Biochimica et Biophysica Acta, 984 (1989) 143–150
Elsevier

BBAMEM 74520

Study of the transcortin binding to human endometrium plasma membrane

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(Received 13 February 1989)
(Revised manuscript received 25 May 1989)

Key words: Transcortin; Plasma membrane; (Human endometrium)

Transcortin complexed with progesterone was shown to bind specifically to the plasma membrane of human decidual endometrium. The binding reaction was characterized by a high affinity (an apparent K_d value was $(1.0 \pm 0.2) \cdot 10^{-10}$ mol/l) and high selectivity: such human serum proteins as albumin, orosomucoid, transferrin, thyroxine-binding globulin and sex hormone-binding globulin did not compete with transcortin for the membrane binding sites. Transcortin binding to the membrane was steroid-dependent: transcortin-cortisol complex bound to the membranes substantially more weakly than transcortin-progesterone, and specific binding of transcortin devoid of steroid was not detected. Using a radioimmunoassay, we have measured the concentration of endogenous transcortin in highly purified membrane preparations solubilized with sodium cholate. It was found that an extensive washing of decidual strips with a physiological buffer prior to the membrane isolation resulted in a decrease of the endogenous transcortin level along with an increase of the specific membrane binding of exogenous ^{125}I-labeled transcortin. Affinity chromatography on immobilized transcortin was used to isolate transcortin-binding components from ^{125}I-labeled, cholate-solubilized plasma membrane of decidual endometrium. Along with lipid components, the structure of which was not investigated, a ^{125}I-labeled transcortin-binding sialoglycoprotein with a minimal M_r of 20.0 ± 1.5 kDa and a p*I* of approx. 3.3 was detected. In the presence of transcortin, this sialoglycoprotein could be precipitated with a monospecific antitranscortin antiserum. Using hydroxylapatite as a separating agent, the interaction of transcortin and the membrane sialoglycoprotein in model systems containing the two proteins and various steroid hormones was studied. It was found that the membrane sialoglycoprotein displayed a higher affinity for transcortin-progesterone than for transcortin-cortisol (the K_d values were, respectively, $2 \cdot 10^{-11}$ and $7 \cdot 10^{-11}$ mol/l) and it did not bind transcortin complexed with testosterone.

Introduction

During the past several years, convincing evidence has been presented that steroid-binding glycoproteins of human serum, namely, transcortin and sex hormone-binding globulin (SHBG) interact with specific, high-affinity binding sites located in the plasma membranes of steroid-hormone target cells [1–7]. Although the physiological significance of this phenomenon is now somewhat obscure, it is reasonable to assume that these glycoproteins are involved in a guided transport of steroid hormones to/into the target cells [4].

Examination of the transcortin and SHBG interactions with the plasma membranes of human syncytiotrophoblast and endometrium is interesting and important for a number of reasons. First, syncytiotrophoblast (as well as the whole placenta) and decidual endometrium represent unique examples of the tissues available from a normal, healthy human organism through a normal parturition or surgical abortion. Second, both trophoblast and endometrium are well-known target tissues for steroid hormones. A large fraction of these hormones is bound to transcortin or SHBG in the blood, and the levels of hormone-glycoprotein complexes increase during gestation [8]. Third, the trophoblast, a tissue of fetal origin, is in direct contact with the maternal blood. The syncytiotrophoblast brush border is an important component of the placental barrier, which is involved in the selective exchange of various

Abbreviation: SHBG, sex hormone-binding globulin.

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substances, including steroid hormones and serum proteins, between the maternal and fetal circulation [9]. Decidual endometrium, a tissue of the maternal origin, is located in close proximity to syncytiotrophoblast, the two tissues being separated with the placental (maternal) blood. It is obvious that a variety of the processes of steroid hormone and/or serum protein uptake could occur in the trophoblast-blood-endometrium system, and it could have a variety of the physiological consequences.

