J. Steroid Biochem. Molec. Biol. Vol. 38, No. 1, pp. 43–47, 1991
Printed in Great Britain.

0960-0760/91 $3.00 + 0.00

TRANSCORTIN IN RAT KIDNEY: SUBCELLULAR DISTRIBUTION OF TRANSCORTIN-SYNTHESIZING POLYRIBOSOMES

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(Received 9 March 1990; received for publication 5 September 1990)

Summary—The [^{3}H]corticosterone–transcortin complexes from kidney cytosol show elution positions on DEAE–cellulose identical to serum transcortin.

The incorporation of ^{14}C-labeled amino acids into anti-transcortin-precipitable material of kidney slices has been measured and compared with that of serum transcortin. It was established that kidney synthesized transcortin with an apparent molecular weight of 66 kDa on SDS–electrophoresis which resembles serum corticosteroid-binding globulin.

Studies on the binding of [^{125}I]anti-transcortin-IgG to membrane-bound rat kidney polyribosomes revealed an association of [^{125}I]anti-transcortin-IgG with a discrete polyribosome fraction in the heavy polyribosome region; free polyribosomes were devoid of antigenic material able to bind antibodies to transcortin.

INTRODUCTION

Transcortin (corticosteroid binding globulin or CBG) is the major specific transport glycoprotein for glucocorticoids in the blood of almost all vertebrate species [1]. The primary role of transcortin might be to protect circulating glucocorticoids from metabolic conversions and to regulate the physiologically active free fraction [2]. Evidence accumulates in the literature that transcortin may interact directly with the plasma membrane of some cells [3, 4]. These binding sites may be involved in the translocation of steroid hormones across cell membranes. Interaction of corticosterone with cytosolic transcortin-like proteins has been demonstrated in numerous mammalian tissues [5–8]. It is tempting to speculate that these extra- and intracellular steroid binding components may play an important role in the modulation of the entry of steroids into the cells and in the delivery of the hormone to cytosolic receptors.

The precise mechanism of the subcellular synthesis of transcortin is not fully understood. It is still doubtful whether these proteins are synthesized in the kidney *de novo* or represent serum proteins which have been transported from the extracellular fluid. In order to solve this question the level of newly synthesized transcortin from labeled amino acids was determined in kidney tissue using an immunochemical technique. The aim of the present work was also to identify polyribosome populations involved in transcortin biosynthesis in the normal rat kidney tissues.

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EXPERIMENTAL

Chemicals

[1α, 2α(n)-^{3}H]Corticosterone 50 Ci/mmol was purchased from Amersham International. Unlabeled corticosterone, activated charcoal, Tris (hydroxymethyl) aminomethane, 2-mercaptoethanol, *N*-(2-hydroxyethyl) piperazine-*N'*-2-ethanesulfonic acid Na-salt, DEAE–cellulose and chloramine T were obtained from Serva Feinbiochemica. Sephadex G-50, Sephadex G-200, Dextran T-500 and all chemicals for electrophoresis were from Pharmacia Fine Chemicals. Cycloheximide and amino acids were obtained from Sigma Chemicals. All other chemicals were of reagent grade or better.

Purification of transcortin and preparation of antibodies

Rat serum transcortin was isolated by means of salt precipitation and chromatographic techniques [9]. Repeated chromatography of rat serum proteins of DEAE–cellulose,

hydroxylapatite and subsequent gel-filtration through Sephadex G-200 resulted in pure transcortin as judged by electrophoresis on polyacrylamide gel with sodium dodecylsulfate. The rabbit antiserum was raised against highly purified rat transcortin preparations according to Vaitukaitis *et al.* [10]. Purification of the IgG fraction to electrophoretic homogeneity was performed by means of ion-exchange chromatography [11] and subsequent purification of IgG fraction was performed on transcortin–Sepharose sorbent prepared according to the manufacturer. The purity of the eluted IgG preparation was assayed both by SDS–polyacrylamide electrophoresis and by double immunodiffusion techniques. The purified IgG was iodinated with [^{125}I] by the choloramine-T method [12].

