In Vitro Polarized Transport of L-Phenylalanine in Human Nasal Epithelium and Partial Characterization of the Amino Acid Transporters Involved

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Purpose. The purpose of this study was to provide functional and molecular evidence to support the existence of large neutral amino acid transporters in human nasal epithelium using nasal primary cell culture model.

Methods. L-Phenylalanine was used as a model substrate to characterize carrier-mediated permeation of amino acids across human nasal epithelium. The influence of temperature, concentration, other amino acids, metabolic/transport inhibitors, and polarity/stereoselectivity on transport of the model compound was investigated. Reverse transcriptase polymerase chain reaction was used for molecular characterization of the existence of the transporters.

Results. The transport of L-phenylalanine across the human nasal epithelium was polarized (apical → basolateral >> basolateral → apical), saturable (K_m = 1.23 mM; V_{max} = 805.1 nmol/mg protein/min) and stereo-selective (permeation of L-phenylalanine >> D-Phenylalanine). Its permeation was significantly (<0.05) reduced by cationic, small and large neutral amino acids, oubain, amiloride, sodium-free medium, and temperature lowering. Reverse transcriptase polymerase chain reaction revealed the presence of the broad-scope cationic-dependent amino acid transporter gene (y^+LAT-2) in the human nasal epithelium.

Conclusions. Based on the results of this study, one may postulate that the human nasal epithelium expresses L-amino acid transporters. More studies are necessary for detailed characterization of the transporters.

KEY WORDS: human nasal epithelium; nasal cell culture; active transport; cation-modulated broad-scope amino acid transporters; L-phenylalanine.

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INTRODUCTION

The evaluation of membrane–protein transporters, their transport characteristics and kinetic parameters, localization, and regulation is crucial to a better understanding of solute uptake process and in turn, the optimization of delivery of drugs that are substrates of these transporters (e.g. L-dopa, dopamine, amino acid prodrugs; Ref. 1). In addition to physicochemical properties of a drug molecule, physiologic features, including the expression of active transport systems, such as amino acid transporters, affect the nasal absorption of drugs and prodrugs (2,3).

Studies conducted in the early 1970s (4,5) on the nasal absorption of amino acids suggested the existence of active transport systems for amino acids in nasal mucosa of rodents. Using L-tyrosine, L-phenylalanine, and L-tryptophan, respectively, subsequent studies established compelling evidence to support the expression of amino acid transporters in nasal epithelium of rats (6–9). With the aid of *in situ* rat perfusion model, these active transport systems have been shown to be potential molecular targets for systemic delivery of drugs via the nasal route (2,3). A summary of literature data on nasal permeation of amino acids is presented in Table I.

The future prospects of systemic delivery of amino acid-linked drugs administered nasally in humans will depend on the expression and characteristics of amino acid transporters in human nasal epithelium. Although virtually nothing is known about human nasal amino acid transporters, the human bronchial epithelium amino acid transport system has been functionally characterized (10).

Amino acids cross the plasma membrane of cells via sodium-dependent and sodium-independent carriers (11). In the past few years, the use of new molecular and kinetic experimental approaches has unveiled a more complex picture, involving various clearly distinct transporters that show differences in structure, substrate specificity, mechanism, site of expression, and regulation (12). Current evidence suggests that heterodimeric amino acid transporters that consist of two different heavy chains (rBAT and 4F2hc/CD98) form a large family of antiporters that are involved in the intercellular and interorgan transfer of amino acids. Furthermore, seven different light chain cDNAs that exist in a heterodimeric form with the heavy chains have been characterized. They include LAT-1 and LAT-2, encoding two isoforms corresponding to system L; y^+LAT-1 and y^+LAT-2, encoding two isoforms of system y^+L; xCT, encoding a glutamate/cystine antiporter; asc, encoding system asc and $b^{0,+}$AT, encoding the light chain of system $b^{0,+}$ (13).

The aim of this study was to provide functional and molecular evidence to support the existence of large neutral amino acid transporters in human nasal epithelium using human nasal primary cell culture system. Details of the morphologic, biochemical, and functional characteristics of the cell culture system used for this study can be found in previous publications (14–17). Essentially, the cells consisted of differentiated monolayer of pseudo-stratified columnar-shaped epithelium with ciliated and nonciliated microvillar cells.

