Pharmaceutical Research, Vol. 7, No. 12, 1990

Report

Mechanism of L-α-Methyldopa Transport Through a Monolayer of Polarized Human Intestinal Epithelial Cells (Caco-2)

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Received February 6, 1990; accepted June 13, 1990

The Caco-2 model system (Hidalgo *et al., Gastroenterology,* 96:736–749, 1989), which is a monolayer of polarized intestinal epithelial cells grown onto a porous polycarbonate membrane, was used to study the mechanism of transcellular transport of an antihypertensive agent, L-α-methyldopa (L-α-MD). The results showed that the transport of L-α-MD was pH, glucose, concentration, and temperature dependent, and it could be inhibited by metabolic inhibitors (e.g., 2,4-dinitrophenol) and by amino acids (e.g., L-phenylalanine) which have an affinity for the large neutral amino acid (LNAA) carrier. In addition, the apparent kinetic constants describing the transcellular transport of L-α-MD were altered depending on the time interval between feeding the cells and the transport experiments (postfeeding time, PFT). The apparent maximum carrier flux (J_{max}) of L-α-MD was significantly increased (from 155 to 547 pmol/mg protein/min) when PFT was prolonged from 8.5 to 56 hr. These results indicated that the transcellular transport of L-α-MD through the polarized Caco-2 cell monolayer was carrier mediated via the LNAA carrier. The similarities in the characteristics of L-α-MD transport exhibited by the Caco-2 model system and other intestinal models *in vitro* further substantiate the usefulness of this cell culture model for studying the intestinal transport of nutrients and drugs.

KEY WORDS: Caco-2; cell monolayers; intestinal; transport mechanism; L-α-methyldopa; amino acid carrier.

INTRODUCTION

L-α-Methyldopa (L-α-MD; Aldomet), an aromatic amino acid, is a commonly prescribed antihypertensive drug (1). After being absorbed orally, L-α-MD can produce its antihypertensive effects only after it has been transported across the blood–brain barrier and converted to its active metabolites (2–4). The transport of L-α-MD across the blood–brain barrier was shown to be mediated by the large neutral amino acid (LNAA) carrier (2).

The systemic availability of orally administered L-α-MD is poor (approximately 25%) and variable (a factor of three) (5–7). The poor bioavailability is not due to the intraluminal degradation since it has been shown that unabsorbed L-α-MD is recovered intact in the faeces (8,9). In order to elucidate the reasons for the problematic bioavailability, various investigators (10–12) have studied the mechanism of L-α-MD transport. The conclusion derived from these studies was that the transport (uptake) of L-α-MD across the intestinal mucosa is mediated by the LNAA carrier. Furthermore, the rate of transport of L-α-MD was shown to be slower than that of natural substrates (e.g., L-phenylalanine; L-Phe). The slower rate of transport (low membrane permeability) of L-α-MD is probably due to the presence of the α-methyl group, which is known to hinder the transport of amino acids (e.g., α-aminoisobutyric acid) (13). Studies have also shown that L-α-MD undergoes significant first-pass metabolism, with as much as 50% of the absorbed drug being metabolized (6,7); the major metabolic pathway is sulfate conjugation catalyzed by phenol sulfotransferase (14). Based on the results of these studies, the poor bioavailability of L-α-MD can be attributed to both low membrane permeability and first-pass metabolism. The highly variable bioavailability of L-α-MD is probably due to the presence of varying amounts of amino acids in the diet which can compete with L-α-MD for the LNAA carrier.

The information described above concerning the mechanism of L-α-MD transport through the intestinal mucosa has been obtained using several intestinal model systems including (a) whole-animal systemic absorption studies (5–9), (b) intestinal perfusion studies (11), and (c) everted intestinal ring studies (10). However, each of these models has inherent problems. For example, a major problem with intestinal perfusion and whole-animal experiments is the high degree of variability. The everted intestinal ring preparation has limited viability and does not take into account the polarity of the intestinal mucosa.

