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Evidence for the Presence of Specific Binding Sites for Corticoids in Mouse Liver Plasma Membranes

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Summary. The specific binding of [^{3}H]cortisol to plasma membranes purified from mouse liver, studied by the ultrafiltration method, shows the existence of specific binding sites for cortisol. The kinetic parameters of this binding are K_D = 4.4 nM and B_{max} = 685 fmol/mg protein in presence of 1 μM of corticosterone. With respect to the binding of 4 nM [^{3}H]cortisol to the membrane, the affinities of the steroids decreased in the following order: deoxycorticosterone > corticosterone > progesterone > cortisol > prednisolone > testosterone > 20β-hydroxyprogesterone > cortisone. Estradiol, dexamethasone, ouabain and triamcinolone acetonide do not have affinity for this binding site. Neither Ca^{2+} nor Mg^{2+} affected the binding of [^{3}H]cortisol to the plasma membranes. Likewise, the presence of agonists and antagonists of alpha and beta-adrenergic receptors did not modify the binding of [^{3}H]cortisol. The results suggest that the plasma membrane binding site characterized is more specific for corticoids and is different from nuclear glucocorticoid and progesterone receptors.

Key Words glucocorticoids · cortisol · steroids · binding sites · plasma membrane · mouse liver

Introduction

The mechanism of steroid hormone action in a target cell has been assumed to be initiated by the binding of hormone to the receptor in the cytosol. The interaction with the nuclear chromatin induces the expression of specific genes.

However, it has been demonstrated recently that this initial binding process occurs exclusively in the nuclear compartment (King & Greene, 1984; Perrot-Applanat et al., 1986). In this way, Grote, Voight and Sekeris (1983) reported the absence of correlation between binding of glucocorticoids to cytosolic receptors and enzyme induction.

It is usually believed that steroid hormones penetrate into the cell by passive diffusion (Giorgi, 1980; Lovell-Smith & García-Webb, 1986). This model is based on the lipophilic nature of steroid molecules and on their affinity for lipid constituents of the plasma membranes (Giorgi & Stein, 1981). However, other investigations in this field of transmembrane movement suggest that there exists another possible process of steroid transport into the target cells. Milgrom, Atger and Baulieu (1973) proposed an active mode of transport of estradiol into uterine cells. Pietras and Szego (1977) characterized the estradiol binding to membrane of uterine cells. Zanker, Prokscha and Blumel (1980) suggested the existence of an estrogen membrane receptor of low affinity and high capacity on breast cancer cells. Pietras and Szego (1980) characterized estrogen receptors in subfractions of hepatic cell plasma membranes. Moreover, it has been shown that steroids penetrate into the target cells not by free diffusion, but by specific transport mechanisms (Rao, 1981; Szego & Pietras, 1981; Allera & Rao, 1986). Other works have confirmed the existence of specific binding sites in plasma membranes for glucocorticoids (Suyemitsu & Terayama, 1975; Rao et al., 1976, 1977; Koch et al., 1978; Fant, Harbison & Harrison, 1979; Allera, Rao & Breuer, 1980; Towle & Sze, 1983; Savart & Cabillic, 1985; Allera & Rao, 1986).

Previous studies in our laboratory have shown that cortisol, estradiol and testosterone have a glycogenolytic effect on chicken liver (Egaña, Sancho & Macarulla, 1981; Díez et al., 1984; Sancho et al., 1986; Sanchez-Bueno et al., 1987). This effect is previous to and independent of protein synthesis

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activation, and it is accompanied by a decrease in the cAMP levels and by an increase in cGMP.

Recently, we have characterized a specific binding site for cortisol in chicken liver plasma membranes (Trueba et al., 1987). Also, it has been shown that cortisol has the same physiological effect on mouse liver (Vallejo et al., 1986). From these results, the possibility of finding glucocorticoid binding sites in plasma membranes purified from mouse liver has been studied. This cortisol-membrane interaction could explain the early glycogenolytic effect previously mentioned.

Materials and Methods

Chemicals

[1,2,6,7-^{3}H]Cortisol (91 Ci/mmol) was obtained from Amersham International (England). Corticosterone, cortisol, cortisone, deoxycorticosterone, 20β-hydroxyprogesterone, dexamethasone, estradiol, ouabain, prednisolone, progesterone, triamcinolone acetonide, propranolol and HEPES were purchased from Sigma Chemical (St. Louis, MO). Phentolamine was kindly donated by Ciba-Geigy laboratories (Barcelona, Spain). PPO, POPOP and scintillation grade toluene were supplied by Scharlau (Barcelona, Spain). All buffering components were obtained from Merck (Darmstadt, Germany).

