Regulation of expression of transcobalamin II receptor in the rat

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Surface and intracellular membrane distribution and hormonal regulation of transcobalamin II receptor (TC II-R) activity and protein levels have been studied in an effort to understand its regulation of expression in the rat. TC II-R activity and the levels of the 62 kDa monomeric and 124 kDa dimeric forms of TC II-R were highest in the rat kidney and intestine, and in these tissues the receptor expression was not dependent upon the post-natal development of the rat. TC II-R expression was uniform in the various regions of the gut. Surface membrane distribution of TC II-R in the kidney revealed the expression of the 124 kDa dimer form of TC II-R in the apical and basolateral membranes in the ratio of 1:10. Further subcellular distribution of TC II-R in the kidney revealed the expression of the 124 kDa dimer in the intermicrovillar clefts and clathrin-coated vesicles and the 62 kDa monomer in the microsomes. Neither the monomer nor the dimer could be detected in the early endosomes or lysosomes. Membrane TC II-R activity and TC II-R protein levels and cobalamin (Cbl; vitamin B_{12}) transport *in vivo* were inhibited by about 90% in adrenalectomized rats and all three returned to normal levels by oral treatment of these animals with cortisone acetate. In contrast, thyroidectomy or experimentally induced diabetes had no effect on TC II-R activity or Cbl transport. Based on these observations, we suggest that TC II-R expression is not developmentally or regionally regulated in rat renal and intestinal membranes and its expression in the kidney is asymmetrically distributed between the apical (10%) and basolateral (90%) membranes. In addition, our results also show that the dimerization of TC II-R is a post-microsomal event and that the expression of TC II-R and plasma Cbl transport is regulated by cortisone.

INTRODUCTION

The absorption and transport of dietary cobalamin (Cbl; vitamin B_{12}) is mediated by two receptor-mediated events [1]. First, dietary Cbl bound to gastric intrinsic factor (IF) is internalized by an apical membrane receptor [intrinsic factor cobalamin receptor (IFCR)], a 180–200 kDa protein [2,3] expressed in the microvillar pits of the ileal mucosa [4]. Secondly, the plasma transport of absorbed Cbl bound to the plasma Cbl binder, transcobalamin II (TC II), is mediated by TC II receptor (TC II-R) [5]. The importance of expression of the two Cbl-binding proteins, IF and TC II and their cell surface receptors is borne out by the observation that defective absorption and tissue transport of Cbl result in its intracellular deficiency, causing a failure in the synthesis and utilization of Cbl as a coenzyme [6]. Prolonged Cbl deficiency caused by defective cellular uptake is manifest as megaloblastic anaemia, impaired immune defence and intestinal and neurological disorders [7].

Although many aspects of tissue and cellular expression of IF, TC II and IFCR have been studied [8,9], very little or no information is available on the regulation of expression of TC II-R. Previous studies have identified saturable specific-binding of TC II–Cbl to various tissue-derived membranes using tissues across species [10–12]. These earlier studies have suggested that TC II-R activity is expressed in many tissues in all species assayed. In support of the observation of multiple tissue expression of TC II-R activity are recent studies that have identified TC II-R protein expression as a dimer of 124 kDa in many human tissue-derived membranes [13]. Interestingly, the highest level of TC II-R expression in human tissue membranes occurs in the kidney with lower levels of expression in other tissues including, intestine, liver and placenta [13]. Although multiple tissue expression of TC II-R is an expected finding due to its role in delivering Cbl bound to TC II to all tissues/cells, it is not known whether, within the same species, TC II-R activity and protein expression are regulated. Thus, the present studies were carried out to examine the regulation of TC II-R expression in a normal developing rat model and in hormonally modulated adult rats.

The results of the present study show that in the rat, steady-state TC II-R expression occurs at various levels in tissue membranes, with similar high levels of expression in the kidney and intestine. In these tissues TC II-R levels were uniform during the post-natal development of the rat and along the horizontal axis of the gut. In addition, these studies also show that in the rat kidney, TC II-R is expressed as a dimer in much higher amounts in the basolateral membranes relative to apical membranes. Intracellularly, TC II-R is expressed as a dimer in clathrin-coated vesicles and as a monomer of 62 kDa in the microsomes, with little or no expression in the early endosomes and lysosomes. Furthermore, adrenal but not thyroid or insulin deficiency decreased tissue membrane expression of TC II-R and plasma transport of Cbl to tissues.

