A high-affinity and specific carrier-mediated mechanism for uptake of thiamine pyrophosphate by human colonic epithelial cells

Svetlana M. Nabokina and Hamid M. Said

Departments of Medicine and Physiology/Biophysics, University of California, Irvine, and Department of Veterans Affairs Medical Center, Long Beach, California

Submitted 16 April 2012; accepted in final form 21 May 2012

Nabokina SM, Said HM. A high-affinity and specific carrier-mediated mechanism for uptake of thiamine pyrophosphate by human colonic epithelial cells. Am J Physiol Gastrointest Liver Physiol 303: G389–G395, 2012. First published May 24, 2012; doi:10.1152/ajpgi.00151.2012.—All mammals require exogenous sources of thiamine (vitamin B1), as they lack the ability to synthesize the vitamin. These sources are dietary and bacterial (the latter is in reference to the vitamin, which is synthesized by the normal microflora of the large intestine). Bacterially generated thiamine exists in the free, as well as the pyrophosphorylated [thiamine pyrophosphate (TPP)], form. With no (or very little) phosphatase activity in the colon, we hypothesized that the bacterially generated TPP can also be taken up by colonicocytes. To test this hypothesis, we examined [3H]TPP uptake in the human-derived, nontransformed colonic epithelial NCM460 cells and purified apical membrane vesicles isolated from the colon of human organ donors. Uptake of TPP by NCM460 cells occurred without metabolic alterations in the transported substrate and 1) was pH- and Na+-independent, but energy-dependent, 2) was saturable as a function of concentration (apparent $K_m = 0.157 \pm 0.028 \mu M$), 3) was highly specific for TPP and not affected by free thiamine (or its analogs) or by thiamine monophosphate and unrelated folate derivatives, 4) was adaptively regulated by extracellular substrate (TPP) level via what appears to be a transcriptionally mediated mechanism(s), and 5) appeared to be influenced by an intracellular $Ca^{2+}$/calmodulin-mediated regulatory pathway. These findings suggest the involvement of a carrier-mediated mechanism for TPP uptake by colonic NCM460 cells, which was further confirmed by results from studies of native human colonic apical membrane vesicles. The results also suggest that the bacterially synthesized TPP in the large intestine is bioavailable and may contribute to overall body homeostasis of vitamin B1 and, especially, to the cellular nutrition of the local colonicocytes.

thiamine pyrophosphate uptake; colonicocytes

THIAMINE (vitamin B1), a water-soluble micronutrient, is absolutely required for normal functions of all living cells. Thiamine in its diprophosphorylated form [i.e., thiamine pyrophosphate (TPP)] is an indispensable cofactor for oxidative metabolism, as it acts as a cofactor for several enzymes involved in carbohydrate and energy metabolism, including pyruvate dehydrogenase, $\alpha$-ketoglutarate dehydrogenase, branched-chain $\alpha$-ketoacid dehydrogenase, and transketolase (reviewed in Refs. 3, 5, and 28). Also, because thiamine bridges the glycolytic and pentose phosphate pathways (which generate chemical reducing power in the cell), it plays a role in reducing cellular oxidative stress (8, 15). Furthermore, it appears to play an important role in maintaining normal mitochondrial function and organization (4). Thus, deficiency of this micronutri...
to another. Evidence for efflux of TPP from mammalian (murine leukemia) cells via the reduced folate carrier RFC1 has also been reported (32).

Thus our aim in this study was to determine whether human colonic epithelial cells can take up TPP directly from the intestinal lumen and, if so, to determine the mechanism involved in this uptake and its regulation. We used as models nontransformed human-derived colonic epithelial NCM460 cells [a well-established model for studying colonocyte physiology (20, 21, 25–27)] and native purified apical membrane vesicles isolated from the mucosa of the proximal colon of human organ donors (7, 19). Our data demonstrate, for the first time, that human colonocytes possess a specific, high-affinity, and regulated carrier-mediated system for TPP uptake.

