Articles

Interaction of Rat Liver Glucocorticoid Receptor with Heparin†

Alessandro Weisz, Giovanni Alfredo Puca, Maria Teresa Masucci, Carlo Masi, Rita Pagnotta, Antonella Petrillo, and Vincenzo Sica*

ABSTRACT: When rat liver cytosol containing [^{3}H]dexamethasone–glucocorticoid receptor complex is exposed to immobilized heparin (Sepharose–heparin; Seph–hep) the steroid receptor complex binds to the substituted Sepharose avidly [K_d = 3.5 (±1.7) × 10^{-10} M], and 80–90% of the receptor present is adsorbed to the solid phase after 40 min at 0 °C. The binding is enhanced by Mn^{2+} (10 mM) and Mg^{2+}, whereas Ca^{2+} and Sr^{2+} are ineffective. Sodium molybdate (10 mM) does not influence the reaction but enhances receptor stability. Moreover, binding of the receptor to Seph–hep is dependent on the ionic strength of the medium, because binding is totally reversed by 300 mM KCl. The bound [^{3}H]dexamethasone–receptor complex can be recovered from Seph–hep with solutions (4 mg/mL) of heparin (95% release), dextran sulfate (88%), and chondroitin sulfate (63%); total calf liver RNA is less effective (9%), whereas dextran, D-glucosamine, *N*-acetyl-D-glucosamine, D-glucuronic acid, and sheared calf thymus DNA are totally ineffective (<3%). Both "native" and temperature "transformed" forms of the glucocorticoid receptor interact with immobilized heparin. These results strongly suggest that the receptor site that binds heparin is distinct from that binding DNA. An immediate application of this newly found ability of the glucocorticoid receptor to interact with heparin is the use of Seph–hep for affinity chromatography purification of the glucocorticoid receptor. A purification of 10-fold, with a recovery of 55–65%, can be achieved by using either 4 mg/mL heparin or 300 mM KCl to elute [^{3}H]dexamethasone–receptor bound to the resin.

Considerable progress has been made in the past 20 years in our knowledge of the interaction of steroid hormones with their target cells, and the main steps of the mechanism of action of these hormones have been elucidated (Gorski & Gannon, 1976; Jensen & De Sombre, 1973; Liao, 1975; O'Malley & Means, 1974; Lan et al., 1984). The action of steroids is initiated by binding to intracellular receptor proteins located in the cytoplasm of target cells. After binding of the hormone, receptors undergo some modifications and translocate to the nucleus, where they interact with nuclear component(s). Although the detailed mechanism is not clear, this interaction appears to cause an increase in the production of certain species of RNA and in the synthesis of proteins that mediate the hormonal action.

From the above described model of the mechanism of action of steroid hormones it seems that at least two functionally distinct domains must be present in the steroid receptor molecules: one is the portion that binds the specific hormone, another is the region binding to the chromatin and/or to other nuclear component(s). Although this model still holds to criticisms, a consistent set of data is accumulating that tend to demonstrate that this may be an oversimplificiation. In the case of the glucocorticoid receptor, for example, a third "immunoreactive" domain has been identified (Carlstedt-Duke et al., 1982), and although its function is not clear, mutated receptors, lacking this portion of the molecule, are inactive (Stevenss & Stevens, 1981; Gehring & Hotz, 1983). Moreover, it has been found that this receptor, when extracted in low ionic strength buffers, may be associated with RNA, thus implying that an RNA binding site may be also present (Rossini & Barbiroli, 1983; Tymoczko & Phillips, 1983; Weisz & Lan, 1983).

In the last few years it has been found that enzymes which act on nucleic acids (Sternbach et al., 1975; Bickle et al., 1977; Jaehning et al., 1977; Teissere et al., 1977; Brennessel et al., 1978; Falco et al., 1978; Pflugfelder & Sonnenbichler, 1978; Spindler et al., 1978; Zillig et al., 1978), protein synthesis factors (Waldman et al., 1975; Slobin, 1976; Hradec & Dusek, 1978; Hradec & Kriz, 1978), and steroid receptors (Molinari et al., 1977; Sica & Bresciani, 1979; Mulder et al., 1979; Mainwaring & Johnson, 1980; Yang et al., 1982) interact with heparin, a natural polyanionic mucopolysaccharide which is present in mammals.