We have previously studied the recognition system for SHBG-estrogen complexes in the human endometrial plasma membrane [2,4] and demonstrated the presence of two classes of specific binding site for transcortin and its pregnancy-associated variant in the plasma membrane of human syncytiotrophoblast [6]. In the present work we report our findings on the specific interaction of transcortin-steroid complexes with the endometrium plasma membrane and on the nature of the membrane binding sites.

Materials and Methods

Progesterone, cortisol, testosterone and human serum proteins (albumin, transferrin and orosomucoid) were purchased from Sigma (St. Louis, MO, U.S.A.). The techniques described previously were used for the isolation of transcortin [10], SHBG [11] and thyroxine-binding globulin [12] from human postpartum serum. Transcortin was radioiodinated using Iodogen (Pierce, Rockford, IL, U.S.A.) [13]. The specific activity of the preparations of ^{125}I-transcortin was 45–65 μCi/μg, their radiochemical purity was greater than 99%. The immunochemical purity tested with a monospecific anti-transcortin antiserum was about 100%.

Isolation of subcellular fractions

Fresh human decidual tissue from therapeutic abortion (7–12 weeks of pregnancy) performed in a local hospital was stored in ice-cold Krebs buffer for approx. 3–4 h. Endometrial strips (dissected to pieces of 0.5–1 cm) were washed with three portions (200 ml each) of the same buffer ('first washing') and then with 1.5–2 l of slowly running Krebs buffer for 18 h at 4°C with continuous stirring ('second washing'). After that, the subcellular fractions were isolated as previously described [4]. In some experiments, crude plasma membrane preparations obtained by differential centrifugation of the tissue homogenate were used instead of the highly purified membranes obtained by density-gradient centrifugation. In order to study the effect of tissue washings on the endogenous transcortin content of the membrane solubilizates and ^{125}I-transcortin binding to the membrane, a sample of endometrial strips was divided into three portions. One of these portions was not washed before the plasma membrane preparation, another portion was subjected only to the 'first washing' and the last portion was subjected to both 'first' and 'second washings'. Plasma membranes were then isolated from all three tissue portions in parallel.

Binding assay

To study the binding of transcortin-progesterone complex to the endometrium plasma membrane, analytical samples (a final vol of 1.0 ml) were prepared that contained the membrane suspension (approx. 0.5 mg membrane protein) in Krebs buffer with the addition of 1 mg/ml ovalbumin and $5 \cdot 10^{-7}$ M progesterone and variable concentrations of ^{125}I-transcortin. At this concentration, progesterone saturated transcortin at all the glycoprotein concentrations used (see below). In order to determine the background adsorption level, analytical samples in a parallel set of tube contained a 500-fold molar excess of unlabeled transcortin. After incubation of the samples for 17 h at 2–4°C with gentle shaking, the membranes were sedimented by centrifugation at $15\,000 \times g$ for 20 min at 4°C. The supernatant was discarded, and the membrane-bound radioactivity was measured with a RIA Gamma counter (LKB-Wallac, Turku, Finland). All determinations were carried out in triplicate; analysis differing by more than 10% were rejected. The binding parameters were calculated using the Scatchard graphic analysis [14].

Radioimmunoassay

To determine the transcortin content of endometrium plasma membrane, membrane preparations (5–10 mg protein) were solubilized by shaking at room temperature with 1 ml of 5% sodium cholate (Serva, Heidelberg, F.R.G.) followed by centrifugation for 15 min at $25\,000 \times g$. Aliquots of the supernatant (50 μl) were taken and radioimmunoassay for transcortin was performed as earlier described [15] with slight modifications. Antiserum was diluted and standard samples were prepared so as to determine transcortin concentrations in the range of 0.005–1 mg/l. For each membrane preparation, nonspecific adsorption of the tracer was measured using blank samples which did not contain antiserum. It was subtracted from the radioactivity precipitated with antibodies when calculating the transcortin concentrations.