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Table I. Summary of Literature Information on Nasal Absorption of Amino Acids

Amino acids investigated	Nasal absorption characteristics	Animal species	Year of publication/references
Glycine	Autoradiographic study on septum	Rabbits (*in vivo*)	1972 (4)
Arginine	Amino acids acummulated in cell cytoplasm Accumulation blocked by –SH blocking agents		
Arginine Glycine Serine Proline	Neutral amino acids (glycine and serine) were best absorbed Arginine and proline were less absorbed Molecular weight and isoelectric pH were the main determinants for absorption	Rabbits (*in vivo*)	1973 (5)
L-Tyrosine	Absorption was pH independent (4.0 and 7.4) Absorption occurred via an active and staurable process	Rats (*in situ*)	1985 (7)
L-Tyrosine L-Phenylalanine	Absorption occurred via an active and staurable process Absorption was inhibited by oubain	Rats (*in situ*)	1988 (6)
L- and D-Phenylalanine	Bioavailability = 96.3% for L-Phenylalanine and 35.2% for D-Phe Saturation of absorption occurred at high doses	Rats (*in vivo*)	1991 (8)
Dopamine	Direct nasal to brain delivery	Mice (*in vivo*)	2000 (18)
L-Dopa	Direct nasal to brain delivery Bioavailability = 90%	Rats (*in vivo*)	2000 (19)

MATERIALS AND METHODS

Chemicals

L-Phenylalanine, D-phenylalanine, L-leucine, L-tyrosine, L-alanine, and L-glycine were purchased from Fluka (Buchs, Germany). Oubain, amiloride, bovine serum albumin, pronase, GeneElute RNA purification kit, platinum TaqDNA polymerase, primer sequences for 4F2hc/CD98, y^+LAT-1, y^+LAT-2, Coomasie brilliant blue G-250, and DNase I were supplied by Sigma (St. Louis, MO, USA). DMEM-F12 1/1, Ultroser G, NU-serum, phosphate-buffered saline and Hanks' balanced salt, streptomycin/penicillin, physiologic saline, random primer, RNase guard, and superscript II were from Life Technologies (Paisley, UK). The gene ladder (100 bp GeneRuler) used was from MBI Fermentas (St.Leon-Rot, Germany).

Cell Culture

Ultrastructurally normal human nasal epithelial tissues obtained from patients were cultured as earlier described for derivatized collagen matrix on Cellagen membranes (14–17). The culture was maintained in DMEM F12 1/1 (supplemented with Ultroser G [2.0%], choleratoxin [10 ng/mL], streptomycin [100 μg/mL] and penicillin [100 IU/mL]) at air-liquid interface until used for transport studies (8–10 days postseeding) or for mRNA extraction (4 days postseeding).

Functional Studies

Transport Studies

L-Phenylalanine (large neutral amino acid) was used as a model substrate to characterize carrier-mediated transport of amino acids across the nasal epithelium. The influence of L-phenylalanine concentration (0.01–5 mM), temperature (4°C, 37°C), other amino acids (L-lysine, L-glycine, L-leucine, L-alanine, L-tyrosine), metabolic/transport inhibitors (sodium azide, oubain, amiloride, Na^+-free medium), and polarity/stereo-selectivity on the transport of L-phenylalanine were investigated.

Before the transport studies, the cells were rinsed twice and preincubated with transport medium (TM; Hanks' balanced salt supplemented with HEPES buffer [10 mM] and 25 mM glucose, pH 7.4) for 15 min at 37°C. For inhibition studies, an additional 15-min preincubation with the inhibitor was allowed. Except for oubain, which was applied to the basolateral side, all other test compounds were applied apically. For studies involving sodium-free transport medium, sodium chloride was completely replaced with teramethylammonium chloride and the pH of the solution was adjusted to 7.4 (20).

Table II. Primers Used for Polymerase Chain Reaction (17)

Gene	Oligonucleotide sequence	Nucleotide number	Product size (bp)
y^+LAT-1	Forward: 5′-CAGCACTGAGTATGAAGTGG-3′	295–314	186
	backward: 5′-TATATGAGCACACCCTTGGG-3′	461–480	
y^+LAT-2	Forward: 5′-ACCCACCTACCATCTTGTCC-3′	291–310	119
	backward: 5′-CATTCAGCAGGGAGATCTCC-3′	390–409	
4Fhc/CD98hc	Forward: 5′-GAATGAGTTAGAGCCCGAGA-3′	151–170	269
	backward: 5′-CGATTATGACCACGGCACCA-3′	400–419	

4Fhc/CD98hc, heavy chain of CD98 surface antigen; y^+LAT-1, system y^+L-amino acid transporter-1, y^+LAT-2, system y^+L-amino acid transporter-2.