MATERIALS AND METHODS

Chemicals

Custom-made [3H]TPP ([3H]TPP, specific activity 1.3 Ci/mmol, radiochemical purity 99.9%) was obtained from Moravek Biochemicals (Brea, CA). [3H]-labeled biotin ([3H]biotin, specific activity 60 Ci/mmol, radiochemical purity 99.0%) and [3H]-labeled riboflavin ([3H]RF, specific activity 21.2 Ci/mmol, radiochemical purity 99.9%) were obtained from American Radiolabeled Chemicals (St. Louis, MO) and Moravek Biochemicals, respectively. Unlabeled TPP and thiamine analogs were purchased from Sigma. All other chemicals and reagents were purchased from commercial sources and were of analytical grade.

Cell Culture and Uptake Studies

The human-derived nontransformed colonic epithelial cell line NCM460 (INCELL, San Antonio, TX) was maintained and subcultured as described by us previously (26, 27). For uptake studies, NCM460 cells were trypsinized and plated onto 12-well plates (~2 × 10⁶ cells/well) in Ham’s F-12 culture medium (Invitrogen) supplemented with 20% (vol/vol) FBS and antibiotics. For assays of TPP oversupplementation effect, cells were grown for 4 days in Ham’s F-12 medium including 1 mM unlabeled TPP, with medium changed every day. Uptake studies were performed on confluent cell monolayers (between passages 29 and 44) 2–4 days following confluence. Uptake was examined in cells incubated at 37°C in Krebs-Ringer (KR) buffer containing (in mM) 123 NaCl, 4.93 KCl, 1.23 MgSO₄, 0.85 CaCl₂, 5 glucose, 5 glutamine, 10 HEPES, and 10 MES (pH 7.4). Although colonocytes are known to express no (or little) alkaline phosphatases (2, 9, 11), which we also confirmed by demonstration of no metabolism in the TPP taken up and the TPP left in the incubation medium (see RESULTS), we added the alkaline phosphatase inhibitor as an additional layer of precaution [phenylalanine (10 mM) did not affect TPP uptake; data not shown]. [3H]TPP was added to the incubation medium at the onset of the uptake experiment. The reaction was terminated after 3 min (unless otherwise stated) by the addition of 2 ml of ice-cold KR buffer, and the cells were immediately aspirated and washed with ice-cold KR buffer. Cells were lysed with 1 ml of 1 N NaOH, lysates were neutralized with HCl, and radioactivity was assessed in a liquid scintillation counter.

Chemical stability of [3H]TPP in the incubation buffer and the metabolic forms of the intracellular radioactivity following uptake were examined under the same conditions used for the uptake experiments. After incubation of NCM460 cells in 1 ml of KR buffer containing [3H]TPP (0.23 μM) for 3 min (unless otherwise indicated) at 37°C, the forms of extracellular or intracellular [3H] radioactivity were identified by thin-layer chromatography (TLC), as described previously (33). For assessment of chemical stability of TPP, 100% trichloroacetic acid (15 μl) was added to the aliquot (250 μl) of extracellular medium or cells, and the cells were subjected to centrifugation and several extractions with water-saturated ethyl ether. For determination of the metabolic forms of the intracellular radioactivity, cells were processed as described above (see Cell Culture and Uptake Studies). The sample (~20 μl) was mixed with the unlabeled thiamine, thiamine monophosphate (TMP), and TPP (to check the separation of thiamine derivatives under UV light), applied to the TLC plate (20 × 20 cm plate coated with a 250-μm layer of silica gel; Whatman), and run using a solvent system of diethanolamine-methanol-formic acid-67 mM dibasic sodium phosphate (1:15:5:5). Silica gel was cut into equal-sized (~1-cm) pieces, and distribution of radioactivity was assessed in a liquid scintillation counter.

Data Presentation and Statistical Analysis

Values are means ± SE of multiple individual uptake determinations and are expressed in picomoles or femtomoles per milligram of protein per unit of time. Student’s t-test was used in statistical analysis, with statistical significance set at 0.01 (P < 0.01). Kinetic parameters of the saturable TPP uptake process [i.e., maximal velocity (Vmax) and apparent Michaelis-Menten constant (Km)] were calculated as described by Wilkinson (31). Uptake by the saturable component was calculated at each concentration by subtraction of uptake by simple diffusion [from the slope of the line between the point of origin and uptake at high pharmacological concentration of TPP (1 mM)] from total uptake.

RESULTS

Uptake of TPP by NCM460 Cells: General Characteristics of the Process

Uptake of TPP (0.23 μM) by NCM460 cells as a function of time was studied and found to be linear (r = 0.99) for up to 10 min of incubation and occurred at 8.57 ± 1.17 pmol/mg protein (Fig. 1). A 3-min incubation time (initial rate) was used in all subsequent experiments.

