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# Properties and serum levels of pregnancy-associated variant of human transcortin

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**The amino acid composition, N- and C-terminal amino acid sequences, and the basic physicochemical and immunochemical properties of the recently discovered pregnancy-associated molecular variant of human transcortin (Strel'chyonok, O.A., Avvakumov, G.V. and Akhrem, A.A. (1984) Carbohydr. Res. 134, 133–140) have been found to be identical to those of transcortin from normal donor serum. This suggests the identity of polypeptide moieties of the two glycoproteins. The transcortin variant has a lower isoelectric point (3.5–4.1) than normal transcortin (3.6–4.2), and different electrophoretic mobility in low-porosity polyacrylamide gel (one band versus two for normal transcortin). These differences can be reasonably explained by different organization of the carbohydrate moieties of these glycoproteins due to diverse post-translational modification of a single polypeptide chain. The levels of transcortin variant in the maternal venous serum throughout normal gestation (447 donors in all) and on the fifth day after delivery, as well as in umbilical cord serum and extracts of term placenta, have been measured by a radioimmune assay. Analysis of the data obtained allowed us to conclude that the biosynthesis of pregnancy-associated transcortin variant occurs in some organ of the maternal organism rather than in the feto-placental system, and it is a characteristic of pregnancy as a unique physiological state of the female organism rather than a phenomenon caused by individual features of certain women. We assume that the transcortin variant takes part in the guided transport of corticosteroids and/or progestins into some tissues that develop in the course of gestation.**

## Introduction

In the human and other species, the protein content of the maternal blood serum in pregnancy differs from that of normal serum: the levels of certain globulins increase, and some new proteins of the feto-placental origin appear [1]. Corticosteroid-binding globulin or transcortin is an example of the former globulins: during gestation its level increases 2–3-fold [2]; the structure and properties of transcortin assumingly remain unaltered [3,4]. It has recently been found, however, that the preparations of pure transcortin isolated from pooled human retroplacental serum contain approx. 10% of a molecular variant with carbohydrate structures different from those of transcortin from normal serum [5].

This fact served as an impetus for the present work, in which we have attempted to clarify the following questions. To what extent are the structure and properties of the transcortin variant different from those of normal transcortin? What is

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the origin of transcortin variant? Is it a common globulin of retroplacental serum or an abnormal transcortin modification from a few donors whose sera had contributed to the pooled one? Where is the transcortin variant synthesized: in the maternal circulation or in the feto-placental system? What are the dynamics of the transcortin-variant level in the maternal serum throughout normal gestation?

The data obtained suggest that the polypeptide components of transcortin and its variant are identical. The biosynthesis of the transcortin variant is a characteristic of pregnancy as a unique physiological state of the female organism rather than a phenomenon due to genetic or other individual features.

## Materials and Methods

Cortisol, progesterone and methyl α-D-glucopyranoside were purchased from Serva (Heidelberg, F.R.G.), Sepharose CL-4B and Con A-Sepharose from Pharmacia (Uppsala, Sweden), and [1,2,6,7-$^3$H]cortisol and [1,2,6,7-$^3$H]progesterone from Amersham International (Amersham, U.K.).

Transcortin was isolated from human postpartum blood serum as previously described [6], and affinity chromatography on immobilized concanavalin A was used to isolate pregnancy-associated transcortin variant [5]. The glycoprotein preparations were desalted by gel filtration, freeze-dried and stored at −20 °C.

Electrophoretic experiments were performed using a GE-2/4 instrument (Pharmacia). Standard electrophoretic procedures were carried out as earlier described [7,8], and electrophoresis in low-porosity polyacrylamide gel was performed as described by Mickelson et al. [9]. Isoelectrofocusing of the glycoproteins was carried out using 7.5% polyacrylamide gel containing 1.8% of Servalyt 2–4 and 0.2% of Servalyt 3–7 (Serva). For the determination of the pH gradient along a gel slab, it was dissected into 0.5-cm pieces, Servalyts were eluted using 1 ml of distilled water per piece, and the pH of the solutions obtained was measured.