Transport studies were initiated by adding 250 μL of test solutions to the donor compartment. At predetermined time points (0–60 min), 100-μL aliquots were sampled from the acceptor compartment (750 μL) and were replaced immediately with an equal volume of TM. All cell monolayers used were checked for epithelial integrity before and after the experiments by measuring transepithelial electrical resistance (TEER) and sodium fluorescein (1 mg/mL) transport during 1 h at the end of the experiment. The mean TEER values before and after experiment were 301.4 ± 77.6 and 437.0 ± 105 $\Omega.cm^2$ (n = 66), respectively. The amount of sodium fluorescein that permeated the cell monolayers during 1 h at the end of the experiment was ≤1.0%. The TEER values and fluorescein permeation confirmed the monolayer integrity of the cells used for this study and these results concurred with initial studies conducted to validate use of this cell culture model for drug delivery studies (14–16). At the end of the permeation studies, the protein content of the cells was estimated using the method of Bradford (21).

Apparent permeability coefficients [P_{app} (cm/s)] for L-phenylalanine transport was calculated using the following equation:

$$P_{app} = \frac{dQ}{dt} \times \frac{V}{AC_0}$$

Where, $(dQ)/(dt)$ (μg/s) = steady rate of appearance of L-phenylalanine to the basolateral side, C_0 (μg/mL) = initial concentration in the apical chamber, A (cm^2) = effective growth surface area of the insert (0.785), and V (mL) = volume of the acceptor compartment.

To estimate the kinetic parameters (K_m, V_{max}) for the active transport component, the data obtained were fitted to Michaelis–Menten equation after correcting for passive diffusion by subtracting the rate constant for passive permeation at 4°C using the equation below:

$$V = \frac{V_{max}[S]}{K_m + [S]} + K_d[S]$$

Where, K_m = Michaelis–Menten constant (μM), V_{max} = maximum L-phenylalanine transport rate (nmol/min/mg protein), [S] = L-phenylalanine concentration (μM), V = rate of apical to basolateral transport of L-phenylalanine (nmol/min/mg protein), and K_d = rate constant for L-phenylalanine passive diffusion (μM/min).

Unless otherwise stated, each experiment was carried out in triplicate using different cell monolayers and the data were expressed as mean ± SD. Bonferroni's one-way analysis of variance was used to estimate the effects of inhibitors and other amino acids on the L-phenylalanine transport relative to cells not treated with inhibitors. The level of significance was set at $p < 0.05$.

Sample Analysis

Sodium fluorescein was analyzed using a diode array spectrophotometer (HP 8452A, Hewlett Packard, Palo Alto, CA, USA) at a wavelength of 490 nm. L-Phenylalanine was assayed isocratically by high-performance liquid chromatography using Hypersil BDS C18 5 μm, 250 × 4.6 mm column (Thermo Hypersil, Cheshire, UK). The mobile phase used was a 10:90 (v/v) mixture of methanol and acetate buffer (0.05 M, pH 4.0). The flow rate and detection wavelength was set at 1.3 mL/min and 235 nm, respectively.

Molecular and Genetic Studies

Messenger RNA (mRNA) Isolation and Purification

mRNA was isolated and purified from the epithelial cells by hybrid capture using GeneElute direct mRNA miniprep kit according to the manufacturer's protocol. For the mRNA isolation, approximately 10^7 cells were lysed with mortar and pestle at 4°C. Additionally, the cells were homogenized by passing them through homogenization columns. Ribonuclease (RNase) that may exist in the cells was eliminated at this stage after a 10-min proteinase K digestion. Subsequently, NaCl was added, and polyadenylated RNA [Poly $(A)^+$ RNA] captured onto oligo (dT) polystyrene beads during a 10-min incubation. For further enrichment, RNA was released from the beads into fresh lysis solution and recaptured with the original beads. After washing three times in spin column, purified mRNA was eluted in 100 μL of 10 mM Tris-HCL, pH 7.4 buffer.

To reduce possible DNA contamination, the extracted mRNA (200 μL) was treated with amplification grade DNase I (20 μL) in a sample mix containing 10× reaction buffer (20 μL) according to the manufacturer's protocol. Thereafter, the samples were quantitatively assessed using Gene/Quant Pro RNA/DNA calculator (Amersham Pharmacia Biotech, Roosendaal, The Netherlands) prior to storage at –70°C.

Codon DNA (cDNA) Synthesis and Reverse Transcriptase Polymerase Chain Reaction (PCR)

The cDNA synthesis for the mRNA extracted from the cultured cells were performed at 42°C for 1 h using 150 ng of the RNA (6 μL) in the presence of a 250 μg/μL random primer (1 μL), 10 mM dNTP mix (1 μL), Milli Q water (4 μL), 5× first-strand buffer (4 μL), 0.1 M DTT (2 μL), 40 U/μL RNaseOUT (1 μL), and superscript II (1 μL) in a 20-μL reaction volume.

