective dose of molybdate, we treated samples

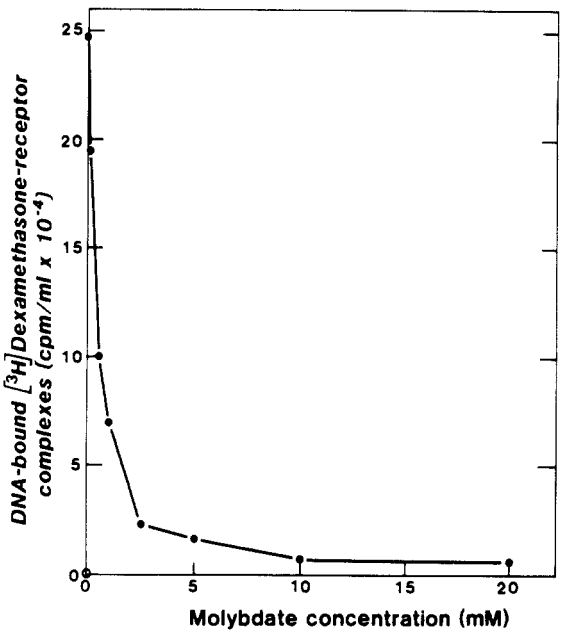

FIGURE 6: Molybdate concentration curve for the inhibition of transformation of [^{3}H]dexamethasone-bound receptor. Sodium molybdate, at the concentrations indicated, was added to [^{3}H]dexamethasone-labeled cytosol for 30 min prior to a 60-min exposure to 0.4 M KCl at 0–4 °C. To measure transformation, 0.4-mL samples of labeled cytosol were diluted 1:5 in buffer and applied to DNA–cellulose columns.

of cytosol with increasing concentrations of molybdate prior to exposure to 0.4 M KCl for 60 min. As can be seen in Figure 6, 5–10 mM molybdate gives maximal inhibition of transformation of this receptor.

The effect of molybdate on the receptor transformation was reversible. Receptor treated with KCl in the presence of molybdate was not transformed, but following the removal of molybdate by dialysis and a seond salt treatment, the receptor was transformed to the DNA-binding form (data not shown).

Effect of Sodium Molybdate on the Sedimentation Profiles of Cytoplasmic Glucocorticoid Receptor. In contrast to reported differences in the sedimentation profile of transformed and nontransformed estrogen receptors, there is no discernible difference between temperature-transformed and nontransformed mammary glucocorticoid receptor when centrifuged on gradients containing KCl (Figure 5). However, since molybdate appears to exert its effect on the transformation reaction by interacting with the nontransformed receptor and not the transformed receptor (Table I), we decided to examine the sedimentation profile of transformed and nontransformed receptors in the presence of molybdate. As shown in Figure 7, dexamethasone–receptor complex in native cytosol sediments at 6–7 S (6.8 ± 0.2 S; mean ± SE, n = 8) in sucrose gradients without KCl (Figure 7A); with the addition of molybdate to cytosol, the complex sediments at 7–8 S (7.6 ± 0.3 S; mean ± SE, n = 5) (Figure 7A). In fact, whether or not molybdate is added to cytosol, the 7–8 S form of the receptor is seen as long as the sucrose gradients contain molybdate (Figure 7A). Similarly, whether or not molybdate is added to cytosol, the receptor sediments at 7–8 S on gradients containing molybdate and also 0.4 M KCl (Figure 7B). The appearance of the receptor from untreated cytosol as 7–8 S on gradients containing 0.4 M KCl is dependent on the presence of molybdate in the gradients since in its absence the receptor sediments as 4 S (4.3 ± 0.04 S; mean ± SE, n = 6) (Figure 7B). However, as shown in Figure 7C, even when molybdate is in the gradients, it is ineffective in preventing the formation of 4 S when the cytosol has been treated with 0.4 M KCl prior to its ex-

