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GENERAL REVIEW

SPECIFIC STEROID-BINDING GLYCOPROTEINS OF HUMAN BLOOD PLASMA: NOVEL DATA ON THEIR STRUCTURE AND FUNCTION

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Summary—In this review, the modern data on the polypeptide and carbohydrate structures of human corticosteroid-binding globulin (CBG) and sex hormone-binding globulin (SHBG) as well as on the biochemical properties and biological functions of these steroid-binding glycoproteins are discussed.

INTRODUCTION

In human and animal blood plasma, steroid hormones are largely bound to specific binding glycoproteins. In humans, two major glycoproteins of this class, transcortin (also called corticosteroid-binding globulin, CBG) and sex hormone-binding globulin (SHBG), bind specifically four classes of steroid hormones: glucocorticoids and progestins (CBG) and androgens and estrogens (SHBG). These glycoproteins as well as related human and animal proteins have been extensively studied for about 30 years. Many interesting reviews of this subject are available (e.g. see the excellent books by Professor Ulrich Westphal[1, 2] and recent review articles [3–6]).

Publication of the present review is, in our opinion, justified by a need for systematic consideration of the interesting and important novel data obtained during the past 5–7 years, which significantly expand our knowledge of the CBG and SHBG molecular properties and allow a deeper insight into the physiological role of hormone-binding serum glycoproteins. In accordance with this aim, the works published after 1983 are mainly discussed. Results from experiments performed on laboratory animals are considered only in the cases when they are useful for better understanding of the structure and function of the human steroid-binding glycoproteins.

MOLECULAR PROPERTIES OF CBG AND SHBG

The most important advantage in the studies of specific steroid-binding globulins of human plasma achieved in the past decade is determination of their polypeptide and carbohydrate structures as well as cDNA sequences and the SHBG gene structure.

Chronologically, the carbohydrate structures of these glycoproteins were firstly determined. Research performed in our laboratory showed that both CBG and SHBG contain sugar chains, the structures of which are typical for the serum glycoproteins of mammals. Only *N*-linked sugar chains of the *N*-acetyllactosamine type (approx. 5 mol per mol glycoprotein) are encountered in CBG [7, 8]. Three of these chains are biantennary (Fig. 1A) and two are triantennary (Fig. 1B) oligosaccharides. In the SHBG molecule, there are two biantennary *N*-linked sugar chains of the above type (Fig. 1A) and one *O*-linked chain (Fig. 1C) [9]. Pronounced microheterogeneity is characteristic of the carbohydrate moieties of both glycoproteins, the *N*-acetylneuraminic acid and fucose content of the individual sugar chains being variable. But, even so, each carbohydrate moiety has a specific structural organization that differs CBG and SHBG from each other and from related serum glycoproteins, e.g. human thyroxine-binding globulin [10]. (In particular, this offers a chemical basis for the involvement of the steroid-binding glycoprotein carbohydrates in the formation of specific determinants for biological recognition of these glycoproteins). As shown below, our data on the number and structure of the CBG and SHBG oligosaccharide chains have been confirmed by others in the course of determination of the amino acid sequences of these glycoproteins.

The known *N*-terminal sequence of human CBG [11, 12] made it possible to deduce the complete primary structure of its polypeptide chain from the sequence of hepatic and pulmonary cDNAs [13]. The liver CBG cDNA was found to code a 405-amino

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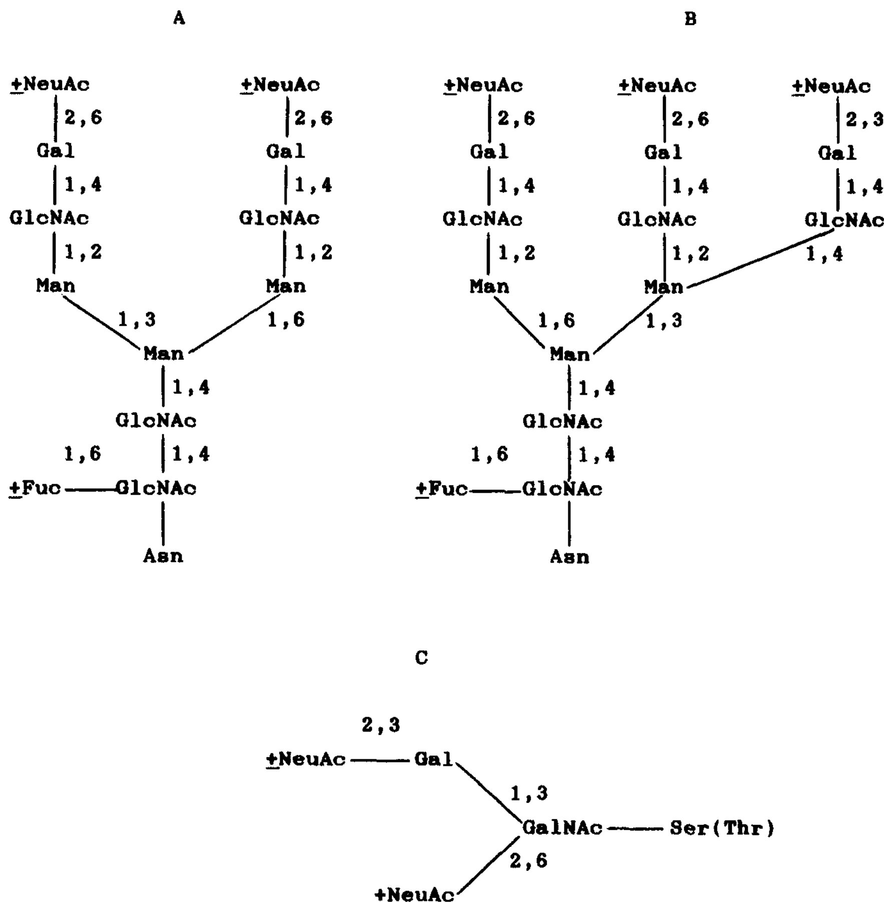

Fig. 1. Structure of the carbohydrate chains of human CBG and SHBG: biantennary (A) and triantennary (B) *N*-linked oligosaccharides of the *N*-acetyllactosamine type and *O*-linked oligosaccharide (C) [7–10].

acid (M_r 45,149) polypeptide. The *N*-terminal sequence of 22 residues, that precedes the known *N*-terminal sequence of human CBG, represents a hydrophobic leader fragment. The mature CBG polypeptide chain consists of 383 amino acid residues (M_r 42,646). It contains two cysteine residues, which are not linked in a disulfide bridge, and six consensus sequences (-Asn-X-Ser/Thr-, where X is not Pro [14]) for the attachment of *N*-linked oligosaccharide chains. According to the available information and secondary structure predictions [15] five of these sites of glycosylation are indeed utilized.

Comparison of the nucleotide and deduced amino acid sequences of human CBG with published sequences of several human steroid-binding proteins, steroid receptors included, showed no significant homologies [13]. However, the CBG precursor amino acid sequence was found to be strongly similar to the sequences of human thyroxine-binding globulin precursor and other members of superfamily 260 (serine protease inhibitors), especially alpha-1-antichymotrypsin precursor (more than 40% identity in a 373-amino acid overlap).

Knowledge of the CBG cDNA sequence will probably facilitate investigation of the abnormal transcortin variants with a reduced affinity for cortisol discovered by Van Baelen *et al.*[16] and Robinson and Hammond[17].

