Mechanism and regulation of human intestinal niacin uptake

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Submitted 11 January 2005; accepted in final form 15 February 2005

Nabokina, Svetlana M., Moti L. Kashyap, and Hamid M. Said. Mechanism and regulation of human intestinal niacin uptake. Am J Physiol Cell Physiol 289:C97–C103, 2005—The mechanism of uptake of dietary niacin (nicotinic acid) by intestinal epithelial cells is not well understood, and nothing is known about regulation of the uptake process. In this investigation, we used human-derived intestinal epithelial Caco-2 cells and purified intestinal brush-border membrane vesicles (BBMVs) isolated from human organ donors to assess niacin uptake. Our findings show niacin uptake by Caco-2 cells to be temperature and energy dependent; 2 Na+ independent, but highly dependent on extracellular acidic pH; 3 saturable as a function of concentration, with an apparent $K_m$ of 0.53 ± 0.08 μM; 4 severely inhibited by the membrane-impermeable sulphydryl group of reagents; and 5 highly specific for niacin but not affected by monosaccharides. A marked trans stimulation in [3H]niacin efflux from preloaded Caco-2 cells by unlabelled niacin in the incubation buffer was also observed. These findings suggest the involvement of a specialized, pH-dependent, carrier-mediated mechanism for human intestinal niacin uptake. This suggestion was confirmed in studies with native human intestinal BBMVs. We also examined possible regulation of niacin uptake by Caco-2 cells via specific intracellular regulatory pathways. The results show that while the PKA-, PKC-, and Ca2+/calmodulin-mediated regulatory pathways play no role in regulating niacin uptake, a role for a protein tyrosine kinase (PTK)-mediated pathway is apparent. The results of these studies show for the first time the existence of a specialized, acidic pH-dependent, carrier-mediated system of niacin uptake by human intestinal epithelial cells that operates at the micromolar (physiological) range of niacin. The results also suggest the possible involvement of a PTK-mediated pathway in the regulation of niacin uptake.

NIACIN (NICOTINIC ACID), a water-soluble vitamin, acts as a precursor to the synthesis of the coenzymes NAD and NADP, which are involved in important reactions that maintain the redox state of cells (e.g., glycolysis, pentose phosphate shunt) (8, 9). In pharmacological doses, niacin reduces plasma triglycerides, cholesterol, and atherogenic apolipoprotein B (apoB)-containing lipoproteins and increases antiatherogenic apoA-I-containing high-density lipoprotein level, thus preventing atherosclerotic cardiovascular disease (10, 11). Humans have access to niacin from endogenous and exogenous sources (1, 9). The endogenous source of niacin is provided via the metabolic conversion of amino acid tryptophan to niacin. The exogenous source is provided through the diet by absorption in the intestine.

The mechanism involved in the intestinal absorption of dietary niacin, and its regulation, is not well understood. Previous studies in laboratory animals have reported the mechanism to function either via simple diffusion of the undissociated form of nicotinic acid (according to the pH partition hypothesis and assisted by acid microclimate at the luminal surface of the intestine) (3) or via a carrier-mediated mechanism (4, 14, 18, 20). The latter studies, however, reported an apparent Michaelis-Menten constant ($K_m$) for the carrier-mediated process from 3.52 to 17.0 mM. The high apparent $K_m$ values reported in the previous studies raise a concern regarding the physiological relevance of the described system, because intestinal luminal concentration of niacin under physiological conditions is estimated to be in the micromolar but not the millimolar range (6, 19).