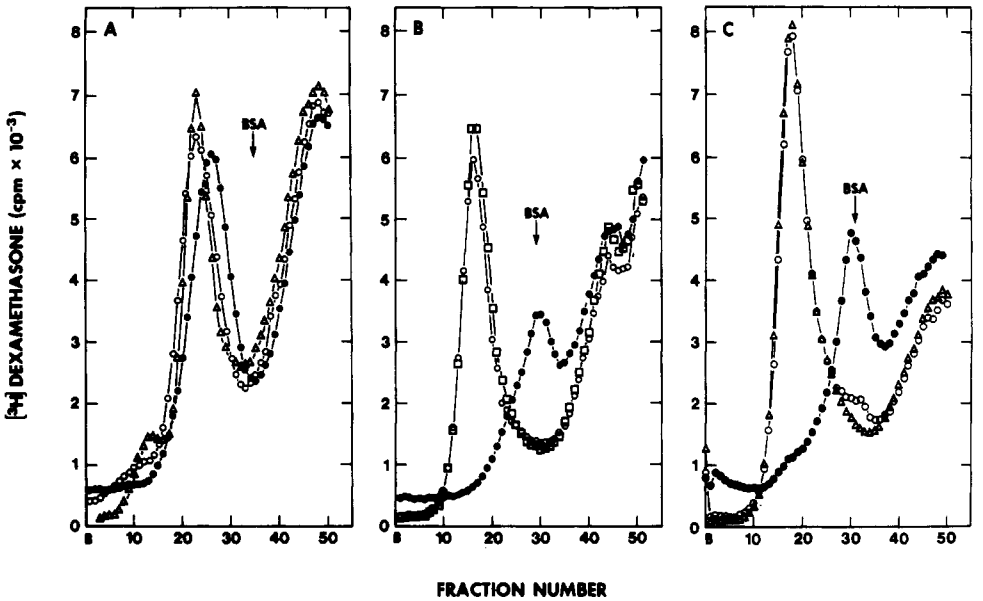

FIGURE 7: Effect of sodium molybdate and KCl on the sedimentation profiles of cytoplasmic glucocorticoid–receptor complex. Cytosol incubated with [^{3}H]dexamethasone was subjected to various treatments as specified prior to DCC treatment and centrifugation at 4 °C on sucrose gradients prepared in phosphate buffer as is or with additions as specified. The gradients without KCl (panel A) were centrifuged at 189000g for 17 h while those with 0.4 M KCl (panels B and C) were centrifuged at 216000g for 22 h. In all cases, B refers to the bottom of the gradient. (A) (●) Untreated cytosol on gradient without molybdate; (△) molybdate-treated cytosol on gradient without molybdate; (○) untreated cytosol on gradient with molybdate. (B) (●) Untreated cytosol on gradient with 0.4 M KCl; (○) untreated cytosol on gradient with molybdate and 0.4 M KCl; (□) molybdate-treated cytosol on gradient with molybdate and 0.4 M KCl. (C) All gradients had molybdate and 0.4 M KCl. (△) Molybdate-treated cytosol; (○) cytosol pretreated with molybdate for 15 min and then exposed to 0.4 M KCl for 1 h; (●) cytosol treated with 0.4 M KCl for 1 h prior to treatment with molybdate for 15 min. The arrow indicates the sedimentation of the standard bovine serum albumin.