The primary structure of human SHBG was determined by direct sequencing of overlapping peptide fragments of this glycoprotein [18]. The SHBG polypeptide chain was found to be composed of 373 amino acid residues (M_r 40,499). It contains two disulfide bonds and, in agreement with our findings (see above), three sugar chains, one *O*-linked and two *N*-linked ones, are attached to it. These authors assumed that, apart from the variability in the carbohydrate structures, SHBG microheterogeneity could be due to the amino-terminal differences (lacking of the terminal leucine and penultimate Arg-Pro by a part of the glycoprotein molecules) and/or the rearrangement of the two available Asn-Gly bonds with the formation of free carboxyl groups.

Sequencing of cDNA for SHBG isolated from a phage lambda gt11 human liver cDNA library [19] confirmed the SHBG primary structure estimated by direct polypeptide sequencing [18] and supported the presence of two sites for *N*-glycosylation and one site for *O*-glycosylation in the SHBG polypeptide chain. The leader peptide fragment was found to be composed of 8 residues. Similar results were obtained in two other works [20, 21]. Gershagen *et al.*[21] found

Table 1. Physicochemical properties of human CBG and its pregnancy-associated molecular variant

Parameter	CBG	Pregnancy-associated variant
M_r	55,000	55,000
R_f: PAGE under standard non-denaturing conditions	0.62	0.62
R_f: PAGE in low-porosity gel under non-denaturing conditions	0.43, 0.46	0.46
pI	3.6–4.2 (6 bands)	3.5–4.1 (6 bands)
$A^{1\%}_{280\,nm,\,1\,cm}$	6.9	6.9
K_a: cortisol, 4°C (l/mol)	$(4.5 \pm 0.5) \cdot 10^8$	$(4.0 \pm 0.8) \cdot 10^8$
K_a: progesterone, 4°C (l/mol)	$(7.2 \pm 0.6) \cdot 10^8$	$(7.3 \pm 0.4) \cdot 10^8$
Binding capacity (mol steroid bound per mol glycoprotein)	0.96	0.96
K_a: antiserum to CBG (l/mol)	$(1.0 \pm 0.2) \cdot 10^9$	
K_a: antiserum to the CBG variant (l/mol)	$(1.8 \pm 0.3) \cdot 10^9$	

Data from Ref. [28].

the homology of human SHBG to human/bovine protein S, a vitamin K-dependent component of the anticoagulation system.

The authors of the above works [18, 19] identified polypeptide chain regions which are likely to be involved in the formation of steroid-binding site.

As in the case of CBG, neither internal homology in the SHBG sequence nor a homologous relationship to other steroid-binding proteins including human glucocorticoid and estrogen receptors were found, the only exception being rat testis androgen-binding protein (68% sequence identity with SHBG without gaps) [18]. Similarity of the molecular properties of human androgen-binding protein and SHBG [22, 23] as well as the identity of their immunochemical properties [24] evidently indicated to even greater homology between the two human proteins. Indeed, it has been recently demonstrated [25] that human SHBG and androgen-binding protein are coded by the same gene consisting of eight exons.

Microheterogeneity of the CBG and SHBG carbohydrate moieties (see above) suggests the existence of molecular variants, i.e. homogeneous populations of the molecules that differ from each other only by some details of their carbohydrate structures [26]. Existence of such molecular variants was considered [27, 28] as a plausible explanation of an apparent heterogeneity observed under electrophoresis of the highly purified CBG and SHBG preparations. (In both cases, isolation and characterization of the electrophoretic variants revealed that their basic physico-chemical properties were similar). However, in this particular case the above explanation sounds inconvincing, since it was shown [27, 29] that desialylation or even almost complete deglycosylation of CBG and SHBG did not result in the disappearance of the multiple electrophoretic bands. (For SHBG, an opposite result has also been reported [25]).

A comparative study of CBG preparations isolated from normal donor serum and from human retroplacental serum [30] revealed the presence, in the retroplacental serum, of a CBG molecular variant, all five sugar chains of which were triantennary *N*-linked oligosaccharides of the *N*-acetyllactosamine type (see Fig. 1B). Basic physico-chemical and immunochemical properties of this variant were found to be similar to those of normal CBG (Table 1). This suggests the identity of the polypeptide chains of the two glycoproteins. (Similar molecular variant of thyroxine-binding globulin was also observed [30].)

Since the CBG molecular variant contains only triantennary sugar chains of the *N*-acetyllactosamine type, unlike normal transcortin and the most of the human serum glycoproteins it does not bind to concanavalin A. Accordingly, upon chromatography on this lectin immobilized on a water-insoluble matrix it is eluted in a run-through fraction [30]. Based upon this fact as well as upon similarity of the immunochemical properties of CBG and its variant, we developed an analytical technique for the measurement of the CBG variant in human serum and other body fluids. Using this technique, we have analyzed some 600 samples of serum from individual normal donors (both male and female) and pregnant women as well as samples of retroplacental serum, umbilical cord serum and placental extracts [31]. Little or zero concentrations of the CBG variant were found in normal donor serum. In the venous sera of pregnant women, this variant appeared at an early stage of gestation (Fig. 2). Beginning from the sixth lunar month of normal gestation, it was found in all the serum samples investigated. At term, the CBG variant level in the maternal blood was close to that in the retroplacental serum (Table 2) and accounted for 7–14% of the total CBG blood level. The absence of the CBG variant in the umbilical cord serum and placental extracts indicates that its biosynthesis occurs in some organ of the maternal organism and not in feto-placental unit.

Collectively, the above findings allowed us to classify [31] the CBG molecular variant within the group of pregnancy-associated proteins [32]. It should be emphasized, however, that unlike other proteins of this group biosynthesis of the pregnancy-associated

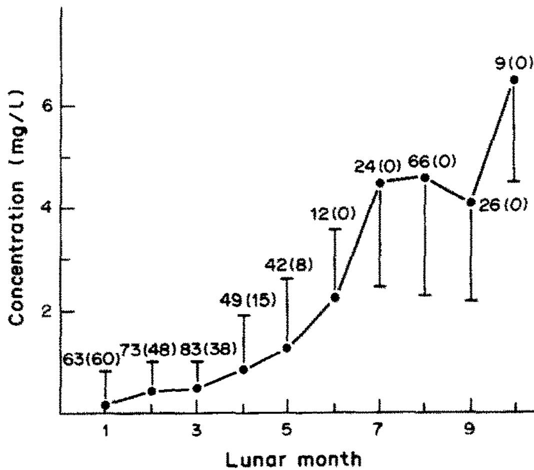

Fig. 2. Concentration of pregnancy-associated CBG variant (mean values (circles) and SD (bars)) in maternal venous blood serum throughout normal gestations. Figures indicate a number of subjects at a given term of pregnancy and, in parentheses, number of subjects in whose sera the concentration of the CBG variant was lower than the sensitivity of the radioimmunoassay used, i.e. 0.2 mg/l. From Avvakumov and Strel'chyonok[31].

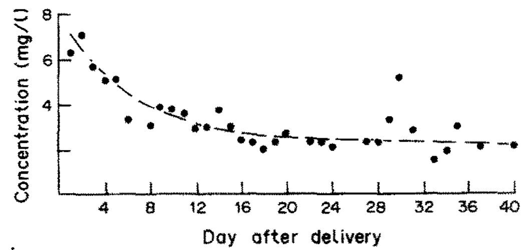

Fig. 3. Concentration of pregnancy-associated CBG variant in maternal venous blood serum in the postpartum period. Each point represents a mean value of 3–10 determinations (typical values of coefficient of variation were 20–30%) with the exception of the points corresponding to days 15, 17, 28–34, 37 and 40, each of which represents a single determination. The total number of the subjects studied was 131.

