Mechanism of thiamine uptake by human colonocytes: studies with cultured colonic epithelial cell line NCM460

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Received 23 January 2001; accepted in final form 14 March 2001

Said, Hamid M., Alvaro Ortiz, Veedamali S. Subramanian, Ellis J. Neufeld, Mary Pat Moyer, and Pradeep K. Dudeja. Mechanism of thiamine uptake by human colonocytes: studies with cultured colonic epithelial cell line NCM460. Am J Physiol Gastrointest Liver Physiol 281: G144–G150, 2001.—Thiamine (vitamin B1) is essential for normal cellular functions and growth. Mammals cannot synthesize thiamine and thus must obtain the vitamin via intestinal absorption. The intestine is exposed to a dietary thiamine source and a bacterial source in which the vitamin is synthesized by the normal microflora of the large intestine. Very little is known about thiamine uptake in the large intestine. The aim of this study was, therefore, to address this issue. Our results with human-derived colonic epithelial NCM460 cells as a model system showed thiamine uptake to be: 1) temperature- and energy dependent, 2) Na+/calmodulin-mediated, 3) increased with increasing buffer pH from 5 to 8 and after cell acidification but inhibited by amiloride, 4) saturable as a function of concentration, 5) inhibited by thiamine structural analogs but not by unrelated organic cations, and 6) inhibited by modulators of a Ca2+/calmodulin-mediated pathway. NCM460 cells and native human colonic mucosa expressed the recently cloned human thiamine transporter THTR-1 (product of the SLC19A2 gene) at both mRNA and protein levels. These results demonstrate for the first time the involvement of a specialized carrier-mediated mechanism for thiamine uptake (21, 23, 26). As to the bacterial source of thiamine, previous studies have shown that the normal microflora of the large intestine synthesize considerable amounts of thiamine and thus must obtain the vitamin from exogenous sources via intestinal absorption. The intestine, therefore, plays a critical role in regulating body thiamine homeostasis. Thus understanding the mechanism and regulation of the intestinal thiamine absorption process is of significant nutritional importance. The intestine is exposed to thiamine from two sources, a dietary source and a bacterial source in which the vitamin is synthesized by the normal microflora of the large intestine (11, 12, 19). Absorption of dietary thiamine has been the subject of intense investigations over the past two decades (see Refs. 21 and 26 and references therein). With a variety of human and animal small intestinal preparations, these investigations demonstrated the involvement of a specialized carrier-mediated mechanism for thiamine uptake (21, 23, 26). As to the bacterial source of thiamine, previous studies showed that the normal microflora of the large intestine synthesize considerable amounts of thiamine and thus must obtain the vitamin from exogenous sources via intestinal absorption.

THIAMINE (vitamin B1), a water-soluble micronutrient, is essential for normal cellular functions, growth, and development. Thiamine in its coenzyme form, thiamine pyrophosphate, plays a critical role in normal carbohydrate metabolism, in which it participates in the decarboxylation of pyruvic and α-ketoglutaric acids and in the utilization of pentose in the hexose monophosphate shunt (2). Thiamine deficiency in humans leads to a variety of clinical abnormalities including cardiovascular disorders (e.g., peripheral vasodilation, biventricular myocardial failure, edema, and potentially acute fulminating cardiovascular collapse) and neurological disorders (e.g., confusion, disordered ocular motility, neuropathy, and ataxia of gait) (2, 31, 34). Thiamine deficiency represents a significant nutritional problem and occurs under variety of conditions such as in alcoholics (in whom impairment in the intestinal absorption process of the vitamin is believed to be a contributing factor; Refs. 17, 30, 33, 34), in patients with thiamine-responsive megaloblastic anemia (in whom impairment in membrane thiamine transport is believed to be the cause; Refs. 4, 7, 16, 22), and in patients with diabetes mellitus (27) and celiac diseases (32) and those on long-term therapy with diuretic medications (28).