posure to molybdate. The experiments described in Figure 7 were also repeated with cytosol containing varying concentrations of KCl, including that at near physiological ionic strength (0.15 and 0.2 M KCl). In these experiments, upon centrifugation on sucrose gradients containing molybdate and 0.4 M KCl, with increasing concentration of KCl in the cytosol, there was a progressive increase in the proportion of 4 S and a concomitant decrease in the 7–8 S forms of the receptor; at 0.2 M KCl, the receptor was distributed approximately equally between the 8 S and 4 S forms (data not shown). Since the DNA-binding experiments shown in Figure 8A reveal that at 0.2 M KCl approximately one-half of the receptor is in the transformed state, it appears that the changes in the sedimentation profiles associated with the transformation of the receptor occur when the receptor is isolated in buffers both of low and at near physiological ionic strengths. Thus these data taken together with the data on transformation reaction shown in Table I suggest that the sedimentation coefficient of the nontransformed glucocorticoid receptor is 7–8 S while that of the transformed receptor is 4 S. It also appears that similar to estrogen receptors, the transformed glucocorticoid receptor can be distinguished from the nontransformed receptor by sucrose gradient analysis with the difference that molybdate is necessary to permit the resolution of the two forms of the glucocorticoid receptor.

Similarity of Conditions for Receptor Inactivation and Transformation. We have previously published the results of studies on KCl- and temperature-mediated inactivation[1] of mammary glucocorticoid receptor (McBlain & Shyamala, 1980). In this case, treatment in the absence of hormone promotes a loss of the steroid binding capacity of the receptor. The salt conditions for these two processes, inactivation and transformation, are compared in Figure 8A. For transformation, [^{3}H]dexamethasone-labeled cytosol was treated with increasing concentrations of KCl for 60 min and assayed for binding to DNA–cellulose. A maximal capacity of steroid–

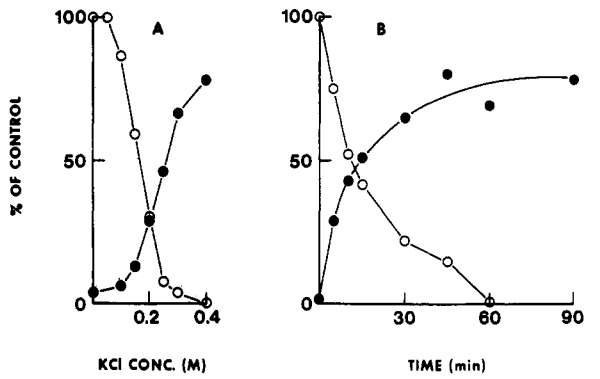

FIGURE 8: (A) KCl concentration curve for the transformation of [^{3}H]dexamethasone-bound receptor or inactivation of steroid-free receptor. For transformation (●), lactating mouse mammary gland cytosol was prepared and labeled as described under Materials and Methods. Samples of cytosol (0.5 mL) received a 0.25 volume of buffer containing KCl to give the indicated final concentration. After 60 min at 0–4 °C, cytosol was diluted 1:5 with buffer and applied to columns of DNA–cellulose. For inactivation (○), steroid-free cytosol was exposed to increasing concentrations of KCl for 60 min at 0–4 °C and then diluted 1:5 with buffer. [^{3}H]Dexamethasone (50 nm) ± a 100-fold excess of unlabeled dexamethasone was added to the samples which were incubated for 4 h and assayed for steroid binding by the DCC method. (B) Time course for the transformation of [^{3}H]dexamethasone-bound receptor or the inactivation of steroid-free receptor exposed to 0.4 M KCl. For transformation (●), cytosol labeled with 50 nm [^{3}H]dexamethasone for 3 h was treated with 0.4 M KCl, and at the times indicated, the samples were diluted 1:5 with buffer and applied to DNA–cellulose columns. For inactivation (○), steroid-free cytosol was exposed to 0.4 M KCl at 0–4 °C. At the times indicated, samples were diluted 1:5 with buffer and incubated a further 4 h with 50 nM [^{3}H]dexamethasone ± a 100-fold excess of unlabeled dexamethasone. Specifically bound steroid was determined by the DCC method.

receptor complex to bind to DNA–cellulose is observed after exposure to about 0.4 M KCl. Also shown in Figure 8A, for comparison, is the KCl concentration requirement for the

inactivation of steroid-free receptor described earlier (McBlain & Shyamala, 1980), and this, too, is maximal at about 0.4 M.

