Tissue distribution of the receptor for plasma retinol-binding protein

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The tissue distribution of the retinol-binding-protein receptor has been studied by using a cell-free binding assay. High binding activity was found in placenta, retina pigment epithelial cells, bone marrow and kidneys. Specific binding activity was also found in the small intestines, spleen and liver, and to a lesser extent in lung. Scatchard analysis revealed that the difference in binding activity was due to variations in receptor level and not affinity changes. When the kidneys were separated into cortex and medulla we found that almost all the specific binding activity present in kidneys was recovered in the cortex. The choroid plexus, an important site in the delivery of nutrients to the cerebrospinal fluid, expressed very high binding activity. The

pineal gland, which has been shown to store vitamin A, also showed high binding activity. Testes from immature animals showed higher binding activity than testes from mature rabbits. Cultured undifferentiated kidney keratinocytes showed about 40 times higher binding activity than differentiated cells. Skin fibroblasts demonstrated no binding activity. In conclusion, the data presented in this report show that the level of the retinol-binding-protein receptor varies considerably between cell types. The observed tissue distribution of the receptor agrees well with the present knowledge on retinol function and metabolism by various cells.

INTRODUCTION

Vitamin A is an important regulator of growth and differentiation in most cells of the body. To fulfil this regulatory role, the physiological concentration of retinol in plasma is regulated within a narrow range. Thus in an individual with adequate vitamin A status most cells are exposed to about 2 μ M retinol bound to a specific plasma protein, the retinol-binding protein (RBP) [1,2]. RBP is synthesized mainly in the liver parenchymal cells, but also in other cells and tissues such as liver stellate cells [3], Sertoli cells [4], kidney [5], placenta (visceral yolk sac of rat) [6] and adipose tissue [5]. It has a molecular mass of 21 kDa and contains one binding site for its ligand, retinol [7]. The protein has several antiparallel β -sheets forming a hydrophobic cleft for ligand binding [8]. In plasma, RBP forms a 76 kDa complex with transthyretin (TTR) which is large enough not to be lost in the glomeruli [7].

After cellular uptake, most of the retinol is recycled to plasma, and only a small proportion is converted into retinoic acid, the active metabolite [9]. Several aspects of this processing, such as the mechanism for cellular uptake, have not yet been elucidated in detail. Although some early data suggested the existence of a specific RBP receptor, this has been questioned by several researchers [7,10–14]. However, it now seems appropriate to conclude from recent evidence that a specific RBP receptor is involved in the cellular uptake of retinol. The strongest evidence supporting this includes selective uptake of iodinated RBP by a subgroup of cells *in vivo* [15–19], and specific binding to cells [20–25] and membrane preparations [26–28] *in vitro*. Furthermore, specific binding of RBP was demonstrated to the putative receptor solubilized from liver membranes [29]. Recently, a putative RBP receptor of molecular mass 63 kDa was cloned from retina pigment epithelial cells. This protein does not, however, enhance RBP uptake in transfected cells [30,31].

Experiments with both isolated cells [23] and membrane preparations [26,28] have shown that the RBP–receptor complex dissociates with a half-life of less than 10 min. Thus repeated washing of cells or membrane preparations to reduce non-

specific binding is also likely to displace the specific binding. Several attempts to demonstrate a specific RBP receptor may have been hindered by these characteristics.

Sivaprasadarao and Findlay [26] developed a method that separates receptor-bound and free RBP in seconds by a centrifugation through oil. We have now used this method, which minimizes the dissociation problem, to study the tissue distribution of the RBP receptor. We demonstrate specific RBP receptors in several organs and tissues relevant for vitamin A metabolism and function.

MATERIALS AND METHODS

Chemicals

Na¹²⁵I was purchased from Dupont–New England Nuclear (Boston, MA, U.S.A.). Enzymobeads were from Bio-Rad Laboratories (Richmond, CA, U.S.A.). Immunodiffusion plates for measuring RBP concentrations and alkaline phosphatase substrate were obtained from Behring (Marburg, Germany) and Sigma Chemical Company (Poole, Dorset, U.K.) respectively.

Animals, tissues and cells

The animals [male Chinchilla rabbits (2.0–3.5 kg) and Wistar rats (270–350 g)] were fed on an ordinary pellet diet (EWAR Sverige AB, Södertelje, Sweden) which contained 3.3 mg of retinol/kg for the rabbits and 3.6 mg of retinol/kg for the rats. Pig brain was purchased from Fellesslakteriet, Oslo, Norway. Full-term human placentas were obtained from Aker Hospital, Oslo, Norway.

