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International Journal of Pharmaceutics 247 (2002) 115–125

international journal of pharmaceutics

www.elsevier.com/locate/ijpharm

Carrier mediated uptake of L-tyrosine and its competitive inhibition by model tyrosine linked compounds in a rabbit corneal cell line (SIRC)—strategy for the design of transporter/receptor targeted prodrugs

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Received 29 August 2001; received in revised form 6 February 2002; accepted 17 July 2002

Abstract

The objective of this study was to investigate the presence of amino acid transporters on the corneal epithelium and to enhance corneal drug absorption through prodrug modification targeted to the amino acid transporters. SIRC was used as a model cell line representing the corneal epithelium. Uptake studies were carried out using [^{3}H] L-tyrosine at 37 °C. Temperature, energy and pH dependence studies were carried out. The uptake seems to be composed of a major saturable and minor non-saturable component ($V_{max} = 2.9 \pm 0.62$ nmoles/min/mg protein, $K_m = 71 \pm 21$ μM, $K_d = 2.6 \pm 0.6$ nl/min/mg protein). No significant inhibition of uptake was observed in the presence of metabolic inhibitors or in the absence of sodium. Competitive inhibition studies were performed in the presence of various amino acids and model tyrosine conjugates (*p*-nitro and *p*-chloro benzyl ether conjugate of L-tyrosine). Uptake was inhibited by neutral aromatic and large neutral aliphatic amino acids. L-Tyrosine uptake was inhibited by its ether conjugates in a concentration dependent manner suggesting that these compounds may be sharing the same transport mechanism. This study provides biochemical evidence of the presence of a large neutral amino acid transport system on the corneal epithelium, which may be utilized to enhance the corneal drug transport.

Keywords: Prodrugs; SIRC; Cornea; Amino acid transporter; Tyrosine

1. Introduction

Permeation of drugs across the cornea represents a major barrier in the delivery of ocular therapeutics (Grass and Robinson, 1988; Sieg and Robinson, 1975). The corneal epithelium is composed of five to six layers of columnar epithelium

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0378-5173/02/$ - see front matter
PII: S0378-5173(02)00405-2

with tight junctions thereby restricting paracellular diffusion. Underneath the epithelial layer is the stroma, which is composed of more than 90% water and presents a barrier to the passage of hydrophobic compounds. Thus a balance between the lipophilicity and the hydrophilicity has to be maintained for a compound to possess optimal permeability (Mitra and Mikkelson, 1988). Several strategies have been developed to overcome poor corneal transport, of which prodrug approach represents a very promising strategy. Prodrug design for ocular therapeutics has generally aimed at improving permeability of the drug by increasing the lipophilicity of the drug (Chang et al., 1987; Shih and Lee, 1990). However, only limited success has been achieved with this approach. Increasing lipophilicity to a certain degree does lower epithelial resistance. However continued increase in lipophilicity lowers permeation across cornea (Narurkar and Mitra, 1989). Owing to their enhanced lipophilicity these compounds possess poor aqueous solubility and hence present problems in their formulation into eye drops.

Recently, a significant amount of work has been reported on the expression of nutrient transporters in various tissues (amino acid, peptide, nucleoside and glucose transporters) (Kanai et al., 1998; Shen et al., 1999). Several reports from our and other laboratories have described prodrug design aimed at increasing drug permeability via carrier mediated transport mechanisms (Oh et al., 1999; Tsuji and Tamai, 1996). This is achieved by chemical conjugation of a parent compound with a nutrient moiety such that the conjugate may be transported by the carrier system across the cell membrane. Prodrugs designed to resemble these nutrients structurally are expected to be absorbed via specific carrier proteins. For example, conjugation with L-tyrosine has been reported to be a successful strategy in enhancing the transport of an anti-cytomegaloviral agent phosphonoformate, which is transported by means of amino acid transport system across porcine brain microvessel endothelial cells (Walker et al., 1994). Similarly the valine ester of acyclovir—Valacyclovir, which is known to be transported via peptide transporters in the small intestine, was found to have higher permeation across the intestinal mucosa as compared to the parent molecule acyclovir (de Vrueh et al., 1998; Han et al., 1998). Previous reports from our laboratory have also revealed that this strategy can be successfully applied to increase the permeability of poorly absorbed drugs across the nasal mucosa (Yang et al., 2001).