EXPERIMENTAL

Materials

The following were commercially purchased from the sources indicated: [^{57}Co]Cbl (15μCi/μg; Amersham, Arlington Heights, IL, U.S.A.), cellulose nitrate membranes used in immunoblotting (Schleicher and Schull, Keene, NH, U.S.A.) and ^{125}I-Protein A (> 30 μCi/μg; ICN Radiochemicals, Irvine, CA, U.S.A.). TC II used for the TC II-R binding assays was partially purified from human plasma [14]. IF used for uptake of IF–[^{57}Co]Cbl by rats *in vivo* and for IFCR binding assays was purified from rat

Abbreviations used: Cbl, cobalamin (vitamin B_{12}); ER, endoplasmic reticulum; TC II, transcobalamin II; TC II-R, transcobalamin II receptor; IF, intrinsic factor; IFCR, intrinsic factor cobalamin receptor.

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stomach as described earlier [15]. Antiserum to human placental TC II-R was prepared as described earlier [13].

Animals

Adrenalectomized and thyroidectomized rats and their respective sham-operated control rats and normal rats not subjected to any type of surgical procedure were purchased from SASCO (Omaha, NE, U.S.A.). The surgery was performed on adult rats (24 days old). After 2 days, some adrenalectomized rats were treated orally with cortisone acetate (10 μg/ml) added to drinking water that also contained 0.9 M NaCl, while others were allowed to drink normal saline. The feeding regime was for 6 days. Some thyroidectomized rats were treated with intraperitoneal injections of thyroxine (1 μg/g body weight) daily for 6 days. Male Sprague–Dawley rats (200–225 g) were anaesthetized with methoxyflurane and injected via the tail vein with 50 mg/kg of streptozotocin freshly reconstituted with 0.9% saline. A control group of rats were treated with sham injections of 0.9% saline. All animals were given 10% sucrose solution during the first 24 h, after which they were fed *ad libitum*. The onset of diabetes was ascertained after 48 h by measuring blood glucose which was found to be between 3.25 and 4.0 mg/ml. The rats were treated for a minimum of 25 days after the onset of diabetes with insulin and the glucose levels were monitored. Only those rats whose glucose levels were persistently reduced to between 1.10 and 1.25 mg/ml were used as models of successfully treated animals. Untreated rats and those rats that responded well to insulin and sham-injected control rats were killed and their intestines removed, washed in ice-cold saline and the mucosa were scraped and homogenized in 10 mM Tris/HCl buffer containing 140 mM NaCl and used for IFCR and TC II-R activity determination. Pregnant rats were purchased from SASCO and following the birth, the pups were allowed to nurse with the mother. Suckling rats (4-, 12- and 16- day-old) and post-weaned rats (20- and 30-day-old rats) were killed, their intestines and kidneys removed, cleaned and homogenized as before. In some adult rats, the washed intestines were separated into their anatomical regions and the mucosa were scraped and homogenized.

Assays

TC II-R activity using human TC II–[^{57}Co]Cbl (2 pmol) was assayed in the Triton X-100 extract of total tissue homogenates (10%, w/v) by the DEAE-Sephadex method of Seligman and Allen [16]. Immunoblotting of the Triton X-100 pellet using TC II-R antiserum confirmed that 100% of TC II-R was solubilized from the total membranes. IFCR activities in the homogenates were assayed using rat IF–[^{57}Co]Cbl (2 pmol) [2]. The protein concentrations in the various fractions were determined by the Bradford assay [17] using BSA as the standard.

Immunoblotting

Tissue homogenates (5–50 μg of protein to detect the dimer and 50–500 μg of protein to detect the monomer) from various tissues or isolated membrane fractions (5–200 μg of protein) were separated under non-reducing conditions on 7.5% SDS/PAGE. The separated proteins were transferred to cellulose nitrate filters. The transfer time varied between 45 and 90 min depending on whether the experiment was designed to detect the TC II-R monomer of 62 kDa or the dimer of 124 kDa. The results shown in Figures 1, 3 and 5 were generated using a transfer time of 90 min, where only the more abundant dimer can be detected. The results shown in Figures 2 and 4 include transfer times of both 45 and 90 min. The filters were then probed with diluted (1:2000) antiserum to human placental TC II-R as described previously [13]. The intensity of TC II-R detected by immunoblotting was quantified on an Ambis-radioimaging system and was found to be linear between 100 and 1200 ng of pure TC II-R. This linearity corresponded to image intensity counts between 0.006 and 1×10^6 counts or arbitrary defined units of 0.24–40 (1 unit = 25000 counts).