The physiological role, if any, of steroid receptor–heparin interaction is not yet understood; nevertheless this property has been very useful in inhibiting the aggregation of estrogen receptor with other proteins in the cytosol (Sica & Bresciani, 1979). Moreover, heparin covalently linked to agarose has been a very helpful tool for the purification of estrogen receptor (Molinari et al., 1977; Sica & Bresciani, 1979).

In this study it is demonstrated that rat liver cytosolic glucocorticoid receptor binds to heparin with very high apparent affinity. This property is common to the "native" and "transformed" forms of this receptor and permits its easy and fast partial purification. Since this ability of the glucocorticoid receptor to interact with heparin may reflect important and unrecognized physiological functions, different hypotheses are considered for discussion.

Materials and Methods

All reagents were of analytical grade. [1,2,4,*N*-^{3}H]Dexamethasone (9α-fluoro-16α-methyl-11α,17α,21-trihydroxypregna-1,4-diene-3,20-dione; specific activity 40 Ci/mmol) was from Amersham. Unlabeled dexamethasone, Trizma base [tris(hydroxymethyl)aminomethane (Tris)] ethylenediaminetetraacetic acid (EDTA), sodium molybdate, heparin (grade I, specific activity 160.9 USP units/mg), calf thymus DNA, total calf liver RNA, D-glucuronic acid, and D-glucosamine were purchased from Sigma, dithiothreitol (DTT)

† From the Institute of General Pathology and Oncology, University of Naples, 1st School of Medicine, Naples, Italy. *Received February 23, 1984.* Research supported by CNR, special project "Control of Neoplastic Growth", Grant 82-00390-96, and NIH Contract NO1-CB-64074.

0006-2960/84/0423-5393$01.50/0

was from Calbiochem, Sepharose 4B-CL and Dextran T70 were from Pharmacia, Norit A was from Matheson Coleman & Bell, sucrose, "ultrapure" grade, was from Schwarz/Mann, DE-52 was from Whatman, and *N*-acetyl-D-glucosamine, dextran sulfate, and chondroitin sulfate were from Serva.

Cytosol Preparation. Intact Sprague-Dawley rats were killed by cervical dislocation. The livers were perfused in situ via portal vein with ice-cold TSMGD buffer (20 mM Tris, pH 7.4, 134 mM NaCl, 10 mM $MgCl_2$, 0.1 mM DTT, 10% glycerol), removed, placed in TSED buffer (50 mM Tris, pH 7.4, 0.25 M sucrose, 1 mM EDTA, and 2 mM DTT), rinsed, minced in small pieces, and gently homogenized in 2 volumes of TSED buffer with a Ultraturrax tissue homogenizer. Homogenization was obtained with six 15-s bursts at 30 V, intercalated by 60-s period of cooling. This procedure was necessary to avoid receptor proteolysis due to the release of lysosomal enzymes, as described by Carlstedt-Duke et al. (1977).

The homogenate was centrifuged for 45 min at 300000*g*, in a Spinco L5-75 ultracentrifuge. After centrifugation the supernatant (cytosol) was collected, avoiding the fat layer.

Determination of [^{3}H]Dexamethasone Binding Capacity. The samples to be tested were brought to 0.5 mL with TSED buffer containing [^{3}H]dexamethasone to a final concentration of 6.25×10^{-9} M and incubated for 150 min at 20 °C or overnight (18–20 h) at 4 °C. Separation of free from macromolecule-bound hormone was accomplished by incubating the samples with an equal volume of dextran-coated charcoal suspension (DCC; 0.05% Dextran T70, 0.5% Norit A) for 15 min at 4 °C. After centrifugation at 5000*g* for 5 min, 0.5 mL of supernatant was added to 10 mL of Ready Solv MP (Beckman) scintillation cocktail, and the radioactivity was measured in a Beckman LS 7500 scintillation spectrometer, with an efficiency of 35–45%. The nonspecific binding was measured by incubating samples with and without 200-fold molar excess of radioinert dexamethasone.

Preparation of Heparin–Sepharose. The affinity matrix was prepared with Sigma grade I heparin and Sepharose 4B-CL as described previously (Sica & Bresciani, 1979).

