Mechanism of thiamine uptake by human jejunal brush-border membrane vesicles

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1Department of Medicine, West Side Veterans Affairs Medical Center and University of Illinois at Chicago, Chicago, Illinois 60612; and 2Departments of Medicine and Physiology/Biophysics, University of California Irvine, and Veterans Affairs Medical Center, Long Beach, California 90822

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Dudeja, Pradeep K., Sangeeta Tyagi, Reena J. Kavilaveettil, Ravinder Gill, and Hamid M. Said. Mechanism of thiamine uptake by human jejunal brush-border membrane vesicles. Am J Physiol Cell Physiol 281: C786–C792, 2001.—Thiamine, a water-soluble vitamin, is essential for normal cellular functions, growth and development. Thiamine deficiency leads to significant clinical problems and occurs under a variety of conditions. To date, however, little is known about the mechanism of thiamine absorption in the native human small intestine. The objective of this study was, therefore, to characterize the mechanism of thiamine transport across the brush-border membrane (BBM) of human small intestine. With the use of purified BBM vesicles (BBMV) isolated from the jejunum of organ donors, thiamine uptake was found to be 1) independent of Na+ but markedly stimulated by an outwardly directed H+ gradient (pH 5.5 in/pH 7.5 out); 2) competitively inhibited by the cation transport inhibitor amiloride (inhibitor constant of 0.12 mM); 3) sensitive to temperature and osmolarity of the incubation medium; 4) significantly inhibited by thiamine structural analogs (amiprol, oxythiamine, and pyrithiamine), but not by unrelated organic cations (tetraethylamonium, N-methylpyridinium, or choline); 5) not affected by the addition of ATP to the inside and outside of the BBMV; 6) potential insensitive; and 7) saturable as a function of thiamine concentration with an apparent Michaelis-Menten constant of 0.61 ± 0.08 μM and a maximal velocity of 1.00 ± 0.47 pmol·mg protein⁻¹·10⁻¹ s⁻¹. Carrier-mediated thiamine uptake was also found in BBMV of human ileum. These data demonstrate the existence of a Na+-independent, pH-dependent, amiloride-sensitive, electroneutral carrier-mediated mechanism for thiamine absorption in native human small intestinal BBMV.

thiamine transporter; human small intestine; brush-border membranes

THIAMINE (vitamin B1), a water-soluble vitamin, plays a vital role in many metabolic reactions and is thus essential for normal cellular functions, growth, and development (21). Thiamine deficiency in humans occurs under different conditions (alcoholism, diabetes mellitus, celiac disease, aging) and leads to a variety of clinical abnormalities, including cardiovascular and neurological disorders (21). Additionally, in alcoholic and celiac disease patients, this deficiency has been suggested to be due to impairment of intestinal absorption of thiamine (21).

Humans and other mammals cannot synthesize thiamine and thus depend on the exogenous supply of the vitamin via intestinal absorption (21). Dietary thiamine exists mainly in the phosphorylated forms (predominantly as thiamine pyrophosphate (TPP)), which are hydrolyzed to free thiamine before absorption in the small intestine (12, 16, 20). The mechanism of absorption of dietary thiamine in the small intestine has been studied in animal models using a variety of intestinal preparations. The absorption was found to involve a specialized carrier-mediated system (5, 9). Furthermore, the thiamine transported across the intestinal epithelia has been shown to undergo some degree of phosphorylation inside the enterocytes (mainly to TPP) via the action of the cytoplasmic thiamine pyrophosphokinase (2, 12, 16). The thiamine that exits from the enterocyte was shown, However, to be in the form of free thiamine (4, 16).