Isolation of Plasma Membranes

Livers were perfused and removed from Swiss male mice weighing about 30 g, homogenized in 4 vol 0.25 M sucrose-5 mM Tris buffer, pH 8.0, and separated into cell fractions as described (Aronson & Touster, 1974). Plasma membranes were purified by centrifugation in sucrose gradient for 16 hr at 75,500 $\times$ g. The plasma membrane fractions were usually washed with buffer (140 mM NaCl, 5.4 mM KCl, 1.8 mM $CaCl_2$, 0.8 mM $MgCl_2$ and 25 mM Tris/HCl, pH 7.4) except in the experiments indicated. After centrifugation at 78,000 $\times$ g the pellets were suspended in the incubation buffer (140 mM NaCl, 5.4 mM KCl, 1.8 mM $CaCl_2$, 0.8 mM $MgCl_2$, 5.0 mM glucose and 25 mM Tris/HCl, pH 7.4) for use in binding studies.

Purified plasma membranes were stored at -80°C in an ultra low temperature freezer, Selecta Conbatemp model (Barcelona, Spain). Proteins were determined by the method of Lowry et al. (1951) using bovine serum albumin as standard.

Electron Microscopy

The negative staining technique has been applied to the study of plasma membranes (Munn, 1974). The plasma membrane suspension was diluted with incubation buffer without glucose, to a final protein concentration of 2 mg/ml. Osmium tetroxide in phosphate buffer was added in the rate 1 : 1. After 30 min at 4°C, a small aliquot was sprayed onto the grid. An equally small drop of 2% sodium phosphotungstate was applied to the grid, and the excess of liquid was removed by touching the edge with a piece of filter paper. The samples were examined in a Zeiss E.M. 10CR electron microscope.

Marker Enzyme Analysis of the Plasma Membrane Fragments

The plasma membrane fragments were tested for specific activities of the following enzymes: 5′-nucleotidase (Aronson & Touster, 1974), Mg^{2+} and Na^+-K^+-ATPase (Quigley & Gotterer, 1969), phosphodiesterase I, glucose-6-phosphatase (Aronson & Touster, 1974) and LDH (Bergmeyer & Bernt, 1974). The first four enzymes are markers for the plasma membrane and the last two are specific for the endoplasmic reticulum and cytosol.

Binding Assay of [^{3}H]Cortisol

Incubation mixtures for binding studies contained 0.1 ml of purified plasma membrane fraction (0.2 mg protein/ml final concentration), 0.1 ml of [^{3}H]cortisol and 0.8 ml of incubation buffer. The presence of specific cortisol binding sites was determined by the difference between the radioactivity bound in the presence of an excess of unlabeled corticosterone (nonspecific binding) and the radioactivity bound to the membranes incubated with [^{3}H]cortisol only (total binding).

Incubation assays were performed in an ice bath. At the end of the incubation period, three 800-μl aliquots were filtered through GF/C fiber filter (Whatman, England) placed in a twelve-place filter manifold (Millipore, Bedford, MA). Filters were immediately washed with 10 ml of ice-cold incubation buffer without glucose. The dried filters were placed in vials and counted in 5 ml of scintillation cocktail (4 g PPO, 0.05 g POPOP, 1 liter toluene) in a Packard Tricarb 2000 CA model with an efficiency of 65%.

The SEM of several samples did not exceed 10% in any case. Estimation of the number of binding sites and of the dissociation constant was calculated with statistic program No. 15-1 of the personal computer FX-720P Casio.

Results

Purity of the Plasma Membrane Fraction

The plasma membrane fraction integrity has been checked by means of electron microscopy technique and marker enzyme analysis.

Electron microscopic examination of the plasma membrane fractions indicated that they contained primarily vesicular elements and neither mitochondria nor rough endoplasmatic reticulum were observed in the preparation (Fig. 1).

The results of marker enzyme analysis for whole liver homogenates, microsomes and membrane fragments are summarized in Table 1. The specific activities of marker enzymes for plasma membranes were much enhanced in the purified plasma membranes as compared with the other fractions. On the other hand, the marker enzyme for the endoplasmic reticulum and cytosol fractions was poorly detected on the purified plasma membranes.