For PCR amplification, primer sequences for human 4F2hc/CD98hc, y^+LAT-1, and y^+LAT-2 were used (Table II) as described by Boyd *et al.* (20). Conditions for amplification were as follows: 94°C for 3 min, 60°C for 1 min, and 72°C for 2 min; then 36 cycles of 94°C for 1 min, 60°C for 1 min, and 72°C for 2 min; followed by a 10-min final extension at 72°C. The studies were conducted in a 50-μL reaction mixture containing 10× PCR buffer, without Mg (5 μL), 10 mM dNTP mixture (1 μL), 50 mM $MgCl_2$ (1.5 μL), 10 μM primer mix (1 μL), template DNA (2 μL), Platinum Taq DNA polymerase (0.2 μL), and autoclaved MilliQ water (39.3 μL). As a negative control, mRNA was omitted from some of the reaction mixtures and was replaced with water.

Samples were subsequently separated in a 2% agarose gel by electrophoresis at 80 v for 2 h, stained with ethidium-bromide and visualized using uv illuminator (Biochrom, Cambridge, UK). The quantity of samples loaded to each lane of the gel was 20 μL. GeneRuler (100 bp DNA ladder) was used to assess the size of the cDNAs.

RESULTS AND DISCUSSION

The present study examined the possible existence of large neutral amino acid transporters in human nasal epithe-

lium. Because the expression of such transporter proteins may be species-specific, the human nasal primary cell culture system was used to probe their expression in the human nasal epithelium using L-phenylalanine as a model substrate. L-phenylalanine was selected as a model amino acid because this molecule is hydrophilic and can only traverse the epithelium paracellularly via the aqueous pores by passive diffusion or transcellularly by active transport. These characteristics result in remarkable polarity in transport, with the apical to basolateral epithelial permeation of the compound much higher than basolateral to apical transport. Moreover, L-phenylalanine has extensively been used to characterize amino acid transport in other epithelia (23,24). It was not necessary to use amino acid-linked drugs for this study because the amino acid is the moiety recognized by the transporters. Several permeation characteristics can be used to functionally discriminate between active and passive transport processes involving amino acids across absorptive epithelia. Such attributes can be used to demonstrate the existence of these transporters in an epithelium. Polarity in transport is often observed with active transport processes because of preferential transport of substrates in one direction. Active transport process requires energy, and can therefore be slowed down by a drop in temperature, metabolic inhibitors, oxygen deprivation, structural analogues and a change in Na^+ gradient. Furthermore, active transport of amino acids is usually concentration-dependent as saturation of the carriers occurs at higher substrate concentration (25). Recently, molecular and genetic approaches have been shown to offer more definitive tools to probe the existence of amino acid transporters in various epithelia (12).

Functional Studies

Figure 1 summarizes the effect of concentration on L-phenylalanine transport across the human nasal cell monolayers. The results indicate that the permeability of L-phenylalanine was concentration dependent, with the permeability of 0.01 mM being significantly higher than that of 5.0 mM ($p < 0.01$) because of transporter saturation. The affinity

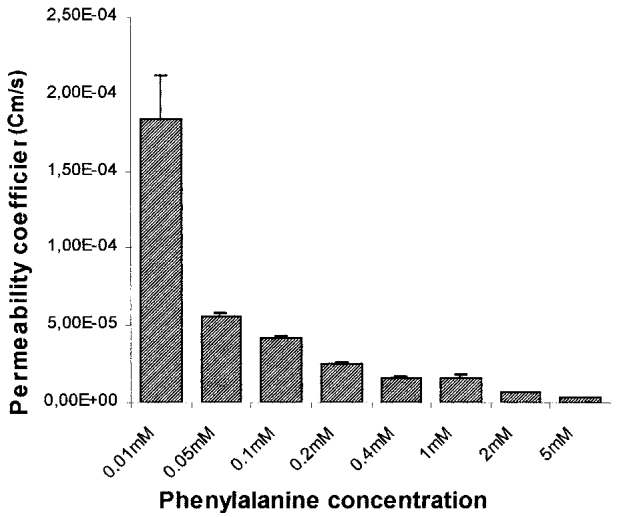

Fig. 1. Effect of concentration on L-phenylalanine transport across human nasal cell monolayers. Each point represents the mean ± SD, n = 3.