The development of the Caco-2 model system (15),

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which is derived from a human colonic carcinoma cell line (Caco-2), has made it possible to study the mechanism of transcellular transport at the microscopic (cellular) level in a highly viable, polarized intestinal cell line. The Caco-2 model system has been used previously to study the transcellular transport of nutrients (16–19), including large neutral amino acids (16). Studies by Hidalgo and Borchardt (16) have shown that Caco-2 cells have a LNAA carrier system capable of mediating the transcellular transport of amino acids like L-Phe. Therefore, the purpose of this study was to delineate the transport characteristics of L-α-MD in the Caco-2 model system and to determine whether these transport characteristics are consistent with those observed with other intestinal model systems.

MATERIALS AND METHODS

Materials

[^{14}C]-L-α-MD (7.5 mCi/mmol) and L-α-MD were gifts from Dr. Joseph A. Fix of INTERx-Merck Sharp and Dohme Research Laboratories (Lawrence, KS). [^{14}C]-Mannitol (55 mCi/mmol) was purchased from American Radiolabelled Chemicals, Inc. (St. Louis, MO). Cell culture media and reagents for culturing Caco-2 cells were the same as those reported previously (15). Dulbecco's phosphate buffer solution (D-PBS; powder form), Hank's balanced salt solution (HBSS; powder form), D-(−)-arabinose, *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid (HEPES), 2-[*N*-morpholino]-ethanesulfonic acid (MES), L-aspartic acid (L-Asp), L-lysine (L-Lys), L-Phe, L-dopa, L-proline (L-Pro), sodium bicarbonate, sodium azide (SA), 2-deoxyglucose (DG), and 2,4-dinitrophenol (2,4-DNP) were purchased from Sigma Chemical Co. (St. Louis, MO). Other chemicals were used as received.

Preparation of Experimental Media

The incubation solutions for transport experiments were prepared as described below. For solutions containing no glucose, D-PBS solutions were prepared from D-PBS powder according to the manufacturer's suggestion and supplemented with 25 m*M* arabinose, 0.9 m*M* calcium chloride, 5 m*M* sodium bicarbonate, and 11 m*M* Mes for pH 5.5 and pH 6.5 solutions or 11 m*M* Hepes for pH 7.4 solution. For solutions containing glucose, HBSS solutions were prepared from HBSS powder according to the manufacturer's suggestion and supplemented with 25 m*M* glucose, 11 m*M* Hepes, and 5 m*M* sodium bicarbonate. The pHs of various solutions were adjusted by using either HCl or NaOH. The osmolarity of the incubation media was approximately 330 mOsm/kg.

Cell Culturing Conditions and Quality Control

Caco-2 cells were plated and grown according to previously published procedures (15). All cells used in this study were between passage 69 and passage 79. The monolayers used in this study were 19–22 days postseeding or 13–16 days postconfluence. The quality of the monolayer was controlled by checking the paracellular transport of [^{14}C]mannitol and transepithelial electrical resistance (TEER). The normal TEER values typically range from 350 to 500 Ω-cm^2 and [^{14}C]mannitol leakage is normally less than 1% per hr per well.

Protocol for Transport Studies

A standard transport experiment was performed as follows: 1.5 ml of test solution (37°C) containing the compound of interest in an appropriate buffer was loaded onto the apical (AP) side (donor side) of a cell insert (Transwell, CoStar, Cambridge, MA). The insert was then partially immersed into a well containing 2.5 ml of an appropriate buffer (release medium) at 37°C. The insert was then moved according to a time course (typically 5, 10, 15, 25, 40, 60 min) from one well to the other in a six-well cell culture cluster (CoStar, Cambridge, MA). The total radioactivity appearing on the basolateral (BL) side (receiver side) was then determined by using a liquid scintillation counter (LSC-5801, Beckman, Palo Alto, CA). At the end of each experiment, the cell monolayer was washed at least twice with excess ice-cold HBSS and treated with 1.5 ml of 1% (w/v) Triton X-100 in 0.3 *N* NaOH. After incubation overnight at ambient temperature, the cells were scraped gently from the polycarbonate membrane and homogenized by incubating the suspension at 37°C for 30 min. A portion of the homogenate (50 μl) was used for the determination of protein by Bradford's method (20) using bovine serum albumin (Sigma Chem. Co.) as a standard.