Figure 8B contains the time course for the transformation by 0.4 M KCl of dexamethasone-bound receptor, and it can be seen that the transformation reaction is complete in about 60 min. The inactivation of steroid-free receptor (Figure 8B) also is complete in about 60 min. Thus, the transformation of steroid-bound receptor has the same requirements for KCl and time as does the inactivation of the steroid-free receptor. Similarly, the concentration of molybdate required to inhibit transformation (Figure 6) is the same as that required to inhibit inactivation (McBlain & Shyamala, 1980). The temperature requirement for transformation also is similar to that required for inactivation (McBlain & Shyamala, 1980), but concomitant degradation of receptor during the transformation process complicates the comparison between the temperature-induced transformation and inactivation reactions.

Discussion

In this paper, we have demonstrated that the cytoplasmic glucocorticoid–receptor complex from lactating mouse mammary glands can be transformed, by high ionic strength or elevated temperature, to a form which will bind to DNA–cellulose or ATP–Sepharose. The binding of transformed receptor to both DNA and ATP resins has previously been shown for the progesterone receptor of chick oviduct (Miller & Toft, 1978; Nishigori & Toft, 1980). By criteria used here, high salt appears to be a more effective condition than elevated temperature for the in vitro transformation reaction, and the transformation seems similar to that previously shown for other glucocorticoid receptors, including those of rat liver (Milgrom et al., 1973; Kalimi et al., 1975; Goidl et al., 1977; Bailly et al., 1978, 1980) and rat hepatoma cells (Higgins et al., 1973; Simons et al., 1976). We have also shown that the dissociation of steroid from nontransformed receptor is faster than that from temperature-transformed receptor. These results are similar to those obtained previously for estrogen receptor (Weichman & Notides, 1977, 1979; Shyamala & Leonard, 1980) and indicate that it may be possible to use steroid dissociation kinetics to differentiate between nontransformed and transformed glucocorticoid receptors. However, the magnitude of the difference in steroid dissociating components for the glucocorticoid receptor reported here is less than that previously reported for estrogen receptor (Weichman & Notides, 1979). The relative dissociation rates are temperature sensitive (Weichman & Notides, 1979), and our findings may reflect differences in tissue- and receptor-specific energy requirements for the competing processes of steroid dissociation and receptor transformation.

Previous studies on glucocorticoid receptor transformation have revealed that unlike the rodent uterine estrogen receptor, both transformed and nontransformed glucocorticoid receptors sediment as 4 S on sucrose gradients containing 0.4 M KCl. Our data show that the 4 S entity can be generated even when cytosol is not exposed to 0.4 M KCl and thus is a product of prolonged exposure of the receptor to KCl during centrifugation. Thus, in previous studies, the "4 S" species obtained with nontransformed receptor might indeed have represented the transformed receptor, i.e., receptor transformed during centrifugation, and this might have been the reason for not detecting any differences in the sedimentation profiles of transformed vs. nontransformed receptors. The use of molybdate as shown in the present studies prevents the conversion of the receptor to 4 S during centrifugation, and thus, in its presence, the transformed receptor (receptor exposed to 0.4 M KCl prior to layering on gradients) and "nontransformed" receptor (receptor in untreated cytosol) exhibit different rates of sedimentation on gradients containing 0.4 M KCl. Therefore, it appears that as with estrogen receptor, upon transformation, glucocorticoid receptors also undergo changes in their physical state which can be analyzed by sucrose gradient centrifugation.