Humans and other mammals cannot synthesize thiamine and thus must obtain the vitamin from exogenous sources via intestinal absorption. The intestine, therefore, plays a critical role in regulating body thiamine homeostasis. Thus understanding the mechanism and regulation of the intestinal thiamine absorption process is of significant nutritional importance. The intestine is exposed to thiamine from two sources, a dietary source and a bacterial source in which the vitamin is synthesized by the normal microflora of the large intestine (11, 12, 19). Absorption of dietary thiamine has been the subject of intense investigations over the past two decades (see Refs. 21 and 26 and references therein). With a variety of human and animal small intestinal preparations, these investigations demonstrated the involvement of a specialized carrier-mediated mechanism for thiamine uptake (21, 23, 26). As to the bacterial source of thiamine, previous studies showed that the normal microflora of the large intestine synthesize considerable amounts of thiamine and thus must obtain the vitamin from exogenous sources via intestinal absorption.

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that up to 50% of this thiamine exists in the free, absorbable form (11, 12, 19). In addition, human and rat large intestine is capable of absorbing thiamine from its lumens (13, 14, 19). Nothing, however, is known about the uptake mechanism involved. Recent studies from our laboratory (5, 15, 24, 25) showed that human colonocytes are able to transport a number of other water-soluble vitamins that are also synthesized by the normal microflora of the large intestine. Specialized carrier-mediated systems were shown to be involved in the uptake of folate, biotin, pantothenic acid, and riboflavin by human colonocytes (5, 15, 24, 25). The aim of this study was, therefore, to determine whether human colonocytes also possess a carrier system for thiamine uptake and, if so, to delineate the characteristics of such a transport system. To do this, we used the human-derived cultured colonic epithelial cell line NCM460 (18) as a model system. The results demonstrated, for the first time, that human colonocytes do indeed possess a specialized, carrier-mediated mechanism for thiamine uptake. This provides further support for the notion that this source of thiamine may contribute to host nutrition and especially toward cellular nutrition of local colonocytes.

MATERIALS AND METHODS

Custom-made \(^{3}H\)-labeled thiamine (sp. act. 559 Bq/μmol; radiochemical purity >98%) was purchased from American Radiolabeled Chemicals (St. Louis, MO). NCM460 cells and M3:10 growth medium were obtained from INCELL (San Antonio, TX). Trypsin, fetal bovine serum, and other cell culture reagents were obtained from Life Technologies (Grand Island, NY). All other chemicals and reagents used in this study were of analytical grade and were obtained from commercial sources.

NCM460 cells were grown and subcultured as described by us previously (15, 24, 25). Cells were used between passages 29 and 39. Uptake studies were performed on confluent monolayers 3–5 days after confluence. Uptake of thiamine was examined in cells incubated in Kreb’s-Ringer (K-R) buffer (in mM: 133 NaCl, 4.93 KCl, 1.23 MgSO\(_4\), 0.85 CaCl\(_2\), 5 glucose, 5 glutamine, 10 HEPES, and 10 MES, pH 7.4, unless otherwise specified) at 37°C. Labeled and unlabeled thiamine were added to the incubation medium at the onset of the uptake experiment. In certain experiments, cells were pre-treated with the compound under study for a specific period of time before the addition of \(^{3}H\)thiamine and the start of the uptake experiments. Uptake was examined over a period of 3 min, i.e., initial rate (unless otherwise specified), and the reaction was terminated by the addition of 2 ml of ice-cold buffer followed by immediate aspiration. Cells were then rinsed twice with ice-cold buffer and digested with 1 ml of 1 N NaOH, neutralized with HCl, and then counted for radioactivity. Protein contents of cell digest were measured on parallel wells by using a Bio-Rad kit (Richmond, VA).

The metabolic form of the \(^{3}H\) radioactivity taken up by NCM460 monolayers after 3- and 10-min incubation in the presence of 150 nM \(^{3}H\)thiamine was determined by cellulose-precocated TLC. In this study, cells were washed three times with K-R buffer after uptake, suspended in 75% ethanol, and homogenized. The supernatant was then applied onto the TLC plate and run using a solvent system of isopropanol-acetate buffer (0.5 M, pH 4.5)-water (65:15:20 vol/vol/vol).