In contrast to the available information regarding thiamine transport in the small intestine of animal models, little is known about the mechanism of thiamine transport in the human small intestine. In vivo studies in healthy humans performed by analyzing serum and urinary radioactivity levels after oral administration of [3H]thiamine (22) have suggested the involvement of a carrier-mediated system for the intestinal absorption process. This suggestion was confirmed by subsequent studies using human intestinal surgical and biopsy specimens (10, 15, 17) and more recently in studies using the human-derived cultured intestinal epithelial cells Caco-2 (19). Although these studies have provided important information regarding the human intestinal thiamine uptake process at the tissue/cellular level and its regulation, no study is

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available describing the mechanism of thiamine transport across the individual membrane domains of the polarized human intestinal absorptive cells, i.e., the brush-border membrane (BBM) and the basolateral membrane (BLM) domains. For a substrate that undergoes some degree of intracellular metabolism, as is the case with thiamine (2, 12, 16), such studies are best performed using purified membrane vesicle preparations that are devoid of intracellular components. Using purified BBM vesicle (BBMV) preparations isolated from the small intestine of organ donors, the current study was, therefore, undertaken to elucidate the mechanism of thiamine transport across the luminal BBM. The results demonstrated the existence of a Na\(^{+}\)-independent, pH-dependent, amiloride-sensitive, carrier-mediated exchange process for thiamine uptake across the human intestinal BBM.

**MATERIALS AND METHODS**

\[^{3}H\]thiamine (sp act 10 Ci/mmol; radiochemical purity > 97\%) was obtained from American Radiolabeled Chemicals (St. Louis, MO). Unlabeled thiamine, amprolium, oxythiamine, pyrithiamine, tetraethylammonium (TEA), N-methyl-nicotinamide (NMN), valinomycin, and amiloride were obtained from Sigma Chemical (St. Louis, MO). All other chemicals and reagents were obtained from either Fisher Scientific (Fairlawn, NJ) or Sigma Chemical (unless otherwise stated) and were of the highest purity available. To determine the degree of thiamine metabolism after uptake by intestinal BBMV, a thin-layer chromatography procedure employing cellulose gel-precoated plates and a solvent system of isopropanol/0.5 M acetate buffer (pH 4.5)/water (65/15/20, vol/vol/vol) was used (11).

**Isolation of human small intestinal BBMV and \[^{3}H\]thiamine uptake studies.** These investigations were approved by the Institutional Review Board of the University of Illinois at Chicago. Small intestines from 10–12 healthy adult organ donors were obtained after the harvest of transplantable organs. The upper one-third jejunum and lower one-third ileum sections of the small bowel were cleaned with an ice-cold 0.9% NaCl solution, and scraped mucosa was frozen away at −80°C and utilized for membrane preparations. Jejunal and ileal BBM were purified from the frozen mucosal preparations isolated from the small intestine of organ donors, the current study was, therefore, undertaken to elucidate the mechanism of thiamine transport across the luminal BBM. The results demonstrated the existence of a Na\(^{+}\)-independent, pH-dependent, amiloride-sensitive, carrier-mediated exchange process for thiamine uptake across the human intestinal BBM.

**RESULTS**

**Effect of Na\(^{+}\) and the presence and absence of a pH gradient on thiamine uptake as a function of time.** To examine if the thiamine uptake in the human jejunal BBMV involved a Na\(^{+}\)-dependent process similar to a number of other nutrient transporters, our initial studies were performed using Student’s t-test. Kinetic parameters of the saturable component of thiamine binding to filters and/or vesicles by subtracting radioactivity present in time 0 vesicle blank.

Data presented in this study are means ± SE of 3–7 independent preparations. Kinetic parameters of the saturable component of thiamine binding to filters and/or vesicles by subtracting radioactivity present in time 0 vesicle blank.

Table 1. Effect of an inwardly directed Na\(^{+}\) or K\(^{+}\) gradient on \[^{3}H\]thiamine uptake

<table>
<thead>
<tr>
<th>Time, min</th>
<th>Inward Na(^{+}) Gradient</th>
<th>Inward K(^{+}) Gradient</th>
</tr>
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<tbody>
<tr>
<td>0.17</td>
<td>0.057 ± 0.014</td>
<td>0.047 ± 0.011</td>
</tr>
<tr>
<td>1.0</td>
<td>0.070 ± 0.011</td>
<td>0.065 ± 0.015</td>
</tr>
<tr>
<td>90</td>
<td>0.139 ± 0.065</td>
<td>0.147 ± 0.075</td>
</tr>
</tbody>
</table>