Studies on cytoplasmic glucocorticoid receptors of various target tissues have revealed a salt-dependent dissociation of steroid–receptor complexes; in all these studies, exposure of cytosol labeled with glucocorticoid to 0.3–0.4 M KCl results in the generation of a 4–5 S receptor complex with a reduced molecular weight as compared to the complex in native cytosol (Sherman et al., 1979; Middlebrook & Aronow, 1977; Carlstedt-Duke et al., 1977). Extensive studies on various glucocorticoid receptors have revealed the physical states of these receptors to be strikingly similar (Munck & Leung, 1977), and thus, even though in these present studies we have not estimated the molecular weights of the various forms of steroid–receptor complexes, it is reasonable to assume that as with other glucocorticoid receptors the 4 S dexamethasone–receptor complex generated by the addition of 0.4 M KCl is the result of a salt-dependent dissociation of the larger steroid–receptor complex in the native cytosol. It is interesting to note therefore that the 4 S form generated from cytosol labeled with steroid and exposed to 0.4 M KCl has the steroid binding site intact while under identical conditions ligand-free receptor loses its dexamethasone binding capacity.

We have previously shown that high ionic strength or elevated temperature could inactivate the steroid-free receptor to a form incapable of binding steroid (McBlain & Shyamala, 1980). The detailed KCl and temperature requirements for the transformation of steroid-bound receptor reported herein are very similar to the conditions effecting the inactivation of steroid-free receptor. The simplest explanation for the in vitro effects of salt or temperature on the glucocorticoid receptor would be that a conformational change has occurred under the conditions of high ionic strength or elevated temperature to cause steric hindrance at the steroid binding site; such hindrance could slow the dissociation of steroid from steroid-bound receptor and prevent steroid binding of steroid-free receptor. Thus, it is likely that the processes of in vitro transformation of cytoplasmic glucocorticoid–receptor complex and the in vitro inactivation of ligand-free glucocorticoid receptor are related; such a possibility is reinforced by the fact that the two processes have a common inhibitor, sodium molybdate.

Recently, reports on molybdate inhibition of transformation of chick progesterone receptor (Nishigori & Toft, 1980), hepatic glucocorticoid receptor (Leach et al., 1979), and uterine estrogen receptor (Mauck & Notides, 1980; Shyamala & Leonard, 1980) have been published, and our results concur with these. Furthermore, it has been found that chemically related tungstate and vanadate can block temperature-induced transformation of avian progesterone receptor (Nishigori & Toft, 1980; Nishigori et al., 1980), and tungstate blocks hepatic glucocorticoid receptor transformation (Leach et al., 1979). The mechanism by which these related anions affect steroid receptor inactivation or transformation is unknown at present; it has been suggested that molybdate may act by inhibiting a critical phosphatase (Shyamala & Leonard, 1980; Nishigori & Toft, 1980; Neilsen et al., 1977; Sando et al., 1979), interacting with a heavy metal (Nishigori & Toft, 1980), interacting with sulfhydryl groups (Leach et al., 1979; Nishigori & Toft, 1980), or complexing with a phosphate group on or

associated with the receptor (Leach et al., 1979; Nishigori & Toft, 1980).

The ability of molybdate to stabilize steroid-free glucocorticoid receptors of other tissues has also been documented (Neilsen et al., 1977; Mierendorf & Mueller, 1979; Miras & Harrison, 1979). The molybdate inhibition of transformation of mammary glucocorticoid receptor is correlated with the maintenance or generation of the heavier 7–8 S form of the receptor. Conversely, transformation is accompanied with the production of the 4 S receptor species. Thus, it appears that the role of molybdate in receptor inactivation and transformation may lie in its ability to counteract the mechanisms by which the 4S receptor is formed.

If the processes of inactivation of ligand-free receptor and transformation of ligand-bound receptor to the nuclear form were indeed related, then the same process could enable the receptor to exist in an equilibrium between forms able and unable to bind steroids and forms able and unable to undergo nuclear translocation. That is, a single process could modulate cellular responsiveness to glucocorticoids by regulating steroid binding and nuclear translocation of cytoplasmic receptors. It has been suggested that dephosphorylation may either directly or indirectly play a role in receptor function (Leach et al., 1979; Sando et al., 1979) and, of course, the counter-reaction, phosphorylation, would be important as well. In this regard, it has been found that ATP or a related substance may be required for normal receptor activity in whole cells (Munck et al., 1972; Wheeler et al., 1981). More recently, it has been reported that the observed transformation of receptor is not an in vitro artifact since it occurs in whole cells (Munck & Foley, 1979) or in vivo (Markovic & Litwack, 1980); however, it is not known if receptor inactivation is of physiological significance or if the inactivation or transformation process is reversible. Obviously, a great deal of research will be required to elucidate the complete molecular basis for receptor inactivation or transformation.