For the amino acid analysis, the glycoproteins were hydrolyzed with 5.7 M hydrochloric acid for 24 h at 110 °C. Amino acids were then quantified with an LKB 3201 instrument (LKB, Bromma, Sweden). Hydrolysis of the glycoproteins with mercaptoethanesulfonic acid [10] was used for the determination of tryptophan content. Oxidation of the glycoproteins with performic acid and the determination of cysteic acid were carried out as previously described [11]. The N-terminal amino acid sequences of transcortin and its variant were determined using an APS 240 Solid Phase Sequencer (Rank Hilger, Westwood Margate, U.K.). The glycoproteins were covalently linked to aminopropyl glass via ε-aminogroups of lysine residues using the *p*-phenylenediisocyanate technique [12]. Carboxypeptidase Y (Pierce, Rockford, IL, U.S.A.) was used for the determination of C-terminal amino acid residues of the glycoproteins [13].

The parameters of hormone binding to transcortin and its variant were determined by equilibrium dialysis technique [9]. The radioactivity of $^3$H-labeled steroids was measured using scintillation liquid ZhS-7 (Reakhim, U.S.S.R.) and a liquid-scintillation counter Mark III (Tracor Europa, Bilthoven, The Netherlands).

Glycoproteins were radioiodinated using carrier-free Na$^{125}$I (Izotop, Leningrad, U.S.S.R.) and Iodogen (Pierce) as earlier described [14]. The $^{125}$I-labeled glycoproteins were purified by gel filtration and affinity chromatography on immobilized cortisol [6]. A Ria Gamma counter (LKB-Wallac, Turku, Finland) was used for measuring $^{125}$I radioactivity.

For preparation of antisera, adult male New Zealand rabbits were immunized with either transcortin or its pregnancy-associated variant. The antisera obtained were monospecific as indicated by radial immunodiffusion in agar gel [15], these were lyophilized and stored at −20 °C. To assay the interaction of the glycoproteins with antisera, $^{125}$I-transcortin or $^{125}$I-labeled transcortin variant (30 000–40 000 cpm per tube) and increasing concentrations of the corresponding unlabeled glycoprotein were incubated with an antiserum, diluted to bind 70–80% of the tracer in the absence of unlabeled competitors, for 3 h at room temperature (18—23 °C). Subsequently, poly(ethylene glycol) 6000 (Serva) was added in each tube to a final concentration of 12–18%. Immunoglobulins and the bound antigen were then precipitated by centrifugation for 10 min at 1500–2500 × *g*. The

supernatant was discarded, and the radioactivity of the precipitates was measured. The binding parameters were calculated as previously described [16]; these are given in the text as means ± S.D. of three to four determinations from independent experiments.

Samples of venous blood serum from normally gestating women at different terms through pregnancy were kindly presented from the Byelorussian Research Institute of Mother and Child Health (Minsk, U.S.S.R.); other serum samples (see Results and Discussion) were collected at local hospitals. For the preparation of placenta extracts, 100 g (wet weight) of trophoblast tissue from freshly delivered normal human placenta was sectioned into approx. 0.5-cm pieces and thoroughly washed with isotonic solution of calcium chloride and then with saline. The tissue was homogenized in three volumes of saline using a blender (Waring, New Hartford, CT, U.S.A.). The homogenate was filtered through several layers of gauze, and the insoluble subcellular fractions were precipitated by centrifugation, first at $10000 \times g$ for 20 min, and then at $100000 \times g$ for 90 min. The final supernatant was concentrated to a volume of 30 ml by ultrafiltration through a PM-30 membrane (Amicon, Lexington, MA, U.S.A.).

The technique for quantification of pregnancy-associated transcortin variant in samples of serum or placenta extracts will be published elsewhere. Briefly, a sample (0.02–0.05 ml) was applied on a microcolumn prepared from a disposable pipette tip and containing 0.25–0.50 ml of Con A-Sepharose. The transcortin variant (along with some other serum proteins which were not retarded in the column) was eluted with 0.05 M Tris-HCl (pH 7.0)/0.15 M NaCl/0.01 M $CaCl_2$/0.01 M $MgCl_2$/0.01 M $MnCl_2$ [5]. In the eluate obtained, pregnancy-associated transcortin variant was quantified using the radioimmunological procedure described above, with slight modifications. Appropriate solutions of pure human transcortin were used as standard samples to calibrate the assay.