Values are in pmol/mg protein and are means ± SE for 3 separate experiments. Brush-border membrane vesicle (BBMV) were pre-loaded with 280 mM mannitol and 20 mM Tris/HEPES (pH 7.5) or 80 mM mannitol, 100 mM K-gluconate, and 20 mM Tris/HEPES (pH 7.5). The uptake was stopped at various time points using 5 ml of ice-cold stop solution containing 280 mM mannitol and 20 mM Tris/HEPES, pH 7.5. The diluted sample was filtered using a rapid filtration technique, employing 0.45-μm nitrocellulose filters. Filters were further washed twice with 5 ml ice-cold stop solution. The filters were then dissolved in Filtercount, and the radioactivity was measured in a Packard TR1600 liquid scintillation counter (Packard, Downers Grove, IL). All values were corrected for nonspecific \[^{3}H\]thiamine binding to filters and/or vesicles by subtracting radioactivity present in time 0 vesicle blank.
BBMV demonstrated a time-dependent increase and was linear for up to 15 s of incubation time. \[^{3}H\]thiamine uptake exhibited an overshoot phenomenon, as the peak uptake at the 15-s time point was markedly higher compared with the 90-min equilibrium uptake. The uptake of thiamine at conditions of pH 7.5\textsubscript{in}/pH 7.5\textsubscript{out} was not significantly different from uptake at pH 5.5\textsubscript{in}/pH 5.5\textsubscript{out} but was lower than uptake at pH 5.5\textsubscript{in}/pH 7.5\textsubscript{out} (data not shown). These data, therefore, clearly indicate the existence of a pH gradient-dependent uptake process for thiamine across the human jejunal BBM.

Effect of temperature and incubation medium osmolality on \[^{3}H\]thiamine uptake. In this study, we examined the effect of temperature on the H\textsuperscript{+} gradient-driven uptake of thiamine (pH 5.5\textsubscript{in}/pH 7.5\textsubscript{out}) by jejunal BBMV. The results showed that thiamine uptake was markedly higher at 37°C compared with uptake at 0°C (0.12 ± 0.08 at 0°C and 1.11 ± 0.04 at 37°C, expressed as pmol·mg protein\textsuperscript{-1}·10 s\textsuperscript{-1}, n = 3, P < 0.001).

To differentiate the nonspecific binding of \[^{3}H\]thiamine to the vesicular membrane from its transport into the intravesicular space, the effect of varying medium osmolarities on \[^{3}H\]thiamine uptake was examined. The osmolarity of the incubation medium was altered by increasing the concentration of the mannitol. As shown in Fig. 2, \[^{3}H\]thiamine uptake into the vesicles at the equilibrium time (90 min) was sequentially reduced parallel to increasing the osmolarity of the incubation media. These data indicated that the vesicles were intact and responsive to variations in medium osmolarity, and \[^{3}H\]thiamine associated with the membrane vesicles was predominantly due to its uptake into the closed intravesicular space rather than due to nonspecific binding to the membrane surface. A linear relationship between the uptake and the reciprocal of osmolarity was seen (Fig. 2). Extrapolation of the straight line to zero (infinite osmolarity) indicated that the binding to the membrane surface of vesicles was minimal (~10%).

In another preliminary study, we examined the metabolic form of the radioactivity taken up by human jejunal BBMV after a 90-min incubation with 0.42 µM \[^{3}H\]thiamine using a thin-layer chromatography procedure as described in MATERIALS AND METHODS (11). The results showed 96% of the radioactivity taken up by the BBMV to be in the form of intact thiamine.

Effect of ATP on \[^{3}H\]thiamine uptake. Previous studies by Laforenza et al. (13) have shown the thiamine transport across rat small intestinal basolateral membranes to be ATP dependent. To examine whether thiamine uptake in our human jejunal BBM preparations depends on ATP, the effect of 1 mM Mg\textsuperscript{2+}-ATP was examined in this study. As shown in Fig. 3, ATP in the extravesicular medium and intravesicular medium failed to influence thiamine uptake at different time intervals, indicating that thiamine uptake across the human jejunal BBMV does not utilize ATP and thus does not display primary active transport.