Cytosol preparation and ion-exchange chromatography

The kidneys were perfused *in situ* with 0.9% cold NaCl and homogenized in 2 vol of buffer A containing 20 mM Tris–HCl, pH 7.5, 25 mM KCl, 2 mM 2-mercaptoethanol and 10% glycerol. The homogenate was centrifuged at 105,000 *g* for 1 h at 4°C. For steroid hormone receptor inactivation the supernatant was immediately incubated at 40°C for 30 min. The cytosol and serum (2.5%) were incubated with 15 nM [^{3}H]corticosterone in the presence or absence of a 1000-fold excess of radioinert corticosterone for 2 h at 4°C. One ml of the prelabeled cytosol or serum was applied on DEAE–cellulose [1.0 × 7.0 cm). The column was washed with 10 ml buffer followed by elution with 40 ml of a linear 0.002–0.4 M KH_2PO_4 gradient.

Assay of transcortin biosynthesis

This was performed using kidney slices as described previously [13]. 2.5 g kidney slices were incubated in 5 ml of Hanks' balanced salt solution containing 10 mM HEPES buffer, pH 7.4 and 0.5 mCi of [^{14}C]protein hydrolyzate under 95% O_2/5% CO_2 at 37°C for 4 h. Kidney slices were homogenized in incubation medium and cytosol was prepared by centrifugation at 105,000 *g* for 1 h. Total incorporation of radioactivity into protein was assayed by trichloracetic acid precipitation. For precipitation of the newly synthesized [^{14}C]transcortin 10 μg of the pure cold transcortin and 600 μg of IgG fraction of rabbit antiserum were added to 2 ml of the cytosol and incubated at room temperature for 1 h and left at 4°C overnight. The immunoprecipitates were washed three times with PBS (10 mM phosphate buffer–145 mM NaCl, pH 7.4) and then solubilized with 0.2 ml of 5 mM Tris–HCl, pH 6.8, containing 1% SDS and 1% mercaptoethanol for SDS–polyacrylamide gel electrophoresis.

Isolation of polyribosome fractions

Kidney tissues were homogenized with 3 vol of buffer B (50 mM Tris–HCl, pH 7.4, 25 mM KCl, 5 mM $MgCl_2$) containing 250 mM sucrose, 200 μg/ml heparin and 20 μg/ml cycloheximide. Free and membrane-bound polyribosomes were prepared from rat kidney as described previously [13] by centrifugation on discontinuous sucrose density gradients. The homogenate was centrifuged at 10,000 *g* for 10 min and the pellets were discarded. Postmitochondrial supernatant was layered over discontinuous 2.0–2.5 M sucrose gradient in buffer A and centrifuged at 150,000 *g* for 4 h. Membrane-bound polyribosomes were treated with 0.5% sodium deoxycholate and 2% Triton X-100 at 4°C for 1 h as well as free polyribosomes. After recentrifugation through 2 M sucrose the two polyribosomal fractions were resuspended in buffer A.

Sedimentation analysis of polyribosomes

For identification of transcortin-synthesizing polyribosomes kidney polysomes (3 A_{260} units) were incubated with 5 μg [^{125}I]anti-transcortin-IgG at 4°C for 1 h in a total volume of 0.5 ml. The specificity of binding was determined by incubating polysomes with [^{125}I]anti-transcortin-IgG in the presence of unlabeled IgG. After the incubation the suspensions were layered on a linear 10–40% sucrose gradient in buffer B and centrifuged at 200,000 *g* for 90 min at 4°C. Gradient fractions (11 drops) were withdrawn from the bottom of each tube and adsorbance at 260 nm monitored. Radioactivity in polyribosomal fractions was measured by a gamma-scintillation spectrometer.

RESULTS

The quantity of bound [^{3}H]corticosterone to transcortin in the renal cytosol and diluted serum (2.5%) was determined by charcoal adsorption. It was found that in heated (30 min at 40°C) renal cytosol non-specific binding of [^{3}H]corticosterone ranged from 10 to 20% of total binding and was less than 2% in serum

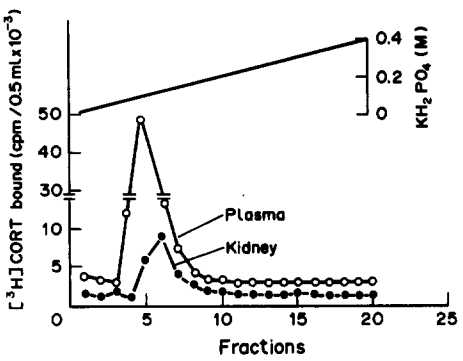

Fig. 1. DEAE–cellulose chromatography of [^{3}H]corticosterone–transcortin complexes in kidney cytosol and serum. Renal cytosol and serum were heated at 40°C for 30 min and then incubated with 15 nM [^{3}H]corticosterone for 2 h at 4°C. One ml of the samples was loaded onto the column. The column were washed with 10 ml buffer to remove unbound complexes and free steroids. Adsorbed complexes were eluted with a 30 ml linear 0.002–0.4 M KH_2PO_4 gradient in buffer A. Two ml fractions were collected and 0.5 ml aliquots were used for determination of radioactivity.