constant (K_m) and the maximum velocity (V_{max}) for the transport were 1.23 mM and 805.1 nmol/mg protein/min, respectively. The K_m for L-phenylalanine transport across the human nasal epithelium was found to be approximately two to three times higher than (0.4–0.68 mM) reported for L-phenylalanine, L-tyrosine, and L-tryptophan in rats (6,9). A comparison of the absolute K_m values obtained in this study with that obtained using nasal *in situ* perfusion model (6,9) may not be warranted. Apart from species-related and methodological differences, the kinetic parameters determined in this study are lump-sum kinetic constants, describing the entire transcellular processes, including both apical uptake and basolateral efflux (14), whereas the K_m values described for the rat *in situ* model accounted for cellular uptake process alone. However, the K_m values for L-phenylalanine transport obtained in this study (1.23 mM) is within the 0.23–8.8 mM range for intestinal preparations from several species (26).

To gain further insight into the transport mechanisms of amino acids across human nasal mucosa, and their molecular structural requirements, the effects of Na^+ ions, temperature, other amino acids, metabolic and transport inhibitors on L-phenylalanine transport was examined. Also polarity in transport of L-phenylalanine was investigated. The results of these studies are summarized in Fig. 2 and Table III.

Figure 2A shows that while 32.7 ± 5.4% of L-phenylalanine was transported from the apical to basolateral compartment; the basolateral to apical transport was less than 5.0%. Figure 2B indicates that the apical to basolateral transport was stereo-selective, with the transport of L-phenylalanine being approximately six times higher than that of D-Phe. These observations suggest that the transport of L-phenylalanine across the human nasal epithelium occurred via carrier-mediated processes. The evidence for carrier-mediated process was further accentuated by the inhibitory effect of temperature lowering on L-phenylalanine transport (Fig. 2C). The cumulative amount of L-phenylalanine transported at 37°C was more than 30%, whereas the amount transported at 4°C was 10 times less.

In the human nasal epithelium, Na^+ ions, passively followed by water, are absorbed by both amiloride-sensitive Na^+ channels and amiloride-insensitive Na^+ conductance in the apical membrane (20). The absorbed Na^+ ions are extruded via a basolateral Na^+-K^+ATPase. Thus, in the human airway epithelia, Na^+ absorption from the airway lumen to the blood is the dominant active transport system and may be the driving force for nasal absorption of amino acids. This hypothesis was investigated by conducting the transport of L-phenylalanine in the presence of oubain (Na^+-K^+ ATPase inhibitor), amiloride (Na^+ channel blocker) and Na^+-free medium.

Amiloride (100 μM) inhibited L-phenylalanine transport by 59.0 ± 1.0% whereas addition of 100 μM oubain to the basolateral side of the cells and elimination of Na^+ ions from the transport medium almost abolished the L-phenylalanine transport (Fig. 2D and Table III). These results strongly suggested that the electrogenic transport of Na^+ ions across the human nasal epithelium was the driving force for the nasal permeation of the amino acid.

Further characterization of the active transport processes involved, showed that an inhibitor of oxidative phosphorylation (sodium azide) had no inhibitory effect on L-phenylalanine transport. This may be the result of glucose

