Comparative analysis of ontogenic changes in renal and intestinal biotin transport in the rat

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Nabokina, Svetlana M., Veedamali S. Subramanian, and Hamid M. Said. Comparative analysis of ontogenic changes in renal and intestinal biotin transport in the rat. Am J Physiol Renal Physiol 284: F737–F742, 2003; 10.1152/ajprenal.00364.2002.—Biotin, an essential water-soluble micronutrient, cannot be synthesized by mammals; rather, it is obtained from exogenous sources via uptake by intestinal epithelia. Renal epithelia reclaim the vitamin that is filtered in the glomeruli. Both epithelia take up biotin via the sodium-dependent multivitamin transporter (SMVT). Little is known about ontogenic regulation of the renal and intestinal biotin transport processes and about the mechanism(s) involved in any such regulation. In this study, we sought to examine and compare ontogenic aspects of the renal and intestinal biotin uptake processes using purified brush-border membrane vesicles (BBMV) isolated from the kidney cortex and jejunum of suckling and adult rats. Clear ontogenic changes were observed in the intestinal biotin uptake process, which were mediated via changes in V_{max} and apparent K_{m}. Parallel changes were also seen in protein, mRNA, and transcription rate of SMVT as indicated by results of Western blotting, RT-PCR, and nuclear run-on assays, respectively. In contrast, biotin uptake by renal BBMV did not show ontogenic changes; i.e., it was similar in suckling and adult rats. Also, the levels of SMVT protein and mRNA were similar in the kidneys of both age groups. These data show that biotin uptake by renal and intestinal epithelial cells responds differently to ontogenic regulation. In addition, the ontogenic changes observed in the intestinal biotin uptake process involve the entry step of the vitamin at the BBM and appear to be mediated via a transcriptional mechanism(s).

ontogenic regulation; renal biotin uptake; intestinal biotin uptake

Biotin (vitamin H) is an essential micronutrient that acts as a coenzyme for five mammalian carboxylases that catalyze essential steps in four pathways involving fatty acid biosynthesis, gluconeogenesis, and catabolism of certain amino acids and fatty acids (5, 29). The importance of biotin for human normal health and well-being is underscored by the serious clinical abnormalities that result from its deficiency, which include neurological disorders, growth retardation, and dermal abnormalities (5, 10, 29, 36). Biotin deficiency and suboptimal levels are being reported with increased frequency in recent years and occur in a variety of conditions, including inborn errors of biotin metabolism/transport, after chronic use of anticonvulsant drugs, after long-term use of parenteral nutrition, and during pregnancy (10, 13, 29, 32, 36).

Humans and other mammals cannot synthesize biotin and thus must obtain the vitamin from exogenous sources. Intestinal epithelial cells absorb dietary biotin as well as the biotin that is synthesized by the normal microflora of the large intestine (22, 27). Renal epithelial cells reabsorb biotin that is filtered into the urine. Thus intestinal and renal epithelial cells play a central role in maintaining and regulating normal biotin body homeostasis. Previous studies have shown the transport process of biotin in both renal and intestinal epithelia to be via a specialized, Na⁺-dependent, carrier-mediated mechanism (3, 4, 7, 14, 23–25). The Na⁺-dependent uptake was found to be located at the brush-border membrane (BBM) of these epithelial cells (14, 25, 26). The molecular identity of the transport system has been recently determined after cloning of the cDNA from human, rat, and rabbit tissues (8, 15, 16). This biotin uptake system was also found to be able to transport two other nutrients, namely, the unrelated vitamin pantothenic acid and the metabolically important substrate lipoate (8, 15, 16, 17, 22). For this reason, the uptake system has been named the sodium-dependent multivitamin transport (SMVT). So far, SMVT appears to be the only significant biotin carrier identified in intestinal and renal epithelia.