Carrier mediated processes are however relatively unexplored particularly in the case of ocular therapeutics. Presence of active transport systems on the conjunctiva have recently been reported (Horibe et al., 1998). Conjunctiva is reported to express a large number of transporter proteins including amino acid transporters, MCT, nucleosides etc. which are believed to play a role in the absorption of drugs through conjunctiva after topical administration (Hosoya et al., 1997, 1998; Kompella et al., 1995). But so far not many people have considered utilizing carrier mediated transport mechanisms on the corneal epithelium to improve drug transport. There are reports about the existence of carrier mediated nutrient transport systems on the cornea and most of them are believed to be present on the corneal endothelium. However little information is available about their existence on the corneal epithelium. As corneal epithelium is the primary barrier to the absorption of drugs after topical administration, the presence of these transporters on the corneal epithelium will offer newer strategies for the design of transporter-targeted prodrugs having enhanced permeability.

The purpose of this study was to examine the presence of any amino acid transport systems on the corneal epithelium, characterize the transport processes and study feasibility of enhancing corneal drug absorption using prodrug approach targeting the amino acid transporters. The hypothesis behind this approach is that a poorly permeable drug will be transported across the cornea to a greater extent after it is conjugated to an amino acid. While the conjugate is being transported across the cornea it will be acted upon by various enzymes in the cornea and aqueous humor, which will cleave it back to the parent drug and the non-toxic amino acid. An enzymatically stable ether linkage was used in this study to link the model compounds to L-tyrosine to avoid complication due to enzymatic degradation and to examine the feasibility of utilizing amino acid conjugates as a

"Trojan horse" to enhance trans-corneal permeation. Various substituted benzyl alcohols were selected as model compounds and the conjugates were synthesized according to our previously published methods (Yang and Mitra, 2001). The structures of these model conjugates as well as the corresponding parent compounds are shown in Fig. 1.

In-vitro uptake studies were carried out using a rabbit corneal cell line (SIRC), which is a well-established cell culture model for corneal epithelium. This cell line has been employed extensively in studies of corneal physiology (Korbmacher et al., 1988), immunology (Grabner et al., 1983), toxicology (Phillips et al., 1966) and more recently as an in-vitro cell culture model for assessing corneal transport of drug molecules (Goskonda et al., 1999). Drug transport studies using this cell line have been reported to exhibit good correlation to in-vitro transport using intact cornea (Goskonda et al., 2000). The cell line displays morphological properties similar to the corneal epithelium in-vivo and thus is a good in-vitro cell culture model for the corneal epithelium (Hutak et al., 1997).

2. Materials and methods

2.1. Materials

The synthesis of the ether conjugates of L-tyrosine has already been described in previous reports from our laboratory (Yang and Mitra, 2001). L-Tyrosine [ring-3, 5-^{3}H] was procured from NEN life sciences (Boston, MA). ^{14}C-L-phenylalanine (0.1 μCi/μl) was purchased from Amersham. L-Tyrosine, D-tyrosine, all unlabelled amino acids, choline chloride, Triton X-100, HEPES, D-glucose, 2,4-dinitrophenol, sodium azide, ouabain, *N*-ethyl maleimide (NEM) and all other chemicals were purchased from Sigma Chemical Co (St. Louis, MO). All the chemicals were products of special reagent grade and used without further purification.

2.2. Cell culture

Rabbit corneal cell line (SIRC) was procured from ATCC at passage 400 and used in the present study between passages 410 and 425. The culture media consisted of minimum essential medium (MEM) (Gibcoryl, Grand Island, NY), 1–10% fetal bovine serum (FBS) (JRH Life Sciences, Lenexa, KS), lactalbumin, HEPES, sodium bicarbonate, penicillin (100 μg/ml) and streptomycin (100 μg/ml)(Sigma Chemical Co., St. Louis, MO). Medium was replaced every alternate day. Cells were maintained at 37 °C, in a humidified atmosphere of 5% CO_2 and 90% relative humidity. For uptake studies the cells were plated at a density of 500 000 cells/well on 12 well culture plates (Costar, Corning, NY) and maintained at 37 °C. Medium containing 10% FBS was used for the first 5 days and subsequently cells were cultured in a 1% serum containing medium to promote cell differentiation (Lentz et al., 2000).