Experiments which investigated the effects of pH, glucose, and inhibitors were performed in basically the same way as those described above except that the compositions of the incubation media were adjusted according to the experimental needs (described in either the text or the legends to the figures).

L-α-MD Stability Test

The chemical stability of the compound was tested both in blank media, in media preincubated for 1 hr with cell monolayers, and in media containing Caco-2 cell homogenates. The cell homogenates were made according to standard procedures from 22-day-old Caco-2 cells after the cells were detached and scraped from the polycarbonate membrane. The test was performed by spiking L-α-MD in these media and following the disappearance of L-α-MD by HPLC after the samples were treated to remove protein, if necessary.

HPLC Analysis of L-α-MD

The HPLC system used to detect L-α-MD was as follows: column, cation exchange (SCX, 10 × 250 mm, Whatman, Maidstone, England); solvent system, 90% ammonium phosphate-phosphoric acid buffer (pH 2.5, 0.01 *M*) and 10% acetonitrile; flow rate, 1.5 ml/min; and detection wavelength, 268 nm. The retention time was 5.8 min for L-α-MD and the sensitivity of detection was 100 ng/ml.

Data Analysis

The steady-state transcellular fluxes (pmol/mg protein/min) of L-α-MD were calculated from the linear portion of "time" versus "amount transported" curves by linear re-

gression. The steady-state flux is defined as the flux which is independent of time change. After subtracting the flux at 4°C from the flux at 37°C, the steady-state carrier-mediated flux (37–4°C flux) at a particular substrate concentration was obtained. These fluxes were then used in the determination of K_m and J_{max} values by using nonlinear regression.

Apparent permeabilities were calculated using the area of the cell insert (Transwell) membrane, 4.71 cm^2, as the surface area of the transcellular flux. The area of the insert membrane was used in the calculations of permeabilities since the protein concentrations were shown to be relatively constant from insert to insert (<15% intrabatch and <10% interbatch differences).

RESULTS

Various experiments were designed to demonstrate that the transport of L-α-MD across the Caco-2 cell monolayers is carrier mediated. These experiments investigated the effects of time, concentration, pH, temperature, and competitive species on L-α-MD transport. To ensure that these experiments would lead to reliable and reproducible results, experimental conditions (e.g., composition of the experimental media, postfeeding time, etc.) were first optimized. Presented below are the results of these studies.

Selection of Experimental Media

Both the pH of the media and the presence and absence of glucose have been shown to affect the transport of amino acids (13,21–23). Therefore, the optimization of these conditions was considered to be critical to obtaining data useful for analyzing the transcellular transport of L-α-MD. The selection of experimental media was further complicated by the fact that L-α-MD is more stable in a slightly acidic environment (24). To investigate the effects of pH and glucose on L-α-MD transport, a series of preliminary experiments was performed. The results from these studies are summarized in Table I. The results indicated that the transcellular transport of L-α-MD was affected by the pH of the media, the pH gradient between the AP and the BL sides, and the presence and absence of glucose and a glucose gradient.

Table I. Effects of pH and Glucose on Transcellular Flux of L-α-MD Across Caco-2 Cell Monolayers

Apical conditions	Basolateral conditions	Flux ± SD (pmol/mg protein/min)[a]
pH 5.5 (no glu)[b]	pH 7.4 (glu)[c]	25 ± 0.4
pH 6.5 (glu)	pH 7.4 (glu)	13 ± 1
pH 6.5 (glu)	pH 7.4 (no glu)	12 ± 0.7
pH 6.5 (no glu)	pH 7.4 (glu)	19 ± 0.7
pH 6.5 (no glu)	pH 7.4 (no glu)	20 ± 0.1
pH 7.4 (no glu)	pH 7.4 (glu)	19 ± 0.2
pH 7.4 (no glu)	pH 5.5 (glu)	18 ± 0.2

[a] Transcellular flux of L-α-MD at a concentration of 0.1 mM and a PFT of 24 hr.