The concentration of heparin bound to the gel was calculated to be between 0.35 and 0.60 mg/g of dry gel by measuring the difference between the concentrations of heparin in the coupling solution before and after the reaction.

Receptor "Transformation" and Analysis on DEAE-cellulose. Five milliliters of cytosol, preincubated with [^{3}H]-dexamethasone, was heated at 20 °C for 30 min or kept in an ice bath. After treatment with DCC, the samples were analyzed on 3-mL DE-52 columns, equilibrated in TSED buffer, as described by Sakaue & Thompson (1977). Elution of bound receptor was achieved with a linear KCl gradient from 0 to 400 mM.

The two peaks of radioactivity eluted respectively at 40 (peak I) and 130 (peak II) mM KCl. Peak I contained prevalently transformed receptor and peak II native receptor as tested with the DNA–cellulose binding assay described by Kalimi et al. (1975).

Protein Assay. Protein determinations were performed with the Bio-Rad protein assay based on the work of Bradford (1976). In the case of samples containing heparin, which interfere with this assay, protein content was calculated by measurement of the optical density.

Results

To test the ability of glucocorticoid receptor to interact with immobilized heparin, rat liver cytosol was first incubated with a saturating concentration of [^{3}H]dexamethasone and, following removal of the excess unbound steroid by DCC treatment, was diluted with homogenization buffer to obtain the desired concentration of receptor. Subsequently, 0.2 mL of sample was incubated for 1 h at 4 °C with 0.1 g of Sepharose–heparin under gentle agitation. After centrifugation, the radioactivity of the supernatants was measured, and the amount of receptor bound to the resin was calculated. The results shown in Figure 1 suggest that this is a high-affinity interaction with an apparent dissociation constant (K_d) of 3.5 ($\pm$1.7) $\times 10^{-10}$ M. The Scatchard plot shown in Figure 1B demonstrates that the binding reaction is saturable and that under these conditions this batch of Sepharose–heparin is able to bind up to 15 nmol of receptor/g of resin.

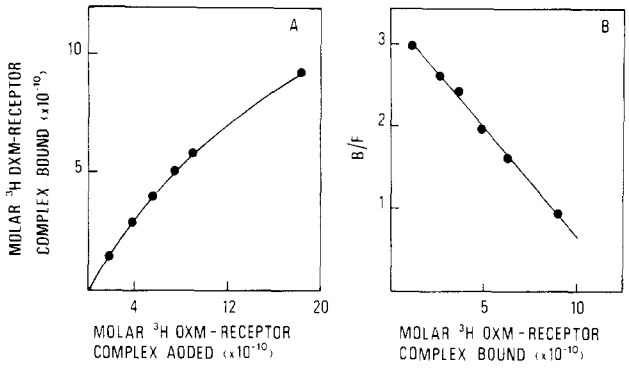

FIGURE 1: Affinity of the glucocorticoid receptor for immobilized heparin. (A) Duplicate 100-mg aliquots of Sepharose–heparin were incubated for 1 h at 4 °C with the indicated amounts of rat liver glucocorticoid receptor. These were prepared by scalar dilution in a final volume of 0.2 mL of cytosol prepared as described under Materials and Methods and charged overnight at 4 °C with 6.25×10^{-9} M [^{3}H]dexamethasone (cytosol specific binding activity 40407 dpm/0.1 mL). At the end of the incubation, samples were centrifuged at 2000*g*, and aliquots of 0.1 mL of supernatant were assayed for radioactivity. (B) Data plotted according to Scatchard.

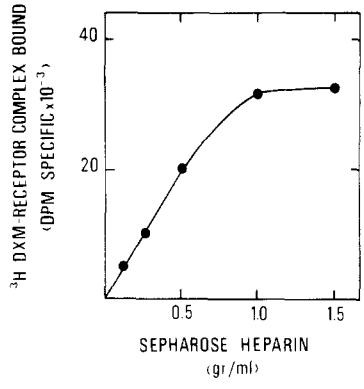

FIGURE 2: Optimization of the interaction of glucocorticoid receptor with Sepharose–heparin. To 0.1-mL duplicate aliquots of cytosol, presaturated with [^{3}H]dexamethasone, was added Sepharose–heparin to the indicated concentration. After 1 h at 4 °C the samples were centrifuged, and the receptor present in the supernatants was measured by DCC adsorption. Specific dpm/0.1 mL of cytosol was 40420.