Chemically, nicotinic acid is a monocarboxylic acid with a pKa of 4.9. Transport of monocarboxylic acids (i.e., lactate, pyruvate, and the ketone bodies acetoacetate and β-hydroxybutyrate) is mediated in mammalian cells by a family of monocarboxylate transporters (MCTs) (7). MCTs are low-affinity carriers that display apparent $K_m$ values in the millimolar range (7). Previous studies have reported inhibition of niacin uptake by monocarboxylic acids (18, 20), raising the possibility of involvement of the MCT systems in niacin uptake. Our aims in the present study were to investigate the mechanism of niacin uptake in the human intestine using as models cultured, human-derived intestinal epithelial Caco-2 cells and purified isolated brush-border membrane vesicles (BBMVs) isolated from the jejunum of organ donors. Our results demonstrate for the first time the existence of a highly specialized, acidic pH-dependent, high-affinity, carrier-mediated system for niacin uptake by human intestinal epithelial cells. Also, evidence was obtained suggesting that the system may be under the regulation of an intracellular protein tyrosine kinase (PTK)-mediated pathway.

MATERIALS AND METHODS

[5,6-3H]Nicotinic acid (specific activity, 50 Ci/mmol; radiocative purity, 99.0%) was obtained from American Radiolabeled Chemical (St. Louis, MO). All other chemicals and reagents used in this study were of analytical quality and were obtained from Sigma (St. Louis, MO) or Fisher Scientific (Tustin, CA).

Cell Culture and Uptake Studies

Human-derived intestinal epithelial Caco-2 cells (passage 20; American Type Culture Collection, Manassas, VA; these cells were derived from colorectal adenocarcinoma obtained from a 72-year-old Caucasian male) were grown in Dulbecco’s modified Eagle’s medium supplemented with 10% (vol/vol) fetal bovine serum in 75-cm² plastic flasks at 37°C in a 5% CO2-95% air atmosphere, with medium changed every 2–3 days. The cells were subcultured and plated onto 24-well plates. Uptake studies were performed on confluent cell monolayers (between passages 23 and 36) 3–5 days after confluence. Cells were fed fresh incubation medium the day before uptake experiments were performed.

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Data Presentation and Statistical Analysis

Fig. 1. Uptake of nicotinic acid by Caco-2 cells as a function of time. Confluent monolayers of Caco-2 cells were incubated at 37°C in Krebs-Ringer buffer, pH 5.0, for different time intervals. [3H]nicotinic acid (6 nM), together with 0.1 µM unlabeled nicotinic acid, was added to the incubation medium at the start of uptake. Each data point represents mean ± SE of four to six separate uptake determinations. When not shown, error bars are smaller than the symbol. y = 0.159x + 0.140; R = 0.995.

[3H]Nicotinic acid uptake was examined in Caco-2 cells incubated in Krebs-Ringer buffer containing (in mM) 133 NaCl, 4.93 KCl, 1.23 MgSO4, 0.85 CaCl2, 5 glucose, 5 glutamine, 10 HEPES, and 10 MES, pH 5.0 (unless otherwise stated). [3H]Nicotinic acid was added to the incubation medium at the onset of the uptake experiment, and the reaction was terminated after 3 min (unless otherwise stated) by the addition of 1 ml of ice-cold buffer, followed by immediate aspiration. Cells were then rinsed twice with ice-cold buffer and lysed with 1 ml of 1 N NaOH. Lysates were neutralized with HCl, and then radioactivity was measured using a scintillation counter. The protein content of cell digests was measured in parallel wells using the Bio-Rad DC protein assay kit (Bio-Rad, Hercules, CA).

The metabolic form of the transported substrate after 3-, 7-, and 10-min incubation with 60 nM [3H]nicotinic acid was determined from the slope of the line between the point of origin and uptake at high pharmacological concentration of niacin; 1 mM) from total uptake.

RESULTS

General Characteristics of Uptake of Nicotinic Acid by Caco-2 Cells

The time-dependent uptake of nicotinic acid (0.106 µM) by Caco-2 cells at pH 5.0 was examined. Uptake was found to be linear up to 10 min at the rate of 159 fmol·mg of protein−1·min−1 (Fig. 1). Thus 3-min incubation was chosen as the standard incubation time for which to study the effect of different factors and/or conditions on the initial rate of niacin uptake. Uptake of a high niacin concentration, 10 µM, also was found to be linear for the selected incubation time of 3 min (data not shown).