Effect of transmembrane potential on \[^{3}H\]thiamine uptake. To determine whether a H\textsuperscript{+} gradient-stimulated transport is an electroneutral process rather than a membrane potential-dependent mechanism, the effect of K\textsuperscript{+}/valinomycin-induced membrane potential on thiamine uptake was determined. The \[^{3}H\]thiamine uptake was measured after imposition of an intravesicular negative or positive membrane potential and compared with uptake under voltage-clamped conditions. As shown in Table 2, the changes in transmem-

![Fig. 1. Effect of pH gradient on time course of \[^{3}H\]thiamine uptake. Jejunal brush-border membrane vesicles (BBMV) preloaded with 280 mM mannitol and 20 mM Tris/2-(N-morpholino)ethanesulfonic acid (MES) (pH 5.5) were incubated in a reaction medium containing 280 mM mannitol, and either 20 mM Tris/MES (pH 5.5) or 20 mM Tris/HEPES (pH 7.5) and 0.1 µM \[^{3}H\]thiamine (final concn). Uptake was determined at 25°C for the indicated time periods. Values are means ± SE for 3 separate membrane preparations.](http://ajpcell.physiology.org/)
brane potential did not affect thiamine uptake in these membranes.

**Effect of amiloride on H\(^+\) gradient-stimulated \([3H]\)thiamine uptake.** Previous studies have shown that amiloride inhibits thiamine uptake by the cultured intestinal epithelial cell line Caco-2 (19), as well as in neuroblastoma cells (1). Therefore, to further characterize the mechanism of inhibition of amiloride on the uptake of the monovalent cation thiamine, the effect of increasing concentrations of amiloride (0.05–1.0 mM) on H\(^+\) gradient-driven \([3H]\)thiamine uptake by human jejunum BBMV was determined. The results, presented in Fig. 4, demonstrated a dose-dependent inhibition of \([3H]\)thiamine uptake into these vesicles by amiloride. An inhibitor constant of \(\approx 0.12\) mM for amiloride was calculated.

**Effect of thiamine structural analogs and unrelated organic cations on H\(^+\) gradient-stimulated \([3H]\)thiamine uptake.** To assess the specificity of the H\(^+\) gradient-dependent thiamine uptake process, the effects of the various structural analogs, i.e., amprolium, cold thiamine, and pyrithiamine, on \([3H]\)thiamine uptake were examined. As shown in Fig. 5, there was a significant inhibition (70–75%) of the H\(^+\) gradient-stimulated thiamine uptake into the vesicles in the presence of the various structural analogs (50 \(\mu\)M) in the incubation medium.

### Table 2. Effect of membrane potential on \([3H]\)thiamine uptake

<table>
<thead>
<tr>
<th>Groups</th>
<th>([3H])thiamine Uptake, pmol/mg protein (\times 10^{-15}) s(^{-1})</th>
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<tbody>
<tr>
<td>Voltage clamped</td>
<td></td>
</tr>
<tr>
<td>(K_{\text{out}}^{+} = K_{\text{in}}^{+} + \text{valinomycin})</td>
<td>0.042 ± 0.010</td>
</tr>
<tr>
<td>Inside positive</td>
<td></td>
</tr>
<tr>
<td>(K_{\text{out}}^{+} &gt; K_{\text{in}}^{+} + \text{valinomycin})</td>
<td>0.045 ± 0.015</td>
</tr>
<tr>
<td>Inside negative</td>
<td></td>
</tr>
<tr>
<td>(K_{\text{out}}^{+} &lt; K_{\text{in}}^{+} + \text{valinomycin})</td>
<td>0.035 ± 0.012</td>
</tr>
</tbody>
</table>

Values are means ± SE for 3 separate experiments. BBMV were preloaded with either 280 mM mannitol and 20 mM Tris/MES (pH 5.5) or 180 mM mannitol, 50 mM K-glucurate, and 20 mM Tris/MES (pH 5.5). \([3H]\)thiamine uptake was determined at 25°C by incubating the vesicles in reaction medium consisting of either 50 mM K-glucurate, 180 mM mannitol, 20 mM Tris/HEPES (pH 7.5), and 0.1 \(\mu\)M \([3H]\)thiamine or 280 mM mannitol, 20 mM Tris/HEPES (pH 7.5), and 0.1 \(\mu\)M \([3H]\)thiamine + 20 \(\mu\)M valinomycin.
Thiamine exists as a monovalent cation at the pH range of 5 to 7.4. Thus to further distinguish between the thiamine uptake system of jejunal BBM and that of the previously characterized organic cations, the effect of various organic cations, e.g., TEA, NMN, and choline, on the uptake of [3H]thiamine was investigated. As shown in Fig. 6, the presence of various organic cations in the extravesicular medium (50 μM) failed to inhibit thiamine uptake into the human jejunal BBM.