CBG variant is due to an altered post-translational modification of the polypeptide chain of a normal serum glycoprotein rather than to an activation of a certain gene.

Study of the dynamics of pregnancy-associated CBG variant level in the maternal venous serum after delivery (Fig. 3) supported the assumption on its biosynthesis in the maternal organism. The total CBG level rapidly decreased to the values which are characteristic of the normal donor serum (data not shown), while the level of pregnancy-associated variant remained rather high. As seen from Fig. 3, this variant did not disappear even to day 40 after the parturition. These data suggest that either the half-life of the CBG variant in the circulation is significantly longer than that of normal CBG (about 5 days [33]) or factors initiating its biosynthesis are still existing in the female organism after delivery. In our opinion, the latter assumption sounds more reasonable, albeit some elongation of the circulatory life of the glycoprotein variants with more branched/more sialylated sugar chains has been reported [34].

Elucidation of the exact tissue in which the CBG variant biosynthesis occurs and the factors controlling this process must await further investigation. It is important that the very fact of the appearance (or dramatic increase) of the CBG variant in pregnancy disagrees with the widespread concept [2] ascribing a passive role to the serum hormone-binding glycoproteins in the mechanisms of steroid hormone action. The CBG carbohydrates do not participate in the steroid binding (see data presented in Ref. [27] and Table 1) and, consequently, are not involved in the CBG functioning as a "depot" for steroid hormones. Assumingly, our findings on the differential binding of CBG molecular variants to human syncytiotrophoblast plasma membrane (see below) will help to clarify the biological significance of the pregnancy-associated variant.

Since the presence of sugar chains with different number of branch-points does not cover all the kinds of microheterogeneity inherent in CBG and SHBG (see above), further investigations may reveal additional molecular variants of these glycoproteins.

An interesting and important property of human CBG and SHBG is their ability to form polymeric molecules. (It should be emphasized that the formation of CBG oligomers and the SHBG subunit structure represent two very different phenomena). The CBG polymerization initiated by the action of various agents and resulted in the protein inactivation was a subject of several investigations [35–38]. Mickelson *et al.*[27] showed that CBG could reversibly form a dimer that retains full steroid-binding activity. It binds two steroid molecules per dimeric

Table 2. Concentrations of pregnancy-associated CBG variant (mg/l) in serum samples from individual patients

Patient	Serum: Maternal venous at term	Retroplacental	Umbilical cord	Maternal venous on day 5 after delivery
A	6.7	7.0	0	6.1
B	10.0	9.5	0	9.5
C	7.0	6.9	0	4.0
D	5.7	5.9	0	3.6
E	7.1	6.5	0	5.0

Data from Ref. [28].

molecule and displays the same affinity for cortisol as the protomer.

More recently, Mueller and Potter[39] described the reversible CBG polymerization in the human plasma. The degree of polymerization diminished on dilution and by addition of sodium dodecyl sulfate or dithiothreitol but not by cortisol. These authors also found [40] that, at physiological CBG concentrations in the plasma, the number of binding sites and affinity for cortisol are substantially less than those of the purified and very dilute protein. These findings are in agreement with an observation made by Siiteri *et al.*[4] that the CBG affinity for [^{3}H]cortisol increases on dilution of human plasma. In our opinion, these results indicate negative cooperativity of the steroid binding at physiological CBG concentrations in the plasma. Dilution of the plasma leads to a partial depolymerization of the glycoprotein which, in turn, decreases the cooperativity of the binding and rises an apparent association constant. Another explanation of the above findings may lie in the fact that free fatty acids, which occur in the blood, influence the steroid-binding activity of CBG [41] (as well as SHBG [42]).

Researchers from several laboratories reported [43–47] that, unlike CBG, SHBG has a subunit structure. A functional SHBG species was suggested [43, 47] to have one steroid-binding site per two subunits which are composed of identical polypeptide and slightly different carbohydrate chains. Under SDS-electrophoresis in polyacrylamide gel, highly purified human SHBG migrated as two bands with slightly different molecular masses, traces of a third band being also visible [43]. Khan *et al.* demonstrated [28] that the observed heterogeneity was not due to the SHBG isolation from pooled serum: similar electrophoretic patterns were obtained with glycoprotein preparations from sera of individual men and women as well as with those from sera of pregnant women. When studying the interindividual variations of the SHBG subunits, Gershagen *et al.*[48] found that in 20% of the donor serum samples an SHBG variant occurred. This variant resolved into three major bands on SDS-electrophoresis, the extra band being heavier than the heavy band of the common, double-banded SHBG. Since the relative amounts of the SHBG electrophoretic variants varied with the glycoprotein preparations, the existence of both homodimeric and heterodimeric molecules was suggested [47]. Chemical basis for the different electrophoretic behavior of the SHBG subunits is still obscure. Cheng *et al.*[29] showed that deglycosylation of the glycoprotein did not alter the distribution of the protomeric forms of the subunits. As mentioned above, an opposite result has been reported by Gershagen[25]. Probably, both the carbohydrate and polypeptide structures of the SHBG subunits are slightly different due to post-translational modification of the polypeptide. It should be noted that the data of a few works [49–51] are consistent with the presence of one binding site per a monomeric rather than dimeric SHBG molecule.

As far as we are aware, reaction of the SHBG polymerization has not been investigated, while such an investigation would clarify in what functional form SHBG exists under physiological conditions. Namkung and Petra (as cited in Ref. [47]) have offered a negative cooperativity mechanism according to which steroid binding sites occur in each SHBG subunit but only half of them could be utilized since "binding of a steroid molecule to one of the subunits induces a conformation change in the other preventing a second steroid molecule from binding".

We would like to briefly report data of our preliminary experiments on the interaction of [^{125}I]SHBG with unlabeled SHBG immobilized on agarose. We detected specific binding of the labeled glycoprotein to the sorbent with a K_d of 10^{-12}–10^{-11} M. Neither specific binding of other ^{125}I-labeled proteins of human serum (albumin, transcortin and thyroxine-binding globulin) to immobilized SHBG nor the labeled SHBG binding to the indicated proteins immobilized on agarose was detected. The above K_d value suggests that at physiological concentrations (near 10^{-7} M [2]) SHBG exists as a dimer. If so, our data on the SHBG interaction with human endometrial plasma membrane at low concentrations of both SHBG and estradiol (see below) could be treated as circumstantial evidence for the steroid-binding activity of the monomeric SHBG and thus for the above negative cooperativity mechanism.

Immunochemical properties of the steroid-binding glycoproteins of human serum have been extensively studied and found to be determined by the structure of polypeptide components of the glycoproteins, since deglycosylation of CBG [27] and SHBG [29, 52] did not alter these properties. Moreover, the immunochemical properties of SHBG are close to those of androgen-binding protein [24], the glycosylation of the two proteins being undoubtedly different [22–41]. Analogously, monospecific antisera to normal human CBG and pregnancy-associated CBG variant do not distinguish the two glycoproteins (see above).