Previous studies have shown that a variety of renal and intestinal transport events undergo ontogenic changes during early stages of life (2, 11, 28, 34, 35). These changes, however, do not follow a unified pattern and in certain cases are tissue specific in nature (9, 11, 19, 34). To date, however, there is nothing known about the effect of ontogeny on renal biotin transport. As to the intestinal biotin uptake process, previous studies from our laboratory have shown that transport of the vitamin across intact intestinal epithelia undergoes clear ontogenic changes during the early stages of life (21). However, little is known about the molecular mechanism(s) that mediate these changes in intestinal biotin transport. Whether these changes involve the transport step of biotin at the entry level of BBM of the polarized enterocyte is unclear. In this study, we examined the effect of ontogeny on biotin transport

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across the BBM of renal and intestinal epithelia using isolated purified BBM vesicle (BBMV) preparations. We also investigated the molecular mechanism(s) that mediates the effect(s). Our results showed that, while biotin transport across the intestinal BBM undergoes ontogenic changes, no such changes occur in the biotin uptake process across renal BBM. In addition, our results suggest that the ontogenic changes observed in intestinal biotin transport involve transcriptional mechanism(s).

**MATERIALS AND METHODS**

**Chemicals and animals.** d-[8,9-3H]biotin (specific activity 51 Ci/mmol; radiochemical purity >97%) was obtained from PerkinElmer Life Science (Boston, MA). All other chemicals, reagents, and kits used in this study were of analytical/ molecular biology grade and were obtained from commercial sources. Cellulose nitrate filters (0.45-μm pore size) for use in uptake studies with renal and intestinal BBMV were purchased from Sartorius Filters (Hayward, CA).

Suckling (13–14 days old) and adult rats (65–70 days old) Sprague-Dawley rats were used in the study (Harlan Sprague Dawley, Indianapolis, IN). Mothers and adult rats were fed Purina Chow diet ad libitum and had free access to water. The National Council’s guidelines for the care and use of laboratory animals were followed, and all studies were approved by our Animal Studies Subcommittee.

**Isolation of renal and intestinal BBMV and transport investigations.** Rats were euthanized with CO2, and their kidneys and small intestine were removed. BBMV were then isolated from the renal cortex and jejunum using validated procedures previously established in our laboratory (25, 26, 33). The suitability of renal BBMV for studying ontogeny of a transport process has been previously established (11, 35). This has been confirmed in our laboratory by demonstrating a similar enrichment in the activity of the BBM marker enzyme alkaline phosphatase in the final BBMV preparations compared with initial cortical homogenates of the rat groups (8–10 fold for both ages). Similarly, intestinal BBMV have been validated and used by us and others to study ontogenic regulation (1, 2, 6, 20). Isolated BBMV were preloaded with a buffer of (in mM) 280 mannitol and 20 Tris-HEPES, pH 7.4, and were incubated in a buffer of (in mM) 140 mannitol, 170 NaCl, 17 Tris-HEPES, and 17 Tris-Mes, pH 7.4, in the presence of [3H]biotin. Uptake studies were performed at 10 s (initial rate) (24, 25) at 37°C using a rapid-filtration technique described previously (12, 24, 25).

**Western blot analysis.** BBMV were isolated in the presence of 2 mM phenylmethylsulfonyl fluoride, 1 μg/ml aprotinin, and 0.5 μg/ml leupeptin as reported previously by us (18). BBMV proteins (150 μg) were resolved on 7.5% SDS-PAGE and electrophoretically transferred onto nitrocellulose membranes (Amersham Pharmacia Biotech). For Western blot analysis, the membrane was blocked with 5% nonfat dry milk in PBS containing 0.1% Tween 20 and then incubated with specific rabbit polyclonal anti-peptide antibodies raised against the LHYACRGWGHVHELLMADR peptide, which corresponds to amino acids 43–64 of the rat SMVT sequence (Alpha Diagnostic, San Antonio, TX). Immunodetection was performed using goat anti-rabbit IgG secondary antibodies conjugated to horseradish peroxidase and an enhanced chemiluminescence kit (Amersham, Arlington Heights, IL). Specific bands were quantitated using the Eagle Eye II System (Stratagene, La Jolla, CA).