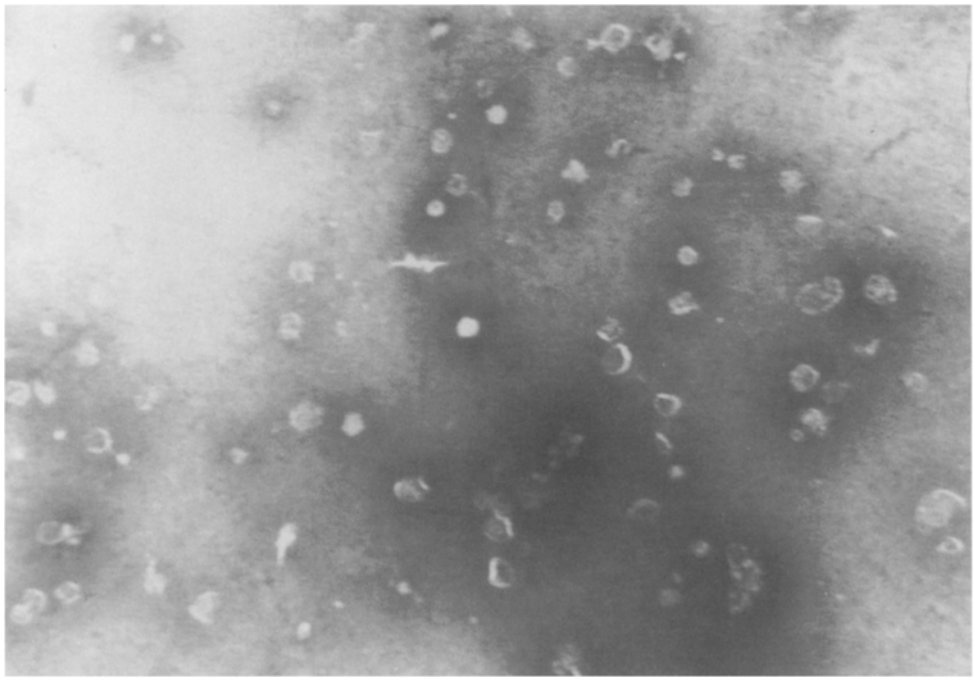

Fig. 1. Electron micrograph of isolated mouse liver plasma membrane preparations. Membranes were fixed in 1% OsO_4. This fraction consists primarily of membranous vesicles. Magnification: ×36,000

Table 1. Marker enzyme analysis of mouse liver plasma membranes[a]

	Homogenate	Microsomes	Plasma membranes
5′-Nucleotidase[b]	1.8 ± 0.2	1.4 ± 0.1	10.6 ± 0.9
Mg^{2+}-ATPase[b]	2.7 ± 0.3	4.8 ± 0.4	23.4 ± 2.1
Na^+,K^+-ATPase[b]	0.4 ± 0.1	0.8 ± 0.2	2.5 ± 0.3
Phosphodiesterase I[b]	0.85 ± 0.2	—	13.7 ± 1.1
Glucose-6-phosphatase[b]	11.4 ± 2.1	50.7 ± 4.5	6.6 ± 0.7
LDH[b]	57.0 ± 2.5	—	Not detected

[a] Mouse liver tissue was homogenized as described in Materials and Methods. Plasma membranes were prepared by the Aronson and Touster (1974) technique. Marker enzymes were analyzed in the crude homogenate, in microsomes and in the membrane preparations. Values are presented as mean ± SE of three determinations.

[b] μmol of inorganic phosphate release/mg protein per hour.

Effect of Buffering Agent, Ionic Strength, Cation Contents and Temperature

A systematic investigation was carried out to characterize the cortisol binding sites in mouse liver plasma membrane.

Buffering Agent Selection

Association kinetics were also performed with the aim of selecting a suitable buffer for the incubations at pH 7.4. The buffers assayed were (in mM): 25 glycil-glycine, 25 HEPES (N-2-hydroxyethylpiperazine-N′-2-ethanesulfonic acid), 25 Tris, 25 BES (2-[bis (2-hydroxyethyl) amino] ethanesulfonic acid) and 25 imidazol. The maximum binding obtained is 152 fmol/mg protein for the 25 mM Tris buffer, for a [^{3}H]cortisol concentration of 4 nM after 90 min of incubation. With other buffers, the maximum binding obtained was: 106 fmol/mg protein with glycil-glycine, 113 fmol/mg for imidazol, 120 fmol/mg with HEPES and 128 fmol/mg for BES. Therefore, Tris buffer was chosen for further experiments.

Table 2. Influence of the ionic strength on [^{3}H]cortisol binding to the plasma membrane

[NaCl] (mM)	[^{3}H]Cortisol bound (fmol/mg protein)		
	A	B	C
0	142	132	150
25	132	151	182
50	134	150	181
75	162	180	200
100	174	182	188
400	178	186	192

Buffer A: 25 mM Tris + 5 mM glucose. Buffer B: 25 mM Tris + 5 mM glucose + 5.4 mM KCl. Buffer C: 25 mM Tris + 5 mM glucose + 5.4 mM KCl + 1.8 mM $CaCl_2$ + 0.8 mM $MgCl_2$. 4 nM [^{3}H]cortisol were used in incubation at 2°C for 90 min. Each point represents the mean of three determinations.