Transport studies *in vivo*

Rat IF–[^{57}Co]Cbl complex (3.5 pmol in 1 ml of normal saline) was administered orally to adrenalectomized rats treated with and without cortisone acetate, thyroidectomized rats treated with and without thyroxine and diabetic rats treated with and without insulin and their respective sham-operated controls with a small feeding tube as described previously [18]. The animals were killed 1 or 6 h after the administration of the ligand to study the intestinal uptake or tissue transport of [^{57}Co]Cbl respectively. The entire length of the intestine and the kidneys were killed. To remove the contents the intestine was washed with ice-cold saline, cut open longitudinally and cut into 5–7 cm pieces. The mucosal sides of the pieces were exposed to pH 5.5/EDTA buffer for 15 min to remove any ligand that might still be bound to IFCR. The intestinal pieces were then washed once in pH 7.4 buffer and blotted dry. Mucosa was then scraped from each piece, pooled and counted in a gamma counter to measure [^{57}Co]Cbl radioactivity. The kidneys were washed in PBS, cut into small pieces and counted for the transported radioactive Cbl.

Isolation of rat renal intracellular and surface membranes

Rat renal cortical light endosomes were prepared by differential Percoll gradient centrifugation [19]. This fraction was slightly lighter than the basolateral membranes and was characterized by the homogeneity of the entrapped marker, fluorescein dextran, administered to rats by intravenous injection and H^+ ATPase [20], and the *in vitro* reconstituted homotypic fusion assay [21]. Co-localization on a particle-by-particle basis of modest amounts (1–2%) of apically derived enzymes and glycoproteins, such as γ-glutamyl transpeptidase and leucine aminopeptidase, along with entrapped markers, suggested that the light endosomes were derived from the apical domain. Heavy endosomes were also isolated by Percoll gradient centrifugation and magnesium precipitation [19,22]. This fraction which was much heavier than basolateral membranes and consisted of intermicrovillar clefts [23] and intermicrovillar cleft elements such as clathrin, actin and the glycoproteins, gp 280 and gp 330 (Heymann antigen), were present in this fraction. The clathrin-coated vesicles were prepared by sucrose gradient centrifugation and negative lectin selection [21]. Rat renal lysosomes were prepared by Percoll gradient centrifugation [24]. The apical brush-border and basolateral membranes from rat renal cortical slices were prepared by the Mg^{2+} precipitation method [25]. The apical membrane was enriched for the apical membrane markers, 15-fold with IFCR and 14-fold with alkaline phosphatase, with a 20–25% recovery of both these markers. The apical membranes were contaminated with less than 2% of Na^+/K^+ ATPase, a marker for the basolateral membranes. The basolateral membranes prepared were enriched 12-fold for Na^+/K^+ ATPase with a recovery of 12% and contained less than 1–2% of the apical markers, alkaline phosphatase and γ-glutamyl transpeptidase. Rat renal microsomes were prepared from a 5% (w/v) homogenate of cortical slices prepared in 0.25 M sucrose containing 5 mM EDTA and 10 mM sodium phosphate buffer, pH 7.4. The post-mitochondrial supernatant was centrifuged at 150000 ***g*** for 2 h

and the pellet obtained was washed in the same buffer once and the re-pelleted membranes were used as microsomes.

RESULTS AND DISCUSSION

Tissue expression of TC II-R activity and protein in the rat

Initial TC II-R activity measurements in adult rats (Table 1) revealed that TC II-R activity was present in all the rat tissues tested, but the relative specific activity expressed per mg of protein or per g of tissue of the receptor was uniformly high in the rat intestine and kidney and lower in liver, lung, heart and colon. The relative specific activity expressed per g of tissue was higher by about 7-fold in heart, 10-fold in liver, 12-fold in lung and 33-fold in colon (Table 1). In contrast to multiple tissue expression of TC II-R, IFCR, which promotes the uptake of Cbl bound to gastric IF, is expressed in the intestine [26], kidney [27], visceral yolk sac [28] of the rat and in human fetal colon [29], but not in other tissues. Similar high levels of TC II-R activity in the rat kidney and intestine and low levels in rat liver is a surprising finding. Liver is thought to be a storage organ for Cbl and hence, one would expect higher TC II-R activity in the liver to facilitate the initial bulk plasma uptake of absorbed Cbl to be followed later by supply to other tissues that need Cbl. On the basis of total tissue weight, rat liver has about 5 nmol of TC II-R compared with about 8 nmol in the kidney and 10–12 nmol in the total mucosa of the gut. Thus, liver expressed about one-half to one-third of the activity expressed in the kidney and intestine respectively. In contrast to rat, TC II-R tissue distribution in humans has revealed [13] much higher levels of expression of TC II-R in the kidney followed by placenta, intestine and liver. These studies show that the relative tissue expression of TC II-R across species varies. The physiological implication of higher levels of TC II-R expression in the intestine and kidney of the rat may be that more Cbl bound to plasma TC II is delivered directly to these two organs than any other organ in this species. This suggestion may help explain why in the rat most of the absorbed Cbl is retained in the kidney [30]. In support of these observations are studies [31,32] that have suggested that in the rat, the kidney acts as a storage site for Cbl. The high levels of TC II-R expression in the intestine may help in facilitating the Cbl needs of rapidly proliferating enterocytes along the entire horizontal axis of the gut. In support of this suggestion was the finding that TC II-R activity was uniformly distributed along the entire length of the gut, including duodenum, jejunum and ileum (Table 1).