A similar technique was used to evaluate the contamination with transcortin of the human serum proteins which were used for the study of selectivity of the transcortin–membrane interaction. It was found that none of these proteins contained transcortin contaminations.

Search for transcortin-binding membrane components

Endometrium plasma membrane preparations were radioiodinated using Iodobeads (Pierce) [16]. With this aim, membranes (150–200 mg protein) were suspended

in 5 ml of Krebs buffer and incubated overnight with $Na^{125}I$ (2–3 μCi) and six Iodobeads at 4°C with constant stirring. The Iodobeads were then taken out, the membranes were sedimented by centrifugation (25000 × g, 15 min) and washed three times with Krebs buffer containing 0.15 M KI (50 ml) to remove nonreacted radionuclide. The labeled membrane pellets were then suspended in Krebs buffer (a final volume of 2 ml) and solubilized by adding 2 ml of 10% sodium cholate solution in water and incubating this mixture for 2 h at 4°C with shaking. The mixture was then centrifuged, the supernatant was 10-fold diluted with Krebs buffer containing 1 mg/ml ovalbumin and $1 \cdot 10^{-6}$ M progesterone (standard buffer) and applied on a column packed with transcortin-Sepharose.

The affinity sorbent was prepared by immobilization of transcortin on BrCN-activated Sepharose 4B (Pharmacia, Uppsala, Sweden) [17]. The transcortin content of different sorbent preparations varied from 0.5 to 2.0 mg per ml settled gel volume as determined by their capacity for cortisol binding.

A column packed with approx. 3.6 ml of the sorbent equilibrated with the standard buffer was used for affinity chromatography. The membrane solubilizate was applied on the column at a rate of 2 ml/h. To remove nonspecifically adsorbed radioactive material, the column was extensively washed with the standard buffer until the radioactivity was decreased to 20000–50000 cpm per ml eluate.

Elution of transcortin-binding membrane components was then achieved using one of the following two procedures. In one procedure, the column was washed with the transcortin solution (1 mg/ml) in the standard buffer, i.e., with transcortin-progesterone complex. The other procedure involved washing of the column with a buffer, which did not contain any steroid, to remove progesterone, filling it with a buffer containing $2 \cdot 10^{-4}$ M testosterone, incubation for 2–10 h at room temperature and washing with the latter buffer. In all cases the elution rate was 4 ml/h. Fractions of 0.4 ml were collected and their radioactivity was measured. Fractions that contained biospecifically eluted labeled substances were combined, desalted by filtration through Bio-Gel P-6 (Bio-Rad, Richmond, CA, U.S.A.), freeze-dried and stored at −18°C for no longer than 2–3 days.

For electrophoresis, either under non-denaturing conditions or in the presence of sodium dodecylsulfate (Sigma), 5, 10 and 15% polyacrylamide gels were used. Isoelectrofocusing was performed using 7.5% polyacrylamide gel and Servalyts 3–10 and 2–4 (Serva). After that, the gel rods were cut into 0.5 cm (electrophoresis) or 0.25 cm (isoelectrofocusing) bands, and the ^{125}I radioactivity of these bands was measured.

In special experiments, prior to electrophoresis or isoelectrofocusing, the affinity eluates were treated with Pronase (Serva, 1 mg per each 100000 cpm of the eluate radioactivity) for 30 min at 25°C or with neuraminidase (EC 3.2.1.18) immobilized on agarose (Sigma) for 1 h at 37°C.