[b] No glu—glucose absent; 25 mM D-(−)-arabinose was used to compensate for the osmotic pressure difference.

[c] Glu—glucose present; concentration = 25 mM.

Based on the results shown in Table I and considering the chemical stability of L-α-MD, the following conditions were selected for all subsequent experiments (except pH effect): AP, pH 6.5; BL, pH 7.4; glucose absent from the AP side; and glucose present on the BL side. Using an HPLC assay, L-α-MD was also shown to be chemically stable (>95%) and biologically stable (>91%) for the time periods used (< or =60 min) under the selected conditions (data not shown). These conditions were also selected in part to mimic the *in vivo* conditions, where the AP side is slightly acidic and glucose is always present on the BL side in the small intestine.

Time-Dependent Transcellular Transport

The results shown in Fig. 1 indicate that the transcellular transport of L-α-MD increases linearly with time after an initial lag period (e.g., 4 min for 37°C) for all the concentrations studied (0.01–10 mM). Such relationships were observed for transport studies performed at both 4 and 37°C, however, there was no lag time observed for transport of L-α-MD at 4°C. The steady-state fluxes were determined from the linear portion (e.g., 15 to 60 min) of the curves by linear regression.

Temperature-Dependent Transcellular Transport

The transcellular transport of L-α-MD was shown to be temperature dependent (Figs. 1 and 2). Furthermore, the temperature dependence of L-α-MD was more significant at 0.01 mM than at 2.5 mM (Fig. 2), indicating that the transport was mostly an energy-dependent process at lower concentrations (<K_m). The less significant effect of temperature on the transcellular transport of L-α-MD at 2.5 mM indicates

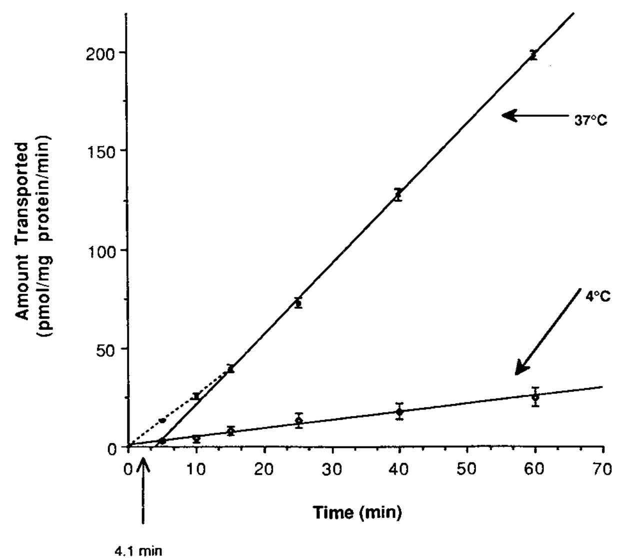

Fig. 1. Time course of L-α-MD transport at 37 and 4°C in Caco-2 cell monolayers. In this set of experiments, the concentration of L-α-MD was 0.01 mM, each experiment was performed on a different Caco-2 cell monolayer, and each point in the figure is the average of four experiments at a specific time point. The experimental medium was pH 6.5 without glucose on the AP side and pH 7.4 with glucose on the BL side. The PFT was approximately 24 hr. The error bars represent the standard deviation of the mean.