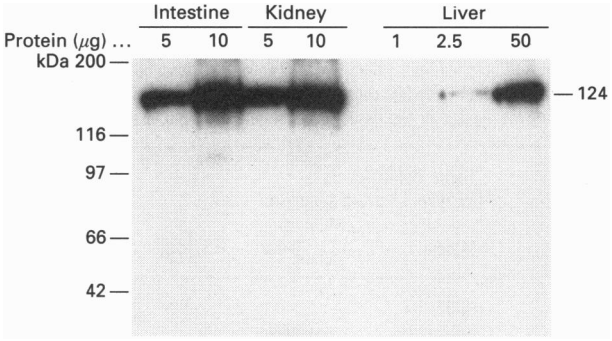

Figure 1 Tissue distribution of TC II-R protein

Indicated amounts of protein from tissue homogenates of rat kidney, liver and intestine were separated on non-reducing SDS/PAGE (7.5%), transferred for 90 min to cellulose nitrate membranes, probed with diluted TC II-R antiserum and ^{125}I-Protein A, and the bands were visualized following autoradiography.

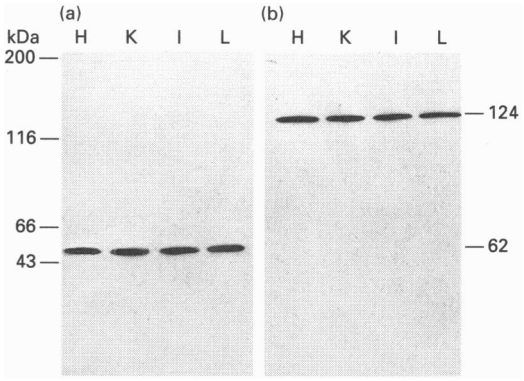

Figure 2 Immunoblot analysis of TC II-R monomer and dimer distribution in rat tissue homogenates

Total homogenates from indicated tissues (H, heart; K, kidney; I, intestine; and L, liver) were subjected to SDS/PAGE (7.5%), transferred to cellulose nitrate membranes for 45 min (**a**) or 90 min (**b**) and probed with antiserum to TC II-R and ^{125}I-Protein A and the bands obtained by autoradiography were quantified as described in the Experimental section. The homogenate protein used for monomer (**a**) detection was, 50 μg for kidney and intestinal homogenates and 360 and 500 μg for heart and liver homogenates. To detect the dimer (**b**) 5 μg of intestinal and kidney and 35 and 50 μg of heart and liver homogenate protein were used.

Table 1 Relative distribution of TC II-R activity in rat tissues

TC II-R activity present in the Triton X-100 (1%) extracts of 10% (w/v) homogenates of the indicated tissues was determined by the DEAE-Sephadex method [16]. The values reported are the average of three assays from each tissue obtained from 6–8 rats.

	TC II-R activity (TC II–[^{57}Co]Cbl bound)	
Tissue	(pmol/mg of protein)	(nmol/g of tissue)
Kidney	110±7	4.2±0.04
Intestine (total)	115±10	5.1±0.06
Duodenum	110±6	4.3±0.04
Jejunum	120±10	4.7±0.03
Ileum	109±9	4.2±0.05
Liver	8±1	0.42±0.02
Heart	15±1.5	0.66±0.05
Lung	10±0.7	0.37±0.04
Colon	4±0.06	0.13±0.015

The activity pattern of TC II-R in rat tissues noted above correlated well with TC II-R protein distribution (Figure 1). While the immunoblotting of total homogenate protein revealed the 124 kDa dimer form of the receptor with as little as 5 μg of protein from the kidney and intestine, about 50 μg of protein from the liver was needed to detect TC II-R in this tissue. It is interesting to note that in all the tissues, the size of TC II-R expressed is 124 kDa, similar to the size of TC II-R expressed in human tissue membranes [13]. These results show that the expression of the TC II-R dimer of 124 kDa in the plasma membranes of all tissues is a common phenomenon in all species and that TC II-R from human and rat share common epitopes. Cell-free translation of rat kidney mRNA yielded a translation product of 45 kDa (results not shown) similar in size to the primary translation product obtained by translating human kidney mRNA. The mature human TC II-R is a 62 kDa monomer

Table 2 Immunblot analysis of the distribution of TC II-R monomer and dimer in rat tissues

The autoradiograph obtained from Figure 2 was quantified as described in the Experimental section. One arbitrary unit = 25000 counts obtained on the Ambis-radioimaging system. The units shown are the average of two tissue homogenate immunoblot experiments.