The effect of uptake buffer pH on the initial rate of TPP uptake by NCM460 cells was examined. The results showed TPP (0.23 μM) uptake to be pH-independent over the pH range 5.5–8.0 (Fig. 2). All subsequent studies were performed at buffer pH 7.4.

The role of Na⁺ in TPP uptake by NCM460 cells was studied by replacement of Na⁺ in the uptake buffer with an equimolar concentration (123 mM) of the monovalent cations K⁺ and choline or with inert mannitol. No inhibition of the initial rate of TPP (0.23 μM) uptake was found upon Na⁺...
In a related study, we also determined the metabolic forms of the transported radioactivity following a 3-min (initial rate) incubation of NCM460 cells with $[^3\text{H}]\text{TPP} (0.23 \, \mu\text{M})$. The results showed that the majority ($\sim 96\%$) of the transported substrate was in the form of intact TPP.

**Involvement of a Carrier-Mediated System for TPP Transport in NCM460 Cells and Native Human Colon**

We examined the effect of unlabeled TPP (1 mM) on the initial rate of $[^3\text{H}]\text{TPP} (0.23 \, \mu\text{M})$ uptake by NCM460 cells. The results showed significant ($P < 0.01$) reduction of TPP uptake in the presence of unlabeled TPP (22.23 ± 0.37 and 0.08 ± 0.01 pmol·mg protein$^{-1}\cdot$min$^{-1}$ for control and 1 mM TPP, respectively).

To establish the physiological relevance of these findings to the native human intestine, we examined the effect of unlabeled TPP (1 mM) on the initial rate of TPP (0.38 μM) uptake by purified apical membrane vesicles isolated from epithelial cells of proximal human colon. The results showed significant ($P < 0.01$) inhibition of TPP transport by unlabeled TPP (2.71 ± 0.08 and 0.65 ± 0.01 pmol·mg protein$^{-1}\cdot$min$^{-1}$ for control and unlabeled TPP, respectively). These results provide the physiological validation for our observations with NCM460 cells with regard to the involvement of a carrier-mediated system for TPP transport.

To further confirm the existence of a carrier-mediated system for TPP uptake in colonocytes, we looked for the possible existence of saturability in the initial rate (3 min) of TPP uptake by NCM460 cells as a function of substrate concentration. The results (Fig. 3) showed the uptake to indeed include a saturable component with kinetic parameters of 0.157 ± 0.028 μM for apparent $K_m$ and 22.31 ± 0.98 pmol·mg protein$^{-1}\cdot$min$^{-1}$ for $V_{\text{max}}$. These data further confirm the existence of a carrier-mediated mechanism for TPP uptake by human colonocytes.

**Specificity of the TPP Uptake System of NCM460 Cells**

To determine the specificity of the TPP uptake system, we examined the effect of unlabeled TPP and a wide spectrum of its structural analogs, including TMP, thiamine, pyrithiamine, and amiloride (each at 1 mM) on the initial rate of TPP (0.23 μM) uptake by NCM460 cells was examined. TPP uptake was significantly ($P < 0.01$) inhibited by both compounds, with amiloride showing a more prominent inhibitory effect (24.44 ± 0.29, 21.68 ± 0.33, and 10.81 ± 0.29 pmol·mg protein$^{-1}\cdot$min$^{-1}$ for control, amiloride, and probenecid, respectively).

Although colonocytes are known to express no (or little) alkaline phosphatase activity (2, 9, 11), we estimated chemical stability of TPP in the uptake buffer (and its possible metabolism after exposure to cells) under the same conditions used in the uptake experiments. NCM460 cells were incubated in KR buffer containing $[^3\text{H}]\text{TPP} (0.23 \, \mu\text{M})$ for 3 min (initial rate) at 37°C; then the forms of extracellular $^3\text{H}$ radioactivity were identified by TLC (see MATERIALS AND METHODS). Cells incubated in the presence of inhibitor of intestinal alkaline phosphatases (phenylalanine, 10 mM) served as a control. The results showed that $\sim 97\%$ and $\sim 98\%$ of the extracellular $^3\text{H}$ radioactivity was in the form of intact TPP after 3 min of exposure to cells in the presence and absence of phenylalanine, respectively. We also estimated the effect of prolonged (60 min) exposure of $[^3\text{H}]\text{TPP} (0.23 \, \mu\text{M})$ to NCM460 cells in the absence of phenylalanine. The results showed that $\sim 95\%$ of the extracellular radioactivity existed in the form of intact TPP. These data clearly point to the absence of detectable degradation of $[^3\text{H}]\text{TPP}$ as a result of contact with NCM460 cells.