Semiquantitative RT-PCR and Northern blot analysis. Three micrograms of poly(A)\(^{+}\) RNA isolated from NCM460 cells were primed with gene-specific primer (to synthesize the first-strand cDNA) by using a SuperScript First-Strand Synthesis for RT-PCR kit (Life Technologies) as described by the manufacturer. Two specific primers spanning the entire open reading frame (ORF) of the SLC19A2 cdNA sequence that was cloned from human fibroblasts, skeletal muscle, placenta, and brain (forward primer, 5'–CGCGCCCGGTAGTGATGTT–3'; reverse primer, 5'–GCTGCTGTTAGTCACAGAAAT–3'); Refs. 4, 6, 7, 16) were used to search for expression of the SLC19A2 gene (the product of which is the THTR-1 protein) in NCM460 cells. PCR conditions were denaturation at 94°C for 2.5 min, 9 cycles of 94°C denaturation for 30 s, 56°C annealing for 30 s, and 68°C extension for 2 min, followed by 30 cycles of 94°C denaturation for 30 s, 56°C annealing for 30 s, 68°C extension for 2 min with an increment of an additional 5 s for every cycle, and a final 7-min extension at 68°C. The PCR product was separated through 0.7% agarose gel with images captured by using an Eagle Eye (Stratagene, La Jolla, CA). The amplified RT-PCR product was normalized to the amplified \(\beta\)-actin RT-PCR products. The nucleotide sequence of the identified PCR product was confirmed by sequencing using a commercial vendor (Seqwright, Houston, TX).

Northern blot analysis was performed as described by us previously (20) by using randomly labeled full-length ORF of the human SLC19A2 cdNA that was recently cloned in our laboratory from human intestinal epithelial Caco-2 cells (unpublished observations; GenBank accession no. AF272359) and poly(A\(^{+}\) cdNA from normal human colonic mucosa (Clontech). Data were normalized relative to human \(\beta\)-actin.

Western blot analysis. The membranous fraction was isolated from NCM460 cells by homogenization of cells in buffer containing (in mM) 300 mannitol, 5 EGTA, 12 Tris-HCl, pH 7.1, and 1 phenylmethylsulfonyl fluoride and then centrifuged at 3,000 g for 15 min. The supernatant was centrifuged at 20,000 g for 30 min. The resulting pellet (150 μg protein) was treated with Laemmli sample buffer and resolved on a 10% SDS-polyacrylamide gel. After electrophoresis, the proteins were electroblotted onto Immob-Blot polyvinylidene difluoride membrane (Bio-Rad, Hercules, CA) overnight. The blots were washed twice in PBS-Tween 20 for 10 min, blocked with 5% dried milk in PBS-Tween 20 for 1 h at room temperature, and washed with PBS-Tween 20. They were then probed with anti-human THTR-1 polyclonal antibodies [1:25,000 diluted in 1% dried milk-PBS-Tween 20 (Sigma)] for 1 h at room temperature, washed twice in 1% dried milk-PBS-Tween 20, and reacted with goat anti-rabbit IgG conjugated to horseradish peroxidase (Sigma) (1:5,000 diluted in 1% dried milk-PBS-Tween 20) overnight. The blots were then treated with Laemmli sample buffer and resolved on a 10% SDS-polyacrylamide gel. After electrophoresis, the proteins were electroblotted onto Immob-Blot polyvinylidene difluoride membrane (Bio-Rad, Hercules, CA) overnight. The blots were washed twice in PBS-Tween 20 for 10 min, blocked with 5% dried milk in PBS-Tween 20 for 1 h at room temperature, and washed with PBS-Tween 20. They were then probed with anti-human THTR-1 polyclonal antibodies [1:25,000 diluted in 1% dried milk-PBS-Tween 20 (Sigma)] for 1 h at room temperature, washed twice in 1% dried milk-PBS-Tween 20, and reacted with goat anti-rabbit IgG conjugated to horseradish peroxidase (Sigma) (1:5,000 diluted in 1% dried milk-PBS-Tween 20) for 1 h at room temperature. The blots were finally washed twice in PBS for 10 min, and color was developed by using an ECL kit (Amersham). Specific bands were quantitated by using Eagle Eye Software (Stratagene). A similar procedure was used in the case of native human colonic apical membrane preparations isolated as described by us previously (5).