When the binding of ligand-free receptor was tested, there was inactivation of the receptor that had interacted with the resin, since no steroid binding activity could be recovered (data not shown).

The binding reaction is completed in 40–60 min of batchwise incubation at 4 °C. The amount of Sepharose–heparin per unit of cytosol volume required for optimal glucocorticoid receptor binding is 1 g/mL (Figure 2) with 80–90% of the receptor present bound by the resin. Higher concentrations of Sepharose–heparin increased the nonspecific binding of proteins, without increasing the amount of receptor bound (not shown).

Table I: Effect of Cations on the Binding of Glucocorticoid Receptor to Sepharose–Heparin[a]

	[^{3}H]dexamethasone–receptor complex bound (dpm)	%
1 mM NaEDTA	40 120 ± 1620	100
5 mM $CaCl_2$	42 780 ± 1980	107
5 mM $MgCl_2$	47 910 ± 2080	119
5 mM $MnCl_2$	54 630 ± 1550	136
5 mM $SrCl_2$	36 050 ± 2130	90

[a] Aliquots of 0.2 mL of cytosol in TSED buffer, presaturated with 6.25×10^{-9} M [^{3}Hdexamethasone ± 200-fold excess of dexamethasone and treated with DCC, were incubated for 1 h at 4 °C with 100 mg of Sepharose–heparin in the presence of the indicated salts. After centrifugation, 0.1-mL aliquots of supernatant were assayed for radioactivity. The given values are means (±SD) of three experiments done in duplicate and were corrected for nonspecific binding.

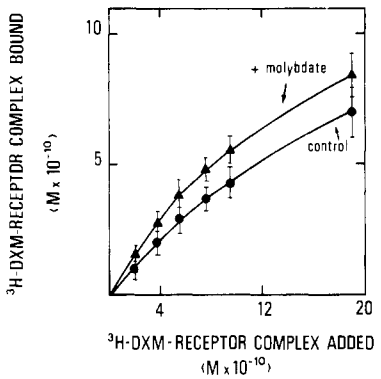

FIGURE 3: Effect of sodium molybdate on the interaction of glucocorticoid receptor with Sepharose–heparin. Rat liver cytosol, presaturated with [^{3}H]dexamethasone and DCC treated, either was incubated directly with Sepharose–heparin (●) or was first brought to 10 mM sodium molybdate (▲). The experiment was carried out as described in the legend of Figure 1. Specific dpm/0.1 mL of cytosol = 42 210.

Since cations are able to influence the interaction between proteins and polyanions such as DNA, RNA, and heparin, the effect of millimolar concentrations of different cations on the binding of the cytosolic glucocorticoid receptor to heparin was tested. As shown in Table I, the most effective is Mn^{2+} followed by Mg^{2+}. Ca^{2+} and Sr^{2+} are ineffective under these conditions. The optimal concentration of Mn^{2+} was found to be between 10 and 12 mM. At this concentration the cation increased the amount of glucocorticoid receptor bound by 40% (not shown).

Sodium molybdate is a salt that influence greatly the physicochemical parameters of the glucocorticoid receptor, increasing its stability (Weisz et al., 1984). The use of 10 mM sodium molybdate would be very useful in preventing receptor inactivation occurring during purification. For this reason the effects of this ion on the interaction between glucocorticoid receptor and heparin were tested. As shown by the experiment reported in Figure 3, molybdate does not greatly influence the binding reaction. An increase in the amount of [^{3}H]dexamethasone–receptor complex bound to the matrix is evident in the presence of molybdate, but the apparent affinity between the receptor and heparin remains unchanged. The most likely explanation is that this ion stabilizes the steroid binding ability of the receptor, thus increasing the amount of [^{3}H]dexamethasone bound at the end of the reaction. A direct effect on the binding of the glucocorticoid receptor to Sepharose–heparin cannot, however, be excluded.