Preparation of cells

Skin fibroblasts were isolated from rabbits by culturing skin tissues on plastic dishes. They were cultivated in Dulbecco's modified Eagle's medium (Flow Laboratories, McLean, VA,

U.S.A.) supplemented with 10% (v/v) fetal calf serum (Flow Laboratories), penicillin (100 units/ml), streptomycin (0.1 mg/ml), fungizone (2.5 µg/ml), gentamicin (0.2 mg/ml) and L-glutamine (2 mM). Sertoli cells were isolated from 18-week-old rats (immature) and cultivated for 5 days [32] before the experiments were carried out. Human keratinocytes were a gift from Dr. Rune Toftgård, Karolinska Institute, Huddinge, Sweden. They were cultured in serum-free MCDB 153 (Sigma) supplemented with CaCl_2 (70 µM), ethanolamine (0.1 mM), penicillin/streptomycin (10000 units/ml), fungizone (2.5 µg/ml), epidermal growth factor (10 ng/ml), hydrocortisone (0.4 µg/ml), insulin (5 µg/ml) and bovine pituitary extract (100 µg/ml) and induced to differentiate with high Ca^{2+} (1.2 mM) [33], adenine (0.18 nM) and 5% fetal calf serum.

Isolation and labelling of RBP

Human RBP was purified by a modification of the procedure of Vahlquist et al. [34]. An additional gel-filtration step (Sephadex G-75) was included in the final RBP preparation to obtain pure protein, as determined by SDS/PAGE and Coomassie Blue staining [35]. RBP was iodinated using the Enzymobead method as described in the instructions from the manufacturer. The iodinated RBP was used within 4 weeks of labelling.

Preparation of membrane vesicles

Placenta membrane vesicles were prepared from freshly obtained human placenta as described by Booth et al. [36]. The purity of the final preparation was assessed by measuring the 5'-nucleotidase which increased more than 20-fold over that of the initial homogenates. The membranes were stored at -20°C at a concentration of 5–10 mg of protein/ml.

Pig brains were obtained in ice-cold 0.15 M NaCl 10–20 min after slaughter. The cortex, pineal gland and choroid plexus were dissected from the brain and placed in ice-cold buffer (20 mM Tris/HCl, pH 7.5, 150 mM NaCl and 4 mM MgCl_2). The pineal gland was localized in the midline posterior, medial to the superior colliculus. All steps were carried out at 4°C . The tissues were homogenized (5 ml of buffer/g of tissue) by ten strokes in a Potter-Elvehjem homogenizer and centrifuged at 800 g for 5 min to remove debris and unbroken cells. The supernatant was filtered through cheesecloth and centrifuged again at 800 g for 5 min. To isolate membrane vesicles the resulting supernatant was centrifuged at 100000 g for 60 min. The pellet was redissolved in 0.15 M NaCl to a concentration of 5–10 mg of protein/ml. This membrane vesicle preparation was stored at -20°C , for up to 2 weeks without loss of activity.

Membrane vesicles from other tissues were prepared using the same procedure. Intestinal cells were scraped off the duodenum of a rabbit and washed in 0.15 M NaCl. Rabbit retina pigmented epithelium cells were isolated as described for rats [37]. Isolated cells were homogenized using a sonicator before centrifugation. The activity of the plasma-membrane marker 5'-nucleotidase in the final preparation was increased 2–5-fold over that of the initial homogenates.

Preparation of membrane vesicles from cells

Cultured keratinocytes or fibroblasts were scraped off with a rubber 'policeman' and pelleted by centrifugation at 200 g for 10 min. The cell pellet was resuspended in an ice-cold hypotonic 20 mM Tris/HCl buffer, pH 7.5, containing 4 mM MgCl_2 and homogenized by ten strokes in a Dounce homogenizer. The suspension was homogenized further by aspiration through a 25-gauge needle and gentle stirring on ice for 1 h. The subsequent

centrifugation steps for isolation of membrane vesicles were identical with those described for isolation of membrane vesicles from organs.