Immunochemical studies

Analytical samples containing equal quantities of the labeled membrane sialoglycoprotein (approx. 6000 cpm) and variable amounts of unlabeled transcortin ($4 \cdot 10^{-11}$–$1 \cdot 10^{-7}$ M) in 0.25 ml of the standard, progesterone-containing buffer (see above) were incubated for 17 h at 4°C. Monospecific rabbit antiserum to human transcortin was then added and the samples were incubated for an extra 2 h period at the same temperature. The amount of antiserum used was enough to bind more than 95% of transcortin up to a transcortin concentration of $1 \cdot 10^{-9}$ M. After that, 0.02 ml of 0.1% rabbit immunoglobulin solution in 0.05 M sodium phosphate buffer (pH 7.0), followed by 1.0 ml of 12% poly(ethylene glycol) 6000 solution in the same buffer was added into each tube. The samples were vortex shaken and centrifuged (2000 × g, 10 min), and the radioactivity precipitated was measured. To determine the nonspecific precipitation of the labeled membrane sialoglycoprotein, analytical samples without transcortin were processed in a parallel set of tubes.

Transcortin-sialoglycoprotein complexation

In order to study the complexation of the membrane sialoglycoprotein with various transcortin-steroid complexes, model systems containing the two proteins and one of steroid hormones, namely, progesterone, cortisol or testosterone, were prepared and processed as follows. Analytical samples contained equal amounts (approx. 4000 cpm) of ^{125}I-labeled membrane sialoglycoprotein, which had been eluted from the affinity column by substituting progesterone for testosterone and desalted by gel filtration (see above). Various amounts of ^{125}I-transcortin (5000–500000 cpm per tube) were added to the samples to produce the glycoprotein concentrations in the range of $1 \cdot 10^{-11}$–$5 \cdot 10^{-9}$ M. Incubation medium (a final vol of 0.4 ml) was 0.005 M sodium phosphate buffer (pH 6.8) containing 0.3 mg/ml ovalbumin and $1 \cdot 10^{-6}$ M progesterone or cortisol or $1 \cdot 10^{-5}$ M testosterone. Two sets of the blank sample, each containing only one of the labeled biopolymers, either transcortin or membrane sialoglycoprotein, at the same concentration as in paired analytical samples, were prepared in order to determine nonspecific adsorption of transcortin on hydroxylapatite and retention of the membrane sialoglycoprotein by this sorbent. Immediately before the experiments, ^{125}I-transcortin was additionally purified by hydroxylapatite chromatography [18].

After incubating all the samples, both analytical and blank, for 17 h at 4°C, they were applied on microcolumns (0.3 ml) packed with hydroxylapatite and equi-

librated with 0.005 M sodium phosphate buffer. The microcolumns were washed with 1.5 ml of the same buffer, and the radioactivity bound to the adsorbent was measured. To determine the amount of ^{125}I-transcortin complexed with the membrane sialoglycoprotein, radioactivity adsorbed by hydroxylapatite from the two blank samples (see above) ws subtracted from the total radioactivity adsorbed by the sorbent from a corresponding analytical sample. In a typical experiment, the blank adsorption of ^{125}I-transcortin was lower than 15%, whereas adsorption of the labeled membrane sialoglycoprotein was 85–90%. The values of specific binding of transcortin (complexes with a certain steroid) to the membrane sialoglycoprotein at various transcortin concentrations were used for the determination of the equilibrium binding parameters by Scatchard graphic analysis [14].

Results and Discussion

Membrane binding of transcortin-progesterone complex

It is well-known that the development of decidual endometrium is mainly controlled by progesterone. So, first of all we have studied the transcortin–membrane interaction in the presence in the medium of progesterone at a concentration ($5 \cdot 10^{-7}$ M) which was high enough to saturate transcortin with the steroid at all the glycoprotein concentrations used. The specific ^{125}I-transcortin binding was observed [19] in the plasma membrane fraction and was absent in two other particulate subcellular fractions, namely, the nuclear and mitochondrial ones. Further purification of the plasma membrane fraction resulted in a substantial increase of the specific ^{125}I-transcortin binding on the basis of the membrane protein units.