## Results and Discussion

As shown in Table I, the amino acid composition of the transcortin variant is similar to that of transcortin from normal donor serum (the coefficient of correlation is greater 0.9). Both N- and C-terminal sequences of these glycoproteins are identical: Met-Asp-Pro-Asn-Ala-Ala-Tyr-X-Met-Ser-Asn- and -(Val,Glu)-Leu, respectively. The similarity of basic physicochemical properties of transcortin and its variant (Table II) also suggests a close relatedness of their polypeptide components. In particular, these glycoproteins display equal affinities for both cortisol and progesterone, which suggests the same structural organization of their steroid-binding sites. This fact is in line with the previously reported data [9] indicating that the transcortin carbohydrates are not involved in the steroid-hormone binding. Further evidence for the structural similarity of the molecules of trans-

TABLE I

AMINO ACID AND MONOSACCHARIDE COMPOSITION OF HUMAN TRANSCORTIN AND ITS PREGNANCY-ASSOCIATED MOLECULAR VARIANT

Values are means of two determinations. The carbohydrate compositions were determined earlier (see Ref. 5). GlcNAc, *N*-acetylglucosamine; NeuAc, *N*-acetylneuraminic acid.

| Amino acid monosaccharide | Content, mol/mol of glycoprotein | |
|---|---|---|
| | Transcortin | Pregnancy-associated variant |
| Asp | 33.2 | 31.2 |
| Thr | 21.8 | 20.4 |
| Ser | 27.6 | 26.0 |
| Glu | 30.4 | 25.3 |
| Pro | 9.8 | 10.2 |
| Gly | 18.7 | 18.1 |
| Ala | 20.4 | 20.3 |
| Val | 21.5 | 18.9 |
| Cys | 1.9 | 1.9 |
| Met | 9.3 | 9.3 |
| Ile | 17.3 | 15.5 |
| Leu | 32.6 | 30.4 |
| Tyr | 8.5 | 8.7 |
| Phe | 17.2 | 16.2 |
| His | 9.5 | 7.7 |
| Lys | 12.6 | 13.3 |
| Arg | 7.7 | 7.4 |
| Trp | 3.7 | 3.6 |
| Fuc | 1.2 | 1.2 |
| Man | 15.0 | 15.0 |
| Gal | 12.1 | 14.8 |
| GlcNAc | 22.1 | 24.3 |
| NeuAc | 10.7 | 12.7 |

TABLE II

PHYSICOCHEMICAL PROPERTIES OF HUMAN TRANSCORTIN AND ITS PREGNANCY-ASSOCIATED MOLECULAR VARIANT

| Measured parameter | Transcortin | Pregnancy-associated variant |
|---|---|---|
| $M_r$ | 55000 | 55000 |
| $R_F$: electrophoresis in standard non-denaturing conditions | 0.62 | 0.62 |
| $R_F$: electrophoresis in low-porosity gel under non-denaturing conditions | 0.43, 0.46 | 0.46 |
| p$I$ | 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 transcortin (l/mol) | $(1.0 \pm 0.2) \cdot 10^9$ | |
| $K_a$: antiserum to the transcortin variant (l/mol) | $(1.8 \pm 0.3) \cdot 10^9$ | |

cortin and its variant is offered by the identity of their immunochemical properties (Table II).

Though the total amino acid sequences of transcortin and its pregnancy-associated variant are not yet known, the combined data of this work allow us to preliminarily conclude that the polypeptide structures of the two glycoproteins are indistinguishable.

Different behavior of transcortin and its variant under isoelectrofocusing and electrophoresis in low-porosity polyacrylamide gel (Table II) could be reasonably explained by the differences in their carbohydrate structures. Thus, the bands obtained after isoelectrofocusing of the transcortin variant are shifted towards lower pH values with respect to the bands of transcortin from normal serum. This is consistent with the higher sialyl residue content in the former glycoprotein (see Table I). During electrophoresis in low-porosity gel, the transcortin variant migrates as a single band, while transcortin from normal serum migrates as two electrophoretic variants [9] (see Table II). This may be explained as follows. Since transcortin

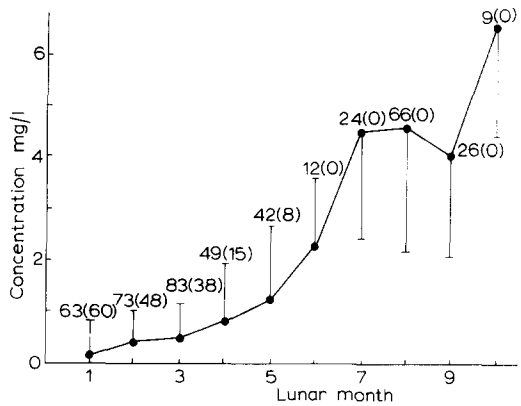


Fig. 1. Concentration of pregnancy-associated transcortin variant (mean values (circles) and S.D. (bars)) in maternal venous blood serum throughout normal gestation. Figures indicate a number of patients at a given term of pregnancy and, in parentheses, number of patients, in whose sera the concentration of the transcortin variant was lower than the sensitivity of the radioimmune assay used, i.e., 0.2 mg/l.