Assay of membrane vesicle receptor binding

^{125}I -RBP binding to membrane vesicle was assayed by using an oil-centrifugation method adapted from that described by Sivaprasadarao and Findlay [26]. Portions of membrane vesicles (50 µl) were incubated with 50 µl of ^{125}I -RBP (about 50000 c.p.m. and 2 nM RBP) in a 20 mM sodium phosphate buffer, pH 7.4, containing 0.1% ovalbumin and 150 mM NaCl. After incubation at room temperature for 15 min the membranes were centrifuged for 2 min at 12500 g, and 200 µl of a precooled phthalate oil mixture (3 parts of dibutyl phthalate and 2 parts of dinonyl phthalate) was added. Membrane-bound ^{125}I -RBP was separated from unbound ^{125}I -RBP by centrifugation for 1 min at 12500 g. Three parallel incubations in the presence or absence of 2 µM unlabelled RBP were carried out to determine specific and non-specific binding. The tubes were then frozen in solid CO_2 , and the bottoms of the tubes containing the pellets were cut off and counted in a Packard γ counter. Scatchard and correlation analyses were carried out using the software program Fig. P (Biosoft, Cambridge, U.K.).

Uptake of ^{125}I -RBP in tissues and organs

Male Wistar rats were anaesthetized and ^{125}I -RBP (1.5×10^6 c.p.m.) was injected in the femoral vein. At 30 min after injection a whole-body perfusion was performed (transcardiac perfusion with PBS) to remove plasma containing radioactivity from the various organs. The different organs and tissues were then removed for analysis.

RESULTS

Binding activity in various tissues

In initial experiments, binding of ^{125}I -RBP to human placenta membrane vesicles was studied. Figure 1 shows the Scatchard

Figure 1 Specific binding of ^{125}I -RBP to placental membrane vesicles and Scatchard analysis of the binding data (inset)

The membranes preparations (1 mg/ml) were incubated with various concentrations of ^{125}I -RBP at room temperature for 15 min. The data were fitted to a Scatchard plot. The calculated K_d and B_{max} were 37.4 nM and 1122 fmol of RBP bound/mg of membrane protein respectively. Values represent means of triplicates.

Table 1 Specific binding of ^{125}I -RBP to membrane vesicles prepared from different tissues

Membrane vesicles were prepared from various rabbit tissues as described in the Materials and methods section. The membrane vesicles (250 μg of protein) were incubated with 2–10 nM retinol- ^{125}I -RBP (50 000 c.p.m.) in the absence (total binding) and presence (non-specific binding) of 2 mM unlabelled retinol-RBP. Specific binding is defined as the difference between total and non-specific binding. The values presented are means $\pm$ S.D. of two to six analyses with triplicate determinations.

Tissue	Specific binding (c.p.m./250 μg of membrane protein)
Placenta	1399 $\pm$ 506
Retina pigment epithelial cells	1068 $\pm$ 394
Bone marrow	719 $\pm$ 104
Kidney	662 $\pm$ 178
Small intestine	399 $\pm$ 210
Spleen	391 $\pm$ 83
Liver	283 $\pm$ 207
Lung	163 $\pm$ 106
Testis	130 $\pm$ 91
Cerebral cortex	77 $\pm$ 109

analysis of binding of ^{125}I -labelled holo-RBP at 22 $^{\circ}\text{C}$ to placenta membrane vesicles. The values calculated for K_d and B_{max} were 37.4 nM and 1122 fmol/mg of membrane protein respectively which are in agreement with earlier published results [26,28,38].

The tissue distribution of the putative RBP receptor was then studied by using this binding assay. Membrane vesicles from rabbit tissues were incubated with 2–10 nM ^{125}I -labelled retinol-RBP in the absence (total binding) and presence (non-specific binding) of excess unlabelled RBP (2 mM) to determine specific binding activity. As shown in Table 1, high specific binding activity was found in placenta, retina pigment epithelial cells, bone marrow and kidneys. Specific binding varied between 23 and 50 % of total binding in these tissues. Specific binding activity was also found in the small intestine, spleen and liver, and to a lesser extent in lung and testis from mature rabbit. Specific binding in spleen and liver were 17 and 15 % of the total binding respectively; about 5 % of the total binding was specific in lung and testis and 2 % in the cortex of the cerebrum.

We then performed a Scatchard analysis of ^{125}I -RBP binding to various membrane preparations; the results are summarized

Table 2 Scatchard analysis of ^{125}I -RBP binding to membrane preparations from different tissues

The membrane preparations (2 mg/ml) were incubated with various concentrations of ^{125}I -RBP at room temperature for 15 min. The data were fitted to a Scatchard plot; they are means $\pm$ S.D.