The anti-human THTR-1 polyclonal antibodies were raised against a synthetic peptide in rabbits by a commercial vendor (Alpha Diagnostic Intl., San Antonio, TX). The design of the antigenic peptide was as follows. The sequence of the THTR-1 protein was searched for any similarities with other known proteins by using a Blast algorithm and a Swissport database. Because the human reduced folate carrier has been reported to have some degree of homology with THTR-1 (4, 6, 7, 16), the sequence of that protein was therefore aligned alongside the THTR-1 by using a Clustal W algorithm to
detect the regions of lowest homology. The regions of THTR-1 protein showing the lowest homology were then analyzed for hydrophilicity (Kyte-Doolittle hydrophathy plot), antigenicity (Hopp/Woods and protrusion index antigenicity profiles), and accessibility. A specific region of the THTR-1 protein that corresponds to amino acids 17–35 of the human sequence (TVLLRTRARVRECWFILTPTA) was determined to be specific for this protein and was used for raising the polyclonal antibodies. We also confirmed the uniqueness of the selected peptide by using the Swissport database to avoid any cross-reactivity of the resulting antibodies with other proteins. The selected peptide was synthesized as keyhole limpet hemocyanin conjugate and was used for immunization in two rabbits.

Data presentation and statistical analysis. Uptake results presented in this paper are means ± SE of multiple uptake determinations and are expressed as picomoles or femtomoles per milligram of protein per unit of time. Statistical differences were analyzed by Student's t-test or ANOVA, with statistical significance being set at $P < 0.05$. Quantitative variations in the absolute amounts of thiamine uptake were observed in certain experiments, and thus appropriate controls were run simultaneously with each set of experiments. Kinetic parameters of the saturable component of thiamine uptake [i.e., maximum velocity ($V_{max}$) and apparent Michaelis-Menten constant ($K_m$)] were calculated by using a computerized model of the Michaelis-Menten equation as described previously by Wilkinson (35).

RESULTS

General characteristics of thiamine uptake by NCM460 cell: uptake as a function of time and effect of temperature, pH, and Na$^+$. Uptake of low (0.1 µM) and high (10 µM) concentrations of thiamine by NCM460 cells was investigated as a function of incubation time at 37°C. At both thiamine concentrations, uptake was linear over the 10-min incubation period and occurred at a rate of 0.21 and 14.98 pmol/mg protein $\cdot$ min$^{-1}$, respectively (Fig. 1). We selected a 3-min period within the linear range as the standard incubation time in all subsequent studies.

In another study, we examined the metabolic form of $^3$H radioactivity taken up by NCM460 cells after incubation of the cells with 150 nM $[^3]$H]thiamine for 3 and 10 min. A TLC system was used in the study as described in MATERIALS AND METHODS. The result showed the majority (97% and 96%, respectively, for 3 and 10 min) of the $^3$H radioactivity taken up by the confluent NCM460 monolayers to be in the form of intact thiamine.

The effect of incubation temperature on uptake of thiamine (30 nM) was also examined. A significant ($P < 0.01$; ANOVA) and progressive decrease in thiamine uptake was observed on decreasing incubation temperature from 37 to 22 to 4°C (103 ± 2.9, 41.6 ± 2.6, and 14.8 ± 1.5 fmol/mg protein for 3 min, respectively).