In another study, we examined the effect of varying incubation buffer pH over the range from 5.0 to 8.0 on the initial rate of niacin (6 nM) uptake by Caco-2 cells. The results (Fig. 2) showed that when the extracellular pH was reduced from 8.0 to 5.0, niacin uptake was dramatically increased, with maximum uptake occurring at pH 5.0. Thus all our experiments were performed at pH 5.0. We also examined the effect of pretreating the cells for 30 min with the protonophore carbonyl cyanide p-trifluoromethoxyphenylhydrazone (FCCP; 50 µM) on the initial rate of niacin (6 nM) uptake by Caco-2 cells. The results show that pretreatment with FCCP led to a dramatic inhibition of niacin uptake (233.67 ± 7.3 and 21.26 ± 0.8 fmol·mg of protein−1·min−1 for control and FCCP-pretreated cells, respectively).

With the use of thin-layer chromatography, the metabolic form of the transported [3H]-labeled niacin after 3-, 7-, and 10-min incubation of Caco-2 cells with 6 nM [3H]niacin was
observed to be mostly (96, 93, and 91% for 3, 7, and 10 min, respectively) in the form of intact niacin.

The effect of incubation temperature on the initial rate of niacin (6 nM) uptake was also investigated. The results showed niacin uptake to be significantly \( (P < 0.01) \) higher at 37°C compared with 27°C (224.8 ± 8.0 and 82.3 ± 0.5 fmol·mg of protein\(^{-1}\)·min\(^{-1} \), respectively), with a calculated \( Q_{10} \) value (ratio of transport rate at 37°C to transport rate at 27°C) of 2.7.

The role of Na\(^+\) dependence in intestinal niacin uptake was tested by examining the effect of isosmotic replacement of Na\(^+\) in the incubation medium with K\(^+\) or Li\(^+\) on the initial rate of niacin (6 nM) uptake. Similar niacin uptake was observed in the presence and absence of Na\(^+\) (233.2 ± 6.5, 225.3 ± 4.8, and 226.9 ± 4.5 fmol·mg of protein\(^{-1}\)·min\(^{-1} \) for control, K\(^+\), and Li\(^+\), respectively). In a related study, we tested the effect of pretreatment (30 min) of cells with the Na\(^+\)-K\(^+\)-ATPase inhibitor ouabain (1 mM) on the initial rate of niacin (6 nM) uptake. Ouabain-pretreated cells showed no significant decrease in niacin uptake (234.74 ± 13.4 and 228.2 ± 12.8 fmol·mg of protein\(^{-1}\)·min\(^{-1} \) for control and after pretreatment with ouabain, respectively). Therefore, uptake of niacin by Caco-2 cells was regarded as a Na\(^+\)-independent process.

We further examined the effect of pretreating (30 min) the cells with the metabolic inhibitors iodoacetate (at 1 mM) and 2,4-dinitrophenol (DNP; 0.5 mM) on the initial rate of niacin (6 nM) uptake. Both compounds were found to cause significant \( (P < 0.01) \) inhibition of niacin uptake (230.54 ± 7.6, 48.14 ± 2.6, and 102.66 ± 8.62 fmol·mg of protein\(^{-1}\)·min\(^{-1} \) for control, iodoacetate, and DNP, respectively).

**Evidence for Existence of a Carrier-Mediated Mechanism for Niacin Uptake by Caco-2 Cells and Purified Native Human Intestinal BBMVs**