Tissue	K_d	B_{max} (fmol RBP bound/mg of protein)
Placenta	37.4 $\pm$ 12.9	1122 $\pm$ 138
Bone-marrow	46.1 $\pm$ 5.3	930 $\pm$ 18
Kidney	47.6 $\pm$ 19.2	619 $\pm$ 80
Liver	32.5 $\pm$ 15.3	295 $\pm$ 66

Table 3 Uptake of intravenously injected ^{125}I -RBP by various rat tissues

^{125}I -RBP (1.5×10^6 c.p.m.; 6 pmol of RBP) was injected into the right femoral vein. After 30 min, the blood contaminants were removed from the different tissues by a whole-body perfusion, and tissues were taken for determination of radioactivity. The values presented are means of two experiments.

Tissue	Uptake (c.p.m./g wet weight)
Bone marrow	11 969
Kidney	72 408
Small intestine	3 636
Spleen	4 211
Liver	3 990
Lung	3 727
Testis	2 105
Cerebral cortex	177

in Table 2. K_d values were at the same level in placenta, kidney, bone marrow and liver. However, the B_{max} values varied between the different organs from 1122 fmol/mg of membrane protein for placenta to 295 fmol/mg of membrane protein for liver. The data suggest that the differences in binding activity shown in Table 1 are a result of the difference in levels of expression of the RBP receptor in the tissues examined.

Tissue uptake of ^{125}I -RBP

In another series of experiments ^{125}I -RBP was injected intravenously into rats. At 30 min after injection whole-body perfusion was performed and tissue samples were collected for determination of ligand uptake. The highest activity was observed in kidneys. Bone marrow, spleen, liver and small intestine also accumulated appreciable amounts of radioactivity (Table 3). We chose to use Enzymobead-labelled RBP in these *in vivo* experiments instead of RBP linked to a non-degradable marker (such as tyramine cellobiose) as Malaba et al. [23] showed recently that this labelling method yields RBP that best retained its binding capacity to liver cells. After 30 min, more than 90 % of the radioactivity taken up in the liver and kidneys was acid precipitable and thus represented protein bound and not radioactivity from degraded RBP.

When these uptake data were plotted against the binding activity found in the cell-free binding assay, the overall correlation between binding *in vitro* and uptake *in vivo* was significant ($R = 0.632$; $P < 0.05$). These data suggest that the tissue uptake of ^{125}I -RBP observed *in vivo* parallels the binding activities measured in the cell-free binding assay *in vitro* in most tissues. It should be added, however, that tissues from different species are compared in this experiment. Further experiment is therefore needed to substantiate this finding.

To ensure that the high uptake observed in kidneys was not due to reduced binding of ^{125}I -RBP to TTR we tested the binding of ^{125}I -RBP to a TTR affinity column. This affinity chromatography revealed that more than 95 % of the RBP bound to TTR.

Uptake and binding activity in kidney

In a previous study Smith et al. [39] reported that ^{125}I -RBP injected intravenously into rats was mainly localized (by using autoradiography) in the cortex of the kidneys. In the next experiment we injected ^{125}I -holo-RBP (500 000 c.p.m.) intravenously into a rabbit and determined uptake in kidneys after

Table 4 Specific binding of ^{125}I -RBP in different organs

Membrane vesicles from the cerebral cortex, choroidal plexus and pineal gland were prepared from pigs. Membrane vesicles were also prepared from testes of immature rabbits (14 weeks old), rats (7 weeks old), mature rabbits (24 weeks old) and isolated rat Sertoli cells. RBP-binding activity to membrane vesicles (250 μg of protein) was tested in the receptor-binding assay. The values for the brain preparations are means $\pm$ S.D. of three analyses with triplicate determinations and those for the testes are means $\pm$ S.D. of two analyses with triplicate determinations except for immature rats which is from triplicate determinations of one rat. Finally, membrane vesicles were prepared from human keratinocytes cultivated in a low- Ca^{2+} medium (undifferentiated) or a high- Ca^{2+} medium (differentiated) as described in the Materials and methods section and used (100 μg of protein) in the receptor-binding assay to determine specific binding. The results are means from triplicates of one typical experiment.