The effect of incubation buffer pH on thiamine uptake was investigated by testing the effect of a gradual increase in buffer pH from 5.0 to 8.0 on the initial rate of vitamin (30 nM) uptake. A gradual increase in thiamine uptake was observed on increasing buffer pH from 5.0 to 8.0; uptake at pH 8.0 was 3.3-fold higher than uptake at pH 5.0 (26.8 ± 2.4, 31.7 ± 2.1, 39 ± 0.8, 54.8 ± 1.5, 59.2 ± 2.9, 75.6 ± 3.1, and 89.1 ± 1.4 fmol/mg protein for 3 min at buffer pH 5.0, 5.5, 6.0, 6.5, 7.0, 7.5, and 8.0, respectively). In a related study, we examined the effect of lowering the intracellular pH of NCM460 cells on the initial rate of influx of thiamine. Incubation buffer pH was maintained at 7.4. Cell acidification was performed by a standard procedure as described previously (29). Briefly, confluent monolayers of NCM460 were incubated for 30 min in K-R buffer in which NaCl was replaced with NH$_4$Cl. This was followed by removal of the buffer, washing the monolayers with KCl-containing K-R buffer (KCl replaced NaCl), and incubating the cells with 30 nM $[^3]$H]thiamine in the same KCl-containing K-R buffer for 3 min. Results were compared with thiamine uptake by cells preincubated for 30 min with a KCl-containing K-R buffer (instead of NH$_4$Cl-containing K-R buffer) with the rest of the cell handling being the same. Significantly ($P < 0.01$) higher uptake was found in the acid-loaded cells compared with control (168.2 ± 7.6 and 123.6 ± 1.1 fmol/mg protein for 3 min, respectively).

The effect of Na$^+$ removal from the incubation medium on thiamine uptake was also examined by studying the effect of isosmotic replacement of Na$^+$ with

![Fig. 1. Uptake of thiamine by NCM460 cells as a function of time. Monolayers were incubated in Krebs-Ringer buffer at 37°C in the presence of 0.1 (A) and 10 (B) µM thiamine. Results are means ± SE of 6–8 separate uptake determinations. When not shown, SE bars are within the symbol size.](http://ajpgi.physiology.org/ by 10.220.32.247 on April 4, 2017)
other monovalent cations (K\(^+\), Li\(^+\), choline, and Tris) or with mannitol on the initial rate of thiamine (30 nM) uptake. The results showed that none of these replacements have any significant effect on thiamine uptake ([111.9 ± 1.9, 118.6 ± 8.0, 121.3 ± 7.0, 118.5 ± 7.3, 109 ± 4.5, and 106.7 ± 5.6 fmol/mg protein for 3 min in the presence of Na\(^+\) (control), K\(^+\), Li\(^+\), choline, Tris, and mannitol, respectively].

We also examined the effect of pretreating (for 30 min) the cells with the Na-K-ATPase inhibitor ouabain (1 mM) on the uptake of thiamine (30 nM). The results showed no effect of this treatment on the vitamin uptake (116.5 ± 2.8 and 114.0 ± 5.6 fmol/mg protein for 3 min, in the absence and presence of ouabain, respectively).

**Uptake as a function of substrate concentration: kinetics of thiamine uptake process.** In this study, we examined the initial rate of thiamine uptake (3 min) by NCM460 cells as a function of increasing the substrate concentration in the incubation medium (0.03–10 µM). Thiamine uptake was found to include a saturable component. Uptake by this component was calculated by subtracting uptake by diffusion from total uptake of the vitamin at each substrate concentration [uptake by diffusion was calculated from the slope of the line between uptake at high pharmacological concentration of thiamine (1 mM) and the point of origin]. Kinetic parameters of the saturable component, i.e., the apparent \(K_m\) and \(V_{max}\), were then determined as described in MATERIALS AND METHODS and found to be 2.33 ± 0.38 µM and 3.76 ± 0.22 fmol/mg protein for 3 min, respectively (Fig. 2).

**Effect of structural analogs and unrelated organic cations on \(^{3}H\)thiamine uptake.** The effect of the thiamine structural analogs oxythiamine and amprolium (both at 25 µM) on the initial rate of \(^{3}H\)thiamine (30 nM) uptake by confluent NCM460 monolayers was tested. The results showed that both structural analogs cause a significant \(P < 0.01\) inhibition in \(^{3}H\)thiamine uptake (114.5 ± 1.1, 86.3 ± 1.1, and 82.4 ± 1.9 fmol/mg protein for 3 min in controls and in the presence of oxythiamine and amprolium, respectively).