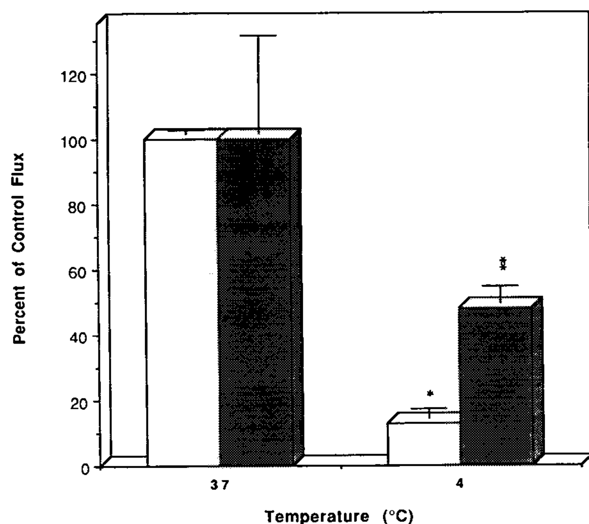

Fig. 2. Effect of temperature on transcellular fluxes of L-α-MD in Caco-2 cell monolayers. The temperature effects were investigated at L-α-MD concentrations of 0.01 mM (open bar) and 2.5 mM (filled bar). Conditions of experimental media for L-α-MD were the same as those in Fig. 1 except that both the release media and the test solutions were cooled to 4°C before the experiments at 4°C were initiated. Each bar was the average of three or four determinations. The asterisks (*, **) indicate that the differences were statistically significant ($P < 0.05$, Student's t test). The PFT was approximately 24 hr. The error bars represent the standard deviation of the mean.

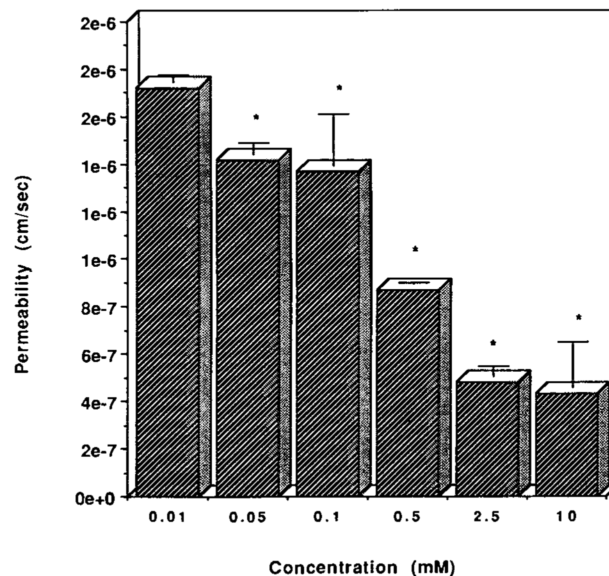

Fig. 3. Concentration-dependent permeabilities of L-α-MD at 37°C in Caco-2 cell monolayers. Each data point is the average of three determinations. Conditions of experimental media are the same as those in Fig. 1. The asterisks (*) indicate that the differences were statistically significant ($P < 0.05$) according to a one-way ANOVA test. The PFT was approximately 24 hr. The error bars represent the standard deviation of the mean.

that a nonsaturable, energy-independent process becomes more important at higher concentrations ($>K_m$) of the solute.

Concentration-Dependent Permeabilities

Generally, concentration-dependent permeability is a valuable indication that the transport is not via passive diffusion. The results shown in Fig. 3 indicate that the permeabilities of L-α-MD were concentration dependent. The highest permeability of L-α-MD was observed with a concentration of 0.01 mM ($P < 0.05$). The permeability of L-α-MD at 0.1 mM was higher than that at 0.5 mM ($P < 0.05$), and the permeability at 0.5 mM was higher than that at 2.5 mM ($P < 0.05$).

Inhibition of L-α-MD Transport

The effects of neutral, acidic, basic, and imino amino acids on the transport of L-α-MD were studied (Table II). The results indicated that both the LNAA L-Phe and the basic amino acid (BAA) L-Lys significantly inhibited the transcellular transport of L-α-MD. Since BAAs can also use the LNAA carrier at neutral pH (21), the results with L-Lys were not unexpected. The imino amino acids, L-Pro and an acidic amino acid, L-Asp, had no inhibitory effects on the transport of L-α-MD (Table II).

To mimic physiological conditions where a mixture of amino acids in the concentration range of 0.1 to 10 mM is commonly present in the small intestine after a normal meal, the effects of a mixture of amino acids on the transcellular transport of L-α-MD were also determined. The results indicated that the amino acid mixture significantly inhibited the transport of L-α-MD (Table II).