Membrane preparation	Specific binding (c.p.m./250 μg of membrane protein)
Brain	
Choroidal plexus	964 $\pm$ 143
Pineal gland	514 $\pm$ 179
Cerebral cortex	80 $\pm$ 23
Testis	
Immature rats	615
Immature rabbits	344 $\pm$ 60
Mature rabbits	130 $\pm$ 90
Sertoli cells (rats)	1013 $\pm$ 336
Skin	
Undifferentiated keratinocytes	1944
Differentiated keratinocytes	44
Skin fibroblasts	0

30 min. When the kidneys were dissected into cortex and medulla, we found that the cortex contained 8327 c.p.m./g wet weight whereas the medulla contained 1767 c.p.m./g wet weight. In both locations more than 95% of the radioactivity was precipitable in 10% trichloroacetic acid, suggesting that it represents protein-bound ^{125}I -RBP and not degradation products such as free ^{125}I which would diffuse rapidly out of the cells.

We then prepared membrane vesicles from the kidney cortex and medulla and determined the binding activity. Specific binding to total kidney was 662 c.p.m./250 μg of membrane protein. Almost all this specific binding activity was recovered in the cortex (1074 c.p.m./250 μg of membrane protein) compared with only negligible binding activity (35 c.p.m./250 μg of membrane protein) in the medulla. These results within an organ (i.e. kidney) show the same relation between high uptake and high specific binding as previously shown for different organs.

Binding activity in the brain

MacDonald et al. [19] have shown that intravenously injected iodinated RBP was localized specifically to the cuboidal epithelial cells of the choroid plexus. The choroid plexus is a primary site of the blood-brain barrier and is therefore important in the delivery of nutrients to the cerebrospinal fluid. Thus we prepared membrane vesicles from the brain cortex, choroid plexus and also from the pineal gland (Table 4). A very high specific binding activity was present in the choroid plexus. The pineal gland, which stores vitamin A in the brain, also showed high binding activity. In contrast, the brain cortex did not bind appreciable amounts of ^{125}I -RBP.

Binding activity in testes

In testes the Sertoli cells form the tight blood-testis barrier. Retinol must cross this barrier and Sertoli cells are therefore expected to express the RBP receptor. We prepared testicular membrane vesicles from 15- and 22-week-old rabbits because these animals reached puberty at about 20 weeks of age. As shown in Table 4, the binding activity in testis from immature animals was two to three times higher than testis from mature rabbits. As immature testis contains a higher proportion of Sertoli cells than germinal cells, these findings suggest that the RBP receptor is expressed predominantly in the former. The activity was therefore measured directly in Sertoli cell membrane vesicles from immature rats. The Sertoli cells were prepared from 18-day-old rats and cultivated for 5 days before analysis. As shown in Table 4, these membrane preparations showed much higher binding activity than membrane vesicles isolated from whole testis.

Binding activity in skin

Retinoic acid is an important modulator of growth and differentiation of several cell types in the skin, including keratinocytes. The retinoic acid is probably formed *in situ* after delivery of retinol from the plasma retinol-RBP complex. We cultivated human keratinocytes in a defined medium and the same medium plus calcium, yielding basal undifferentiated cells and differentiated cells respectively [33]. As shown in Table 4, membrane vesicles isolated from undifferentiated keratinocytes had about 40 times higher binding activity than differentiated cells. It should be noted that the binding activity in undifferentiated keratinocytes is the highest observed in all tissues and cells presented. In contrast, rabbit skin fibroblasts showed no binding activity.

DISCUSSION

By using the binding assay developed by Sivaprasadarao and Findlay [26] we have studied the tissue distribution of the RBP receptor by incubating ^{125}I -RBP with membrane vesicles from various tissues. Separation of bound from unbound RBP by centrifugation through oil has an advantage over many other methods as it is rapid and does not involve prolonged centrifugations or washing steps which may dissociate the RBP-receptor complex. Because of the rapid dissociation of RBP from its receptor, we believe that the method using centrifugation through oil gives the best estimate of the tissue distribution of RBP receptor. Båvik et al. [28] used a cell-free binding-assay which separated bound from unbound ligand by two ultra-centrifugation steps lasting 30 min each. They found highest binding activity in membrane vesicles prepared from retina pigment epithelium cells. Membrane vesicles prepared from liver and kidney expressed only 20% of the binding activity of retina pigment epithelium cells, and membrane vesicles from lung and muscle about 10%. These relative binding activities agree fairly well with our observations, although we detected higher relative binding activity in the kidneys.