Phylogenetic studies of the steroid-binding glycoproteins were performed using monospecific polyclonal antisera to human CBG and SHBG [53, 54] as well as monoclonal antibodies to human CBG [55]. Human CBG was found to be structurally related to CBG of the apes and Old World monkeys, whereas in New World monkeys and Prosimians the steroid-binding activity in the blood plasma and structure of CBG were substantially different. Use of the monoclonal antibodies revealed a highly evolved and a more conserved part in the human CBG molecule. SHBG binding activity and structure among the various primates were found to be similar. Antisera to human SHBG could not differentiate human and ape SHBG and reacted with SHBG of Old World

Table 3. Specific binding of serum binding glycoprotein–hormone complexes to the plasma membranes of human steroid target tissues

Complex	Tissue	K_d^a, mol/l	B_{max}^a, fmol per mg membrane protein	Ref. No.
CBG–cortisol	Liver	$1 \cdot 10^{-6}$	10	[61]
SHBG–estradiol	Decidual endometrium	$3 \cdot 10^{-12}$	10	[68]
CBG–cortisol	Placental syncytiotrophoblast	$2 \cdot 10^{-10}$	150	[79]
CBG–progesterone	Decidual endometrium	$1 \cdot 10^{-10}$	5	[76]
Pregnancy-associated CBG variant–cortisol	Placental syncytiotrophoblast	$3 \cdot 10^{-12}$	3	[79]

[a]Rounded mean values are shown for better comparison.

monkeys and, though rather slightly, with SHBG of New World monkeys.

Various immunochemical techniques for the CBG and SHBG measurement in human serum and other body fluids, which are convenient for research and clinical investigations (e.g. radioimmunological [56–62], immunoradiometric [63, 64] and enzymo-immunological [65] ones) have been developed in several laboratories. These techniques along with some other procedures and commercial radio-diagnostic kits for the determination of CBG and SHBG that became recently available have been used for detailed investigations of the levels of these steroid-binding glycoproteins in serum and other body fluids at various physiological states of the human organism. These investigations demonstrated the validity of the CBG and SHBG determinations for clinical diagnostics and therapy (see, e.g. Refs [66, 67]).

INTERACTIONS OF CBG AND SHBG WITH PLASMA MEMBRANES OF HUMAN CELLS

From a viewpoint of the putative biological functions of the specific hormone-binding glycoproteins of human plasma, the experimental data on the selective, high-affinity interactions of CBG, SHBG and their steroid complexes with the plasma membranes of steroid target cells are of great importance. These data support an active role played by steroid-binding glycoproteins in the physiological mechanisms of steroid hormone action. It should evidently be the plasma membrane delimiting the intracellular compartments from the outer medium, that selectively recognizes and takes up regulatory molecules from the blood. The first and principal stage of the mechanism of hormonal signal transmittance with the involvement of a hormone-binding glycoprotein should be reception of the hormone–glycoprotein complex by specialized binding sites on the cell membrane. The entire process would obviously involve a number of transport stages, which are to be shut down in order to detect the primary act of the membrane reception of the hormone–glycoprotein complex. *In vitro*, it could be most easily performed by studying the specific membrane binding of this complex, labeled either with ^{125}I via the glycoprotein or ^{3}H via the steroid component, at low temperatures (0–4°C) which allows a quasi-equilibrium state of the analytical system to be achieved.

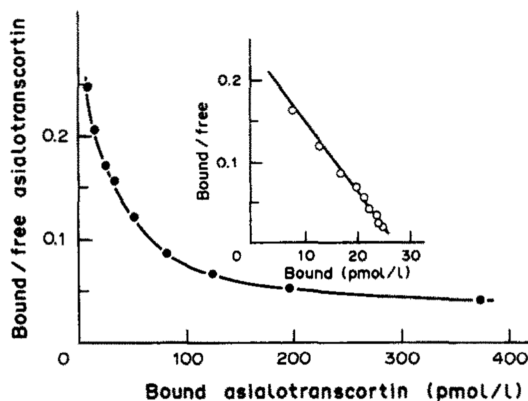

Fig. 4. Scatchard plot of the interaction of asialo-CBG with the human liver plasma membranes. Insert: linearized Scatchard plot of the high-affinity binding [68].

Using such an approach, we have studied the interactions of human CBG, pregnancy-associated CBG variant, SHBG as well as complexes of these glycoproteins with various steroids with the plasma membranes of a number of the human tissues. Results of this work are briefly summarized in Table 3.

It was found [68] that human liver plasma membranes contained not only the classical asialoglycoprotein receptor [68] that bound asialo-CBG with a K_d of $\sim 10^{-8}$ M but also specific binding sites which displayed much higher affinity for asialo-CBG: $K_d = (1.1 \pm 0.2) \cdot 10^{-10}$ M (Fig. 4). Other desialylated glycoproteins from human serum competed with asialo-CBG for these sites only weakly, and there was no typical for asialoglycoprotein receptor dependence [69] of the relative competition ability of an asialoglycoprotein on the number of exposed galactose residues in its molecule [70]. The most prominent observation was that intact CBG and its complex with cortisol competed with [^{125}I]asialo-CBG for the binding to the discovered binding sites [68]. The K_d value calculated from the competition experiments (see Table 3) indicates the specific interaction of intact CBG with the liver membrane.

Hryb *et al.* have stated [71] that the observed competition of intact CBG with asialo-CBG for the liver membrane binding sites could be attributed to the presence of desialylated molecules in the CBG preparations used. Indeed, the molar content of

N-acetylneuraminic acid in the CBG preparations from the human serum has been usually less than that of galactose [8], which indicates a partial desialylation of the glycoprotein. But, as shown above, the discovered binding sites are clearly distinct from the classical asialoglycoprotein receptor with respect to both the affinity for asialo-CBG and selectivity of the binding reaction. Furthermore, saturable binding of ^{125}I-labeled intact CBG–cortisol complex could be observed at glycoprotein concentrations ranging from 10^{-7} to 10^{-5} M (our unpublished data). This is consistent with the K_d value for the membrane binding of intact CBG calculated on the basis of competitive inhibition of [^{125}I]asialo-CBG binding (Table 3).

In vivo, CBG binding to the liver cell membranes will evidently lead to an increase of the local concentrations of CBG and CBG-bound steroids in this tissue. This will facilitate the glucocorticoid uptake by the liver cells even if CBG is not directly involved in the transmembrane steroid transport.

The K_d value for the membrane binding of intact CBG (Table 3) is close to the value of physiological concentration of the glycoprotein in the human blood (about 10^{-6} M [1]). It means that the CBG binding to the liver membrane could be regulated both at the level of the organism as a whole via an alteration of the CBG concentration in the blood and at the organ level through modulation of the density of exposed CBG receptors in the hepatocyte membranes. (Of course, this assumption is valid only if the K_d value determined at 0–4°C is close to that at the physiological temperature).