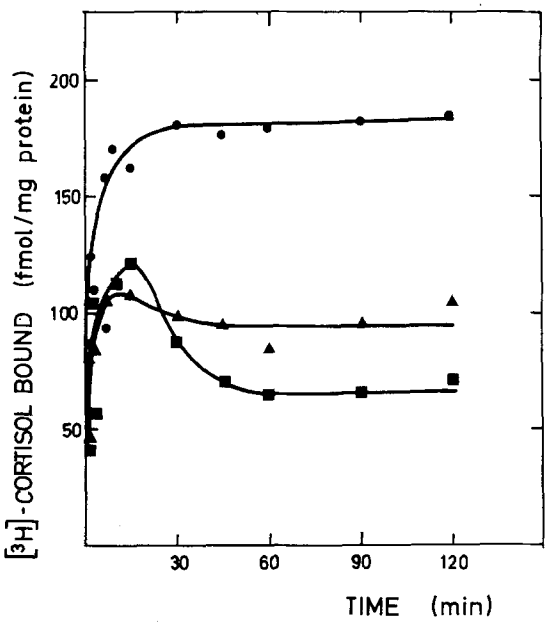

Fig. 2. Association kinetics of 4 nM [^{3}H]cortisol to mouse liver plasma membrane as a function of the incubation time at different temperatures: 2°C (●), 20°C (▲) and 37°C (■). Each point represents the means of four experiments

Ionic Strength and Cation Contents

The influence of the ionic strength on cortisol binding to the plasma membrane has been verified. This assay has been carried out in the presence of different concentrations of NaCl from 0 to 400 mM. In Table 2 it is shown that an increase in NaCl concentration from 0 to 25 mM, using 25 mM Tris + 5 mM glucose + 5.4 mM KCl + 1.8 mM $CaCl_2$ + 0.8 mM $MgCl_2$ as buffer, resulted in a slight binding increase. Higher ionic strength did not affect the cortisol binding. Using 25 mM Tris + 5 mM glucose + 5.4 mM KCl or 25 mM Tris + 5 mM glucose as buffers, the binding was constant with increasing concentration of NaCl until 50 mM. From 50 to 100 mM binding increased and it was not affected by higher NaCl concentrations.

For the assay of the influence of different cation contents on binding, all divalent cation salts were eliminated from the incubation buffer. The $CaCl_2$ and $MgCl_2$ concentrations were varied from 10^{-7} to 10^{-1} M. It was verified that [^{3}H]cortisol binding to the plasma membrane does not change significantly with the different concentrations of Ca^{2+} (159–180 fmol/mg protein) or Mg^{2+} (156–171 fmol/mg protein) used.

Temperature

In order to determine the stability of [^{3}H]cortisol binding with respect to temperature, association kinetics were carried out at 37, 20 and 2°C (ice bath) (Fig. 2). As shown, saturation of cortisol binding was obtained after 60 min in the ice bath. At 20 and 37°C the greatest binding was reached after 15 min, but with less cortisol bound. After this point a dissociation process began. Taking these results into account, all further experiments were performed in an ice bath with a standard incubation time of 90 min.

Kinetics of [^{3}H]Cortisol Binding

A previous experiment was carried out to verify whether [^{3}H]cortisol binding was linear or not when membrane protein concentrations were increased. A linearity was obtained over a range of 0.1–0.7 mg of membrane protein per milliliter. At higher membrane concentrations, the binding was not linear. Assays were routinely conducted at 0.2 mg/ml of protein, which was appropriate within the range of linearity.

The association and dissociation kinetics were carried out in the presence of 4 nM [^{3}H]cortisol. This concentration is higher than that of binding sites (0.14 nM). The free hormone concentration may be considered constant under these conditions, and the formation of the hormone-site complex may be regarded as a pseudo-first order reaction that depends only on the concentration of binding sites.

The association and dissociation kinetic constants were determined from the time course of association and dissociation, according to the review of Weiland and Molinoff (1981). The association

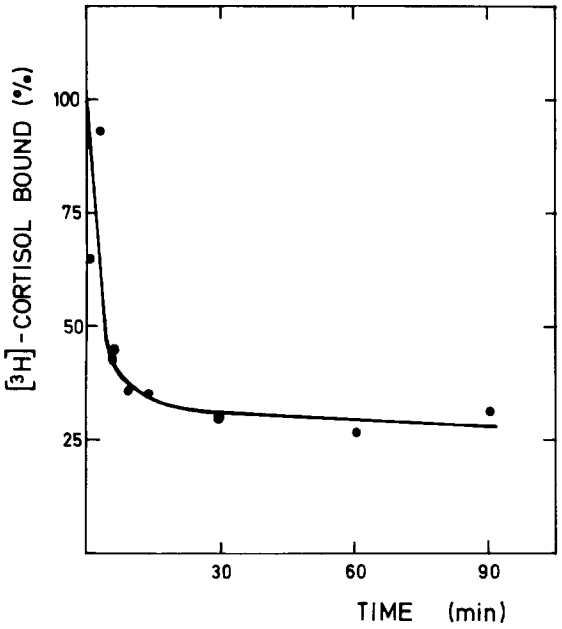

Fig. 3. Dissociation kinetics of 4 nM [^{3}H]cortisol binding to mouse liver plasma membrane in the presence of corticosterone 10^{-6} M. Each point represents the mean of three experiments