contains N-linked sugar chains of two kinds, biantennary and triantennary [17], there is a possibility for the existence of isomeric molecules of this glycoprotein with different distribution of biantennary and triantennary oligosaccharides among the sites of glycosylation in the polypeptide chain. The transcortin variant contains only triantennary N-linked oligosaccharides [5], which eliminates the possibility for the above isomerism. Consequently, the comparative data on the low-porosity gel electrophoresis of transcortin and its variant support and improve a previous assumption [9] of a differing arrangement of carbohydrate moieties of the electrophoretic variants of normal transcortin.

Fig. 1 shows the results of determination of the transcortin variant level in the maternal venous serum throughout normal gestation. As seen from these data, pregnancy-associated transcortin variant has been found in the serum of all the women investigated, beginning from the sixth lunar month of pregnancy, and the mean level of this glycoprotein increases with the time of pregnancy. Consequently, the biosynthesis of transcortin variant is controlled by certain common factor(s), characteristic of pregnancy as a unique physiological state of the female organism. Furthermore, the data on the transcortin variant blood level

throughout normal gestation may have implications in clinical diagnostics.

The biosynthesis of pregnancy-associated transcortin variant seems to occur in some organ of the maternal organism and not in the feto-placental system. The following facts support this assumption (see Table III). First, the transcortin variant level in maternal serum in late pregnancy (cf. Fig. 1) and at term is close to that in retroplacental serum. Second, we have not found this substance in umbilical cord serum. This means that the transcortin variant is not synthesized in the fetus and does not penetrate through the placental barrier. The latter conclusion is of interest in connection with the obscure problem of penetration of the maternal globulins, in general [18], and of transcortin, in particular [19,20], through this barrier. Third, the transcortin variant level in women's serum is little or not decreased on the fifth day after delivery. It is known [21] that the half-life of transcortin in human blood circulation is 4.6–6 days. So, it can be assumed that either the transcortin variant has a longer half-life in the circulation or its biosynthesis continues for some period of time after delivery. In our opinion, the latter assumption seems more likely. Finally, the content of the transcortin variant in concentrated extracts of human placenta (three independent experiments) was lower than the sensitivity of the radioimmune assay used, approx. 0.2 mg/l. This means that a whole term placenta contains less than 0.05 mg of this glycoprotein and, consequently, could hardly be a tissue wherein the biosynthesis of the transcortin variant occurs.

TABLE III

CONCENTRATIONS OF PREGNANCY-ASSOCIATED TRANSCORTIN VARIANT (mg/l) IN SERUM SAMPLES FROM INDIVIDUAL PATIENTS

| Patient | Serum | | | |
|---|---|---|---|---|
| | maternal venous at term | retro-placental | 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 |

According to the adopted classification [22], there are three groups of pregnancy-specific proteins: trophoblast-specific; fetal; and pregnancy-associated. The latter group includes proteins which are synthesized in the maternal organism, the blood levels of which alter significantly during pregnancy. The data obtained in the present work allow us to classify the transcortin variant with this group of proteins: it appears in the maternal circulation at the early stage of pregnancy, its level increases during gestation, and it is synthesized neither in the fetus nor in the placenta. There is, however, a peculiar feature that differentiates the transcortin variant from other representatives of this group. Since the apoglycoprotein glycosylation is not genetically encoded [23], the appearance of a special transcortin variant evidently results not from the activation of biosynthesis of the transcortin polypeptide chain but from an alteration in the process of its post-translational modification.

Elucidation of the physiological significance of pregnancy-associated transcortin variant must await further investigation. It is, however, evident that the very fact of the appearance, in pregnancy, of a special molecular variant displaying the same steroid-binding ability as transcortin from normal serum disagrees with the current concept [24] ascribing a passive role to serum specific binding globulins in the mechanisms of steroid hormone action. In the light of the previous data on the involvement of these globulins in interactions of steroid hormones with plasma membranes of target tissues (e.g., see Refs. 25–27), it can be assumed that pregnancy-associated transcortin variant takes part in a guided transport of corticosteroids and/or progestins to one of the tissues (uterus, decidual endometrium, placenta, etc.) developing during pregnancy.

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