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References

Alberts, B., & Herrick, G. (1971) *Methods Enzymol. 21*, 198–217.

Aronow, L. (1978) *Fed. Proc., Fed. Am. Soc. Exp. Biol. 37*, 162–166.

Atger, M., & Milgrom, E. (1976) *Biochemistry 15*, 4298–4304.

Bailly, A., Savouret, J.-F., Sallas, N., & Milgrom, E. (1978) *Eur. J. Biochem. 88*, 623–632.

Bailly, A., LeFevre, B., Savouret, J.-F., & Milgrom, E. (1980) *J. Biol. Chem. 255*, 2729–2734.

Carlstedt-Duke, J., Gustafsson, J.-A., & Wrange, O. (1977) *Biochim. Biophys. Acta 497*, 507–524.

Castellino, F. J., & Barker, R. (1968) *Biochemistry 7*, 2207–2217.

Cidlowski, J. A., & Munck, A. (1978) *Biochim. Biophys. Acta 543*, 545–555.

Climent, F., Doenecke, D., & Beato, M. (1977) *Biochemistry 16*, 4694–4703.

Colman, P. D., & Feigelson, P. (1976) *Mol. Cell. Endocrinol. 5*, 33–40.

Costello, M. A. (1980) *Diss. Abstr. Int. B 41*, 434.

DeSombre, E. R., Mohla, S., & Jensen, E. V. (1972) *Biochem. Biophys. Res. Commun. 48*, 1601–1608.

Goidl, J. A., Cake, M. H., Dolan, K. P., Parchman, L. G., & Litwack, G. (1977) *Biochemistry 16*, 2125–2130.

Goral, J. E., & Wittliff, J. L. (1975) *Biochemistry 14*, 2944–2952.

Higgins, S. J., Rousseau, G. G., Baxter, J. D., & Tomkins, G. M. (1973) *J. Biol. Chem. 248*, 5866–5872.

Jensen, E. V., & DeSombre, E. R. (1973) *Science (Washington, D.C.) 182*, 126–134.

Juergens, W. G., Stockdale, F. E., Topper, Y. J., & Elias, J. J. (1965) *Proc. Natl. Acad. Sci. U.S.A. 54*, 629–634.

Kalimi, M., Colman, P., & Feigelson, P. (1975) *J. Biol. Chem. 250*, 1080–1086.

Korenman, S. G. (1968) *J. Clin. Endocrinol. Metab. 28*, 127–130.

Leach, K. L., Dahmer, M. K., Hammond, N. D., Sando, J. J., & Pratt, W. B. (1979) *J. Biol. Chem. 254*, 11884–11890.

LeFevre, B. Bailly, A., Sallas, N., & Milgrom, E. (1979) *Biochim. Biophys. Acta 585*, 266–272.

Markovic, R. D., & Litwack, G. (1980) *Arch. Biochem. Biophys. 202*, 374–379.

Martin, R. G., & Ames, B. N. (1961) *J. Biol. Chem. 236*, 1372–1379.

Mauck, L. A., & Notides, A. C. (1980) 62nd Annual Meeting of the Endocrine Society, Washington, D.C., Astract 209.

McBlain, W. A., & Shyamala, G. (1980) *J. Biol. Chem. 255*, 3884–3891.

McBlain, W. A., Toft, D. O., & Shyamala, G. (1980) 62nd Annual Meeting of the Endocrine Society, Washington, D.C., Abstract 171.