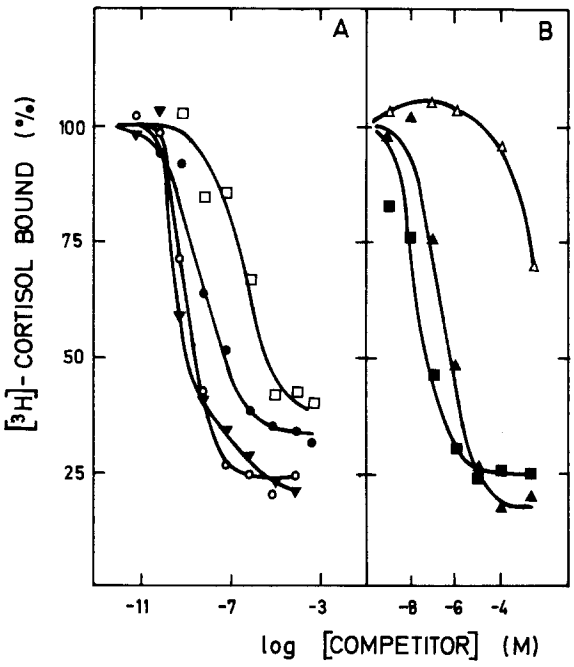

Fig. 4. Displacement of [^{3}H]cortisol bound to mouse liver plasma membrane by different concentrations of the following natural steroids: (*A*) corticosterone (○), deoxycorticosterone (▼), cortisol (●) and cortisone (□). (*B*) Progesterone (■), testosterone (▲) and estradiol (△). [^{3}H]cortisol 4 nM and 180 fmol/mg protein were used as 100% of the binding value. The incubation time was 90 min. Each point represents the mean of three or five experiments

constant (k_{+1}) determined from Fig. 2 was 2.6×10^7 M^{-1} min^{-1}.

The dissociation rate constant was determined in the same experimental conditions as the association kinetics. The value of k_{-1} calculated from the results shown in Fig. 3 was 0.08 min^{-1} and the corresponding dissociation constant was $K_D = k_{-1}/k_{+1} = 3.1$ nM.

Specificity of Cortisol Binding

To assess the specificity of [^{3}H]cortisol binding to plasma membranes, the ability of various steroid hormones to displace cortisol was examined (Figs. 4 and 5). Binding of 4 nM [^{3}H]cortisol was studied in the presence of different concentrations of corticosterone, cortisol, cortisone, deoxycorticosterone, dexamethasone, estradiol, 20β-hydroxyprogesterone, ouabain, prednisolone, progesterone, testosterone and triamcinolone acetonide, ranging from 0.1 nM to 3 mM. Estradiol, dexamethasone, ouabain and triamcinolone acetonide did not displace [^{3}H]cortisol from the binding site. It was also found that cortisone, testosterone, prednisolone and 20β-hydroxyprogesterone were weak competitors. In contrast, the natural steroids deoxycorticosterone, corticosterone, progesterone and cortisol produced a marked displacement of [^{3}H]cortisol bound to the plasma membranes.

Table 3 presents the values of the affinities of

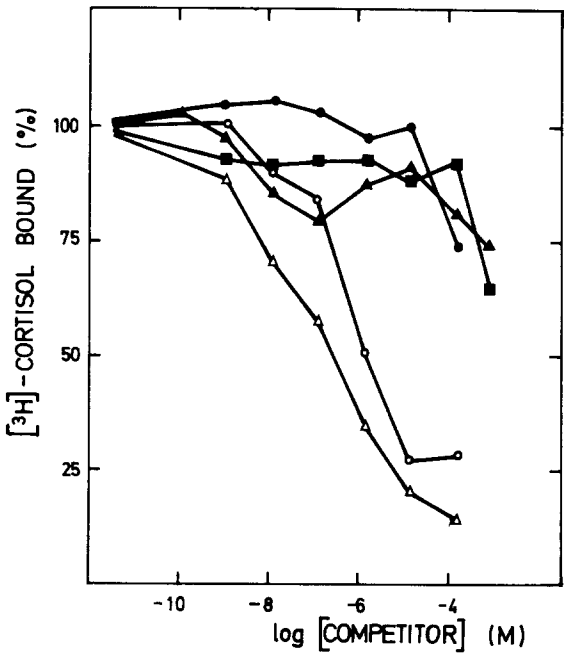

Fig. 5. Displacement of [^{3}H]cortisol bound to mouse liver plasma membrane by increasing concentrations of different synthetic steroids: prednisolone (△), 20β-hydroxyprogesterone (○), dexamethasone (▲), triamcinolone (■) and oubain (●). The same conditions as in Fig. 4