The effects of two metabolic inhibitors on the transcellular transport of L-α-MD were also determined. Both 2,4-dinitrophenol and sodium azide (+2-deoxy-glucose) were

Table II. Effects of Inhibitors on Transcellular Fluxes of L-α-MD (0.01 mM) Across Caco-2 Cell Monolayers

Inhibitor	Concentration (mM)	Control flux[a] ± SD (%)
—	—	100 ± 11
L-α-MD	10	28 ± 8*
L-Phe	1	80 ± 4*
L-Phe	25	46 ± 7*
L-Lys	10	64 ± 2*
L-Pro	10	120 ± 14
L-Asp	10	110 ± 12
Amino acid mixture[b]	2	60 ± 4*
2,4-DNP[c]	1	65 ± 7*
SA + DG[c]	1 + 50	51 ± 12*

[a] The control flux was 3.06 ± 0.34 pmol/mg protein/min when the concentration of L-α-MD was 0.01 mM. The conditions of experimental media were pH 6.5 without glucose at the AP side and pH 7.4 with glucose (25 mM) at the BL side. The metabolic inhibitors were presented at both the AP and the BL sides, while other inhibitors were presented only at the AP side.

[b] The mixture contains 2 mM concentrations of L-Phe, L-Leu, L-Lys, L-Pro, L-Asp, and L-dopa.

[c] Cell monolayers were preincubated with the metabolic inhibitors (same concentrations) for 30 min prior to actual experiments.

* Significant inhibition ($P < 0.05$).

shown to significantly inhibit the transcellular transport of L-α-MD (Table II).

pH-Dependent Fluxes

Transport of L-α-MD was studied at different AP pHs to determine whether the effect of pH could be explained by a passive diffusion process. Experiments were conducted where the BL pH was maintained at 7.4 and the AP pH was 5.5, 6.5, or 7.4. The results showed that the transcellular flux of L-α-MD was slightly pH-dependent (Fig. 4). For example, the transcellular flux of of L-α-MD was approximately 20 to 25% higher at pH 5.5 than at pH 6.5 or pH 7.4 ($P < 0.01$).

Effect of Postfeeding Time

A previous study has shown that the uptake of L-α-MD was significantly increased in fasted animals (10). The present study was designed to determine whether the transport was also affected by the increase in postfeeding time (PFT), which is the time interval between the last feeding and the actual experiment. The results indicated that the transport of L-α-MD (0.01 m*M*) was increased about 7.5 times when the PFT was increased from 5 to 54 hr (results not shown). To investigate further the effects of PFT, experiments were designed to derive the "37–4°C fluxes" versus "concentration" curves at different PFTs. These curves were then fitted by nonlinear regression for the determination of K_m and J_{max} values, which are listed in Table III. The results indicated that the change of PFT from 8.5 to 56 hr significantly increased the J_{max} from 155 to 547 pmol/mg protein/min (3.5 times). The same PFT change also caused a decrease in K_m from 1.97 to 0.96 m*M* (50%). At lower concentrations ($<K_m$), the transcellular flux of L-α-MD is determined by the ratio of J_{max}/K_m. Therefore, it is obvious that the effects of PFT on L-α-MD transport (0.01 m*M*) observed in the preliminary studies mentioned above were due to the change in this ratio (79 to 569, 7.2 times) (Table III).

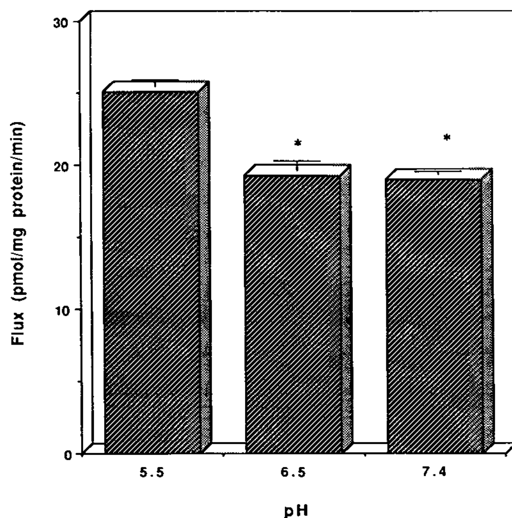

Fig. 4. Effect of the AP pH on transcellular fluxes of L-α-MD in Caco-2 cell monolayers. The concentration of L-α-MD was 0.01 m*M*. Glucose was present on the BL side but not on the AP side. The pH of the BL side was 7.4. Each bar was the average of four experiments. The asterisks (*) indicate that the differences were statistically significant ($P < 0.05$). The PFT was approximately 24 hr. The error bars represent the standard deviation of the mean.