As seen from Fig. 1, the saturable binding of ^{125}I-transcortin-progesterone to the membrane was observed at glycoprotein concentrations below approx. $3 \cdot 10^{-10}$ mol/l. Analysis of the data from six experiments using

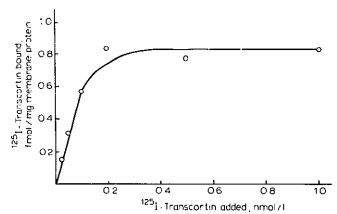

Fig. 1. Concentration dependence of specific binding of ^{125}I-transcortin-progesterone to endometrium plasma membrane. See Materials and Methods for experimental details.

TABLE I

Endogenous transcortin content and ^{125}I-transcortin-binding capacity of various plasma membrane preparations

In both columns values are given in fmol transcortin per mg membrane protein. Washing procedures and plasma membrane preparations are described in Materials and Methods.

Expt. No.	Tissue washings	Endogenous transcortin content	Binding capacity
1.	None	16.0	11.6
	First	13.0	7.7
	First + second	10.0	19.1
2.	None	15.0	0
	First	4.0	0
	First + second	1.0	2.2
3.	None	48.0	0
	First	27.0	1.5
	First + second	7.0	8.4

different membrane preparations gave an apparent equilibrium dissociation constant, $K_d = (1.0 \pm 0.2) \cdot 10^{-10}$ mol/l. The binding capacity of the membrane, B_{max}, was extremely variable for different membrane preparations, the mean value being 3.7 ± 3.2 fmol transcortin per mg membrane protein (range: 0–11.7 fmol/mg; $n = 16$). Such wide scattering of the B_{max} values could be caused by, at least, two reasons. First, a variable number of patients (10–20 in each experiment) at different terms of pregnancy (7–12 weeks), who had been the donors of the tissue specimens for the membrane preparation. Second, a partial occupancy of the membrane binding sites with endogenous transcortin, i.e., transcortin taken up by the tissue from the patients' blood.

Using a radioimmunoassay, we found that the highly purified membrane preparations solubilized with sodium cholate did contain a protein sharing common antigenic determinants with serum transcortin (Table I). Washing of endometrial strips with a physiological buffer prior to the membrane isolation led to a decrease in the level of this protein in the membrane solubilizate, its complete removal being, however, unachievable even with an extensive washing procedure. In parallel with this, the binding capacity of the membranes with respect to exogenous ^{125}I-transcortin tended to increase (see Table I). This suggests that the protein detected by using the radioimmunoassay was endogenous transcortin, at least a fraction of which was bound to the specific membrane sites.

The transcortin–membrane interaction was found to be highly selective: none of the human serum proteins used, except transcortin itself, competed with ^{125}I-transcortin for the binding sites [19]. At this stage of the work, we did not thoroughly examine the steroid-dependence of the transcortin binding to the membrane. An

individual experiment (data not shown) demonstrated that endometrial plasma membrane bound transcortin-cortisol complex with an affinity at least 4-fold lower than for transcortin-progesterone. Also, we did not detect the membrane binding of transcortin devoid of steroid. As we have stated earlier [4], the steroid-dependence of membrane binding of steroid-binding glycoproteins is, evidently, due to the known effect of the steroid complexing on the glycoprotein conformation [20].

Affinity chromatography of membrane components

In a search for transcortin-binding components of the endometrium plasma membrane, we have used an approach based upon non-selective labeling of the membrane components with ^{125}I, solubilization of the labeled membrane preparation and affinity chromatography of the solubilizate on immobilized transcortin.

Membranes were radioiodinated using a solid-phase technique with Iodobeads as oxidizing agent. Under the conditions used (see Materials and Methods), 15–30% of ^{125}I radioactivity added was included in the membranes, depending on the amounts of the membrane material and $Na^{125}I$ used and the duration of the reaction. As shown below, both protein and lipid components of the membrane were labeled.

The labeled membrane preparations were solubilized using sodium cholate. After the separation of an insoluble fraction, which presumably represented fragments of the basal membrane associating in the decidualized endometrium [21], approx. 70% of ^{125}I radioactivity covalently linked to the membrane components was recovered in the solubilizate.