Several works from other laboratories have reported interactions of CBG and SHBG with the plasma membranes of steroid target tissues, which were characterized by apparent K_d values close to the values of physiological concentrations of these glycoproteins in the human blood. Thus, Hryb *et al.*[71] found two classes of specific binding sites for SHBG in the plasma membranes of human prostate (the tissue was obtained from men undergoing prostatectomy for benign prostatic hyperplasia): high-affinity, $K_a = (6.53 \pm 2.32) \cdot 10^7\ M^{-1}$, and low-affinity, $K_a = (1.23 \pm 0.43) \cdot 10^5\ M^{-1}$, ones. The high-affinity sites were specific for SHBG, whereas the low-affinity ones also bound human CBG and transferrin with approximately equal affinities. Specific binding sites for CBG were also found in these membranes [72]. Again, the calculated K_d value was close to the glycoprotein concentration in the blood. Similar results were obtained in the study of rat CBG interaction with the plasma membranes of the rat spleen [73]. Unfortunately, the authors of the above works [71–73] have not investigated steroid-dependence of the glycoprotein–membrane interactions. This makes their conclusions on the involvement of CBG and SHBG in the steroid penetration into the human prostatic cells somewhat speculative. One more remark is related to the correctness of the K_d values determined in these works. As stated above, for a correct determination of the equilibrium binding parameters, a quasi-equilibrium state of the analytical system should be achieved. This means that any other ways of the decomposition of the "glycoprotein–membrane complex", except for its dissociation to the starting components (i.e. glycoprotein in the solution and specific binding site in the membrane), should be excluded or minimized. The authors of the above works [71–73] carried out binding assays at 37°C. As could be clearly seen from the data given in these works, formation of the glycoprotein–membrane complexes was accompanied by side reactions that led to the degradation of either the glycoprotein or the membrane binding site. This is evidenced, e.g. by a dramatic decrease of the membrane-bound radioactivity after incubation for 6 h at 37°C [71]. Besides, it is easy to demonstrate that the determination of the non-specific labeled ligand binding to the membrane by the use of a large excess of the unlabeled ligand is only valid when an equilibrium state of the analytical system is achieved. One could suspect, therefore, that the closeness of the equilibrium (?) K_d values determined under principally non-equilibrium conditions to the values of the physiological concentrations of CBG and SHBG is only fortuitous.

Steroid-dependent CBG binding to rat tissues (kidney, uterus, pituitary and fat) was shown by Kuhn[74]. He demonstrated the presence of one class of specific binding sites for CBG with a K_d of $1.3 \cdot 10^{-6}$ M and concluded that these findings support a mechanism of glucocorticoid uptake with the involvement of CBG.

Thus, the above cases of hormone-binding glycoprotein interactions with the plasma membranes of hormone target tissues probably reflect the existence of CBG- and SHBG-mediated mechanisms of steroid hormone uptake which allow dual regulation: the hormone influx into the cells is sensitive to both changes of the blood level of the corresponding hormone–glycoprotein complex and alterations of the exposed receptor density on the cell membrane.

Experiments using tissues which develop during gestation, namely, decidual endometrium and placenta, showed the possibility of mechanisms with another kind of regulation. As seen from Table 3, the K_d values that characterize CBG and SHBG interactions with the plasma membranes of these tissues are substantially less than the values of normal plasma concentrations of these glycoproteins. Moreover, the glycoprotein binding to these membranes is steroid-dependent (see below). Hence, CBG and SHBG could supply, via membrane receptor systems, endometrial and trophoblastic cells with steroid hormones necessary for the normal development of feto-placental unit irrespectively of alterations in the hormone and glycoprotein levels in the maternal circulation. In a wide range of the concentrations of glycoprotein–hormone complex, such a mechanism

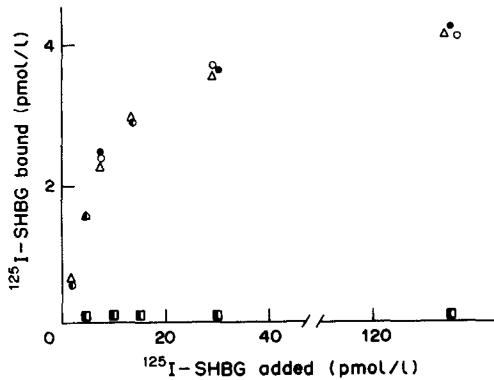

Fig. 5. Specific binding to human decidual endometrium plasma membranes of [^{125}I]SHBG complexed with estradiol (●), estriol (○), estrone (△), testosterone (□) and 5-alpha-dihydrotestosterone (■) [77, 79].

would be sensitive only to the membrane receptor concentration.

A recognition system for SHBG-estrogen complexes was found in the plasma membrane of decidual endometrium [75]. This system is composed of an SHBG-binding component and the well-known estrogen receptor [76]. The endometrial membrane was found [77] to display approximately equal affinities for SHBG complexes with estradiol, estriol and estrone and not to bind the glycoprotein complexed with androgens, testosterone or 5-alpha-dihydrotestosterone (Fig. 5), though the latter steroids also occur at rather high concentrations in the plasma of pregnant women [78].

The following experiments showed that the SHBG-binding membrane component and estrogen membrane receptor function as a unified membrane recognition system for SHBG estrogen complexes [75, 79]. When studying the dependence of [^{125}I]SHBG membrane binding on estradiol concentration in the

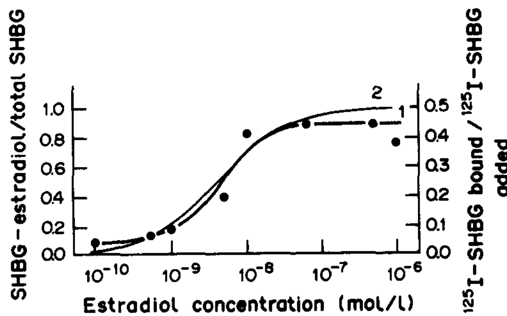

Fig. 6. Equilibrium binding of [^{125}I]SHBG to human decidual endometrium plasma membranes as a function of the estradiol concentration in the medium (curve 1). Each sample contained 4500 cpm of [^{125}I]SHBG, which corresponded to a protein concentration of approx. 4 pmol/l. Increasing concentrations of estradiol were added into the samples prior to the incubation. Each point is the average of two determinations from the same membrane preparation. Curve 2 represents the portion of SHBG associated with estradiol calculated as a function of the steroid concentration in an equilibrated binary system wherein the SHBG concentration is 4 pmol/l. From Strel'chyonok *et al.*[75].

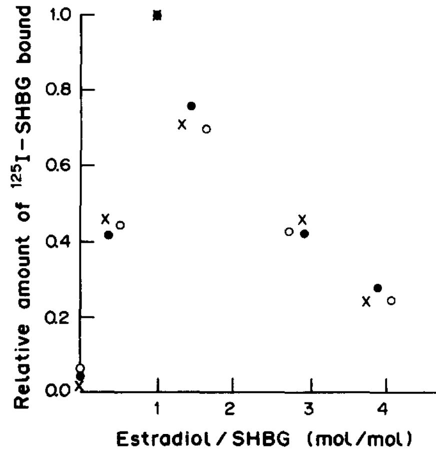

Fig. 7. Relative amount of [^{125}I]SHBG bound to human decidual endometrium plasma membrane as a function of the estradiol/SHBG ratio. Samples contained 1.6 (○), 2.8 (●) or 9.7 (×) pmol/l of [^{125}I]SHBG, and increasing amounts of estradiol were added before the incubation. The relative amount of bound [^{125}I]SHBG was calculated as a ratio of [^{125}I]SHBG bound at a given estradiol/SHBG ratio to the maximum amount of [^{125}I]SHBG bound at a given protein concentration. Each point is the average of two determination. From Strel'chyonok *et al.*[75].