We also examined the effect of the unrelated organic cations tetraethylammonium (TEA) and \(N\)-methylnicotinamide (NMN) (both at 50 µM) on the uptake of cationic thiamine (30 nM) by NCM460 cells. Neither of these compounds was found to have a significant effect on thiamine uptake (135 ± 1.3, 133 ± 1.0, and 136 ± 1.5 fmol/mg protein for 3 min in controls and in the presence of TEA and NMN, respectively).

**Effect of metabolic and membrane transport inhibitors on thiamine uptake.** In this study, we examined the effect of preincubating the cells for 30 min in buffer containing the metabolic inhibitors fluoride (10 mM), iodoacetate (1 mM), or dinitrophenol (DNP, 10 mM) on the initial rate of thiamine (30 nM) uptake. The results showed significant \(P < 0.01\) for all) inhibition in the vitamin uptake by all the tested metabolic inhibitors (116.5 ± 2.8, 65.5 ± 7.3, 86.8 ± 5.6, and 77.6 ± 8.3 fmol/mg protein for 3 min for controls and pretreatment with fluoride, iodoacetate, and DNP, respectively).

In another study, we examined the effect of the membrane transport inhibitors DIDS, probenecid, furosemide, and amiloride (all at 1 mM) on the initial rate of thiamine (30 nM) uptake. With the exception of amiloride, which caused significant \(P < 0.01\) inhibition in thiamine uptake, none of the other tested membrane transport inhibitors significantly affected vitamin uptake (119.5 ± 4.9, 121.3 ± 5.1, 112 ± 3.5, 112.5 ± 4.8, and 82.9 ± 2.3 fmol/mg protein for 3 min for controls and in the presence of furosemide, DIDS, probenecid, and amiloride, respectively).

**Expression of human thiamine transporter THTR-1 in NCM460 cells and in native human colonic mucosa.** The SLC19A2 cDNA has been recently cloned from a number of human tissues including skeletal muscle, brain, and placenta, and its functionality in transporting thiamine has been established by transfection assay (4, 6, 7, 16). In this study, we investigated whether this human thiamine transporter is also expressed in the human-derived NCM460 cells and in native human colonic mucosa at both the mRNA and protein levels. Using a RT-PCR procedure described in MATERIALS AND METHODS, we found clear expression of the SLC19A2 gene in these cells (Fig. 3A). The identity of the cloned cDNA was confirmed by sequencing and found to be identical to that of the SLC19A2 cDNA. Similarly, Northern blot analysis showed expression of the SLC19A2 mRNA in native human colonic mucosa (Fig. 3B). We also tested for expression of the THTR-1 protein in NCM460 cells and in apical membrane preparations isolated by an established procedure (5) from native human colonic mucosa using specific polyclonal antibodies raised in rabbits against a specific peptide of
In this study, we examined the possible involvement of regulation of thiamine uptake by colonic NCM460 cells. The results showed that neither activating [with the use of phorbol-12-myristate-13-acetate (PMA; 10 \( \mu M \)] nor inhibiting [with the use of staurosporin and chelerythrine; both at 10 \( \mu M \)] this signaling pathway significantly affects thiamine uptake (103.8 ± 3.3, 113.5 ± 2.4, 103 ± 6.8, and 105.6 ± 4.2 fmol/mg protein for 3 min for controls and after pretreatment with PMA, staurosporin, and chelerythrine, respectively). Similarly, no role for a PTK-mediated pathway was found because pretreatment of NCM460 with modulators of this pathway failed to significantly affect thiamine (30 nM) uptake (97.2 ± 5.7, 89.5 ± 2.5, and 96.6 ± 1.6 fmol/mg protein for 3 min for controls and after pretreatment with 25 \( \mu M \) genistein and 10 \( \mu M \) tyrophos- 