**Semiquantitative RT-PCR.** A gene-specific primer and 1 μg of poly(A)+ RNA isolated from rat renal cortex and jejunum and were used with a SuperScript RT-PCR kit (Life Technologies) to synthesize first-strand cDNA as described by the manufacturer. To amplify the open reading frame (ORF) of rat SMVT, two gene-specific primers (5′-GAGGATGACTGTCGGAGACAC-3′ and 5′-CAGCTCAACACAGTATGGC-3′), corresponding to the sequence in the ORF of rat SMVT, were used in a PCR, yielding a 150-bp product. The PCR was performed within the linear range of amplification. The conditions for PCR were denaturation at 95°C for 30 s, annealing at 58°C for 30 s, and extension at 72°C for 2 min (20 and 30 cycles for kidney and jejunum, respectively). To amplify the variant II of SMVT mRNA, two gene-specific primers (5′-CAGGGCAATCTGGGATTT-3′ and 5′-GTATAAGGAGGAGCCCA-3′), spanning the sequence in the variant II of rat SMVT mRNA, were used in a PCR, yielding a 463-bp product. The conditions for PCR were denaturation at 95°C for 1 min, annealing at 48°C for 1.5 min, and extension at 76°C for 1.5 min (35 cycles). The products were analyzed on 2% agarose gels, the images were captured using the Eagle Eye II system (Stratagene), and the amplified RT-PCR products were normalized to amplified β-actin controls.

**Nuclear run-on assay.** Nuclei were isolated from jejunum of 10 suckling and 3 adult rats using a modification of the method of Traber et al. (30). The mucosa was suspended in 10 vol of homogenization buffer [(in mM) 250 sucrose, 10 MgCl2, 2 DTT, 1 PMSF, and 10 HEPES, pH 8.0, as well as 2.5% Nonidet P-40], allowed to stand on ice for 1 h, and then homogenized using a Potter-Elvehjem homogenizer at 700 rpm for 10 strokes (adult rats) and 5 strokes (suckling rats). The suspension was filtered through two layers of gauze and centrifuged at 700 g for 5 min, and the pellet was resuspended in 2.5 ml of homogenization buffer. To purify the nuclei, the suspended pellet was layered on the top of a sucrose cushion (2.5 M sucrose, 1 mM MgCl2, 1 mM HEPES, pH 6.8) and centrifuged at 50,000 g for 80 min at 4°C. The nuclear pellet was suspended in a storage buffer [(in mM) 5 MgCl2, 2 DTT, 0.1 EDTA, and 20 HEPES, pH 8.0, as well as 40% glycerol] and stored at −80°C until use.

**Nuclear transcription reactions.** Reactions were carried out using equal amounts of nuclei from suckling and adult rats as described by Traber et al. (30). RNA transcripts were isolated by sequential treatment with deoxyribonuclease and proteinase K, followed by phenol-chloroform extraction, ethanol precipitation, and purification on a Sephadex G-50 spin column (Boehringer Mannheim, Indianapolis, IN). Denatured cDNAs were immobilized on nylon filters and hybridized with the nascent RNA transcripts for 72 h at 42°C with gentle mixing. cDNAs tested for hybridization were the full-length ORF of rat SMVT, cloned in pcDNA3.1 (250 ng), and rat β-actin (positive control) (50 ng). After stringent washings, membranes were exposed to film for 3–5 days at −80°C with intensifying screens. The intensity of the hybridization signal was quantitated using the Eagle Eye II System (Stratagene). Data were normalized relative to rat β-actin.

**Statistical analysis.** Transport studies were the results of multiple separate determinations using different BBMV preparations isolated from various rats on different occasions. Data are as means ± SE expressed as picomoles per milligram protein per unit time. Data were analyzed using Student’s t-test. Kinetic procedures (i.e., maximum velocity (Vmax) and apparent Michaelis constant (Km)) were determined using a computerized model of the Michaelis-Menten equation as described by Wilkinson (31). RT-PCR, Western blotting, and nuclear run-on assays were...
performed on at least three separate occasions using samples from different rats; representative data are presented in this report.

RESULTS

The effect of ontogeny on carrier-mediated biotin transport across the BBM of rat renal cortex and jejunum was examined using purified BBMV preparations isolated from suckling and adult rats. Biotin transport was measured by determining the initial rate of \[^3\text{H}\]biotin uptake as a function of concentration. Kinetic parameters of the biotin carrier-mediated uptake process were then calculated as described in MATERIALS AND METHODS. The results (Fig. 1, A and B) show a significantly (\(P < 0.01\)) higher \(V_{\text{max}}\) in adult compared with suckling rat jejunum (4.7 \(\pm\) 0.33 and 1.9 \(\pm\) 0.10 pmol·mg protein\(^{-1}\)·10 s\(^{-1}\), respectively). A change in the apparent \(K_{\text{m}}\) was also observed, with values being significantly (\(P < 0.01\)) higher in adult compared with suckling rat jejunum (11 \(\pm\) 1.6 and 5.5 \(\pm\) 0.84 \(\mu\)M, respectively). In contrast, the results with renal BBMV (Fig. 1, C and D) showed similar \(V_{\text{max}}\) values for the biotin uptake process in suckling and adult rats (2.2 \(\pm\) 0.12 and 2.1 \(\pm\) 0.08 pmol·mg protein\(^{-1}\)·10 s\(^{-1}\), respectively). In addition, the apparent \(K_{\text{m}}\) was found to be similar in suckling and adult rat renal BBMV (30 \(\pm\) 3.8 and 27 \(\pm\) 2.5 \(\mu\)M, respectively).