medium, we found that, at very low concentration of the labeled glycoprotein (approx. $4 \cdot 10^{-12}$ M), an increase in the estradiol concentration from 10^{-11} M to 10^{-6} M led to an increase in the [^{125}I]SHBG membrane binding which paralleled the SHBG saturation with the steroid (Fig. 6). This means that the endometrium membrane recognizes and binds only SHBG molecules having a particular conformation induced by complexing with estrogen and does not bind SHBG molecules devoid of steroid. Unexpectedly, specific [^{125}I]SHBG membrane binding was also observed at an estradiol concentration which was approximately equal to that of the labeled glycoprotein (i.e. about $4 \cdot 10^{-12}$ M). At such low concentrations of glycoprotein and steroid their complex is almost completely dissociated (K_d is about $4.5 \cdot 10^{-9}$ at 4°C [80]). Further study [79], in which the [^{125}I]SHBG concentration ranged from $1.6 \cdot 10^{-12}$ to $9.7 \cdot 10^{-12}$ M confirmed that the specific membrane binding of the glycoprotein was maximal at an equimolar concentration of estradiol (Fig. 7). At the same time, the observed increase of the [^{3}H]estradiol membrane-binding in the presence of unlabeled SHBG in the medium reached a plateau at an SHBG/estradiol ratio near 1.0 mol/mol (Fig. 8) [79, 81]. Collectively, these findings suggest that the assembly of SHBG–estradiol complex on the plasma membrane of endometrial cells is possible. In other words, SHBG binds to estradiol complexed with the estrogen membrane receptor and, after a conformational transition of the SHBG molecule, is recognized by and binds to the SHBG-binding membrane component. (Such a complex composed of at least four different molecular species, namely, estrogen receptor,

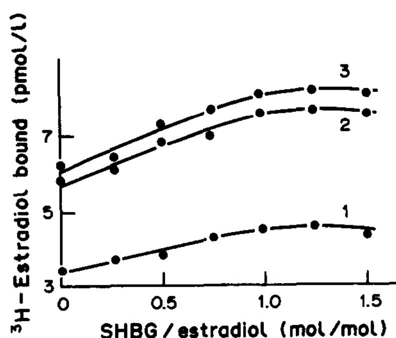

Fig. 8. Dependence of the specific [³H]estradiol binding to human decidual endometrium plasma membrane on the SHBG/estradiol ratio. Samples contained 3 (1), 6 (2) or 12 (3) pmol/l of [³H]estradiol, and increasing amounts of SHBG were added before the incubation. Each point is the average of two determinations. From Avvakumov *et al.*[79].

estradiol, SHBG and SHBG-binding component of the membrane, is, evidently, a kind of the transient state in the multi-step reaction of the SHBG-estradiol binding to the membrane. So, it could be observed only at relatively low temperatures, when transport phenomena are minimized. Nevertheless, the very possibility of its formation gives evidence for the existence of the membrane recognition system for SHBG–estrogen complexes).

An improtant role of the steroid-induced conformational transitions of the SHBG polypeptide chain [2, 82] in the formation of determinants for the membrane recognition of SHBG-steroid complexes stems from the steroid dependence of the glycoprotein membrane binding. The SHBG carbohydrates are also involved in the formation of these determinants. We have found [52] that the SHBG desialylation does not influence the immunochemical properties and steroid-binding activity of the glycoprotein but results in a complete loss of its ability to bind specifically to the endometrium membranes.

Specific binding sites for CBG were also found in the plasma membrane of human decidual endometrium [83] (Table 3). Endometrium is known to be an important target tissue for progesterone. In accordance with this, endometrial cell membrane displayed a higher affinity for CBG complexed with progesterone than with cortisol [84].

Using affinity chromatography of ^{125}I-labeled, cholate-solubilized endometrium membrane preparations on immobilized CBG we have isolated and partially characterized the CBG-binding membrane components [84]. Some of these components are lipids presumably containing unsaturated fatty acid residues. Few CBG-binding proteins were also observed, and basic molecular properties of one of them were determined. It was found to be a sialoglycoprotein with a minimum apparent M_r of $20{,}000 \pm 1{,}500$ and a pI of 3.3. During electrophoresis under non-denaturing conditions, it tended to form a stoichiometric oligomer. Using immunochemical techniques and hydroxylapatite adsorption chromatography, we have demonstrated that the purified membrane sialoglycoprotein binds CBG complexed with progesterone or cortisol (the respective K_d values were $\sim 2 \cdot 10^{-11}$ and $\sim 7 \cdot 10^{-11}$ M) but does not interact with CBG devoid of steroid or complexed with testosterone (Fig. 9). It is evident that, in this case either, selectivity of the membrane binding of the hormone-binding glycoprotein is due to the specific conformational transitions of its molecule induced by steroid binding.

Two classes of specific binding sites for CBG molecular variants were found in the plasma membrane of human placental syncytiotrophblast [85] (Table 3). Binding sites of both classes may differentiate molecular variants with different carbohydrate structures: normal CBG and pregnancy-associated CBG variant. High-affinity sites, occurring in the membrane at a very low concentration (see Table 3),

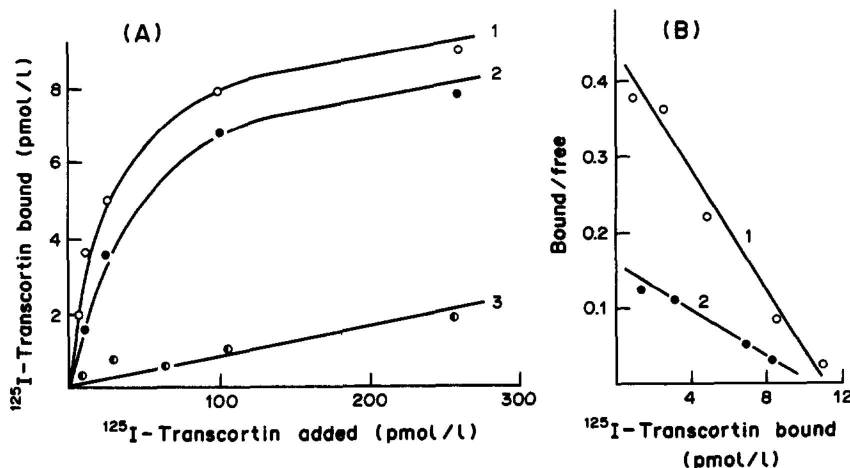

Fig. 9. (A) Equilibrium binding to the 20 kDa sialoglycoprotein, purified from human decidual endometrium plasma membranes, of [^{125}I]CBG complexed with progesterone (1), cortisol (2) or testosterone (3); (B) Scatchard plot of the specific binding of the progesterone (1) and cortisol (2) complexes. From Avvakumov *et al.*[84].

Table 4. Kinetic parameters of the binding of cortisol complexes of CBG and pregnancy-associated CBG variant to the microvesicular fraction derived from human placental syncytiotrophoblast plasma membrane at 23 ± 2°C

Glycoprotein	K_d, mol/l	V_s, s^{-1}
	Concentration range A	
CBG	$2.7 \cdot 10^{-12}$	$1.1 \cdot 10^{-3}$
CBG variant	$0.7 \cdot 10^{-12}$	$4.6 \cdot 10^{-4}$
	Concentration range B	
CBG	$1.0 \cdot 10^{-9}$	$1.2 \cdot 10^{-2}$
CBG variant	$4.0 \cdot 10^{-9}$	$1.7 \cdot 10^{-2}$

Mean values calculated from 2–3 independent experiments are given. The glycoprotein concentrations ranged from 10^{-12} to $6 \cdot 10^{-11}$ M (A) and from $5 \cdot 10^{-10}$ to $1.3 \cdot 10^{-7}$ M (B); data from [86].

display an affinity for the pregnancy-associated variant ($K_d = (3.3 \pm 2.0) \cdot 10^{-12}$ M) higher than that for normal CBG ($K_d = (2.5 \pm 0.7) \cdot 10^{-11}$ M). On the contrary, binding sites of another class, occurring in the membrane at a far greater concentration, show a higher affinity for normal CBG ($K_d = (1.6 \pm 0.6) \cdot 10^{-10}$ M) than for the pregnancy-associated variant ($K_d = (4.5 \pm 1.2) \cdot 10^{-9}$ M). Thus, the existence of the natural CBG molecular variants gave us a unique opportunity to demonstrate the involvement of CBG carbohydrates in the glycoprotein interaction with the plasma membrane of a hormone target tissue without performing any chemical or enzymatic modification of the sugar chains, which could give artifactual results because of a partial CBG denaturation. Again, conformation of the CBG polypeptide chain (and, consequently, the entire CBG molecule), that depends on the structure of a steroid bound, was found to influence the membrane binding: devoid of steroid, both normal CBG and the pregnancy-associated variant did not bind to the membrane (data not shown).