![Fig. 1. Uptake of thiamine pyrophosphate (TPP) by NCM460 cells as a function of time. Confluent monolayers of NCM460 cells were incubated in Krebs-Ringer buffer, pH 7.4, at 37°C. $[^3\text{H}]\text{TPP} (0.23 \, \mu\text{M})$ was added to the incubation medium at the start of uptake. Values are means ± SE of 3–4 separate uptake determinations. SEs are not visible on the scale of the graph.](image1)

![Fig. 2. Effect of incubation buffer pH on initial rate of TPP uptake by NCM460 cells. Confluent monolayers of NCM460 cells were incubated at 37°C in Krebs-Ringer buffer at pH 5.5–8.0. $[^3\text{H}]\text{TPP} (0.23 \, \mu\text{M})$ was added to the incubation medium at the onset of a 3-min incubation (i.e., initial rate). Values are means ± SE of 3–4 separate uptake determinations. Some SEs are not visible on the scale of the graph.](image2)
benfotiamine, oxythiamine, amprolium, and thiamine disulfide (each at 200 µM), on the initial rate of TPP (0.23 µM) uptake by NCM460 cells. The results (Table 1) showed that none of the compounds, other than unlabeled TPP, significantly affected uptake.

We also examined the effect of the organic phosphates, ADP and ATP (200 µM each), on the initial rate of TPP (0.23 µM) uptake by NCM460 cells. No significant inhibition in TPP uptake was observed for these compounds (Table 1).

Because the RFC has been reported to induce efflux of TPP in murine leukemia cells (32), we next determined the possibility of involvement of the RFC system in TPP uptake by NCM460 cells. To do this, we examined the effect of adding high concentrations of the naturally occurring folate derivative 5-formyltetrahydrofolate, the anti-folate methotrexate, and folic acid (each at 200 µM) to the incubation medium on the initial rate of TPP (0.23 µM) uptake by NCM460 cells. The results (Fig. 4) showed that none of these compounds inhibits TPP uptake by NCM460 cells, ruling out the possibility that RFC is involved in TPP uptake by human colonocytes.

### Table 1. Effect of TPP structural analogs and related compounds on the initial rate of [3H]TPP uptake by NCM460 cells

<table>
<thead>
<tr>
<th>Compound (200 µM)</th>
<th>Uptake, pmol·mg protein⁻¹·3 min⁻¹</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>22.81 ± 0.16</td>
<td></td>
</tr>
<tr>
<td>TPP</td>
<td>0.18 ± 0.01</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>TMP</td>
<td>20.95 ± 1.04</td>
<td>NS</td>
</tr>
<tr>
<td>Thiamine</td>
<td>23.66 ± 1.31</td>
<td>NS</td>
</tr>
<tr>
<td>Pyritiamine</td>
<td>21.38 ± 0.31</td>
<td>NS</td>
</tr>
<tr>
<td>Benfotiamine</td>
<td>22.13 ± 0.42</td>
<td>NS</td>
</tr>
<tr>
<td>Oxythiamine</td>
<td>22.61 ± 0.39</td>
<td>NS</td>
</tr>
<tr>
<td>Amprolium</td>
<td>22.98 ± 0.37</td>
<td>NS</td>
</tr>
<tr>
<td>Thiamine disulfide</td>
<td>23.23 ± 0.35</td>
<td>NS</td>
</tr>
<tr>
<td>ADP</td>
<td>22.54 ± 1.38</td>
<td>NS</td>
</tr>
<tr>
<td>ATP</td>
<td>19.31 ± 0.76</td>
<td>NS</td>
</tr>
</tbody>
</table>

Values are means ± SE of 3–6 separate uptake determinations. Monolayers of NCM460 cells were incubated in Krebs-Ringer buffer, pH 7.4, at 37°C. [3H]labeled thiamine pyrophosphate ([3H]TPP, 0.23 µM) and the compound under investigation were added to the incubation medium at onset of 3-min incubation. TMP, thiamine monophosphate; NS, not significant.