Table 3. Affinity of several steroids for the displacement of [^{3}H]cortisol bound to mouse liver plasma membrane

Competitor steroid	IC_{50} (nM)
Deoxycorticosterone	2
Corticosterone	4
Progesterone	40
Cortisol	79
Prednisolone	200
Testosterone	700
20β-hydroxyprogesterone	1120
Cortisone	6300

Incubation of 4 nM [^{3}H]cortisol and plasma membranes was performed in ice bath in the presence of various concentrations of the unlabeled steroid and during 90 min of incubation time. Values for inhibition of binding at each concentration are means of four determinations.

the steroid competitors with respect to IC_{50} (concentration of unlabeled steroid that caused a 50% inhibition of [^{3}H]cortisol binding).

Binding as a Function of [^{3}H]Cortisol Concentration

The specific binding of [^{3}H]cortisol to plasma membranes of liver, determined in the presence of 1 μM corticosterone (steroid of highest affinity), was saturable with respect to the free concentration of the ligand (Fig. 6). Approximately 20 nM of free [^{3}H]cortisol were necessary to reach saturation. When binding data were represented as a Scatchard graph (Scatchard, 1949) the plot obtained was linear (Fig. 7). Values of 4.4 nM for the dissociation constant (K_D) and 685 fmol/mg protein for the maximum capacity of binding (B_{max}) were obtained. The Hill coefficient calculated was 0.96.

Relationship with Other Receptors

In order to determine that the specific binding was not due to a possible contamination of adsorbed cytosolic receptor, the membranes were rigorously washed with 1 M of NaCl and 1 mM EDTA. It is known that most peripheral membrane proteins are solubilized under these conditions (Findlay, 1987), so the possible adsorbed receptors will be completely removed.

The results are showed in Fig. 8. It can be observed that the binding is increased in treated membranes. This increase is explained because the membrane proteins solubilized (about 40%) are not involved in the binding of cortisol, which implies an increase of fmol cortisol/mg protein.

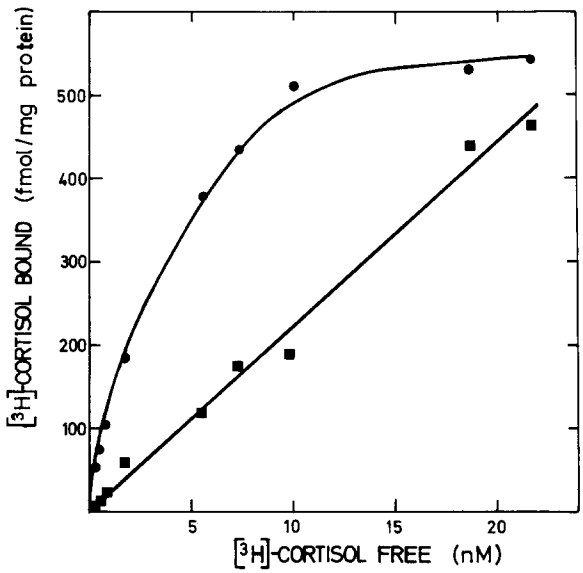

Fig. 6. Equilibrium binding of [^{3}H]cortisol (0.4–40 nM) to mouse liver plasma membrane. Two sets of incubation were carried out simultaneously with the same plasma membrane fraction in the presence and absence of corticosterone 10^{-6} M. The incubation time was 90 min. Specific binding (●) and nonspecific binding (■). Each point represents the mean of four or six experiments

The possible influence of alpha and beta-adrenergic antagonists as mediators on the cortisol binding to plasma membranes was investigated. The concentration range of antagonists has been from 1 nM to 100 μM. The specific binding of [^{3}H]cortisol obtained in the presence of phentolamine remained unmodified because the values of [^{3}H]cortisol bound vary between 89 and 94% with respect to the [^{3}H]cortisol bound in the absence of antagonist. Probably the binding of [^{3}H]cortisol to plasma membranes is not significantly displaced by propranolol. At 10 nM and 1 μM propranolol an 84 and 100% of [^{3}H]cortisol bound are obtained, respectively, being all other values in this range.

Discussion

Plasma membrane binding sites for glucocorticoids have been observed in liver (Suyemitsu & Terayama, 1975), pituitary gland (Koch et al., 1978), skeletal muscle (Savart & Cabillic, 1985) and *Xenopus* oocytes (Sadler & Maller, 1982; Sadler, Bower & Maller, 1985).