In another study, we used Western blot analysis to investigate the level of expression of the biotin transporter SMVT in purified jejunal and renal BBMV preparations isolated from suckling and adults rats (see MATERIALS AND METHODS). Specific polyclonal anti-SMVT antibodies were used in the study. The results (Fig. 2) showed the polyclonal antibodies to recognize two major protein bands (~120 and ~140 kDa) in jejunal BBM of adult rats, whereas only one band (~120 kDa) was
found in jejunal BBM of suckling rats. Immunodetection of both proteins was blocked by pretreatment of the antibody with the synthetic antigenic peptide. With densitometry it was determined that the intensity of the signal of the 120-kDa band increased with maturation (51.5 ± 4.9 and 67.4 ± 3.7 for suckling and adult rats, respectively). The intensity of the signal of the 140-kDa band of adult rat jejunal BBM was estimated at 22.6 ± 1.4. With regard to renal BBM, the results of Western blot analysis (Fig. 3) showed that the anti-SMVT antibodies recognize two protein bands with apparent molecular masses of ~105 and 69 kDa (the latter band has the predicted molecular mass of rat SMVT) (16) in both age groups. No protein bands were detected when the antibody was preincubated with the antigenic peptide. Direct estimation of the intensity of these two specific bands showed similar levels of SMVT protein.

We also determined the level of mRNA expression of SMVT in the jejunum and kidney cortex of suckling and adult rats. We used semiquantitative RT-PCR and gene-specific primers within the ORF of SMVT in the study (see MATERIALS AND METHODS). Data were normalized relative to β-actin. The results showed a 2.61 ± 0.15-fold higher SMVT mRNA level in the jejunum of adult compared with suckling rats (Fig. 4A). In contrast, the level of SMVT mRNA expression was found to be the same in renal cortex of rats in the two age groups (Fig. 4B).

Previous studies in our laboratory have shown the existence of four variants (I, II, III, and IV) for rat SMVT, which have significant heterogeneity at the 5'-untranslated region (8). We have also shown variant II to be the predominant form expressed in adult rat small intestine (8). In this study, we examined the effect of developmental maturation on the level of variant II expression in the small intestine using semiquantitative RT-PCR and variant II-specific primers. The results (Fig. 5A) show a 1.92 ± 0.13-fold higher level of variant II expression in adult compared with suckling rat jejunum. To examine the effect of ontogeny on level of expression of the predominant variant in rat kidney cortex, we first determined the identity of that variant and then examined its level in suckling and adult rat kidney cortex. The results (Fig. 5B) showed variant II to be the only SMVT variant expressed in rat kidney cortex. In addition, kidney cortices of suckling and adult rats were found to express similar levels of this variant.

The results described above suggest possible involvement of transcriptional mechanism(s) in ontogenic regulation of the intestinal biotin uptake process. To further examine this possibility, we performed a nuclear run-on assay using nuclei isolated from suckling and adult rats.
adult rat jejunum and the full-length ORF of rat SMVT in the study. The results (Fig. 6) showed a 1.86 ± 0.07-fold higher nascent transcription rate for SMVT in adult compared with suckling rats.

**DISCUSSION**

The purpose of the present study was to examine and compare ontogenetic aspects of the biotin uptake processes in renal and intestinal epithelia and to determine the mechanism involved in any observed changes. Purified renal and intestinal BBMV isolated from suckling and adult rats were used in the studies. The results showed that while the renal biotin uptake process is not ontogenically regulated, clear evidence was obtained to indicate that the intestinal biotin uptake process is under such ontogenic regulation.