In a related study, we examined the effect of TPP structural analogs, including TMP and thiamine (each at 120 µM) on the initial rate of [3H]TPP (0.38 µM) uptake by apical membrane vesicles isolated from proximal human colon (the effect of unlabeled TPP was also simultaneously tested as a positive control). The results showed no significant inhibition of TPP uptake by unlabeled TMP and thiamine but the expected significant (P < 0.01) inhibition by unlabeled TPP (2.44 ± 0.09, 2.56 ± 0.2, 2.75 ± 0.18, and 0.46 ± 0.01 pmol·mg protein⁻¹·15 s⁻¹ for control, TMP, thiamine, and unlabeled TPP, respectively). These data provide the physiological validation for our observations with NCM460 cells with regard to specificity of the TPP carrier system.

### Regulation of the TPP Uptake Process in NCM460 Cells by Extracellular and Intracellular Factors

**Effect of extracellular substrate level.** We examined the effect of substrate (TPP) level in the extracellular environment on initial rate of [3H]TPP uptake. Figure 5 depicts the effect of maintaining NCM460 cells in TPP-oversupplemented (1 mM TPP) growth medium for 4 days on the initial rate of TPP (0.23 µM) uptake. Uptake by cells maintained in a medium containing a sufficient amount of thiamine (0.9 µM), but lacking TPP, served as a control in these experiments. As shown in Fig. 5, [3H]TPP uptake by cells maintained in TPP-oversupplemented conditions was significantly (P < 0.01) lower than uptake by cells maintained in control medium. Uptake of unrelated vitamins [3H]RF and [3H]biotin was not affected by the TPP level in cell growth medium (Fig. 5), confirming the specificity of the observation.

Since adaptive regulation of a number of vitamin transporters by substrate level has been shown to include transcriptional mechanisms (reviewed in Ref. 28), we aimed to examine the possibility of involvement of transcriptional mechanisms in TPP uptake regulation by substrate level. NCM460 cells grown in TPP-oversupplemented conditions for 4 days (control) were transferred to the medium lacking TPP and treated with the transcriptional inhibitor actinomycin D (0.3 µg/ml) for 24 h. Significant (P < 0.01) induction of TPP (0.23 µM) uptake was observed for cells in TPP-deficient medium compared with those continuously kept in TPP-oversupplemented medium, with the induction being significantly (P < 0.01) inhibited by
actinomycin D (Fig. 6). These data suggest that the adaptive regulation of the TPP transporter system by the substrate level occurs at the transcriptional level.

**Role of the Ca\(^{2+}\)/calmodulin-mediated intracellular regulatory pathway.** Pathways known to affect the nutrient uptake regulation in epithelial cells, including the Ca\(^{2+}\)/calmodulin-mediated, PKC-mediated, cAMP (or PKA)-mediated, nitric oxide (NO)-mediated, and protein tyrosine kinase (PTK)-mediated pathways (6, 10, 13, 16, 23), were chosen for our studies. Possible regulation of TPP uptake in colonocytes by these intracellular pathways was studied by examining the effect of a modulator of a specific pathway on TPP uptake. The modulators of the PKC-mediated (phorbol 12-myristate 13-acetate, 10 \(\mu\)M), cAMP (or PKA)-mediated ( dibutyryl cAMP, 5 mM), NO-mediated (S-nitroso-N-acetylpenicillamine, 1 mM), and PTK-mediated (genistein and tyrphostin A-1, 50 \(\mu\)M each) pathways do not affect TPP uptake by NCM460 cells (data not shown). In contrast, the Ca\(^{2+}\)/calmodulin-mediated intracellular regulatory pathway plays a role in regulation of TPP uptake in NCM460 cells. Pretreating NCM460 cells with calmidazolium (1, 10, and 50 \(\mu\)M), an inhibitor of the Ca\(^{2+}\)/calmodulin-mediated pathway, for 1 h caused a significant (\(P < 0.01\)) concentration-dependent decrease in TPP uptake (Table 2). Another inhibitor of the Ca\(^{2+}\)/calmodulin-mediated pathway, KN62 (50 \(\mu\)M), was also found to significantly (\(P < 0.05\)) inhibit TPP uptake (Table 2). We determined the effect of calmidazolium on the kinetic parameters of TPP uptake. Figure 7 depicts the results of the initial rate of TPP uptake as a function of substrate concentration by NCM460 cells pretreated for 1 h with 25 \(\mu\)M calmidazolium. As shown in Fig. 7, calmidazolium causes a significant (\(P < 0.01\)) inhibition of \(V_{\text{max}}\) (18.96 ± 1.21 and 5.79 ± 0.20 pmol·mg protein\(^{-1}\)·3 min\(^{-1}\) for control and calmidazolium, respectively), as well as a significant (\(P < 0.01\)) inhibition of the affinity for substrate (Table 2).