In these investigations, we examined the initial rate of niacin uptake (i.e., 3 min) as a function of concentration over a wide range of concentrations spanning nanomolar and micromolar ranges (we also included the nanomolar range because recent studies with other water-soluble vitamins such as thiamin and biotin have suggested the existence of a high-affinity system in the nanomolar range in addition to the well-characterized systems that operate at the micromolar ranges of these vitamins; Refs. 5, 17). Our findings show that niacin uptake over the nanomolar range to be linear \( (R = 0.997) \) as a function of concentration (Fig. 3A). On the other hand, saturation was observed in niacin uptake over the micromolar concentration range (Fig. 3B). Kinetic parameters of the saturable component were calculated as described in MATERIALS AND METHODS and found to be 0.53 ± 0.08 \( \mu \)M and 13.32 ± 0.58 pmol·mg protein\(^{-1}\)·min\(^{-1} \) for apparent \( K_m \) and maximal velocity \( (V_{max}) \), respectively. These findings suggest the existence of a carrier-mediated uptake system for niacin that operates in the micromolar range. To confirm this conclusion and to determine the specificity of the niacin uptake system, we examined the effect of unlabeled niacin and that of its structural analogs isonicotinic acid, nicotinamide, isonicotinic acid hydrazide, nicotinyl alcohol, and nicotinuric acid on the initial rate of \([^3H]\)niacin (6 nM) uptake by confluent monolayers of Caco-2 cells. We also examined the effect of the niacin related compound 5-methyl-1H-pyrazole-3-carboxylic acid (which has been shown to act as a high-affinity ligand for the recently described niacin receptor HM74A; Ref. 22) on the initial rate of \([^3H]\)niacin uptake. The results (Table 1) show that none of the compounds other than unlabeled niacin significantly affected niacin uptake.

In other studies, we examined possible trans stimulation of \([^3H]\)niacin transport by unlabeled niacin. For this purpose, we preloaded Caco-2 cells with \([^3H]\)niacin (7 min) and then incubated them in the presence and absence of unlabeled niacin (1 mM) in the incubation buffer. The results show the cell content of \([^3H]\)niacin to be significantly \( (P < 0.01) \) lower in cells incubated in the presence of unlabeled niacin in the incubation medium compared with those incubated in buffer alone (249.15 ± 1.7 and 437.36 ± 3.5 fmol·mg of protein\(^{-1}\)·10 min\(^{-1} \)·incubation, respectively), clearly indicating trans stimulation in niacin uptake.

To establish the relevance of these findings on the existence of a carrier-mediated uptake system for niacin to the native human intestine, we examined the effect of unlabeled niacin (1 mM) on the initial rate of uptake of \([^3H]\)niacin (12 nM) by
Isonicotinic acid 10 214.8
5-Methyl-1H-pyrazole-3-carboxylic acid 20 218.0
Nicotinamide 10 212.6
Isonicotinic acid hydrazide 10 214.8
Nicotinyl alcohol 10 203.4

To test this possibility, we examined the effect of different monocarboxylic acids with a pKa value of 4.9. Previous studies have suggested that niacin may be transported by the MCT systems. As mentioned earlier, niacin is a weak monocarboxylic acid with a pKa value of 4.9. Previous studies have suggested that niacin may be transported by the MCT systems. To test this possibility, we examined the effect of different carboxylic acid with a pKa value of 4.9. Previous studies have suggested that niacin may be transported by the MCT systems. To test this possibility, we examined the effect of different

<table>
<thead>
<tr>
<th>Condition</th>
<th>Concentration, μM</th>
<th>[3H]Niacin Uptake, fmol-mg protein⁻¹ min⁻¹</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unlabeled niacin</td>
<td>20</td>
<td>213.6±3.3</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Isonicotinic acid</td>
<td>10</td>
<td>84.8±1.8</td>
<td>NS</td>
</tr>
<tr>
<td>Nicotinyl alcohol</td>
<td>50</td>
<td>210.4±4.1</td>
<td>NS</td>
</tr>
<tr>
<td>Isocitonic acid hydrazide</td>
<td>50</td>
<td>210.4±3.8</td>
<td>NS</td>
</tr>
<tr>
<td>Nicotinamide</td>
<td>10</td>
<td>212.6±1.0</td>
<td>NS</td>
</tr>
<tr>
<td>Nicotinuric acid</td>
<td>50</td>
<td>210.7±3.5</td>
<td>NS</td>
</tr>
<tr>
<td>5-Methyl-1H-pyrazole-3-carboxylic acid</td>
<td>20</td>
<td>214.8±10.6</td>
<td>NS</td>
</tr>
</tbody>
</table>

Data are means ± SE of 4–10 separate uptake determinations. NS, not significant. Initial rate of [3H]niacin (6 nM) uptake by Caco-2 cells was measured with or without the compound under investigation for 3 min at pH 5.0.