The results of biotin uptake studies performed on renal cortex BBMV showed a similar \( V_{\text{max}} \) and apparent \( K_m \) for the vitamin uptake process in suckling and adult rats. In contrast, a significant increase in the \( V_{\text{max}} \) and apparent \( K_m \) of the vitamin uptake process was observed in jejunal BBMV of adult compared with suckling rats. The latter observations suggest that developmental maturation is associated with an increase in the number (and/or activity) of the intestinal biotin uptake carriers and a decrease in their affinity, respectively. The physiological importance of such changes could be related to the need for efficient biotin absorption in suckling compared with adult rats. The findings described above also demonstrate that the previously reported developmental changes in the transepithelial transport of biotin in intact rat intestinal tissue preparations involve the entry step of the vitamin at the BBM level of the polarized enterocytes.

The lack of changes in biotin uptake kinetics with ontogeny in the rat renal cortex was associated with a lack of change in the level of SMVT protein in suckling and adult rats. It is interesting to mention here, however, that two specific protein bands were observed on a Western blot of renal cortical BBM. One band appeared at ~69 kDa, which is the estimated molecular mass of the SMVT protein calculated from its predicted amino acid sequence, whereas the other appeared at 105 kDa. The latter band suggests that some of the SMVT protein in renal BBM undergoes posttranslational modification (e.g., glycosylation). As to the changes observed in rat intestinal biotin uptake kinetics with maturation, these changes were associated with an increase in the level of the SMVT protein from suckling to adult rats as shown by Western blot analysis. In addition, ontogeny was associated with the appearance on Western blots of an additional band for SMVT in adult rats of ~140 kDa in addition to the 120-kDa band that also existed in suckling rat jejunal BBM. Because the predicted molecular mass of the SMVT protein is 69 kDa, the above findings suggest that the SMVT protein also undergoes posttranslational modifications in the rat intestine. It may also be possible that the 140-kDa band represents a dimer of SMVT. Further studies are required to determine the nature of the posttranslational modifications (and dimerization) of SMVT in renal and intestinal tissues.

We should mention here that the observed changes in molecular mass of SMVT with maturation are not unique to the biotin transporter but have been observed with other carriers (e.g., the intestinal type IIb Na–P_i cotransporter).

The pattern of expression of the SMVT ORF was found to parallel the observed changes in biotin uptake and SMVT protein levels in renal and intestinal BBM. Similar SMVT mRNA levels were found in suckling and adult rat renal cortex, whereas the level was 2.61 ± 0.15-fold higher in the jejunum of adult compared with suckling rats. In previous studies from our laboratory, we have shown that rat SMVT exists in the form of four different variants (I, II, III, and IV). These variants arise from significant heterogeneity in the 5'-untranslated region of SMVT. In addition, variant II was found to be the predominant variant expressed in the small intestine of adult rats. In the present study, we also found variant II to be the predominant form expressed in the renal cortex of adult rats. In addition, the variant II level was found to parallel the level of the SMVT ORF in suckling and adult rats renal and jejunal tissues. Thus while similar levels of variant II expression were found in suckling and adult rat renal cortex, a 1.92 ± 0.13-fold higher level of this variant was found in the jejunum of adult compared with suckling rats.

The above-described changes in intestinal biotin uptake process and SMVT mRNA levels with maturation suggest the involvement of regulation by transcriptional mechanism(s). To further test this possibility, we performed a nuclear run-on assay to determine the transcription rate of SMVT in suckling and adult rat jejunum. The results showed a higher transcription rate for SMVT in adult compared with suckling rats, clearly supporting the suggestion stated above.

The findings that the intestinal, but not the renal, biotin uptake process is subjected to ontogenic changes suggest that the two processes are under different
regulation during maturation. The reason(s) behind this difference between intestinal and renal biotin uptake processes in response to ontogenic regulation is unclear. However, it could reflect the physiological importance of renal biotin reabsorption in both suckling and adult animals. It is interesting to mention here that the tissue-specific ontogenic regulation observed in this study for biotin uptake in renal and intestinal epithelia is not unique to biotin but has also been reported for transport of other substrates (e.g., transport of bile acid in renal and intestinal tissues) (9). In summary, the results of this study show that biotin uptake by intestinal and renal epithelial cells responds differently to ontogenic regulation. In addition, the results suggest that the changes in intestinal biotin uptake with maturation appear to involve regulation by transcriptional mechanism(s).

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