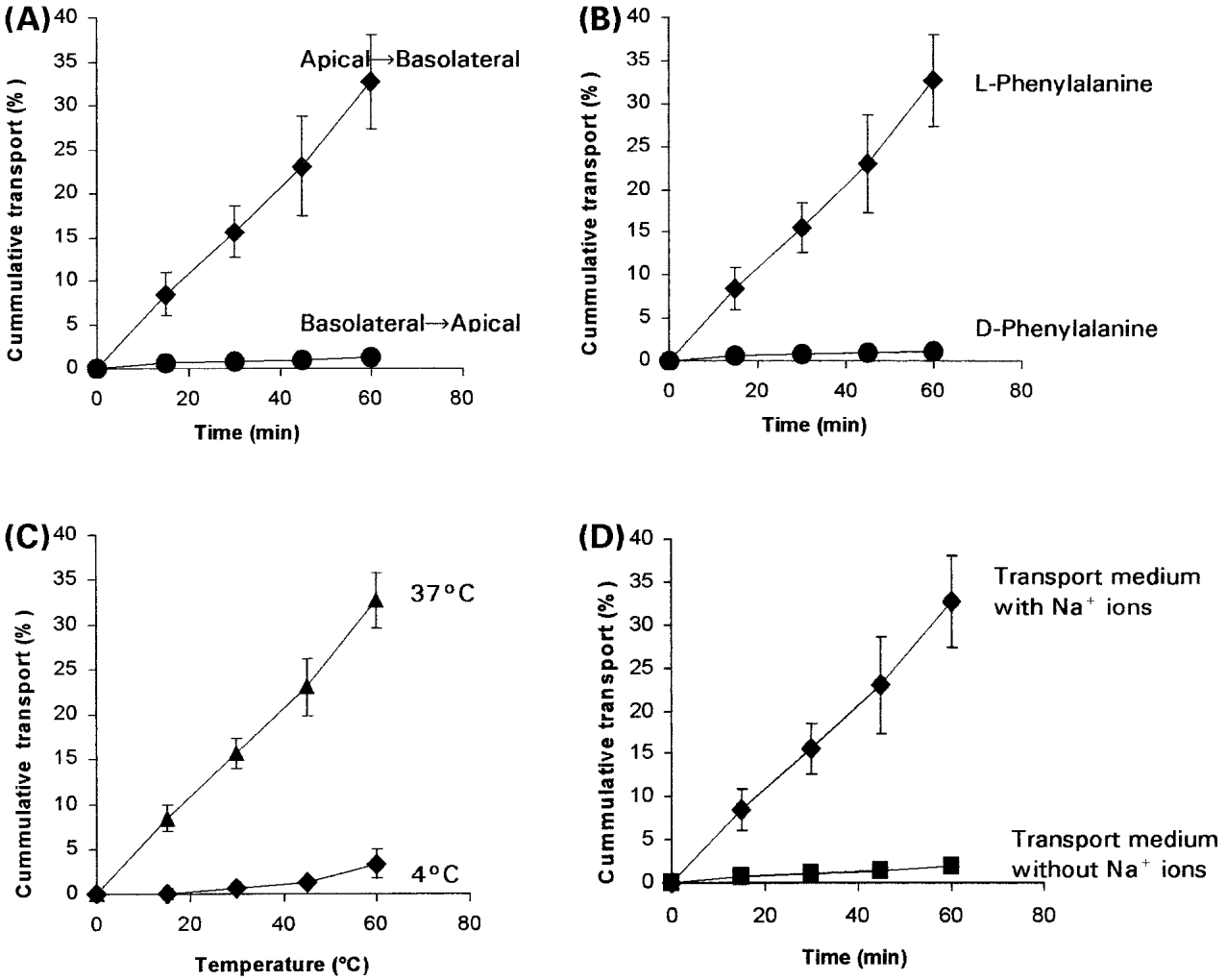

Fig. 2. Polarity in transport (A), stereo-selectivity (B), effects of temperature (C) and Na^+ ions (D) on L-phenylalanine transport across human nasal cell monolayers. Each point represents the mean ± SD, n = 3.

consumption and lactate production in the culture medium. Under our experimental conditions, it was possible that in the presence of the inhibitor, more glucose was oxidized into lactate to produce ATP, thus obliterating the energy depletion effect of sodium azide. However, considering the fact that amiloride, oubain, and sodium free-medium reduced L-phenylalanine transport significantly, while sodium azide had no inhibitory effect suggests that the energy dependency in L-phenylalanine transport across the human nasal epithelium was coupled mainly to Na^+-K^+ATPase and not to Na^+-glucose co-transporter as in the intestinal epithelium. This can be substantiated by the fact that the human nasal epithelium has been shown not to express Na^+–glucose cotransport system, which is known to enhance amino acid permeation by solvent drag and other unknown mechanisms (20,27).

Table III. Effects of Inhibitors and Other Amino Acids on L-Phenylalanine Transport across Human Nasal Cell Monolayers

Inhibitors and amino acids	Concentration	% inhibition ± SD, n = 3
Inhibitors		
Oubain	0.1 mM	90.2 ± 3.2[a]
Amiloride	0.1 mM	59.4 ± 1.1[a]
Sodium azide	5.0 mM	57.0 ± 11.3[b]
Amino acids		
L-Alanine	5.0 mM	91.2 ± 0.8[a]
L-Lysine	5.0 mM	22.0 ± 2.0[a]
L-Leucine	5.0 mM	89.9 ± 2.9[a]
L-Glycine	5.0 mM	66.4 ± 4.2[a]

[a] Statistically significant inhibition ($p < 0.05$).
[b] 57% increase in transport.