The pH for the assay was 7.4, and the temperature was controlled in an ice bath. This pH has also been used for other investigations of steroid binding to plasma membranes (Suyemitsu & Terayama, 1975; Koch et al., 1978; Pietras & Szego, 1980;

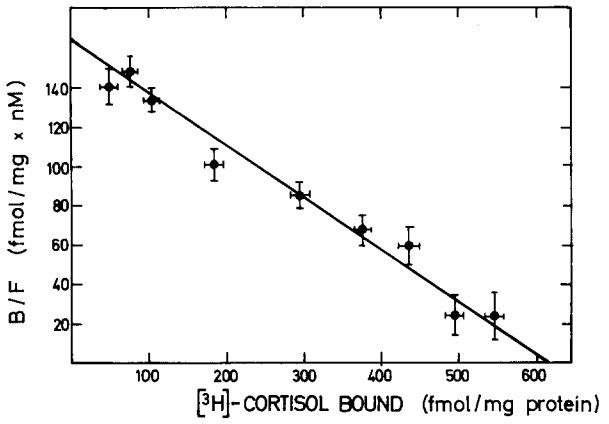

Fig. 7. Scatchard plot calculated from equilibrium binding data of Fig. 6 by linear regression analysis. *B*: bound cortisol (fmol/mg protein); *F*: free cortisol (nM)

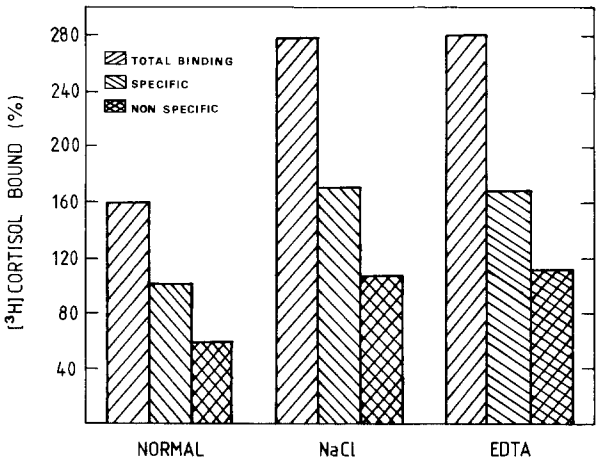

Fig. 8. Binding of 7.7 nM [^{3}H]cortisol to mouse liver plasma membranes. Three sets of experiments were carried out: membranes without treatment (normal) and membranes obtained after special washing with 1 M NaCl, 25 mM Tris/HCl, pH 7.4, or 1 mM EDTA, 25 mM Tris/HCl, pH 7.4. The nonspecific binding was obtained in the presence of 10^{-6} M corticosterone. The incubation time was 90 min. Specific binding to normal plasma membranes is considered to be 100% of binding. Each point represents the mean of three determinations

Haukkamaa, 1984) as well as for binding nuclear receptors (Rousseau, Baxter & Tomkins, 1972; Feigelson, Ramanarayanan-Murthy & Colman, 1978).

The experiment on cortisol binding performed with different buffering agents suggests that the best buffer is Tris/HCl. Ionic strength exerts little influence over cortisol binding. A maximum is reached at a concentration of 100 mM in NaCl. Nevertheless, the influence of ionic strength on ligand binding to the nuclear glucocorticoid receptor has been reported (Blanchardie et al., 1984).

Moreover, a preferential requirement of divalent cation or a dependence on its concentration does not exist. These results differ from those observed for nuclear glucocorticoid receptor in which the presence of 3 mM of Ca^{2+} or Mg^{2+} inhibits nuclear binding (Milgrom et al., 1973).

The binding to plasma membrane is more stable at low-temperature ice baths, which is in concordance with the results found by other authors for glucocorticoid binding to plasma membrane (Suyemitsu & Terayama, 1975; Koch et al., 1978; Pietras & Szego, 1980; Trueba et al., 1987). But it is in contrast with the instability observed at this temperature for the nuclear steroid receptor (Giannopoulos, 1973) and with optimum temperatures of 22 and 37°C for binding of dexamethasone to rat liver microsomes (Omrani et al., 1983) and to skeletal muscle plasma membranes (Savart & Cabillic, 1985), respectively.

At 20 and 37°C the maximum binding reached after 15 min is followed by a decrease when higher incubation times are used (Fig. 2). This plot is similar to that detected for cortisol binding to chicken liver plasma membranes (Trueba et al., 1987). By contrast, Fishman and Fishman (1985) reported that the nuclear estradiol-receptor complex was stabilized at 37°C in cytosol of rat uterus cells by means of a transformation to a nondissociable form. A specific binding decrease is observed in equilibrium studies at 20°C, and specific binding does not exist at 37°C (*data not shown*). So, this decrease in the binding to plasma membrane is probably due to a denaturalization and/or a degradation process of the binding site. From association and dissociation kinetic studies, a value of 3.1 nM has been determined for the dissociation constant, K_D. On the other hand, from equilibrium studies using the Scatchard method a K_D of 4.4 nM has been obtained. This concordance corroborates the validity of the methodology utilized.