Study of the kinetics of the CBG variant interactions with the syncytiotrophoblast membrane at 23 ± 2°C [86] confirmed substantial differences between the two classes of the binding sites (Table 4). This suggests that these sites are the components of two different systems of hormone reception and, probably, transmembrane hormone transport. Investigation of this possibility is now under way in our laboratory.

PHYSIOLOGICAL ROLE OF SPECIFIC HORMONE-BINDING GLYCOPROTEINS

Current concepts of steroid hormone action have been mainly formulated under an influence of the "two-step" model suggested by Jensen *et al.* in 1968[87]. According to this model, steroids penetrate through plasma membrane of every tissue by free diffusion and selectivity of their hormonal action is due to the presence of "cytoplasmic" receptors in the responsive tissues. Upon binding steroids these receptors enter the nucleus and interact with the genome to modulate gene activity.

Results of a number of works carried out in various laboratories in the past several years disagree with the "two-step" model. Thus, it was found that some physiological effects of steroid hormones could be realized at the level of cytoplasm [88] or even plasmalemma [89]. Several authors (see e.g. Refs [90] and [91]) have reported that unoccupied intracellular steroid receptors seem to be localized in the nucleus, not in the cytoplasm. Moreover, convincing evidence has been obtained for the steroid uptake by target cells via a mechanism involving receptor on the outer side of the plasma membrane followed by specific transport of the hormone into the intracellular compartments [76, 92, 93]. So, when considering the hypothetical mechanisms that control the selectivity of steroid hormone action, we should pay attention to the plasma membrane rather than the cytoplasm of the responsive cells.

Most of the suggested models of the physiological action of steroid hormones pay little or no attention to the specific steroid-binding glycoproteins of the blood plasma. It is well known that, in the circulation, steroid (as well as thyroid) hormones are largely bound to proteins (albumin and specific binding glycoproteins) [1–6]. Nevertheless, the idea that only "free", not protein-bound steroids are physiologically active is still wide spread (see e.g. Refs [2] and [5]). Specific binding glycoproteins are conventionally assumed to transport steroid hormones in an inactive form, protecting them from adsorption to the vascular walls and rapid metabolism as well as from any enzymatic and chemical attacks in the plasma. It is supposed that rapid dissociation of the steroid–protein complexes at physiological temperatures compensates the uptake of "free", physiologically active hormones by tissues.

In this review, we will not consider numerous papers that have reported correlations between the biochemical or physiological activity of a steroid hormone and the "free" hormone level. The possibility that, under certain (not too rarely occurring) conditions, the "free" steroid concentrations simply reflect the degree of saturation of the corresponding binding glycoproteins with the steroids, i.e. the concentration of the steroid–glycoprotein complexes, has usually been overlooked in these papers. For example, a substantial rise in the concentrations of both cortisol and CBG is observed in pregnancy. This, in turn, leads to a significant (2–3-fold) increase of the CBG-cortisol complex level which is accompanied by a relatively small increase of the "free" cortisol concentration [94]. Besides, one could hardly assume that the enhanced cortisol and CBG biosynthesis and secretion during gestation have no relation to an enhanced glucocorticoid hormone action on certain tissues. Furthermore, the appearance of high levels of a special, pregnancy-associated CBG variant, having the same steroid-binding ability as normal CBG, could not be explained in the framework of the concepts ascribing to CBG a passive role in steroid hormone action.

Two groups of investigators, namely, Pardridge and co-workers and Ekins and co-workers (see e.g. Refs [95–97] and [98, 99], respectively), developed hypotheses that steroids complexed with the specific binding glycoproteins are readily available for tissues although these glycoproteins are not involved in the hormone penetration into the target cells. Based upon substantially different mathematical models, both groups have shown that binding of low-molecular-weight hormones to the serum glycoproteins enhances (and does not restrict) hormone efflux from the capillary into the tissue. It is important that some of the factors that influence the hormone efflux are "tissue-specific", e.g. the capillary wall permeability for the serum glycoproteins and the presence, in the endothelial cell membranes, of specific receptors facilitating dissociation of the hormone–glycoprotein complexes in the capillary [95–97], the extravascular "free" hormone concentration and the capillary wall permeation rate constant (for hormone) [98, 99]. This suggests that specific binding glycoproteins, while not penetrating the capillary wall and interacting with cells, may selectively deliver steroid hormones to the tissues.

Independently from the traditional viewpoint on biological functions of the serum hormone-binding glycoproteins, various authors have been developing hypotheses during the last 20 years, on an active role played by these glycoproteins in the interactions of low-molecular-weight hormones with the responsive cells. As far as we are aware, such hypotheses were originally offered by Keller *et al.* in 1969 [100] and Winterburn and Phelps in 1972 [101]. More recently, similar hypotheses have been formulated in the works by Bordin and Petra[102], Siiteri *et al.*[4] and Hryb *et al.*[71] as well as in a number of works from several other laboratories. These hypotheses have been supported by certain experimental findings: the hormonal activity of CBG-bound steroids, which was found to be, at least, the same as that of "free" steroids [100, 103], penetration of the hormone-binding glycoproteins into cells [102, 104] and immunocytochemical localization of CBG- and SHBG-like proteins in the steroid target cells [102, 105–114].

The involvement of serum binding globulins in the cellular uptake of biologically important small molecules is now well established [115–118]. It is reasonable to assume that steroid-binding glycoproteins are involved in similar uptake mechanisms. As concluded by Kuhn *et al.* (for the case of CBG-cortisol) [114], this gives several important advantages: "(1) specificity of hormone effects on appropriate cells would be determined by the presence (or absence) of recognition sites for the CBG-cortisol complex on the cell surface membrane, (2) economy of hormone action would be achieved since the CBG-bound cortisol would be protected from metabolism by the ubiquitous steroid-metabolizing enzymes, and (3) modulation of hormonal activity . . . could be achieved at a step before receptor binding by mechanisms analogous to those observed for protein hormones . . .". Of course, one could note that the presence of membrane receptors for steroid hormones as such could facilitate realization of some of the above advantages. The most convincing rationale for the involvement of steroid-binding glycoproteins in steroid hormone action still lies in the fact that the serum levels of the glycoprotein-bound steroids are much higher than those of "free" steroids and, under variable conditions, the levels of protein-bound and "free" steroids often alter in the same direction (and not in opposite directions). Nevertheless, only experimental data could provide conclusive evidence for the validity of this idea. That is why we pay such great attention to the findings on specific binding of steroid–glycoprotein complexes to the plasma membranes of steroid responsive cells. Indeed, these findings unambiguously indicate that the hormone-binding glycoproteins of the human blood plasma control the selectivity of interactions between steroids and target cells and they are likely to be directly involved in the hormone (or hormonal signal) transmission into the cells.