	Tissue distribution (arbitrary units/μg of protein)		
Tissue	Monomer	Dimer	Dimer/monomer
Kidney	0.392	3.111	7.936
Intestine	0.365	3.001	8.220
Heart	0.055	0.540	9.818
Liver	0.037	0.296	8.000

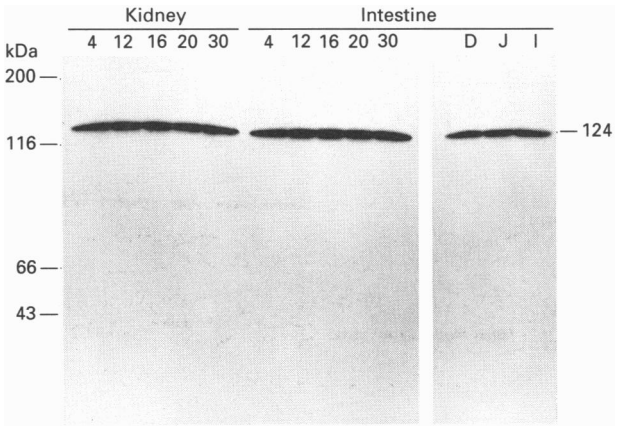

Figure 3 TC II-R protein distribution in the intestinal and renal membranes during post-natal development of the rat

Total mucosal homogenate from the entire length of the intestine or intestinal segments (duodenal, D; jejunal, J; ileal, I) or kidney homogenate (5 μg protein) were subjected to SDS/PAGE (7.5%), transferred to cellulose nitrate for 90 min, probed with diluted antiserum to TC II-R and ^{125}I-Protein A, and the bands visualized by autoradiography. The numbers 4, 12, 16, 20 and 30 indicate the post-natal age in days of rats used in the study.

containing about 33% carbohydrate in the form of both N- and O-linked sugars [13]. Thus, based on the size of the primary translated product and the size of the mature receptor expressed in the membrane, the rat TC II-R also appears to be processed in a very similar fashion to the human receptor. The 62 kDa monomer and the 124 kDa dimer were detected following a transfer time of 45 and 90 min respectively (Figure 2). Quantification of these immunoblots revealed that the level of the dimer was between 8- and 10-fold higher than that of the monomer in all the tissues tested (Table 2). Because of high levels of TC II-R expression in the renal and intestinal membranes, further studies were carried out to study the regulation of expression of TC II-R in these two tissues.

Developmental and regional expression of TC II-R in the rat kidney and intestine

It has been well established [33] that the IF-mediated gastrointestinal absorption of Cbl in the rat is developmentally regulated, with peak expression of both the ligand, IF [34], and its receptor, IFCR [18], occurring around 19–20 days after birth.

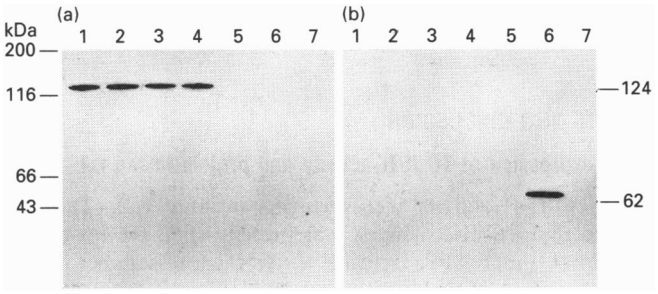

Figure 4 Immunoblot analysis of TC II-R protein distribution in the rat renal surface and intracellular membranes

Different rat kidney membranes isolated were subjected to SDS/PAGE (7.5%), transferred to cellulose nitrate membranes for 90 min (**a**) or 45 min (**b**) to detect the 124 kDa dimer form (**a**) or the 62 kDa monomer form (**b**) of TC II-R respectively. The individual membranes and the protein concentration used were: (**a**) apical (lane 1, 50 μg); basolateral (lane 2, 5 μg); clathrin-coated vesicles (lane 3, 20 μg); intermicrovillar clefts (lane 4, 20 μg); early/light endosomes (lane 5, 200 μg); ER (lane 6, 200 μg); and lysosomes (lane 7, 200 μg). (**b**) Apical (lane 1, 100 μg); basolateral (lane 2, 100 μg); clathrin-coated vesicles (lane 3, 100 μg); intermicrovillar clefts (lane 4, 100 μg); early/light endosomes (lane 5, 200 μg); ER (lane 6, 20 μg); and lysosomes (lanes 7, 200 μg). Other details of immunoblotting and quantification of bands are provided in the Experimental section.