Fig. 4. The effect of unlabeled niacin on the initial rate of uptake of [3H]niacin by purified brush-border membrane vesicles (BBMVs) isolated from the jejunum of human organ donors. BBMVs were preloaded with 280 mM mannitol and 20 mM HEPES-Tris (pH 7.4). The uptake of [3H]niacin (12 nM) was determined at 37°C for 10 s by incubating the BBMVs with 1 mM niacin or without 1 mM niacin (control) in a buffer containing (in mM) 140 mannitol, 100 NaCl, 10 HEPES, and 10 MES at pH 5.0 (A) or pH 7.4 (B). Values represent means ± SE of at least three separate determinations.

INTESTINAL NIAVIN UPTAKE
Table 2. Effect of different monocarboxylic acids on the uptake of [3H]niacin by Caco-2 cells

<table>
<thead>
<tr>
<th>Agent</th>
<th>Concentration, µM</th>
<th>[3H]Niacin Uptake, fmol/mg protein−1·3 min−1</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td></td>
<td>226.1 ± 3.1</td>
<td></td>
</tr>
<tr>
<td>Butyric acid</td>
<td>25</td>
<td>224.1 ± 5.0</td>
<td>NS</td>
</tr>
<tr>
<td>Acetic acid</td>
<td>25</td>
<td>221.3 ± 9.0</td>
<td>NS</td>
</tr>
<tr>
<td>Propionic acid</td>
<td>25</td>
<td>226.5 ± 4.0</td>
<td>NS</td>
</tr>
<tr>
<td>Valproic acid</td>
<td>25</td>
<td>224.4 ± 4.3</td>
<td>NS</td>
</tr>
<tr>
<td>L(+)-lactic acid</td>
<td>25</td>
<td>227.1 ± 1.9</td>
<td>NS</td>
</tr>
<tr>
<td>Pyruvic acid</td>
<td>25</td>
<td>220.1 ± 6.9</td>
<td>NS</td>
</tr>
</tbody>
</table>

Data are means ± SE of 4–9 separate uptake determinations. Initial rate of [3H]niacin (6 nM) uptake by Caco-2 cells was measured with or without the compound under investigation for 3 min at pH 5.0.

Cellular Regulation of Niacin Uptake by Caco-2 Cells

We examined the possible cellular regulation of the niacin uptake process into Caco-2 cells by specific intracellular regulatory pathways. Pathways shown to be involved in the regulation of uptake of other water-soluble vitamins and other nutrients in intestinal and other cellular systems, i.e., protein kinase A (PKA)-, PTK-, protein kinase C (PKC)-, and Ca2+-calmodulin-mediated pathways, (13, 15, 16), were chosen. These studies were performed by pretreating (for 1 h) the cells with specific modulators of the individual pathway, followed by determination of the initial rate of niacin (6 nM) uptake. The results were compared with simultaneously performed control experiments.

The possible involvement of a PTK-mediated pathway was assessed by examining the effect of pretreatment with genistein and tyrphostin A25 on the initial rate of niacin uptake. The results (Table 3) show that both of the inhibitors caused significant inhibition of niacin uptake; the negative control experiments with genistin and tyrphostin A1, however, were without effect. Next, we determined the effect of genistein on the kinetic characteristics of the niacin transport in Caco-2 cells. For this purpose, we measured the initial rate of niacin uptake as a function of concentration in genistein (50 µM)-pretreated cells and compared the results with that of untreated control cells. Our results show (Fig. 5) a significant (P < 0.01) decrease in the Vmax of niacin uptake in genistein-pretreated cells (12.88 ± 0.14 and 7.27 ± 0.25 pmol·mg protein−1·3 min−1 for control and genistein-pretreated cells, respectively), whereas the apparent Km was found to increase slightly with genistein pretreatment (0.52 ± 0.02 µM and 0.67 ± 0.06 µM for control and genistein-pretreated cells, respectively).