Table III. Effect of Postfeeding Time (PFT) on the Kinetics of L-α-MD Transport Across the Caco-2 Cell Monolayers

PFT (hr)	$K_m \pm$ SED[a] (m*M*)	$J_{max} \pm$ SED[a] (pmol/mg protein/min)	Ratio (J_{max}/K_m)
8.5	1.97 ± 0.31	155.4 ± 12.2	79
26	0.70 ± 0.06	156.7 ± 5.2	224
56	0.96 ± 0.07	546.6 ± 15.3	569

[a] Standard error of determination as reported by nonlinear regression program. Regression analysis performed at all three PFTs showed r^2 values of 0.95 or larger.

DISCUSSION

Absorption of L-α-MD has been studied extensively (5–12) and its mechanism of absorption has been described as a carrier-mediated process which utilizes the intestinal LNAA carrier (10–12). However, this conclusion was based on studies with intestinal rings (10), which measure uptake of L-α-MD, and intestinal perfusion studies (11,25), which measure the disappearance of L-α-MD from the intestinal lumen. The actual process of transcellular transport of L-α-MD, e.g., flux of solute from the apical to the basolateral side of a monolayer, has not been investigated. The development of the Caco-2 model system (15) has now provided the opportunity to study the transcellular transport of L-α-MD in a polarized intestinal epithelial model.

To characterize the transcellular transport of L-α-MD, steady-state fluxes across the cell monolayer were determined. The "time" versus "amount transported" curves for L-α-MD were shown to be linear after a brief lag time (Fig. 1), which made the determination of steady-state fluxes easier than in the *in vitro* intestinal ring uptake model, where the "time" versus "amount transported" curves were only initially linear (2 min) (10).

Having determined the fluxes of L-α-MD at different concentrations at 37 and 4°C, the fluxes mediated by the LNAA carrier (i.e., "37–4°C fluxes") were plotted versus L-α-MD concentration and fitted using nonlinear regression, yielding the kinetic parameters for the carrier-mediated process. The J_{max} value for L-α-MD (157 pmol/mg protein/min) was estimated to be three times less than that for L-Phe (498 pmol/mg protein/min) (unpublished results), indicating that the carrier capacity for L-α-MD transport is much smaller than that for the natural LNAAs. In contrast, the K_m value for L-α-MD (0.70 m*M*) is only slightly higher than the K_m value observed for L-Phe (0.34 m*M*; unpublished results). However, these K_m values for L-α-MD and L-Phe are an order of magnitude smaller than those reported using other *in vitro* methods (10,25–27). It is likely that many factors contributed to the differences in the K_m values. Aside from the differences in the species and methodologies, another possible explanation is that the K_m values determined here are lump-sum kinetic constants describing the entire transcellular transport process, including both apical uptake and basolateral efflux. The K_m values determined in other *in*

vitro models reflect primarily the cellular uptake process (10,25–27).

A potential problem with the present method of estimating the kinetic parameters is the lack of proper stirring in the cell insert. Thus, the K_m values could be considerably biased by the existence of a significant aqueous boundary layer (28). The aqueous boundary layer will increase the K_m value for substrates whose transport is diffusion controlled (28). However, it is unlikely that this aqueous boundary layer will have a significant effect on L-α-MD permeation, since it is largely membrane controlled (11,25; present study). Nevertheless, it is still important to reduce the thickness of the aqueous boundary layer in the transport study to minimize the effect of the aqueous bias. Therefore, a novel diffusion apparatus with adequate stirring for the Caco-2 model system has recently been developed in our laboratory (29).