Generally speaking, the involvement of a binding glycoprotein in the interaction of steroid with a target tissue does not mean that the steroid–glycoprotein complex penetrates through the plasma membrane as previously assumed [4, 71, 102, 114]. Analysis of the data on the SHBG–estradiol complex interaction with the recognition system located in the endometrium plasma membrane allowed us to suggest a "shuttle" mechanism [77] (Fig. 10). This suggestion was based on the following facts: (1) the affinities of individual components of the SHBG–estradiol complex for the components of the membrane recognition system is significantly higher than the SHBG affinity for estradiol and (2) SHBG devoid of steroid does not bind to the membrane. Consequently, it is very probable that the receptor of SHBG–estradiol complex is followed by dissociation of this complex on the outer surface of the membrane. Estrogen, bound to its

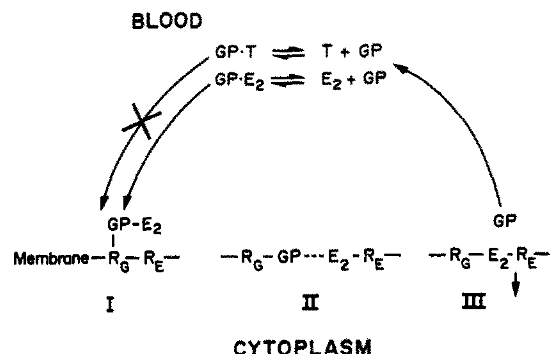

Fig. 10. A hypothetical shuttle mechanism for the SHBG-mediated estradiol (E_2) interaction with the target cell plasma membrane. GP, glycoprotein (SHBG), T, testosterone, R_G and R_E, membrane receptors for SHBG and E_2, respectively.

receptor, is then transported into the cell via the established mechanism [76]. SHBG loses its affinity for the SHBG-binding membrane component because of a conformational transition of the SHBG molecule after dissociation of steroid, leaves the membrane and re-enters the intercellular medium wherein it can complex a new estradiol molecule and transport it to the membrane. We assume that similar mechanisms are realized in the cases of the CBG–progesterone complex interaction with the endometrium membrane [83] and interaction of the pregnancy-associated CBG variant–cortisol complex with the plasma membrane of human syncytiotrophoblast [85].

Penetration of a steroid–glycoprotein complex as a whole through the target cell plasma membrane seems to occur in the case of the interaction of normal CBG–steroid complexes with the plasma membrane of human placental syncytiotrophoblast [86]. This conclusion stems from the similarity of kinetics of the uptake, by syncytiotrophoblast membrane microvesicles, of [^{125}I]CBG in the presence of excess unlabeled cortisol (Table 4) and, vice versa, [^{3}H]cortisol in the presence of excess unlabeled CBG (data not shown) as well as from the selectivity of the membrane binding of CBG–steroid complexes. As mentioned above, CBG devoid of steroid did not interact with the syncytiotrophoblast membrane. But, along with the specific membrane binding of CBG complexed with cortisol, we have observed specific binding of the glycoprotein complexed with corticosterone, progesterone and even with testosterone. It could hardly be assumed that the membrane binding of CBG complexed with the latter two steroids has any physiological significance. It is well known that, in pregnancy, the progesterone biosynthesis occurs in the placenta while CBG-bound testosterone represents only a minute fraction of the circulating steroid [67]. In this case, a putative mechanism for the **CBG** transfer from the maternal to fetal circulation via transcytosis [119] through the trophoblast cells could be suggested. Independent of the steroid structure, specific steroid binding to CBG provides a guarantee that the glycoprotein is in a physiologically active form. (Denatured forms of hormone-binding glycoproteins have been also found in the blood [120]).

Unlike normal CBG, pregnancy-associated CBG variant does not penetrate the syncytiotrophoblast membrane, but cortisol complexed with it can easily penetrate the membrane (our unpublished observation). On the one hand, these findings are in accordance with the absence of the CBG variant in the umbilical cord serum, i.e. on the fetal side of the placental barrier [31]. On the other hand, they agree with the above assumption that this CBG variant functions as a transport "shuttle" which delivers cortisol into the trophoblast cells.

Of course, other mechanisms of the cellular uptake of CBG- or SHBG-bound steroids could also be proposed. However, it should be emphasized that they must be consistent with the existence of membrane receptor components that recognize the glycoprotein molecules complexed with certain steroids. This is principally the difference of such mechanisms from the previous assumption [4] that cell membrane recognizes and binds **steroid** complexed with the glycoprotein, and cellular uptake of the steroid is accompanied with the transmembrane transfer of the binding glycoprotein. (It must be noted that the depth of the CBG binding site [121] will, evidently, prevent the steroid molecule from the membrane recognition except for the case of preliminary weakening of the CBG–steroid complex due to the **CBG** interaction with the membrane).

In the light of the data obtained biological functions of the CBG and SHBG carbohydrate moieties have become clearer. As mentioned above, carbohydrates are not involved in the steroid binding to these glycoproteins. This is consistent with the localization of the sugar chains on the surface of polypeptide globule [122]. Integrity of the SHBG oligosaccharide chains and specific features of the carbohydrate structures of CBG molecular variants were found to be essential for the specific binding of hormone–glycoprotein complexes to the plasma membranes of target cells. Consequently, the glycoprotein sugar chains play an important role in the formation of determinants for the recognition of these complexes by the membrane receptor systems. This conclusion is in line with an assumption made by Winterburn and Phelps[101] that the carbohydrate chains of specific hormone-binding glycoproteins of the blood plasma serve a function of "antigenic determinants" for the recognition of small, hydrophobic hormone molecules by the responsive cells. However, the role of the glycoprotein polypeptide chains should not be underestimated: when binding steroids, the polypeptide chain change its conformation which, in turn, changes the spatial orientation of the sugar chains.

The involvement of serum binding glycoproteins in steroid hormone action on the target cells makes more evident possible similarity between the mechanisms of physiological action of steroid hormones, on the one hand, and peptide and protein hormones, on the other hand, which have been previously discussed by Szego[89]. It could be expected that the interactions of hormone–glycoprotein complexes with the membranes give rise to certain effects that are typical for protein hormones which do not penetrate through the membranes. Otherwise, there appears a possibility for the transmembrane transfer of **hormonal signal** without the transfer of the **hormone** itself. Recently, Nakhla *et al.*[123] have for the first time reported experimental evidence supporting this possibility. These authors have established that the interaction of CBG–steroid complexes with MCF-7 carcinoma cells resulted in the induction of adenylate cyclase activity and the accumulation of cAMP within the cells, i.e. in the activation of a second messenger system, which

is typical for non-penetrating protein and peptide hormones.

The involvement of glycoprotein carbohydrate moieties in the membrane receptor of the hormone–glycoprotein complexes makes possible a differential regulation of the hormone delivery into tissues on the basis of alterations in the carbohydrate structures accompanying changes of the physiological state of human organism. For example, the syncytiotrophoblast membrane displays a higher affinity for pregnancy-associated CBG variant than for normal CBG [85]. On the contrary, the liver plasma membrane shows a higher affinity for normal CBG [12]. Consequently, increase of the CBG variant blood level during gestation would promote the glucocorticoid action on the placenta and not on the liver. Desialylation of the serum glycoproteins, which is known to be sensitive to the physiological state of human organism, inhibits the interaction of hormone–glycoprotein complexes with plasma membranes of the peripheral tissues thus directing steroid transport to the liver.

Finally, the interaction of CBG and SHBG with the cell membranes may have physiological consequences apart from steroid hormone action. For instance, the selective penetration of CBG molecular variants through the placental barrier may be related not only to the hormone transfer from the maternal to the fetal circulation but with the transfer of CBG to fulfill its physiological role in the fetus.

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