2.3. Uptake experiments

2.3.1. Concentration dependence

Uptake studies were performed based on the method of Surendran et al. (1999), with slight modifications. Briefly, at 10–12 days post seeding

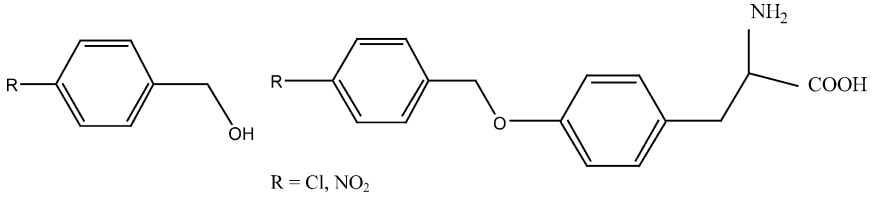

Fig. 1. Structures of the model compounds and their L-tyrosine conjugates.

the cells were rinsed thrice with Dulbecco's phosphate buffered saline i.e. DPBS (130 mM NaCl, 0.03 mM KCl, 7.5 mM Na_2HPO_4, 1.5 mM KH_2PO_4, 1 mM $CaCl_2$, 0.5 mM $MgSO_4$ and 5 mM glucose), pH 7.4 except in pH dependence studies and equilibrated for 1 h with the buffer. In the case of studies determining sodium dependency of the uptake, NaCl and Na_2HPO_4 were substituted with equimolar quantities of choline chloride and K_2HPO_4, respectively. Tyrosine solutions were prepared at different concentrations (1 μM–1 mM) in the buffer containing ^{3}H-L-tyrosine (0.25 μCi/ml). Uptake was initiated by incubating the cells with 2 ml of each drug solution at 37 °C for a suitable time period. At specific time intervals, the drug solution was aspirated off and cells washed three times, 5 min per wash, with 2 ml of ice-cold stop solution (210 mM KCl, 2 mM HEPES) pH 7.4, to stop the cellular uptake. After the washings, cells were lysed by keeping them over night in 1 ml of 0.1% Triton-X solution in 0.3% NaOH. Following overnight incubation, 500 μl of the cell lysate from each well was transferred to scintillation vials containing 5 ml of scintillation cocktail (Fisher Scientific, Fair Lawn, NJ). Samples were analyzed by liquid scintillation counter (Beckman Instruments Inc., Model LS-9000) and the uptake was normalized to the protein content in each well. Cell lysate protein content was determined by the method of Bradford (Bradford, 1976) using bovine serum albumin as the standard (Bio-Rad protein estimation kit, Hercules, CA). Non-specific binding was assessed by carrying out zero time uptake at 4 °C and subtracted from total uptake.

2.3.2. Effect of inhibitors

In the case of experiments using competitive inhibitors (various amino acids and the model conjugates), cells were incubated simultaneously with the unlabeled inhibitor and L-tyrosine and uptake was carried out as described earlier. All the unlabelled amino acids were used at a concentration of 1 mM. Inhibitors for Na^+/K^+-ATPase (Ouabain, 100 μM) and metabolic inhibitors i.e. sodium azide (100 μM) and 2, 4-dinitrophenol (100 μM) were used to further characterize the transport. In these experiments the cells were pre-incubated with the inhibitors for 30 min and rinsed with buffer before initiating uptake.

2.3.3. Treatment with amino acid modifying agents

NEM and dithiothreitol were used as amino acid modifying agents to investigate the amino acid residues present at the substrate-binding site. The cells were pre-incubated for 30 min in DPBS containing 1 mM NEM or 1 mM dithiothreitol and rinsed in buffer before uptake.

2.4. Corneal transport studies

Transport of ^{14}C-L-phenylalanine across freshly excised rabbit cornea was carried out based on the method of Tak et al. (2001). Briefly, corneas obtained from New Zealand albino rabbits weighing 2.0–3.0 kg were used for transport studies. The animals were euthanized by an overdose of pentobarbital through a marginal ear vein. Eyes were then carefully enucleated and washed with ice-cold isotonic phosphate buffer saline (IPBS) pH 7.4 to remove any traces of blood. Subsequently, a small incision was made to the sclera and vitreous humor aspirated using a 1-ml syringe. Cornea was carefully excised, leaving some portion of the sclera attached to it so that mounting on the diffusion apparatus could be facilitated. The lens and iris–ciliary body were then separated from cornea, and the cornea washed immediately with ice-cold DPBS and mounted on a Side-bi-Side™ diffusion half chamber where the temperature was maintained at 34 °C. Transport of ^{14}C-L-phenylalanine (200 μM) was then carried out for 2 h in the absence and presence of 2 mM L-tyrosine. Also, transport of ^{3}H-L-Tyr was determined in the absence and presence of 100 μM chlorobenzyl ether conjugate.