The nature and reversibility of the interaction between rat liver glucocorticoid receptor and heparin were investigated by testing the ability of different compounds to release [^{3}H]-

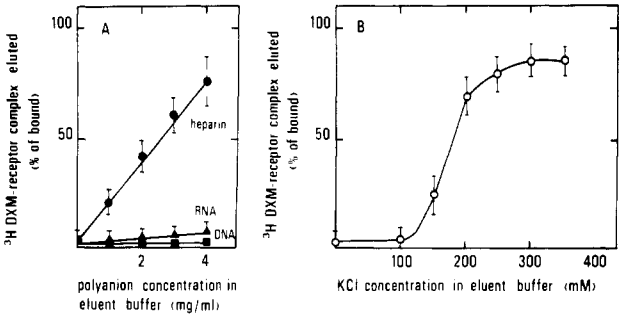

FIGURE 4: Effect of heparin, RNA, DNA, and potassium chloride on the interaction of glucocorticoid receptor with Sepharose–heparin. A total of 1 mL of rat liver cytosol, preincubated with a saturating concentration of [^{3}H]dexamethasone and treated with DCC, was filtered through 1-mL Sepharose–heparin columns. The columns were washed with 5 mL of TSED buffer alone and subsequently with 5 mL of buffer containing heparin (●), total calf liver RNA (▲), sonicated calf thymus DNA (■), (A) or KCl (○) (B), at the indicated concentrations. Radioactivity was measured in 0.1-mL aliquots of the effluent fractions. All columns retained between 68 and 79% of input receptor. Specific dpm/0.1 mL of cytosol was 37 140.

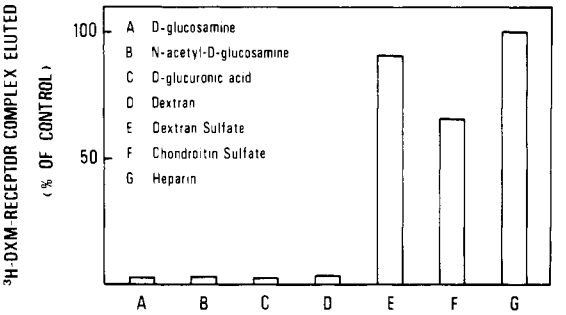

FIGURE 5: Effect of mono- and polysaccharides on the interaction of glucocorticoid receptor with Sepharose–heparin. One-milliliter rat liver cytosol samples, preincubated with 6.25×10^{-9} M [^{3}H]dexamethasone and treated with DCC, were filtered through 1-mL Sepharose–heparin columns. The columns were washed with 5 mL of TSED buffer alone and subsequently with 5 mL of buffer containing 4 mg/mL samples of the indicated substances. Radioactivity was measured in 0.1-mL aliquots of effluent fractions. All columns retained between 75 and 85% of input receptor. Heparin eluted 96% of receptor bound, and specific dpm/0.1 mL of cytosol was 40 045.

dexamethasone–receptor complex previously bound to Sepharose–heparin. In the experiment shown in Figure 4, rat liver cytosol was incubated with [^{3}H]dexamethasone prior to DCC treatment followed by exposure columnwise to Sepharose–heparin. After washing with homogenization buffer, the columns were washed with buffer containing the indicated amounts of KCl, total calf liver RNA, sonicated calf thymus DNA, or heparin. Aliquots of the effluent fractions were tested for bound [^{3}H]dexamethasone. KCl (300 mM) eluted 85% of the bound receptor, whereas 100–150 mM salt induced little receptor release. Thus, the interaction between the glucocorticoid receptor and heparin can withstand buffers of physiological ionic strength. When heparin was used for elution, a linear relationship was observed between its concentration in the elution buffer and the amount of receptor recovered. The aminoglycan (4 mg/mL) induced release of 75–95% of bound receptor. Sheared DNA was totally ineffective, and total cellular RNA induced a very slight, concentration-dependent release of receptor from the columns.