McGrath, C. M. (1971) *J. Natl. Cancer Inst. 47*, 455–467.

Middlebrook, J. L., & Aronow, L. (1977) *Endocrinology (Philadelphia) 100*, 271–282.

Mierendorf, R. C., & Mueller, G. C. (1979) *Mol. Cell. Endocrinol. 13*, 301–316.

Milgrom, E., Atger, M., & Baulieu, E.-E. (1973) *Biochemistry 12*, 5198–5205.

Miller, J. B., & Toft, D. O. (1978) *Biochemistry 17*, 173–177.

Miras, M. E., & Harrison, R. W. (1979) *J. Steroid Biochem. 11*, 1129–1134.

Moudgil, V. K., & Toft, D. O. (1975) *Proc. Natl. Acad. Sci. U.S.A. 72*, 901–905.

Moudgil, V. K., & John, J. K. (1980) *Biochem. J. 190*, 809–819.

Munck, A., & Leung, K. (1977) in *Receptors and Mechanism of Action of Steroid Hormones* (Pasqualini, J. R., Ed.) pp 311–393, Marcel Dekker, New York.

Munck, A., & Foley, R. (1979) *Nature (London) 278*, 752–754.

Munck, A., Wira, C., Young, D. A., Mosher, K. M., Hallahan, C., & Bell, P. A. (1972) *J. Steroid Biochem. 3*, 567–578.

Neilsen, C. J., Sando, J. J., Vogel, W. M., & Pratt, W. B. (1977) *J. Biol. Chem. 252*, 7568–7578.

Nishigori, H., & Toft, D. (1980) *Biochemistry 19*, 77–83.

Nishigori, H., Alker, J., & Toft, D. (1980) *Arch. Biochem. Biophys. 203*, 600–604.

Notides, A. C., & Nielson, S. (1974) *J. Biol. Chem. 249*, 1866–1873.

Ringold, G. M., Yamamoto, K. R., Tomkins, G. M., Bishop, J. M., & Varmus, H. E. (1975) *Cell (Cambridge, Mass.) 6*, 299–305.

Rousseau, G. G., Baxter, J. D., Higgins, S. J., & Tomkins, G. M., (1973) *J. Mol. Biol. 79*, 539–554.

Sando, J. J., Hammond, N. D., Stratford, C. A., & Pratt, W. B. (1979) *J. Biol. Chem. 254*, 4779–4789.
Sherman, M. R., Barzilai, D., Pine, P., & Tuazon, Fe. B. (1979) in *Steroid Hormone Receptor Systems* (Leavitt, W. W., & Clark, J. H., Eds.) pp 357–375, Plenum Press, New York.
Shyamala, G. (1973) *Biochemistry 12*, 3085–3090.
Shyamala, G. (1974) *J. Biol. Chem. 249*, 2160–2163.
Shyamala, G. (1975) *Biochemistry 14*, 437–444.
Shyamala, G., & Dickson, C. (1976) *Nature (London) 262*, 107–112.
Shyamala, G., & Leonard, L. (1980) *J. Biol. Chem. 255*, 6028–6031.
Simons, S. S., Jr., Martinez, H. M., Garcea, R. L., Baxter, J. D., & Tomkins, G. M. (1976) *J. Biol. Chem. 251*, 334–343.
Stockdale, F. E., & Topper, Y. J. (1966) *Proc. Natl. Acad. Sci. U.S.A. 56*, 1283–1289.
Turkington, R. W., Brew, K., Vanaman, J. C., & Hill, R. L. (1968) *J. Biol. Chem. 243*, 3382–3387.
Weichman, B. M., & Notides, A. C. (1977) *J. Biol. Chem. 252*, 8856–8862.
Weichman, B. M., & Notides, A. C. (1979) *Biochemistry 18*, 220–225.
Wheeler, R. H., Leach, K. L., Laforest, A. C., O'Toole, T. E., Wagner, R., & Pratt, W. B. (1981) *J. Biol. Chem. 256*, 434–441.
Wira, C. R., & Munck, A. (1974) *J. Biol. Chem. 249*, 5328–5336.
Young, H. A., Scolnick, E. M., & Parks, W. P. (1975) *J. Biol. Chem. 250*, 3337–3343.