Studies with other amino acids showed that L-lysine (cationic amino acid) and L-glycine (small neutral amino acid) reduced L-phenylalanine transport by approximately 22% and 66%, respectively, whereas L-leucine and L-alanine (large neutral amino acids) had a more remarkable effect on transport of the compound (Table III). The above results implied that in the human nasal epithelium both neutral and cationic amino acids share the same transport system (System $B^{0,+}$, y^+L), though with different affinities. Considering the inhibition of L-phenylalanine transport by cationic, small neutral and large neutral amino acids, and the effect of Na^+ ions, one can speculate that the transport of L-phenylalanine was mediated by either Na^+-dependent broad-scope trans-

porter (System $B^{0,+}$) or cation-modulated broad-scope transporter (y^+L; Refs. 10,12). The results of amino acid inhibition studies agreed with the general features of amino acid transporters which include the existence of multiple systems with overlapping specificities in a single membrane and the fact that no substrate analogue interacts exclusively with a particular transporter system (12).

Molecular Genetics Studies

Because there are currently no molecular or genetic methods to investigate the existence of system $B^{0,+}$ in plasma membrane, these techniques were used to further probe y^+L transporters only.

Recently, amino acid transporter cDNAs (LAT-1, LAT-2, y^+LAT-1, y+LAT-2) were identified as subunits of the heavy chain on the cell surface antigen 4F2hc/CD98 (18). Therefore, it would be necessary to know if any of these genes are expressed in the human nasal epithelium. Functional transport characteristics of LAT-1 and LAT-2 in various epithelia indicate that these transporters are Na^+-independent amino acid transporters (28,29). Considering the fact that the transport of L-phenylalanine across the human nasal epithelium was strictly sodium-dependent, no further studies were conducted to provide molecular evidence for the presence of LAT-1 and LAT-2 in the nasal epithelium.

Before the cDNA synthesis for y^+LAT-1, y^+LAT-2 and 4F2hc/CD98, the mRNA extracted from the cells was quantitatively assessed. The yield was 2.5 μg mRNA/10^7 cells with an optical density ratio (OD_{260}/ OD_{280}) of 2.0. This ratio indicated that the extracted mRNA was pure as the ratio was within the acceptable range of 1.7–2.1. Because extraneous DNA may affect the PCR results by resulting in false bands, the extracted mRNA was treated with amplification grade DNase I to digest any DNA present in the samples. Figure 3 shows the agarose gel separation of cDNAs for 4Fhc/CD98hc, y^+LAT-1, and y^+LAT-2. Only one band (lane 5) could be detected. Matching the band with the GeneRuler indicated that the size of the base pair was 120, which corresponds to that of y^+LAT-2 (119 bp).

Unexpectedly, 4F2hc/CD98 was not detected. The detection of y^+LAT-2 alone indicated that in the cultured cells, these transporters did not exist in a dimeric form with the glycosylated surface antigen, 4F2hc/CD98. It is known that with the exception $B^{0,+}$AT, all light chains identified to date (LAT-1, LAT-2, y^+LAT-1, y^+LAT-2, xCT, asc) function as amino acid transporters when associated with the heavy chain, and the substrate specificity of the heterodimer depends on the nature of the light chain (29). It is, however, not unusual to have intracellular expression of y^+LAT-2 alone without its surface existence in association with 4F2hc/CD98. In the absence of the heavy chain, the light chain remains in