Moreover, the maximum binding capacity is 685 fmol/mg protein, threefold smaller than that obtained for cortisol binding to plasma membranes purified from chicken liver (Trueba et al., 1987).

Therefore, the affinity (K_D) remains constant, 4.5 and 4.4 nM in chicken and mouse, respectively, but the receptor concentration in liver plasma membrane is smaller in mammals.

From the Scatchard plot and Hill coefficient, independent and equivalent between themselves, we infer that only one type of binding site exists.

The K_D values obtained are similar to those determined by other authors for plasma membrane: 1.6 nM for the cortisol binding in rat liver (Suyemitsu & Terayama, 1975); 3.2 nM for the cortico-

sterone binding in rat pituitary gland (Koch et al., 1978) and 0.6 nM for dexamethasone binding to rabbit skeletal muscle (Savart & Cabillic, 1985).

The values of IC_{50} have been determined from displacement studies of cortisol bound to mouse liver plasma membrane (Table 3). The order of affinity for several steroids that can act as competitors is: deoxycorticosterone > corticosterone > progesterone > cortisol > prednisolone > testosterone > 20β-hydroxyprogesterone > cortisone. On the other hand, estradiol, dexamethasone, ouabain and triamcinolone acetonide do not compete with cortisol for the same binding sites.

Dexamethasone and triamcinolone have a high affinity for the nuclear glucocorticoid receptor (Ohl et al., 1982; Groyer et al., 1985), but in our case they do not displace [^{3}H]cortisol from the membrane corticoid binding site. Therefore, we can conclude that this binding site is different from the nuclear receptor. It is not concerned with the nuclear progesterone receptor either, because the latter does not bind cortisol or corticosterone and in our experiments both steroids have a very high affinity for the plasma membrane binding site.

Our results are in agreement with the data obtained by Suyemitsu and Terayama (1975) for cortisone, corticosterone, dexamethasone and estradiol in rat liver plasma membranes, and by Koch et al. (1977) for the competition studies on binding of [^{3}H]corticosterone to rat adenohipophysis plasma membrane with corticosterone, deoxycorticosterone and progesterone.

In conclusion, all these results prove that this binding site for cortisol is a true membrane protein that is not exclusive for cortisol, but could be common for corticoids and other steroids.

On the other hand, the existence of a plasma membrane steroid receptor in *Xenopus* oocytes as a protein of 110,000 daltons (Sadler & Maller, 1982; Sadler et al., 1985) and a glucocorticoid "carrier" in rat liver plasma membrane (Allera & Rao, 1986) have been recently described.

Therefore, the chicken liver plasma membrane results (Trueba et al., 1987) are different from those performed on mouse liver because in chicken dexamethasone has a high affinity for its binding site. Moreover, the affinities for prednisolone and 20β-hydroxyprogesterone are greater in chicken than in mouse.

As ouabain does not compete with the cortisol binding sites, the possibility of considering the digitalis receptor of Na^+,K^+-ATPase as a binding site for cortisol has been rejected. This same conclusion can be reached from the data reported by Labella et al. (1985) for progesterone. This hormone does not bind to the digitalis receptor, even though several derivatives do.

As it has been described that cortisol enhances the β-adrenergic agonist response in thymocytes and mast cells (Durant, Duval & Homo-Delarche, 1983), we thought that cortisol interaction with the membrane might be mediated by the adrenergic receptors. But we have observed that cortisol binding to mouse liver plasma membranes is independent of the alpha and beta-adrenergic receptors.

The biological importance of this receptor or "carrier" in the membrane is due to the fact that the steroid might have produced some physiological effect at that level, for example the oocyte maturation (Maller & Krebs, 1977; Sadler et al., 1986).

In our case, we believe that the molecular process by which cortisol binds to plasma membrane sites probably originated the early glycogenolytic effect previously cited (Díez et al., 1984; Sancho et al., 1986; Vallejo et al., 1986; Sanchez-Bueno et al., 1987). This glycogenolytic effect could be affected by the interaction between the membrane binding site and the steroid. This interaction could produce an alteration of membrane fluidity, phospholipid methylation and/or changes in calcium permeability.

Thus, Drouva et al. (1986) have reported that estradiol activates methylating enzyme(s) involved in the conversion of phosphatidylethanolamine to phosphatidylcholine in rat pituitary membranes.

Thus, in this work we have reported evidence of the existence of a specific binding site for corticoids in plasma membranes from mouse liver. In addition, studies to identify, remove and purify this binding site may originate very interesting results.

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