Free Cbl is also taken up in the absence of IFCR by pinocytosis in suckling rats and the absorbed Cbl is transferred to TC II [18] and delivered to tissues. In addition, TC II expression in rat is not under developmental regulation (N. Li and B. Seetharam, unpublished work). These observations suggest that the Cbl transport machinery involving TC II and its receptor, unlike the IF/IFCR uptake system, is operational at all stages of development. In order to address this issue and to test how soon after birth TC II-R is expressed, immunoblot analysis of intestinal and renal homogenates from rats aged 4–30 days were carried out (Figure 3). The 124 kDa TC II-R expression was uniform in the renal and intestinal tissue throughout the development of the rat. Densitometric scanning of these blots from two separate experiments revealed less than 5–7% difference in the amount of TC II-R protein at different ages of the rat. It is interesting to note that at all stages of development, TC II-R protein expression in the kidney and intestine was the same, coinciding with similar levels of receptor activity expressed in an adult rat (Table 1) or in rats throughout their post-natal development (results not shown). As before, lower levels of the 62 kDa monomer form of TC II-R were also detected throughout the developing rat intestine and kidney and in the various regions of the gut in blots obtained with 45 min transfer time. Interestingly, the ratio of the dimer to monomer in these experiments was also about 9:1. The intestinal expression of TC II-R activity (Table 1) and protein (Figure 3) was uniform in the duodenum, jejunum and ileum. The uniform expression of TC II-R along the horizontal axis of the gut is an interesting and unique observation, since the expression of many functional proteins of the absorptive enterocytes, including IFCR [18], are both regionally and developmentally regulated [35]. The importance of the lack of regional and developmental regulation of TC II-R in the intestine and kidney (Figure 3) and liver (results not shown) underscores the importance of TC II-mediated delivery of Cbl from the circulation to all the tissues throughout the life of an animal.

It is interesting to note that the surface-membrane distribution of TC II-R in the rat kidney revealed the distribution of the 124 kDa dimer of TC II-R in both the apical (Figure 4a, lane 1) and basolateral (Figure 4a, lane 2) membranes. The densitometric

analysis revealed that the intensity was the same using 5 μg (basolateral; 6 units/μg) and 50 μg (apical; 0.57 units/μg) of membrane protein respectively, suggesting a 1:10 apical to basolateral distribution of TC II-R protein in the kidney. These results have confirmed, by demonstrating higher basolateral expression of TC II-R protein, the results from earlier studies that have shown the basolateral enrichment of TC II-R activity in the rat [36] and pig kidney [37]. Although the apical distribution of TC II-R protein and activity was about only 10–15% of the homogenate activity, it does not appear to be due to contamination from either basolateral or intracellular membranes, as very little or no contamination of markers from these membranes were present in the apical membranes (results not shown). Moreover, a similar type of TC II-R activity and protein distribution between the apical and basolateral membranes was also noted in the intestinal mucosa (results not shown). In addition, filter-grown polarized Caco-2 cells exhibit specific TC II–[^{57}Co]Cbl binding to both apical and basolateral surfaces in the ratio of 1:8 and a very similar type of TC II-R distribution has also been noted in these cells using a cell surface-specific biotinylation technique (B. Seetharam and S. Bose, unpublished work). The functional significance of the apical expression of TC II-R, if any, is not known and further studies are needed to address this issue. However, TC II-R was also detected in both the apically derived clathrin-coated vesicles (Figure 4a, lane 3) and intermicrovillar clefts (Figure 4a, lane 4). This finding suggests that the small amount of TC II-R present apically in the kidney may be functional in internalizing TC-II–Cbl. This uptake would minimize the urinary loss of either TC II or TC II–Cbl by facilitating their tubular reabsorption. It is known that TC II–Cbl is filtered and minuscule amounts (< fmol) of TC II have been detected in urine [38].

The 124 kDa dimer form of TC II-R was not detected in early endosomes (Figure 4a, lane 5), endoplasmic reticulum (ER) (lane 6) and the lysosomes (lane 7) using as much as 100–200 μg of protein from these internal membranes. Since the 62 kDa monomer form of TC II-R can be detected on immunoblots when the transfer time was 45 and not 90 min, the time-frame needed to transfer the dimer [13], intracellular distribution of the monomeric form of TC II-R was also investigated (Figure 4b). Using similar or larger amounts of membrane protein (except in the ER fraction) than were used to detect the dimer (Figure 4a), the monomeric 62 kDa form of TC II-R was detected only in the ER with as little as 20 μg of protein, but not in the other intracellular or surface membranes. Previous studies [13] have shown that the formation of non-covalent dimers of TC II-R is mediated by the interaction of the hydrophobic receptor with the annular lipids of the plasma membranes. Further studies on the nature of lipid–TC II-R interactions within the cellular compartments are needed to ascertain the site and the mechanism of dimerization of TC II-R during its vectorial movement from the ER.