In the experiment shown in Figure 5, the same procedure described above was followed. The elution of [^{3}H]dexamethasone–receptor complex from the Sepharose–heparin columns achieved with 4 mg/mL solutions of D-glucosamine, *N*-acetyl-D-glucosamine, D-glucuronic acid, dextran, dextran sulfate, and chondroitin suflate was compared with that in-

Table II: Binding of Native and Temperature Transformed Glucocorticoid Receptor to Sepharose–Heparin[a]

		cpm total	%
0 °C			
peak I[b] (transformed)	control	87.070	100.0
	break-through	7.850	8.1
peak II[b] (native)	control	879.740	100.0
	break-through	113.330	12.9
20 °C			
peak I (transformed)	control	288.270	100.0
	break-through	9.280	3.2
peak II (native)	control	358.850	100.0
	break-through	17.060	4.8

[a] Cytosol samples (10 mL) preincubated with [^{3}H]dexamethasone were either kept in ice or heated at 20 °C for 30 min prior to DCC treatment; 5 mL was then chromatographed on Sepharose–heparin (5 mL bed volume). The receptor not adsorbed to Sepharose–heparin (break-through) or 5 mL of cytosol (control) was then analyzed on DEAE-cellulose chromatography. Results given are representative of two separate experiments. [b] Peaks I and II were named after Sakaue & Thompson (1977). Peak I corresponds to the transformed and peak II to the native forms of glucocorticoid receptor. For details see Materials and Methods.

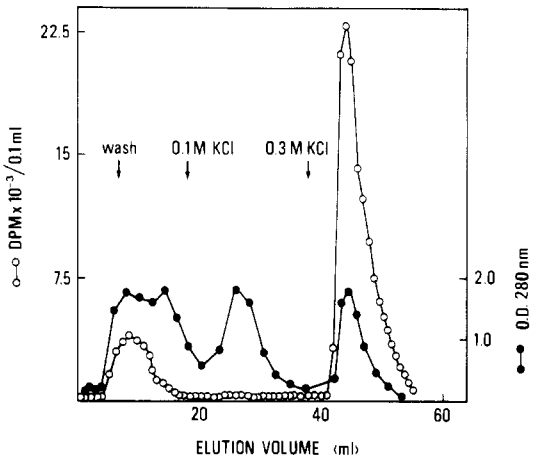

FIGURE 6: Purification of glucocorticoid receptor by means of Sepharose–heparin affinity chromatography. A total of 5 mL of [^{3}H]dexamethasone-labeled cytosol was treated with DCC before applying on a 5-mL Sepharose–heparin column. The column was washed with 10 mL of buffer, 20 mL of buffer plus 0.1 M KCl, and 20 mL of buffer plus 0.3 M KCl; 1-mL fractions were collected and were assayed for bound radioactivity (O) and relative protein content (OD = 280 nm) (●). Specific dpm/0.1 mL of cytosol was 38 990.

duced by an equally concentrated solution of heparin.

The most efficient in inducing the release of the glucocorticoid receptor from the resin is dextran sulfate (88% release), followed by chondroitin sulfate (63%), whereas D-glucosamine, *N*-acetyl-D-glucosamine, D-glucuronic acid, and dextran were completely inefficient (<3%).

Glucocorticoid receptors may exist in vitro in two distinct molecular forms: native (or unactivated) and transformed (or activated) that differ from each other on their physicochemical parameters and on their ability to interact with DNA or nuclei (Schmidt & Litwack, 1982).

Since both forms may be present simultaneously in cytosol, simple binding assay would not give enough information on the ability of each one of these two receptor forms to bind to Sepharose–heparin. For this reason the experiment reported in Table II was performed. Cytosol was incubated either at 0 °C (to reduce the concentration of temperature-transformed receptor to a minimum), or to 20 °C for 30 min (to induce transformation). Aliquots of cytosol so treated were analyzed by DEAE-cellulose chromatography either before or after exposure to Sepharose–heparin. As shown in Table II, both receptor forms do bind to the aminoglycan. It should be noted, however, that since this method does not give any information on the affinity of each receptor form for heparin, affinities could differ between the two forms.

An immediate application of this newly found property of the glucocorticoid receptor is that Sepharose–heparin can be successfully employed for purification of this receptor (Figure 6). At the present time a purification of 10-fold, with a recovery of 55–65% can be easily achieved in a very short time and without exposing the receptor to particularly critical conditions.

Discussion

The results presented in this report demonstrate that rat liver cytosolic glucocorticoid receptor is able to interact with heparin in vitro. The significance of these findings is still unclear; however, the data reveal some interesting possibilities and useful applications.