Possible role of intracellular regulatory pathways in regulation of thiamine uptake by colonic NCM460 cells. In this study, we examined the possible involvement of Ca\(^{2+}\)/calmodulin-), protein kinase C (PKC)-, and protein tyrosine kinase (PTK)-mediated pathways in cellular regulation of the thiamine uptake process by NCM460 cells. The study was performed using specific modulators of these pathways. The role of the Ca\(^{2+}\)/calmodulin-mediated pathway in the regulation of thiamine uptake by NCM460 cells was investigated by examining the effect of pretreating (for 1 h) NCM460 cells with the Ca\(^{2+}\)/calmodulin inhibitor trifluoperazine (TFP; 50 \( \mu M \)) and N-(4-aminobutyl)-5-chloro-2-naphthalenesulfonamide (W13; 100 \( \mu M \)) on the initial rate of uptake of \([^{3}H]\)thiamine (30 nM). The results showed significant (\( P < 0.01 \)) inhibition in thiamine uptake by these compounds (95.2 ± 6.8, 59.2 ± 3.8, and 60.9 ± 3.9 fmol/mg protein for 3 min for controls and in the presence of TFP and W13, respectively). The inhibitory effect of TFP (50 \( \mu M \)) on thiamine uptake appeared to be mediated via a decrease in the \( V_{\text{max}} \) of the thiamine uptake process [2.27 ± 0.13 and 0.79 ± 0.07 fmol/mg protein for 3 min (\( P < 0.01 \)) for control and TFP-treated cells, respectively] and the apparent \( K_{\text{m}} \) [3.61 ± 0.39 and 1.48 ± 0.21 \( \mu M \) (\( P < 0.01 \)), respectively]

The possible role of a PKC-mediated pathway in the regulation of thiamine uptake by these colonic epithelial cells was also investigated by examining the effect of pretreating NCM460 cells (for 1 h) with modulators of this pathway on the vitamin uptake process. The results showed that neither activating [with the use of phorbol-12-myristate-13-acetate (PMA; 10 \( \mu M \)] nor inhibiting [with the use of staurosporin and chelerythrine; both at 10 \( \mu M \)] this signaling pathway significantly affects thiamine uptake (103.8 ± 3.3, 113.5 ± 2.4, 103 ± 6.8, and 105.6 ± 4.2 fmol/mg protein for 3 min for controls and after pretreatment with PMA, staurosporin, and chelerythrine, respectively). Similarly, no role for a PTK-mediated pathway was found because pretreatment of NCM460 with modulators of this pathway failed to significantly affect thiamine (30 nM) uptake (97.2 ± 5.7, 89.5 ± 2.5, and 96.6 ± 1.6 fmol/mg protein for 3 min for controls and after pretreatment with 25 \( \mu M \) genistein and 10 \( \mu M \) tyrophos- 

### DISCUSSION

In this study we examined thiamine uptake by human colonocytes using human-derived, nontransformed cultured colonic epithelial NCM460 cells as an in vitro model system. These cells were chosen for the study because they have been demonstrated to possess characteristics similar to those of native colonocytes, including similar uptake mechanisms (5, 15, 18, 24, 25). The results showed thiamine uptake by these cells to be appreciable and to occur with no metabolic alterations in the transported substrate. The latter finding indicates that transport of thiamine into this type of cells is governed mainly by the transmembrane event
and not by an intracellular metabolic event of the transported molecule. The latter mechanism has been recently suggested for uptake of another water-soluble vitamin (riboflavin) in isolated rat enterocytes (9).