The solubilizate was 10-fold diluted with a buffer that did not contain any detergent, the cholate concentration being thus lowered to 0.5%. At this concentration of the detergent, the transcortin molecules seem to have their native conformation because the steroid-binding ability [18] and immunochemical properties [19] of the glycoprotein are unchanged. A high progesterone concentration ($1 \cdot 10^{-6}$ M) in the diluting buffer was necessary to saturate immobilized transcortin with this steroid when performing the steps of biospecific adsorption of the labeled membrane components onto transcortin-Sepharose and washing of the affinity column.

Extensive washing of the column after the solubilizate application resulted in the elution of labeled substances, which had no affinity for transcortin (usually, about 99.9% of the radioactivity applied in the column), and removal of the detergent. Biospecific elution of the transcortin-binding membrane components was then carried out using one of the two following procedures.

In one procedure, the column was washed with transcortin solution in the standard buffer, i.e., with a solution of transcortin-progesterone complex (Fig. 2A). In various experiments, this washing led to the elution of 0.02–0.1% of the total radioactivity applied on the column. Such a yield of the transcortin-binding components was in line with the extremely low content of the specific binding sites in the membrane (see above).

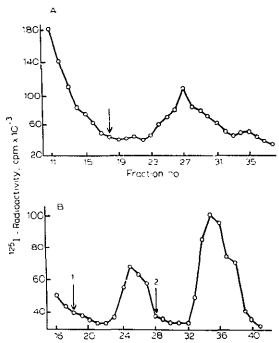

Fig. 2. Biospecific elution of radiolabeled membrane components from the transcortin-Sepharose column. (A) Elution with transcortin-progesterone complex, i.e., with the transcortin solution in a progesterone-containing buffer; (B) elution by substituting progesterone, complexed with immobilized transcortin, for testosterone (1) followed by the elution with transcortin progesterone complex (2). Arrows indicate beginning of the elution with a given agent.

The other elution procedure used the advantage of the dependence of transcortin conformation and, as a consequence, of its affinity for the membrane components on the nature of steroid bound to it. This approach was supported by the data of the preliminary experiment on the steroid-dependence of the transcortin–membrane interaction (see above) and by our earlier observation that endometrium plasma membrane could recognize another steroid-binding serum protein, SHBG, complexed with certain steroids [4]. The elution was performed as follows. First, the column was extensively washed with a buffer that did not contain any steroid to remove progesterone. Then it was filled with a buffer containing testosterone, incubated for 2–5 h at 4° C and, finally, washed with the same buffer. As seen from Fig. 2B, application of this procedure resulted in the elution of a portion of the labeled membrane components. The yield was approx. 30% of that obtained when using the first elution procedure, and an extra washing of the column with the buffer containing transcortin-progesterone led to elution of additional labeled material (Fig. 2B). In our opinion, the incomplete elution of the transcortin-binding components achieved by

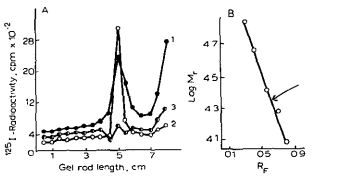

Fig. 3. (A) Electrophoresis in 15% polyacrylamide gel in the presence of sodium dodecylsulfate of the whole affinity eluate (1), trichloroacetic-acid-precipitated components of the eluate (2), and the same components after the treatment with pronase (3); (B) calibration plot for the M_r determination (position of the transcortin-binding membrane sialoglycoprotein is indicated by the arrow).

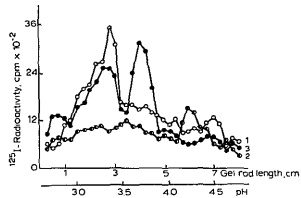

Fig. 4. Isoelectrofocusing of the affinity eluate. (1) Intact eluate; (2) eluate treated with pronase; (3) eluate treated with neuraminidase.

substitution of progesterone, bound to immobilized transcortin, for testosterone was due to hindered conformational transitions of the immobilized glycoprotein molecules.