(data not shown). When corticosterone-labeled cytosol from rat kidney was chromatographed on columns of DEAE–cellulose, one peak of bound radioactivity eluted at 0.08–0.10 M KH_2PO_4 (Fig. 1). These results are consistent with reports that corticosterone binding exists in kidney citosol [7]. A preparation from rat serum transcortin also contained similar corticosterone binding protein peak eluting at the same KH_2PO_4 concentrations. Comparison of two chromatograms shows that the hormone binding component in kidney cytosol is similar to that of rat serum transcortin. However, in this binding experiment it is impossible to exclude

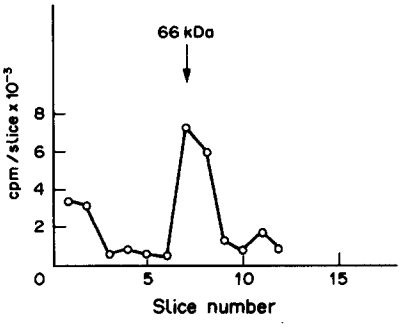

Fig. 2. SDS–polyacrylamide gel electrophoretic analysis of anti-transcortin precipitable material in kidney cytosol. Kidney slices were incubated at 37°C for 3 h with [^{14}C]protein hydrolysate in MEM medium with Hanks' salts. The [^{14}C]transcortin in kidney cytosol was precipitated with specific anti-IgG at 4°C for 18 h and the resulting precipitate was assayed on polyacrylamide gel. Then electrophoresis gels were cut into 2 mm slices and dissolved in 0.4 ml 30% hydrogen peroxyde at 60°C for 18 h. An arrow indicates the position of unlabeled serum transcortin in a parallel gel. The direction of protein migration is from the left to the right.

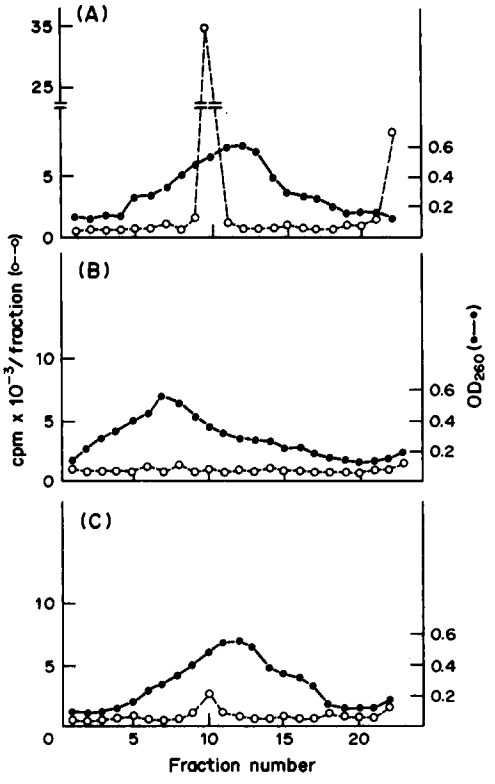

Fig. 3. Sucrose gradient analysis of rat kidney polysomes. Sedimentation profiles and binding of [^{125}I]anti-transcortin-IgG to membrane-bound (A) and free polyribosomes (B); (C) specificity of the [^{125}I]anti-transcortin-IgG binding to rat kidney membrane-bound polyribosomes. Kidney polysomes were isolated as described in the Experimental section. Polysomes (3 A_{260} units) were incubated with 5 μg [^{125}I]anti-transcortin-IgG at 4°C for 1 h. The specificity of the [^{125}I]anti-transcortin-IgG binding was detected by incubating polysomes in the presence or absence of 100 μg unlabeled anti-transcortin-IgG.

the possibility that the observed bound radioactivity was transported from another tissue synthesizing transcortin.

Synthesis of transcortin in rat kidney was examined by incubation of kidney slices with [^{14}C]amino acids. Under the conditions of our experiments material precipitable by anti-transcortin accounted for 0.08–0.2% of the total protein synthesis in kidney slices prepared from normal rats. In order to further substantiate transcortin synthesis by kidney slices, the material precipitable by anti-transcortin was electrophoresed on SDS–polyacrylamide gels. As shown in Fig. 2 the material precipitable by the anti-transcortin in the kidney cytosol gave one prominent radioactive peak with the mobility of serum transcortin.