In view of our results it is less likely that the interaction between heparin and the receptor is purely of electrostatic nature. It should be noted that this interaction is stable in the presence of 100 mM KCl and is disrupted completely only by 300 mM salt. Moreover, the high binding affinity, the apparent saturability, and the complete reversibility with heparin but not, for example, with DNA are signs of the presence in the receptor of a highly specific binding site. In this regard, it has to be noted that the bacterially produced dextran, when sulfated, is more efficient in displacing glucocorticoid receptor bound to heparin than chondroitin sulfate and almost as efficient as heparin (Figure 5). Thus, in view of the fact that non-sulfated dextran and the components of heparin itself (D-glucosamine and D-glucuronic acid) are ineffective, it seems that the major requirement for recognition by the glucocorticoid receptor is the presence of negatively charged groups in large numbers and in the correct spatial configuration.

It is also possible to postulate that the binding of the glucocorticoid receptor to this aminoglycan is indirect, mediated via a cytosolic component aggregated with the receptor. Although possible this would mean that all receptor molecules present in the extract are associated with this component, since more than 90% of the receptor binds to heparin under appropriate conditions, and that the association of the receptor with this hypothetical component is very tight, resisting to relatively highly ionic strength. There is also preliminary evidence that glucocorticoid receptor partially purified by affinity chromatography is still able to bind tightly to heparin (not shown).

On the other hand, the finding that steroid receptors other than the glucocorticoid are able to interact with heparin leads to the more interesting hypothesis that this is in fact a physiologically relevant property of these receptors.

Since several proteins that interact with nucleic acid are also able to bind well to Sepharose–heparin, the possibility arises that the binding to heparin mimics the binding to a nucleic acid. The glucocorticoid receptor, when transformed, is able to bind to DNA; thus, DNA could be an interesting candidate. However, the native, untransformed receptor does not bind to DNA whereas it binds well to heparin, and soluble DNA is unable to extract the receptor previously bound to Sepharose–heparin. The DNA binding site of the glucocorticoid receptor seems thus not to be implied in the recognition of heparin.

More interesting is, instead, the possibility that the ability of the glucocorticoid receptor to bind heparin is a signal of the presence in the receptor of an RNA binding site. In fact

experimental evidence is accumulating that tends to demonstrate that the glucocorticoid receptor is able to bind to RNA (Costello & Sherman, 1980; Chong & Lippman, 1982; Hutchens et al., 1982; Rossini & Barbiroli, 1983; Tymoczko & Phillips, 1983; Weisz & Lan, 1983). When total RNA was used to extract heparin-bound glucocorticoid receptor, a very slight elution was achieved. This, however, could be explained by the fact that only a small fraction of the total RNA may be recognized by the receptor specifically and that this fraction may be diluted by the bulk of the other RNAs. Moreover, the RNA could have been digested by RNase present in the crude cytosol preparations, thus loosing its proper conformation and size or reducing its concentration.

There is finally a last possibility that the glucocorticoid receptor recognizes heparin not only in vitro but also in vivo. Since steroid receptors have been found to bind to membranes and to the cytoskeleton (Parikh et al., 1980; Puca & Sica, 1981; Puca et al., 1981; Szego & Pietras, 1981) and since aminoglycans such as heparin are present in these cellular components, binding of receptor to heparin could mean that steroids act directly at the membrane or cytoskeleton level and/or that these components are involved in the mechanism of steroid hormone action.

Registry No. Mn, 7439-96-5; Mg, 7439-95-4.

References

Bickle, T. A., Pirotta, V., & Imber, R. (1977) *Nucleic Acids Res. 4*, 2561–2572.

Bradford, M. (1976) *Anal. Biochem. 72*, 248–254.

Brennessel, B. A., Buhrer, D. P., & Gottlieb, A. A. (1978) *Anal. Biochem. 87*, 411–417.

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

Carlstedt-Duke, J., Okret, S., Wrange, O., & Gustafsson, J. A. (1982) *Proc. Natl. Acad. Sci. U.S.A. 79*, 4260–4264.

Chong, M. T., & Lippman, M. E. (1982) *J. Biol. Chem. 257*, 2996–3002.

Costello, M. A., & Sherman, M. R. (1980) *Annu. Meet. Endocr. Soc., 62nd*, 174 (Abstract).

Falco, S. C., Zivin, R., & Rothman-Denes, L. B. (1978) *Proc. Natl. Acad. Sci. U.S.A. 75*, 3220–3224.