In the present publications, we have also determined the binding activities in several other tissues and cells. First, we observed that placenta expressed even higher binding activity than the retina epithelium cells, and that the choroid plexus of the brain and Sertoli cells of the testis expressed relatively high binding activity. These are all cell types that have tight junctions and form a barrier between plasma and the interstitial fluid of the tissue. Thus plasma retinol must cross these cells in order to reach the target cells. These data are in agreement with an

observation by MacDonald et al. [19], who suggested that high levels of RBP receptors should be expressed in cells comprising blood-tissue barriers. This conclusion was based on autoradiographic localization of intravenously injected ^{125}I -RBP and the high level of immunoreactive cellular RBP found in cells forming blood-tissue barriers such as Sertoli cells, retina pigment epithelium cells and the cuboid cells of the choroid plexus.

Shingleton et al. [22] reported that uptake of ^3H retinol from the retinol-RBP complex by cultured Sertoli cells was specific and saturable. In our study, testis from immature animals with relatively more Sertoli cells showed higher binding activity than testis from mature rabbits. We also observed that membrane vesicles from isolated Sertoli cells expressed appreciable binding activity.

Several reports have emphasized the importance of retinoids in differentiation of haematopoietic and bone marrow cells [40,41]. For example, it was shown that retinol complexed to RBP efficiently regulated growth and differentiation of myeloid cells [42] and the activation of B-cells [43]. Interestingly, in the present report we detected both high RBP-binding activity and *in vivo* uptake in bone marrow cells.

In kidney, almost all the specific RBP binding activity was recovered in the cortex where the proximal convoluted tubule has brush-border membranes. The high binding to the kidney cortex suggests that RBP primarily filtered in the glomeruli is taken up by the epithelial cells of the proximal convoluted tubuli. In addition, selective uptake of RBP was observed when we injected ^{125}I -RBP intravenously into rats and determined recovery in tissues. These data are in agreement with an earlier observation by Katoh et al. [44] who found that filtered RBP was reabsorbed by the proximal tubular cells and degraded in lysosomes. In addition, an immunohistochemical study by Kato et al. [45] located RBP in the proximal convoluted tubular cells of the kidney.

The pineal gland is an organ that is rich in vitamin A and some retinoid-specific binding proteins [46]. The function of the organ and the role of vitamin A storage, however, remains unknown. Interestingly, in our *in vitro* binding assay we observed that membrane vesicles prepared from the pineal gland express about 7–8 times as much RBP-binding activity as the cerebral cortex.

We also detected high RBP-binding activity in non-differentiated human keratinocytes whereas differentiated keratinocytes showed almost no specific binding. Creek et al. [12] studied binding of ^{125}I -RBP to keratinocytes using culture conditions yielding non-differentiated cells. They did not, however, observe any specific RBP binding. This contradictory result may be due to the extensive washing steps used in the study by Creek et al. [12] in contrast with the more rapid centrifugation-through-oil method used in the present study.

The non-differentiated keratinocytes represent basal cells located next to the capillaries. Our observation that non-differentiated keratinocytes express RBP receptors whereas differentiated keratinocytes do not is interesting, as Törmä et al. [47] suggested that keratinization in the skin is due to a functional vitamin A deficiency, i.e. when the cells migrate towards the upper layer of the epidermis they are exposed to lower levels of retinol-RBP, and therefore will differentiate. The observation that differentiated keratinocytes down-regulate the RBP receptor would correlate with such a regulation of differentiation.

The K_d of RBP-receptor binding found in the present study and in previous studies [23,26,28,38] is well below the RBP level present in plasma and extracellular fluids. These results are based on binding of RBP not complexed to TTR. The RBP-TTR complex may function as a reservoir for RBP releasing more RBP as it binds to the receptor. Alternatively, the RBP-TTR

complex may be the physiological ligand for the receptor. Conflicting results have been published as to the effect of TTR on RBP binding: some studies [28] find no effect of RBP binding whereas others [26] report that TTR inhibits the binding of RBP suggesting that uncomplexed RBP is the physiological ligand.

The results presented in the present report show that the RBP receptor concentration varies considerably from cell type to cell type and organ to organ. Furthermore, the tissue distribution of the receptor observed here correlates with uptake and appears to be physiological and consistent with previous knowledge of retinol function and metabolism by various cells. It is therefore reasonable to conclude that the level of RBP-receptor expression is an important regulator of tissue binding of RBP and thus probably also of uptake of retinol bound to RBP.

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