The sedimentation profiles depicted in Fig. 3 show ribosomal distribution from endoplasmic reticulum and postmitochondrial supernatant of rat kidney tissue. It is shown that more than

90% of ribosomes sedimented in the polyribosomal region of the gradient prepared from both membrane-bound and free fraction. The free polyribosomes showed an increase in the larger polyribosome pool. As can be seen in Fig. 3, after incubation of [^{125}I]anti-transcortin-IgG with kidney membrane-bound polyribosomes, radioactivity is not uniformly distributed, but associated with discrete polyribosome region. These data indicate the functional activity of transcortin mRNA involved in synthesis of nascent polypeptide chains of transcortin molecules on membrane-bound polyribosomes. In order to determine the specificity of the binding reaction of [^{125}I]anti-transcortin-IgG to polyribosomes we have studied binding in the presence of a large excess of unlabeled anti-transcortin-IgG. When polyribosomes were first preincubated with unlabeled antibody, followed by incubation with [^{125}I]anti-transcortin-IgG no radioactive peak was observed in the heavy polyribosomes region. The data suggest that binding is not due to a nonspecific association. In a separate experiment, kidney free polyribosomes were incubated with [^{125}I]anti-transcortin and fractionated by density gradient centrifugation. The binding profile of labeled antibodies to free polyribosomes is shown in Fig. 3. In this case the major part of the radioactivity is distributed almost uniformly throughout the A_{260} profile in the presence or absence of cold anti-transcortin. This finding demonstrates that free polyribosomes are devoid of antigenic material able to bind antibodies to transcortin.

DISCUSSION

Up to now it is generally believed that the liver is the only site of transcortin synthesis. However, several reports have been published which showed the presence of transcortin in some target organs. Immunoreactive transcortin has been detected in the rat uterus, kidney [14] and human lymphocytes [8]. A similar transcortin-like binding protein was found in the rat kidney by Feldman *et al.* [7]. These binding proteins are not only similar in steroid-binding characteristics, but also resemble rat transcortin antigenically [14]. More recently, Hammond *et al.* [15] have isolated and sequenced cDNAs for transcortin from human liver and lung. Their results have demonstrated that transcortin mRNA also exists in the testis and in the kidney. Accordingly, liver is not the only source of transcortin synthesis. In the current experiment it is clear that transcortin is synthesized *de novo* also in kidney tissues. However, our results are in disagreement with those of Smith and Hammond [16], who failed to show transcortin mRNA in the rat kidney tissues. The reason for this discrepancy in our opinion is probably that synthesis of transcortin occurs not in the whole kidney tissue but only in the small populations of kidney cells, surrounding distal and collecting tubules [14].

Earlier our studies have documented the synthesis of transcortin exclusively on membrane-bound rat liver polyribosomes [13]. From the current experiments, the renal transcortin also is synthesized by the polyribosomes attached to rough endoplasmic reticulum. This work is the first direct demonstration of intracellular transcortin biosynthesis in kidney tissue. In this context the presence of nascent transcortin polypeptide chains preferentially at the level of membrane-bound polyribosomes is worth noting. Assuming a secretory pathway of export protein synthesis, one can postulate that the transcortin is secreted from the membrane-bound form to the blood stream without occurring in the soluble phase of the liver and kidney cells. These findings, however, are not in agreement with earlier experiments [5, 7, 17], in which transcortin-like binding activity for corticosterone was found in cytosol. It seems most likely that transcortin occurrence in cytoplasm may be a consequence of receptor-mediated transcortin internalization into kidney cells from extracellular space.

The biological role of subcellular transcortin is not clear at the present time. Early reports demonstrating the presence of transcortin receptor on plasma membranes of a number of target tissues [3] together with our results suggest that this protein evidently could play a more important physiological role in the mechanism of action of steroids than has previously been believed. One can speculate that transcortin may be involved in the selective transfer of glucocorticoid across the plasma membrane and influence the intracellular delivery and transport to the nucleus of steroid bound to protein. In this way transcortin may also affect the biotransformation of steroid hormone by the cytoplasmic or microsomal steroid reductases.

Acknowledgements—We are grateful to Ms Rita Dudaravičiūtė for excellent technical assistance and Ms Vida Bakšytė for typing this manuscript.

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