We also examined potential role of the Ca2+-calmodulin-mediated pathway by examining the effects of pretreatment of cells with inhibitors of the pathway (trifluoperazine, calmidazolium, and KN-62; all at 50 µM concentration) on the initial rate of niacin uptake. None of the tested inhibitors were found to affect niacin uptake (227.8 ± 4.0, 230.4 ± 5.8, 226.8 ± 7.9, and 228.8 ± 8.8 fmol·mg of protein−1·3 min−1 for control, trifluoperazine, calmidazolium, and KN-62, respectively).

The potential role of PKC-mediated pathway in regulating niacin uptake by Caco-2 cells was also examined by testing the effect of pretreating the cells with the PKC activator phorbol 12-myristate 13-acetate (PMA; 10 µM) or the PKC inhibitors staurosporine and chelerythrine (both 1 µM) on the initial rate of niacin uptake. None of the modulators led to a significant effect on niacin uptake (228.0 ± 5.5, 222.8 ± 7.3, 227.7 ± 9.7, and 221.6 ± 5.3 fmol·mg of protein−1·3 min−1 for control, PMA, staurosporine, and chelerythrine, respectively).

We also examined the potential involvement of a cAMP- or PKA-mediated pathway in the regulation of niacin uptake by Caco-2 cells. Pretreatment of cells with a compound known to increase intracellular cAMP level, i.e., dibutyryl cAMP (1 mM) or forskolin (100 µM), as well as an inhibitor of PKA, i.e., H-89 (50 µM), caused no significant effect on niacin uptake (230.8 ± 10.4, 238.2 ± 10.8, 221.0 ± 2.8, and 231.0 ± 9.6 fmol·mg of protein−1·3 min−1 for control, dibutyryl cAMP, H-89, and forskolin, respectively).

DISCUSSION

The aim of the present investigations was to delineate the mechanism and regulation of niacin uptake in the human intestine. Two model systems were used: human-derived intestinal epithelial Caco-2 cells and purified native human...
BBMVs isolated from the jejunum of organ donors. The results clearly established the existence of an acidic pH-dependent, carrier-mediated mechanism that operates in the physiological range of niacin concentrations.

The studies with confluent monolayers of Caco-2 cells showed niacin uptake to be both temperature and energy dependent and to occur with minor metabolic alterations in the transported substrate. Also, the niacin uptake process was Na⁺ independent as indicated by the lack of effect of Na⁺ replacement with other monovalent cations on the initial rate of niacin uptake, as well as by the inability the Na⁺-K⁺-ATPase inhibitor ouabain to affect the substrate uptake. Niacin uptake by Caco-2 cells, however, was found to be highly dependent on acidic incubation buffer pH. Decreasing the incubation buffer pH from 8.0 to 5.0 led to a marked (~5 fold) increase in niacin uptake. This finding suggests the possible involvement of the niacin-H⁺ cotransport system, which is supported by the finding of significant inhibition in niacin uptake after pretreatment of Caco-2 cells with the protonophore FCCP (see also below).