The data shown in Fig. 4 as well as the results reported by Amidon *et al.* (11) illustrate that the transport of L-α-MD is pH dependent, e.g., transport was higher when the pH of the media was less than pH 6.3 (L-α-MD's pI = pH 6.29). These results indicate that transport of L-α-MD cannot be explained by the pH-partition theory since the theory would predict that the transport of L-α-MD, which is a zwitterion and is always charged, should be the same at all three pHs. Therefore, the results suggest that the transport of L-α-MD is not due solely to passive diffusion.

The concentration- and pH-dependent transport of L-α-MD indicates that the transcellular transport of L-α-MD is a carrier-mediated process. To establish further the characteristics of the LNAA carrier in the Caco-2 cell monolayers, experiments were performed to determine the energy dependence of the transport process and the specificity of the carrier for L-α-MD. By studying the transport of L-α-MD at different temperatures, the temperature dependence of this transcellular transport process was established (Figs. 1 and 2). Furthermore, the temperature dependence was more significant at lower concentrations ($<K_m$), where the carrier-mediated process dominated the overall transport process. This result suggests that energy is required for the transport process. To substantiate this hypothesis further, the effects of two metabolic inhibitors on the transcellular transport of L-α-MD were determined. The results showed that the transport of L-α-MD was decreased by 35 and 49% when 2,4-dinitrophenol and sodium azide plus 2-deoxyglucose, respectively, were included in the incubation media (Table II). The effects of both temperature and metabolic inhibitors are similar to the results reported previously by Osiecka *et al.* (10) using a rat intestinal ring preparation.

To determine the structural specificity of the carrier responsible for L-α-MD transport, the effects of neutral, basic, acidic, and imino amino acids on the transport of L-α-MD were determined. The results indicate that the transport of L-α-MD was inhibited by the LNAA L-Phe and the BAA L-Lys, but not by the acidic amino acid L-Asp and the imino amino acid L-Pro. The inhibitory effects of L-Phe on L-α-MD transport in the Caco-2 model are similar to those observed in the intestinal ring preparation (10).

One of the main reasons to characterize the transport of L-α-MD was to determine why the oral bioavailability of L-α-MD is poor and variable. The results reported here indicate that transcellular transport of L-α-MD by the LNAA carrier is significantly slower than that of L-Phe at low concentrations ($<K_m$). This is probably caused by the lower affinity of L-α-MD for the carrier (higher K_m value) and its lower maximum flux. These observations are similar to those made by Osiecka *et al.* (10) and Hu *et al.* (25). To investigate the effects of diet on L-α-MD transport, a mixture of amino acids at physiological concentrations was used to inhibit L-α-MD transport across the Caco-2 monolayer. The results have shown that the amino acid mixture significantly inhibited the transcellular transport of L-α-MD (Table II). It is clear from these results that both the slow transport and the potentially high competition for the carrier by naturally occurring amino acids may contribute to the poor and variable bioavailability of L-α-MD.

Another possible explanation for the variability in the oral absorption of L-α-MD may be the nutritional state of the patient. This could be illustrated in the Caco-2 model system by changing the postfeeding time (PFT), which is the time interval between the last feeding and the actual transport experiment. Changing PFT has been shown to alter significantly the transcellular transport of L-α-MD, which included changes in both the K_m and the $J_{\max}$ values for L-α-MD (Table III). The results showed that the transcellular transport of L-α-MD was significantly increased when the PFT was prolonged, which would be a situation analogous to starvation. Similar results were observed by Osiecka *et al.* (10) using intestinal rings prepared from animals deprived of food for 48 hr.

In conclusion, the transcellular transport of L-α-MD was shown to be time, pH, concentration, glucose, and temperature dependent. Furthermore, the transport of L-α-MD was inhibited by neutral as well as basic amino acids and by metabolic inhibitors. These results illustrated that the transcellular transport of L-α-MD is via an active LNAA carrier in the Caco-2 model system. The results also indicated that the Caco-2 model system is useful for the study of drug transport *in vitro*.

ACKNOWLEDGMENTS

This work is supported by a grant from The Upjohn Company. M.H. would like to thank Dr. Ismael J. Hidalgo for his advice and consultation.

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