Analysis of Chicken Progesterone Receptor Structure Using a Spontaneous Sheep Antibody†

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ABSTRACT: A spontaneous sheep antibody to chick progesterone receptor was characterized and used as a tool to study the receptor structure. The antibody, which is present to some extent in sera from about one-third of the sheep tested, binds to *Staphylococcus aureus* protein A–Sepharose and therefore appears to be an IgG. It is specific for the chick progesterone receptor and does not react with free progesterone or with any of the other proteins tested, including other receptors and corticosteroid binding globulin. The antibody is nonprecipitating and has a very low titer (equivalence point = 2.5 pmol of receptor/mL of serum). The interaction of the receptor with the antibody was measured, and an apparent dissociation constant of 2×10^{-9} M was determined from these studies. The antibody reacts equally well with the two receptor subunits A and B but does not appear to react with the native aggregate form found in the cytosol. Thus, the immunologic site is occluded in the aggregate, and therefore the antibody will be a useful probe for this important region of the proteins. The antibody recognition sites on the receptors were further characterized by analysis of a proteolytic digest of receptors by using an endogenous Ca^{2+}-activated neutral protease. Competition studies using native receptor and receptor digests demonstrated that the antigenic site was not destroyed in the digest and was separated from the hormone binding fragment. We conclude that receptor subunits A and B have a cross-reactive immunologic site on a portion of the molecule other than the hormone binding domain.

The hen oviduct is a target tissue for progesterone. Oviduct cytosol contains a receptor protein made up of the two hormone binding subunits A and B (M_r 79 000 and 106 000, respectively) (Vedeckis et al., 1978). The proteins have kinetically identical progesterone binding sites (Schrader & O'Malley, 1972; Hansen et al., 1976) but differ in their interactions with nuclear constituents (Schrader & O'Malley, 1972; Schrader et al., 1972, 1975). Proteolytic digestion studies (Vedeckis et al., 1980; Sherman et al., 1974, 1976; Sherman & Diaz, 1977) have established the structural similarity of the two proteins, but peptide mapping studies have indicated that they are separate gene products (N. L. Weigel, unpublished experiments). Until now, no antibodies to chick progesterone receptor have been available for analysis of this receptor. In order to analyze their structural and functional domains, we attempted to raise antibodies in sheep to purified progesterone receptor A and B subunits. The approach has been sucessfully undertaken to raise antibodies to calf uterine estrogen receptor (Greene et al., 1977). During these studies, we found that about one-third of the sheep tested had an endogenous immunoglobulin activity of low titer which reacts with the chick progesterone receptor. This activity is reminiscent of "5S-CA" activity described by Fox (1978) but differs in that this immunoglobulin recognizes cytoplasmic receptors. This paper describes the interaction of these sheep sera with the chick receptor subunits and with proteolytic fragments derived from the receptor.

Materials and Methods

[1,2-$^3H_2$]Progesterone (50 Ci/mmol) and [6,7-$^3H_2$(N)]-triamcinolone acetonide (37 Ci/mmol) were purchased from New England Nuclear Co. R5020, [17α-*methyl*-^{3}H]-17α,21-dimethyl-19-nor-4,9-pregnadiene-3,20-dione (55.4 Ci/mmol), was from Roussel-UCLAF, Romaineville, France. Nonradioactive steriods were obtained from Steraloids.

All chemicals were reagent grade. Tris, ammonium sulfate, and sucrose were ultrapure grade from Schwarz/Mann. Ion-exchange resins were from Whatman. Poly(ethylene

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