Uptake of niacin as a function of concentration showed saturation when tested in the micromolar range but not in the nanomolar range. This finding suggests the involvement of a carrier-mediated system for substrate uptake that operates in the former concentration range. The apparent $K_m$ of the saturable process was calculated to be 0.53 μM, suggesting that this system is most likely responsible for the absorption of dietary niacin (estimated to be in the micromolar range; Refs. 6, 19). The existence of a carrier-mediated system for [3H]niacin uptake in Caco-2 cells was further confirmed by the findings of significant cis inhibition and trans stimulation by unlabeled niacin. The relevance of these findings to the native human intestine was also established by demonstrating significant inhibition in [3H]niacin uptake by unlabeled niacin in purified BBMVs isolated from the jejunum of human organ donors. Our studies with intestinal BBMVs have also shown the niacin uptake process to be similarly dependent on very acidic pH. The inability of previous studies to identify the existence of a high-affinity niacin uptake system is most probably due to the use of high working niacin concentrations (18, 20). Previous studies have reported an apparent $K_m$ at 3.52 and 17.0 mM (4, 14, 18, 20), which is rather high, considering that the luminal concentration of niacin was in the micromolar range. It is possible that the latter system (which is not specific for niacin but appears to be shared by other monocarboxylates; Refs. 18 and 20) is responsible for the uptake of pharmacological concentrations of niacin, which are used clinically for the prevention of atherosclerotic cardiovascular disease (10, 11).

The niacin uptake system of Caco-2 cells appears to be highly specific in nature. This conclusion is based on the observations that, with the exception of unlabeled niacin, none of the niacin structural analogs tested (i.e., isonicotinic acid, nicotinuric acid, nicotinamide, nicotinyl alcohol, and isonicotinic acid hydrazide) had an effect on substrate uptake. Also, the related compound 5-methyl-1H-pyrazole-3-carboxylic acid, which represents a high-affinity ligand for the recently described niacin receptor HM74A (22), failed to affect niacin uptake by Caco-2 cells, suggesting that the intestinal Caco-2 uptake process does not involve this receptor.

An interesting observation in this study was the sensitivity of the niacin uptake system of Caco-2 cells to the inhibitory effect of the sulfhydryl group reagent p-CMBS. This inhibition was significantly reversed by treating the cells with the reducing agent dithiothreitol. Because p-CMBS can hardly penetrate the plasma membrane, the possibility exists that the affected sulfhydryl groups are located at the exofacial surface of the cell membrane. None of the other tested membrane transport inhibitors (SITS, probenecid, and amiloride) had an effect on niacin uptake.

Previous studies have suggested that niacin could be transported by the MCT system (7). Our findings in the present study argue against this concept. First, the affinity of most MCT systems is low (reportedly in the millimolar range), while the niacin uptake system described in this study functions in the micromolar range. Second, none of the model (prototype) ligands for these transporters, i.e., lactate, pyruvate, butyrate, and propionate, affected the initial rate of uptake of physiological concentrations of niacin by Caco-2 cells. Third, classic inhibitors of the MCT system, i.e., α-cyano-4-hydroxycinnamate and phloretin (7), also failed to affect carrier-mediated niacin uptake by Caco-2 cells.

To further study the cellular uptake of niacin, we investigated the possible involvement of specific intracellular regulatory pathways in the regulation of niacin uptake by Caco-2 cells. We focused on pathways that have been shown to be involved in the regulation of uptake of other nutrients in intestinal and other epithelial systems (13, 15, 16). Our results show that while no role for PKA-, PKC- and Ca²⁺/calmodulin-mediated pathways is evident, a role for the PTK-mediated pathway is suggested. Inhibitors of this pathway (tyrphostin A25 and genistein, but not their negative controls tyrphostin A1 and genistin, respectively), were found to cause significant inhibition of niacin uptake. The inhibitory effect of one such inhibitor, genistin, was further tested and found to be mediated mainly via a decrease in the $V_{max}$ of the niacin uptake process, suggesting a decrease in the activity (and/or number) of the involved carriers. Further studies are required to determine the exact nature of the inhibitory mechanism involved.

In summary, the results of the present study demonstrate for the first time the existence of a highly specialized, acidic pH-dependent, high-affinity, carrier-mediated system for niacin uptake at the apical membrane of human intestinal epithelial cells. In addition, the results also suggest possible involvement of a PTK-mediated pathway in the regulation of niacin uptake by these cells.

**GRANTS**

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-56061 and DK-58057.

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