It should be emphasized that both elution procedures produced labeled membrane components with similar molecular properties.

Properties of transcortin-binding membrane components

Extraction of the affinity eluates obtained with chloroform/methanol [22] and treating them with trichloroacetic acid showed that about half of the radioactive material was lipid, while the rest was protein. In accordance with these observations, electrophoresis in the presence of sodium dodecylsulfate (Fig. 3A) gave a diffuse zone near the end of the gel rod, which is typical for lipids, and one discrete band that corresponded to a protein with an apparent molecular mass of 20.0 ± 1.5 kDa (mean value ± S.E.; $n = 4$). The protein nature of this substance was proved by the disappearance of the discrete band after pronase digestion of the preparation prior to electrophoresis, the diffuse lipid zone being unchanged (Fig. 3B). Omission of 2-mercaptoethanol from the mixture used for the treatment of the preparations before electrophoresis did not result in the appearance of new bands or shift of the 20 kDa band. This suggests that there are no intra- or intermolecular disulfide bridges in the membrane protein discovered.

Membrane receptors for peptide and protein hormones are known to be biopolymers having a subunit structure [23]. We, therefore, assume that the 20 kDa protein found is one of the subunits that form the membrane binding sites for transcortin. Its behavior during electrophoresis under non-denaturing conditions, namely, formation of a single low-mobility band (R_f approx. 0.1), suggests that this protein can form a polymer of a definite stoichiometry. This fact could also be explained by a little net charge of the protein molecule under the weakly alkaline conditions of electrophoresis. However, isoelectrofocusing (Fig. 4) revealed that this protein is very acidic: its isoelectric point is at pH 3.3. After the treatment of the preparation with neuraminidase, the isoelectric point rose to 3.7 (Fig. 3). This suggests that the transcortin-binding membrane component is a sialoglycoprotein.

Immunochemical experiments (Table II) gave further evidence that the isolated membrane sialoglycoprotein had an affinity for transcortin. It was precipitated by a monospecific antitranscortin antiserum only in the presence of transcortin in the medium. Furthermore, at transcortin concentrations in the analytical samples ranging from $4 \cdot 10^{-11}$ to $1 \cdot 10^{-9}$ mol/l the amount of the precipitated sialoglycoprotein increased with the transcortin concentration. This is in agreement with the concentration range wherein the specific transcortin binding to the endometrium plasma membrane was observed (cf. Fig. 1). When the transcortin concentra-

TABLE II

Precipitation of the ^{125}I-labeled membrane sialoglycoprotein isolated from decidual endometrium plasma membrane by antitranscortin antiserum in the presence of increasing transcortin concentrations

Specific precipitation was calculated by subtracting precipitation in the absence of antiserum from the total precipitation. Results from two independent experiments; in each of them analysis were carried out in triplicate.

Transcortin concentration (mol/l)	Precipitation		
	total cpm	specific	
		cpm	% of total ^{125}I radioactivity added
0 (nonspecific adsorption)	4100	–	–
$5 \cdot 10^{-12}$	4100	0	0
$4 \cdot 10^{-11}$	4400	300	4.4
$2 \cdot 10^{-10}$	4700	600	8.8
$1 \cdot 10^{-9}$	5100	1000	15.7
$1 \cdot 10^{-8}$	4300	200	2.9
$1 \cdot 10^{-7}$	3900	0	0

tion in the medium was increased to $1 \cdot 10^{-7}$ mol/l, which was much greater than the transcortin-binding capacity of the antiserum used, the precipitation of the labeled membrane sialoglycoprotein decreased to the background radioactivity adsorption level. Collectively, these findings indicate that the membrane sialoglycoprotein complexes with transcortin-progesterone in the solution.