**DISCUSSION**

Previous studies have characterized certain aspects of the mechanism of thiamine uptake by human small intestine using surgical and biopsy specimens and cultured intestinal epithelial Caco-2 cells (10, 15, 17, 19). Very little, however, is known about the mechanism of thiamine transport across the individual membrane domains of the functionally polarized human intestinal epithelial cells. In the present study, we examined the mechanism of thiamine transport across the apical BBM of native human enterocytes using purified BBM isolated by a well-validated technique from organ donor jejunal mucosa. This membrane preparation was utilized to avoid possible changes in the metabolic form of the transported thiamine into the intestinal BBM. Our data provided evidence for the existence of a distinct pH-dependent, amiloride-sensitive, carrier-mediated system for an electroneutral thiamine transport process across the human intestinal BBM.

The uptake of thiamine by human jejunal BBM was found to be similar in the presence and absence of a Na⁺ gradient, indicating that the process is Na⁺ independent in nature. An outwardly directed H⁺ gradient (pH 5.5/pH 7.5out), however, was found to result in significant stimulation of the thiamine uptake with a distinct overshoot phenomena being observed during the initial phase of uptake. The overshoot phenomena in thiamine uptake indicated movement of the vitamin against a concentration gradient in the intravesicular space. These data could also be explained by a possible involvement of a thiamine/H⁺ exchange mechanism.

Uptake of thiamine by the human jejunal BBMV was found to involve a carrier-mediated system. This conclusion is based on a number of observations, including temperature dependence of the uptake process, inhibi-
tion by unlabeled thiamine and related compounds, and saturation of the uptake process as a function of increasing the substrate concentration in the incubation medium. Kinetic parameters of the thiamine uptake system in jejunal BBMV were an apparent $K_m$ of $0.61 \pm 0.08 \, \mu M$ and a $V_{\text{max}}$ of $1.00 \pm 0.47 \, \text{pmol} \cdot \text{mg protein}^{-1} \cdot 10^{-1} \, \text{s}^{-1}$. Similarly, saturation in thiamine uptake as a function of concentration was also observed in studies with human ileal BBMV with an apparent $K_m$ of $1.2 \pm 0.5 \, \mu M$ and $V_{\text{max}}$ of $2.2 \pm 0.5 \, \text{pmol} \cdot \text{mg protein}^{-1} \cdot 10^{-1} \, \text{s}^{-1}$. These findings suggest that both the proximal and the distal areas of the human small intestine are capable of transporting thiamine.

The identified pH-dependent uptake system for the monovalent cation thiamine across the human intestinal BBMV was found to be potential insensitive in nature. This finding suggested that the process of thiamine uptake by the human intestinal BBMV was electroneutral in nature and further supported the earlier stated suggestion that a thiamine/H⁺ exchange mechanism may be involved in the uptake process. The carrier-mediated system for thiamine uptake was found to be specific for the vitamin and is different from previous findings on Na⁺/H⁺-independent, electroneutral, carrier-mediated system for thiamine uptake across the human intestinal BBMV. Additionally, this system appears to be inhibited by the diuretic amiloride in a concentration-dependent manner. Further studies to characterize the molecular identity of this transporter and its molecular regulation will be of importance.

In summary, our current results provide strong evidence for the existence of a specific, Na⁺/H⁺-independent, pH-dependent, electroneutral, carrier-mediated system for thiamine transport across the human intestinal BBMV. This study was supported by grants from the Department of Veterans Affairs and by National Institute of Diabetes and Digestive and Kidney Diseases Grants DK-33348, DK-54016, DK-56061, and DK-58057.

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