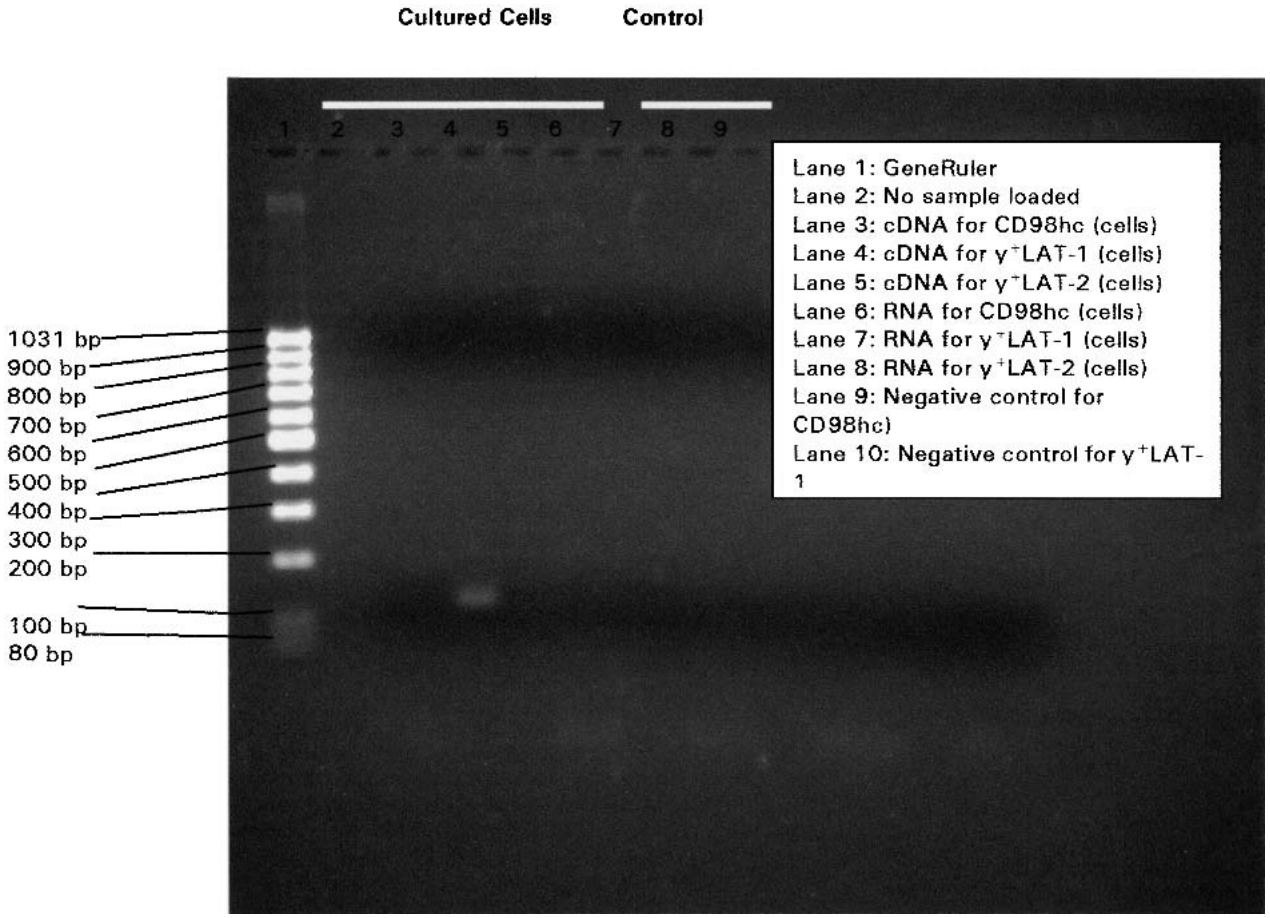

Fig. 3. Agarose gel detection of genes that encode Na^+-dependent L-amino acid transporters in human nasal epithelium. To eliminate artifactual detection of bands, the gel was also loaded with the extracted mRNA and polymerase chain reaction product of water in the presence of reverse transcriptase (negative control).

an intracellular compartment, probably in the endoplasmic reticulum (30). If this is the case with the human nasal epithelium, the L-phenylalanine transport observed might be ascribed to system $B^{0,+}$ because intracellularly located y^+LAT-2 transporters may not contribute significantly to the active transport of the amino acid as surface expression is a *sine-qua-non* to function as an amino acid transporter (30). Nevertheless, it is important to point out that the possibility exists that the transporter (y^+LAT-2) might be co-expressed in the plasma membrane with a yet unidentified heavy chain and as such might have actually been responsible for the transport of the amino acid. Apparently, further studies are required to unravel the nature of interaction among various amino acid transporters in the human nasal epithelium.

CONCLUSIONS

This study demonstrated the usefulness of cultured human nasal epithelium as a model to characterize nasal amino acid absorption. Based on the saturability of L-phenylalanine transport, its temperature and cation dependency, as well as stereo-selectivity, polarity in transport and the influence of metabolic inhibitors and other amino acids, it was apparent that the permeability of L-phenylalanine across the human nasal epithelium occurred mainly by active transport via sodium-dependent amino acid transport systems. The effect of cationic, small and large neutral amino acids and Na^+ ions on L-phenylalanine transport, as well as the detection of y^+LAT-2 gene implicated system y^+L and $B^{0,+}$ transporters. Because y^+LAT-2 was not co-expressed with 4F2hc/CD98, one is not in a position, at this point, to conclude whether y^+LAT-2 or system $B^{0,+}$ or both was responsible for the observed transport. Therefore, further studies are necessary to determine the specific transporters responsible for the carrier-mediated permeation of L-phenylalanine in the human nasal epithelium. As in the bronchial epithelium, the nasal amino acid transporters could be important *in vivo* to maintain low amino acid concentration in the fluid covering the airway surface, especially after leakage of interstitial fluids due to epithelial damage or at birth when fluids and solutes filling the airways have to be rapidly reabsorbed (10). The presence of L-amino acid transporters in human nasal epithelium highlights the potentials of nasal amino acid transporters as molecular targets for systemic delivery of amino acid-linked drugs in humans.

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