Human transcortin is one of a few proteins known to interact with hydroxylapatite so weakly that they are not retained in a column packed with this adsorbent when being dissolved in and eluted by a diluted sodium phosphate buffer (e.g., 0.005 M, pH 6.8) [18]. Unlike transcortin, the membrane sialoglycoprotein was found to adsorb strongly to hydroxylapatite under the above conditions. Thus, when a solution of the labeled sialoglycoprotein preparation in the above buffer was applied on a microcolumn packed with hydroxylapatite (see Materials and Methods for details), about 70% of the radioactivity was retained in the column. Based on the trichloroacetic acid precipitation, this corresponded to the retention of about 90% of the labeled protein. The dramatic difference in the chromatographic behavior of the two glycoproteins allowed us to use hydroxylapatite as an agent to separate 'free' and 'receptor-bound' transcortin fractions, when studying the concentration-dependence of complex formation in model systems. Each of these systems contained a variable amount of ^{125}I-transcortin, a constant quantity of the membrane sialoglycoprotein and a high concentration of a certain steroid. These experiments showed (Fig. 5) that the membrane sialoglycoprotein binds specifically transcortin complexed with progesterone or cortisol but not with testosterone. In agreement with the experiments on the membrane level (see above), the solubilized sialoglycoprotein displayed higher affinity for transcortin-progesterone than for transcortin-cortisol: the respective K_d values were approx. $2 \cdot 10^{-11}$ and $7 \cdot 10^{-11}$ mol/l.

The affinity of the solubilized sialoglycoprotein for transcortin-progesterone is apparently higher than that of the membrane binding sites: the respective K_d values are approx. $2 \cdot 10^{-11}$ and $1 \cdot 10^{-10}$ mol/l. The approximately 5-fold difference between these values could hardly be explained by the use of two different analytical techniques for their determination. Assumingly, it reflects that, when located in the membrane, the transcortin-binding sialoglycoprotein interacts not only with transcortin but also with other membrane components. Alternatively, it could be a subunit of a holoreceptor for transcortin-steroid complexes, which is similar to the recognition system for SHBG-estrogen complexes previously found in the endometrium cell membrane [4].

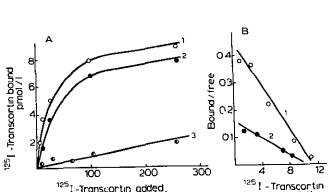

Fig. 5. (A) Equilibrium binding to the membrane sialoglycoprotein of ^{125}I-transcortin complexed with progesterone (1), cortisol (2) or testosterone (3); (B) Scatchard plots for the specific binding of progesterone (1) and cortisol (2) transcortin complexes.

In the present work we have not investigated the origin of the non-proteinous substances that accompanied the transcortin-binding sialoglycoprotein during affinity chromatography of the membrane solubilizate. It can be assumed that these substances are lipids containing unsaturated fatty acid residues, which explains the covalent linking of ^{125}I, and that they are important for the assembly and functioning of the membrane binding sites for transcortin. These lipids seem to be tightly, although noncovalently, bound to the sialoglycoprotein found.

It is possible that, apart from the sialoglycoprotein described in the present work, some other membrane proteins are involved in the specific binding of transcortin, and we could not detect these proteins because they interacted with the affinity resin too weakly (and were eluted during the initial washing of the column) or too tightly (and were not eluted at all) or because they did not contain the radiolabel. However, it should be emphasized that the sialoglycoprotein discovered is the principal transcortin-binding component of the human decidual endometrium plasma membrane. This stems from its high affinity and steroid-dependence of the transcortin binding, which are similar to those displayed by the membrane preparations. Low apparent molecular mass of the membrane sialoglycoprotein, revealed by electrophoresis under denaturing conditions, along with its ability to form a polymer indicates that, in the membrane, several 20 kDa protomers could be involved in the formation of a functionally active transcortin-binding site.

The structure of the transcortin-binding site and the biochemical consequences of the binding of transcortin-steroid complexes to the endometrium plasma membrane must await further investigation. It is, however, evident that the results of the present work support an active role played by transcortin in the mechanism of steroid hormone action.

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