The high level of TC II-R expression in the rat intestine and kidney suggested that TC II-R may mediate a higher flux of Cbl across the basolateral membranes of these two tissues. However, no direct evidence exists that the level of TC II-R expression in any tissue is related to the amount of Cbl transported to that tissue. The up- or down-regulation of TC II-R on the cell surface membranes appears to depend upon the functional status of the cell, its intracellular content of Cbl [39] and on the differentiation status of the cell [14,40]. Other than these factors, which are intrinsic to the differentiation status of the cell or its Cbl content, there are no studies demonstrating whether extrinsic factors regulate TC II-R expression. In order to address this issue, TC II-R activity, protein expression and Cbl transport to the kidney was studied in hormonally modified rats.

Table 3 Effect of adrenalectomy and thyroidectomy on Cbl receptor activities in the rat intestine

IFCR activity was determined using total homogenates with rat IF–[^{57}Co]Cbl (2 pmol) [2] and TC II-R activity was determined using human TC II–[^{57}Co]Cbl (2 pmol) in the Triton X-100 (1%) extracts of the tissue homogenates by the DEAE-Sephadex method [16]. The values reported represent the mean ± S.D. from triplicate assays carried out using at least 6–8 rats in each category.

Animal and treatment	TC II-R (pmol of ligand bound/g of mucosa)	IFCR (pmol of ligand bound/g of mucosa)
Adrenalectomized rats		
Sham-operated controls	4400 ± 75	0.410 ± 0.04
Adrenalectomized	84 ± 10	0.395 ± 0.03
Adrenalectomized + treated with cortisone acetate	3954 ± 110	0.400 ± 0.04
Thyroidectomized rats		
Sham-operated controls	4350 ± 110	0.395 ± 0.034
Thyroidectomized	4145 ± 115	0.130 ± 0.04
Thyroidectomized + treated with thyroxine	4500 ± 95	0.245 ± 0.06
Diabetic rats		
Control	4130 ± 95	0.385 ± 0.04
Diabetic	4000 ± 73	0.410 ± 0.06
Diabetic + treated with insulin	3957 ± 33	0.421 ± 0.045

Effect of adrenalectomy, thyroidectomy and diabetes on the TC II-R expression

TC II-R activity per g of kidney declined by nearly 90% (Table 3), from about 4400 pmol of ligand binding in sham-operated controls to 84 pmol in adrenalectomized rats. The activity was completely restored to normal levels following a 6 day oral treatment of these rats with cortisone acetate. There was no significant change in TC II-R activity in either thyroidectomized or experimentally induced diabetic rats (Table 3). The decline of TC II-R activity in adrenalectomized rats was tissue non-specific and occurred in the intestine and other tissues tested by a similar amount (results not shown). The lack of effect on TC II-R activity in thyroidectomized and diabetic rats clearly demonstrates the specificity of cortisone in regulating TC II-R activity. The specificity of cortisone in regulating TC II-R activity is further borne out by the observation (Table 3) that IFCR activity was unaffected in adrenalectomized rats. However, in thyroidectomized, but not diabetic rats, there was a decline of IFCR activity by nearly 66%. However, it is not certain whether this decline was due to lack of thyroxine, since the activity was not restored completely when the rats were treated with thyroxine. Further studies are needed to address this issue. It is interesting to note that some earlier studies using thyroidectomized rats [41–43] have suggested that Cbl deficiency noted in these rats could be due to defective absorption and transport of Cbl. The results given in Table 3 suggest that the effect, if any, of hypothyroidism could be on the intestinal uptake but not the plasma transport of Cbl.

In order to test whether the loss of TC II-R activity was due to the loss of TC II-R protein, immunoblot analysis was carried out using renal homogenate from sham-operated control, cortisone-treated and untreated adrenalectomized rats (Figure 5). In adrenalectomized rats, the 124 kDa dimer TC II-R protein levels were much lower (Figure 5, lanes 3 and 4) relative to sham-operated controls (lanes 1 and 2) and the protein levels returned to normal (lanes 5 and 6) following treatment of these rats *in vivo*

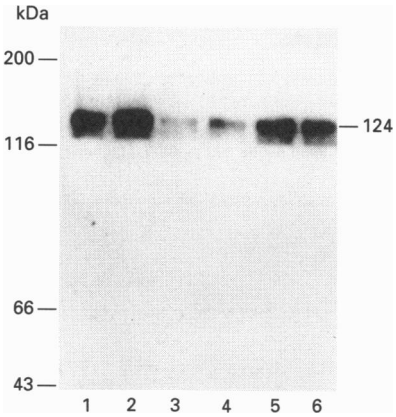

Figure 5 Effect of adrenalectomy on kidney TC II-R protein levels

Renal cortical homogenate (20 μg of protein) from sham-operated control rats (lanes 1 and 2), adrenalectomized rats (lanes 3 and 4) and adrenalectomized rats treated with cortisone acetate (lanes 5 and 6) were separated on non-reducing SDS/PAGE (7.5%), transferred to cellulose nitrate for 90 min and probed with diluted antiserum to human placental TC II-R and ^{125}I-Protein A. The bands were visualized by autoradiography. Other details of hormonal treatment and immunoblotting are provided in the Experimental section.