Gehring, U., & Hotz, A. (1983) *Biochemistry 22*, 4013–4018.

Gorski, J., & Gannon, F. (1976) *Annu. Rev. Physiol. 38*, 425–450.

Hradec, J., & Dusek, Z. (1978) *Biochem. J. 172*, 1–7.

Hradec, J., & Kriz, O. (1978) *Biochem. J. 173*, 349–352.

Hutchens, T. W., Markland, F. S., & Hawkins, E. F. (1982) *Biochem. Biophys. Res. Commun. 105*, 20–27.

Jaehning, J. A., Woods, P. S., & Roeder, R. G. (1977) *J. Biol. Chem. 252*, 8762–8771.

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

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

Lan, N. C., Karin, M., Nguyen, T., Weisz, A., Birnbaum, M. J., Eberhardt, N. L., & Baxter, J. D. (1984) *J. Steroid Biochem. 20*, 77–88.

Liao, S. (1975) *Int. Rev. Cytol. 41*, 87–172.

Mainwaring, W. I. P., & Johnson, A. D. (1980) in *Perspectives in Steroid Receptor Research* (Bresciani, F., Ed.) pp 89–97, Raven Press, New York.

Molinari, A. M., Medici, N., Moncharmont, B., & Puca, G. A. (1977) *Proc. Natl. Acad. Sci. U.S.A. 74*, 4886–4890.

Mulder, E., Foekens, J. A., Peters, M. J., & Van der Molen, H. J. (1979) *FEBS Lett. 97*, 260–264.

O'Malley, B. W., & Means, A. R. (1974) *Science (Washington, D.C.) 183*, 610–620.

Parikh, I., Anderson, W. L., & Neame, P. (1980) *J. Biol. Chem. 255*, 10266–10270.

Pflugfelder, G., & Sonnenbichler, J. (1978) *FEBS Lett. 93*, 361–364.

Puca, G. A., & Sica, V. (1981) *Biochem. Biophys. Res. Commun. 30*, 682–689.

Puca, G. A., Nola, E., Molinari, A. M., Armetta, I., & Sica, V. (1981) *J. Steroid Biochem. 15*, 307–312.

Rossini, G. R., & Barbiroli, B. (1983) *Biochem. Biophys. Res. Commun. 113*, 876–882.

Sakaue, Y., & Thompson, E. B. (1977) *Biochem. Biophys. Res. Commun. 77*, 533–541.

Schmidt, T. J., & Litwack, G. (1982) *Physiol. Rev. 62*, 1131–1192.

Sica, V., & Bresciani, F. (1979) *Biochemistry 18*, 2369–2378.

Slobin, L. I. (1976) *Biochem. Biophys. Res. Commun. 73*, 539–547.

Spindler, S. R., Duester, G. L., D'Alessio, J. M., & Marvin, R. P. (1978) *J. Biol. Chem. 253*, 4669–4675.

Sternbach, H., Engelhardt, R., & Lezius, A. G. (1975) *Eur. J. Biochem. 60*, 51–55.

Stevens, J., & Stevens, J. W. (1981) *Cancer Res. 41*, 125–133.

Szego, C. M., & Pietras, R. J. (1981) *Biochem. Actions Horm. 8*, 307–463.

Teissere, M., Penon, P., Azou, Y., & Richard, J. (1977) *FEBS Lett. 82*, 77–81.

Tymoczko, J. L., & Phillips, M. M. (1983) *Endocrinology (Baltimore) 112*, 142–149.

Waldman, A. A., Marx, G., & Golstein, J. (1975) *Proc. Natl. Acad. Sci. U.S.A. 72*, 2352–2356.

Weisz, A., & Lan, N. C. (1983) *Proc. Int. Symp. J. Steroid Biochem., 6th*, 17 (Abstract).

Weisz, A., Baxter, J. D., & Lan, N. C. (1984) *J. Steroid Biochem. 20*, 289–293.

Yang, C. R., Mester, J., Wolfson, A., Renoir, J., & Baulieu, E. E. (1982) *Biochem. J. 208*, 399–406.

Zillig, W., Stetter, K. O., & Tobien, M. (1978) *Eur. J. Biochem. 91*, 193–199.