Fig. 6. TPP uptake by NCM460 cells grown under TPP-deficient and control conditions: effect of actinomycin D. NCM460 cells grown in TPP-oversupplemented medium (1 \(\mu\)M) for 4 days (control) were transferred to TPP-deficient medium and treated with actinomycin D (0.3 \(\mu\)g/ml) for 24 h. Uptake (for 3 min) was measured in Krebs-Ringer buffer, pH 7.4, in the presence of 0.23 \(\mu\)M \([^{3}\text{H}]\)TPP. Values are means ± SE of 3–6 separate uptake determinations. \(*P < 0.01.\)

Table 2. Effect of modulators of the Ca\(^{2+}\)/calmodulin-mediated intracellular regulatory pathway on \([^{3}\text{H}]\)TPP uptake by NCM460 cells

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Uptake, pmol·mg protein(^{-1})·3 min(^{-1})</th>
<th>(P) Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>23.37 ± 0.89</td>
<td></td>
</tr>
<tr>
<td>Calmidazolium</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 (\mu)M</td>
<td>22.84 ± 0.03</td>
<td>NS</td>
</tr>
<tr>
<td>10 (\mu)M</td>
<td>14.55 ± 0.16</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>50 (\mu)M</td>
<td>6.09 ± 0.11</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>KN62 (50 (\mu)M)</td>
<td>17.77 ± 0.32</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>

Values are means ± SE of 3–4 separate uptake determinations. Monolayers of NCM460 cells were incubated in Krebs-Ringer buffer, pH 7.4, at 37°C. \([^{3}\text{H}]\)TPP (0.23 \(\mu\)M) and the compound under investigation were added to the incubation medium at onset of 3-min incubation.
uptake process in NCM460 cells.

Initial rate of uptake of different concentrations of TPP was then examined in Krebs-Ringer buffer, pH 7.4. Values are means ± SE of 6–9 separate uptake determinations. Some SEs are not visible on the scale of the graph.

0.01) inhibition of apparent $K_m$ (0.14 ± 0.03 and 0.05 ± 0.01 μM for control and calmidazolium, respectively) of the TPP uptake process in NCM460 cells.

DISCUSSION

In contrast to the situation in the small intestine, there is no (or very little) alkaline phosphatase activity in the colon (2, 9, 11). This raises the possibility that the bacterially synthesized TPP in the colonic lumen remains (for the most part) unchanged and, thus, is available for uptake by the colonic epithelial cells. The current study was designed to directly test whether colonic epithelial cells are capable of taking up TPP and, if so, to determine the mechanism involved and how it is regulated. We used nontransformed human-derived colonic epithelial NCM460 cells and native human colonic apical membrane vesicles as models in our investigations (19–21, 25–27).

Our studies with NCM460 cells showed no degradation of TPP in the incubation medium whether phenylalanine (an inhibitor of alkaline phosphatases) was present or absent. Even prolonged (60 min) incubation of the cells with [3H]TPP in the absence of phenylalanine was not associated with detectable hydrolysis of TPP. Furthermore, the metabolic form of the transported TPP following 3 min of incubation of cells with [3H]TPP was mainly intact TPP. These findings clearly show that little, if any, degradation occurs in TPP prior its absorption, as well as after its internalization in colonic epithelial cells. In addition, the data support the belief that no (or little) alkaline phosphatase activity exists in colonocytes.