Table 4 Intestinal uptake and plasma transport of [^{57}Co]Cbl to kidney in sham-operated and hormonally modulated rats

The mucosal uptake and kidney transport were measured 1 and 6 h after the oral administration of the ligand rat IF–[^{57}Co]Cbl (3.5 pmol) respectively. The values reported represent the mean ± S.D. of uptake and transport carried out using 6–8 rats in each category. Other details of hormonal treatment of rats and the tissue Cbl measurements are provided in the Experimental section.

Animals and treatment	Mucosal uptake (fmol/gm)	Kidney transport (fmol/gm)
Sham operated	480 ± 40	400 ± 25
Adrenalectomized	475 ± 40	45 ± 10
Adrenalectomized + cortisone	490 ± 25	380 ± 20
Thyroidectomized	260 ± 20	240 ± 20
Thyroidectomized + thyroxine	390 ± 30	340 ± 20
Diabetic	460 ± 30	400 ± 35
Diabetic + insulin	475 ± 20	410 ± 34

with cortisone acetate. Further studies are needed to examine whether lack of cortisone affects the relative turnover of TC II-R or whether the dramatic decline of TC II-R protein levels is due to an effect on the transcription of the TC II-R gene. Such studies must wait until the gene encoding TC II-R is isolated. The multiple tissue decline of TC II-R in adrenalectomized rats should lead to decreased plasma transport of absorbed Cbl. In order to test this possibility, we measured the kidney levels of [^{57}Co]Cbl following intestinal absorption of orally administered IF–[^{57}Co]Cbl.

Transport of absorbed [^{57}Co]Cbl in adrenalectomized rats

When rat IF–[^{57}Co]Cbl was administered orally to sham-operated and adrenalectomized rats, the mucosal uptake of Cbl in 1 h was the same in both, but the Cbl transported in 6 h to the kidney dramatically declined from about 400 to 40 fmol in the adrenalectomized rats (Table 4). A time of 1 h to study the intestinal uptake of Cbl was chosen since, in 1 h, very little Cbl exits the mucosal cell and is found in either the circulation or in peripheral tissues [44]. When the ligand was administered to adrenalectomized rats that were treated with cortisone for 6 days, the amount of Cbl transported to the kidney was restored to normal values. These results are consistent with the loss of TC II-R activity (Table 3) and protein (Figure 5). The decline in the amount of Cbl transported to the kidney in thyroidectomized rats (Table 4) from about 400 to 240 fmol was due to a decline in the intestinal uptake of IF–Cbl, from about 480 to 260 fmol. This latter observation is consistent with the observed loss of IFCR activity in the thyroidectomized but not in the adrenalectomized rats (Table 3). Treatment with thyroxine restored to some extent not only the uptake of IF–Cbl but also the amount of Cbl transported to the kidney from 240 to 340 fmol (Table 4). In diabetic rats, neither the intestinal uptake nor the transport of Cbl to the kidney was affected (Table 4). One consistent finding in adrenalectomized rats is that in about 12 h following oral administration of IF–Cbl, the mucosal Cbl levels were 112 fmol/g of mucosa compared with about 280 fmol/g of mucosa in cortisone-treated rats. This observation indicates that by 12 h, Cbl is being delivered via TC II to this tissue from the circulation via TC II-R, and the decreased levels of mucosal Cbl in adrenalectomized rats is due to decreased intestinal TC II-R in adrenalectomized rats. For this to occur TC II-R must be expressed in the basolateral membranes of the absorptive enterocyte, an observation confirmed by the detection of > 80% of mucosal TC II-R activity in the lateral membranes (results not shown).

In conclusion, the results of this study have shown that TC II-R expression in the rat occurs in multiple tissues. Although the levels of expression vary in tissues, its expression is not subject to developmental or regional regulation. Furthermore the regulation of expression of TC II-R is controlled by cortisone but not by thyroxine or insulin, and prolonged deficiency of cortisone may affect Cbl homeostasis by inhibiting the plasma-membrane flux of Cbl. Future studies should be directed towards an understanding of the molecular mechanisms of hormonal regulation of TC II-R.

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