Uptake of thiamine by NCM460 cells was found to involve a carrier-mediated system as indicated by the saturation in the substrate uptake as a function of concentration and by the inhibition in thiamine uptake by the thiamine structural analogs oxythiamine and amprolium. This system was found to be specific for cationic thiamine because other unrelated organic cations like TEA and NMN failed to affect the vitamin uptake process. Na\(^+\) in the incubation medium was found to have no role in thiamine uptake by these cells. This is based on the observation that replacing Na\(^+\) in the incubation medium with other monovalent cations failed to affect thiamine uptake. A role for H\(^+\) in thiamine uptake, however, was evident, and a thiamine/H\(^+\) exchange mechanism may be suggested. Evidence to support the latter suggestion includes the marked increase in thiamine uptake on increase of incubation buffer pH from 5.0 to 8.0 (i.e., on decrease of extracellular H\(^+\) concentration) and the significant increase in thiamine uptake on increase of the intracellular H\(^+\) concentration (i.e., after cell acidification). The finding that a Na\(^+\)/H\(^+\) exchange inhibitor, amiloride, also inhibits thiamine uptake by NCM460 cells lends further support to this suggestion. The possible involvement of a thiamine/H\(^+\) exchange mechanism that is sensitive to inhibition by amiloride has also been suggested for the vitamin uptake in other cell types (3, 8, 10, 23).

Recent studies have reported the cloning of a cDNA of a thiamine transport protein (i.e., the so-called human thiamine transporter-1, THTR-1) from several human tissues including skeletal muscle, brain, and placenta (4, 6, 7, 16). Functional identity of the cloned SLC19A2 cDNA was confirmed by expression in HeLa cells, which showed significant induction in thiamine uptake in cDNA-transfected cells compared with controls (6). It is interesting to note here that the characteristics of the expressed THTR-1 carrier protein with regard to pH profile, apparent \(K_m\) (2.5 ± 0.6 \(\mu\)M), and lack of effect of unrelated organic cations on thiamine uptake (6) are similar to those observed for the vitamin transport in NCM460 cells reported in this study. For this reason, we decided to investigate whether NCM460 cells also express the mRNA and protein of the SLC19A2 gene. Semiquantitative RT-PCR using specific primers designed from the sequence of the cloned human SLC19A2 cDNA and Western blotting using specific anti-THTR-1 polyclonal antibodies were used. The results showed that these cells do indeed express the mRNA and protein of the SLC19A2 gene. Similarly, native human colonic mucosa was found by Northern and Western blot analysis to express the mRNA and protein of the SLC19A2 gene, respectively. These findings raise the possibility that THTR-1 carrier protein may be involved in thiamine uptake in human colonocytes. Further studies, however, are required to confirm this suggestion.

Possible regulation of the thiamine uptake process of NCM460 cells by specific intracellular regulatory pathways was also investigated. Our findings indicate that modulation of a calmodulin (but not PKC or PTK)-mediated pathway led to a significant inhibition in thiamine uptake by NCM460 monolayers. The effect of the calmodulin-mediated pathway inhibitor TFP was mediated via a decrease in \(V_{max}\) and the apparent \(K_m\) of the thiamine uptake process. This suggests that the effect is the net result of alterations in activity (and/or number) and affinity of the thiamine uptake system. Further studies are required to determine the exact mechanism through which this intracellular pathway affects the thiamine uptake process in these cells.

The demonstration of existence of a specialized carrier-mediated mechanism for thiamine uptake in human-derived colonic epithelial NCM460 cells, together with the previous findings of specialized carrier systems for the uptake of other water-soluble vitamins (folate, biotin, pantothenic acid, and riboflavin; Refs. 5, 15, 24, 25) that are also synthesized by the normal microflora of the large intestine (11, 12), further highlight the possible importance of this source of vitamins in host nutrition in general and in cellular nutrition of the local colonocytes in particular.

In summary, the results of this study demonstrate for the first time the existence of a specialized carrier-mediated mechanism for thiamine uptake by a human colonic epithelial cell model. This system appears to involve a thiamine/H\(^+\) exchange mechanism and appears to be under the regulation of an intracellular Ca\(^{2+}\)/calmodulin-mediated pathway. Furthermore, NCM460 cells as well as native human colonic mucosa appear to express the mRNA and protein of the SLC19A2 gene.

This study was supported by grants from the Department of Veterans Affairs and the National Institutes of Health (DK-56061, DK-58057, DK-54016, and HL-04184).

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