Uptake of TPP by NCM460 cells was found to be energy-dependent, but it was Na$^+$- and pH-independent. We also obtained evidence indicating the involvement of a carrier-mediated mechanism for TPP uptake by NCM460 cells. This was shown by the significant inhibition of the initial rate of uptake of [3H]TPP by unlabeled TPP and further supported by the observation of saturation in the uptake of TPP as a function of substrate concentration in the incubation medium. An apparent $K_m$ of the saturable TPP uptake system was determined to be 0.157 ± 0.028 μM, suggesting high affinity for the system involved. The physiological relevance of our findings with NCM460 cells with regard to involvement of a carrier-mediated system for TPP uptake was confirmed by the findings with purified native human colonic apical membrane vesicles, which again showed significant inhibition of [3H]TPP uptake by unlabeled TPP.

The functionally identified TPP uptake system in human colonocytes appears to be highly specific for TPP, as neither free thiamine (including a host of its analogs), TMP, the adenine nucleotides (ADP and ATP), nor folate derivatives were able to inhibit the initial rate of [3H]TPP uptake by NCM460 cells. The inability of TMP to inhibit [3H]TPP uptake by NCM460 cells indicates that the TPP uptake system can discriminate between mono- and diphosphorylated thiamine. Also, the inability of 5-methyltetrahydrofolate and methotrexate to inhibit TPP uptake by NCM460 cells indicates that the system involved is not related to the RFC (32). Further studies are required to determine the molecular identity of the colonic TPP uptake system.

We also examined the possible regulation of the TPP uptake process by extracellular and intracellular factors. Our results showed that maintaining NCM460 cells in TPP-over-supplemented growth medium leads to a specific and significant downregulation of the initial rate of [3H]TPP uptake by these cells. This adaptive regulation of the TPP uptake process by extracellular substrate level seems to be mediated, at least in part, at the transcriptional level, as indicated in the study involving the transcriptional inhibitor actinomycin D. We also examined possible regulation of the TPP uptake process of NCM460 cells by intracellular regulatory pathways. Pathways known to play a role in nutrient uptake regulation in epithelial cells, such as the Ca$^{2+}$/calmodulin-mediated, PKC-mediated, cAMP (or PKA)-mediated, NO-mediated, and PTK-mediated pathways (6, 10, 13, 16, 23), were studied. Our results show that modulators of the PKC-, PKA-, NO-, and PTK-mediated signaling pathways were unable to affect TPP uptake by NCM460 cells, suggesting that these pathways do not play an important role in TPP uptake by NCM460 cells. In contrast, a role for the Ca$^{2+}$/calmodulin-mediated intracellular regulatory pathway was evident. Calmidazolium, a widely used modulator of this pathway, caused a significant and concentration-dependent inhibition of TPP uptake, and its effect appeared to be mainly mediated via a significant inhibition of the $V_{\text{max}}$ of the TPP uptake process of NCM460 cells. This observation suggests that the effect of calmidazolium is a result of alterations in the activity (and/or number) of the TPP carrier. The exact mechanism (direct, through an effect on the uptake system, or indirect, through an effect of another entity that then affects the TPP uptake system) through which the Ca$^{2+}$/calmodulin-mediated pathway affects the TPP uptake process in NCM460 cells is not clear, and further studies are required to address this issue.

Finally, it is worth mentioning that recent studies have shown that human thiamine pyrophosphokinase (the cytoplasmic enzyme that converts transported free thiamine to TPP) is barely expressed in the human colon, while it is abundantly expressed in the human small intestine (34). This is most likely because colonic epithelial cells do not need this enzyme, as they import an already made TPP from the lumen. In summary, results of this study demonstrate, for the first time, the existence of a specific and high-affinity carrier-mediated mechanism for TPP uptake by human colonocytes. The results also show that the TPP uptake process is adaptively regulated by the extracellular substrate level and appears to be under the regulation of an intracellular Ca$^{2+}$/calmodulin-mediated pathway.
GRANTS

This study was supported by grants from the National Institutes of Health (DK-55061 and AA-018071) and the Department of Veterans Affairs.

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

S.M.N. and H.M.S. are responsible for conception and design of the research; S.M.N. and H.M.S. performed the experiments; S.M.N. and H.M.S. interpreted the results of the experiments; S.M.N. prepared the figures; S.M.N. and H.M.S. drafted the manuscript; S.M.N. and H.M.S. edited and revised the manuscript; H.M.S. approved the final version of the manuscript.

REFERENCES