2.5. Data treatment

For the carrier-mediated process, the data was fitted to a modified Michaelis–Menten equation expressed by Eq. (1), which takes into account the carrier mediated process (as described by the classical Michaelis–Menten equation) and a non-saturable process.

$$J_T = \frac{J_{max}[C]}{K_m + [C]} + K_d[C] \quad (1)$$

J_T is the total rate of uptake, J_{max} is the maximum uptake rate for the carrier-mediated process; K_m is the concentration at half saturation (Michaelis–Menten constant) and K_d is the rate constant for the non-saturable component.

Data was fitted to this equation using a non-linear least square regression analysis program (Kaleida Graph V3.09). In the case of competitive inhibition experiments involving model ether conjugates the data was fitted to a Lineweaver–Burk transformation of Michaelis–Menten equation, given by Eq. (2).

$$\frac{1}{J} = \frac{1}{J_{max}} + \frac{K_m[1 + [I]/K_i]}{J_{max}} \frac{1}{[C]} \quad (2)$$

K_i values were determined by using Eq. (3) (Walker et al., 1994) derived from the Michaelis–Menten equation for uptake in the presence (J_i) and in the absence (J) of a reversible inhibitor.

$$K_i = \frac{K_m[I]}{\frac{100(K_m + [S])}{100 - R_{100}} - K_m - [S]} \quad (3)$$

$$R_{100} = 100\frac{(J - J_i)}{J}$$

2.6. Statistical analysis

All results were expressed as mean ± standard deviation (SD). Statistical analysis between two groups was carried out using Student's t-test. A difference between mean values was considered significant if the P-value was less than 0.05.

3. Results

3.1. Concentration dependence

L-Tyrosine was rapidly accumulated in the cells with time (Fig. 2 inset). The uptake was linear initially and the slope gradually decreased after 10 min. Therefore all the subsequent studies and kinetic analyses were performed using data collected from initial 5 min uptake studies.

Uptake of L-tyrosine consisted of a major (saturable) carrier mediated process and a minor non-saturable component evident at higher concentrations (Fig. 2). Uptake data was fitted to a modified Michaelis–Menten equation as per Eq. (1) and kinetic parameters of L-tyrosine uptake determined by non-linear regression analysis of the data. V_{max} and K_m values were calculated to be 2.9 ± 0.6 nmoles/min/mg protein (mean ± SD) and 71 ± 21 μM (mean ± SD), respectively. K_d had a value of 2.6 ± 0.6 nl/min/mg protein (mean ± SD). At any given time the uptake by the saturable component was higher than the non-saturable component.

3.2. Effect of metabolic inhibitors and pH

The effects of metabolic inhibitors on the uptake of L-tyrosine were studied to determine whether the uptake required energy expenditure. Sodium azide, a respiratory chain inhibitor and 2,4-dinitrophenol, which blocks oxidative phosphorylation, did not inhibit the uptake (Table 1). Ouabain, an inhibitor of Na^+/K^+-ATPase also did not exhibit significant inhibition of uptake. Further, L-tyrosine uptake was not altered in a sodium free buffer or with changes in the buffer pH.

3.3. Substrate specificity

To investigate the substrate specificity of the carrier involved in the uptake of L-tyrosine, effect of various amino acids including large neutral AA (e.g. phenylalanine, leucine), small neutral AA (e.g. alanine, cysteine), basic AA (e.g. lysine, asparagine) and acidic AA (e.g. glutamic acid) were examined as potential inhibitors. Results shown in Table 2 indicate that large neutral amino acids (LNAA) like L-leucine and L-phenylalanine were able to inhibit the uptake of L-tyrosine to a greater extent as compared to other amino acids. Inhibition with small neutral amino acids like L-alanine was moderate as compared to the LNAA, whereas inhibition due to basic amino acids like L-lysine and acidic amino acids like L-glutamic acid was negligible.

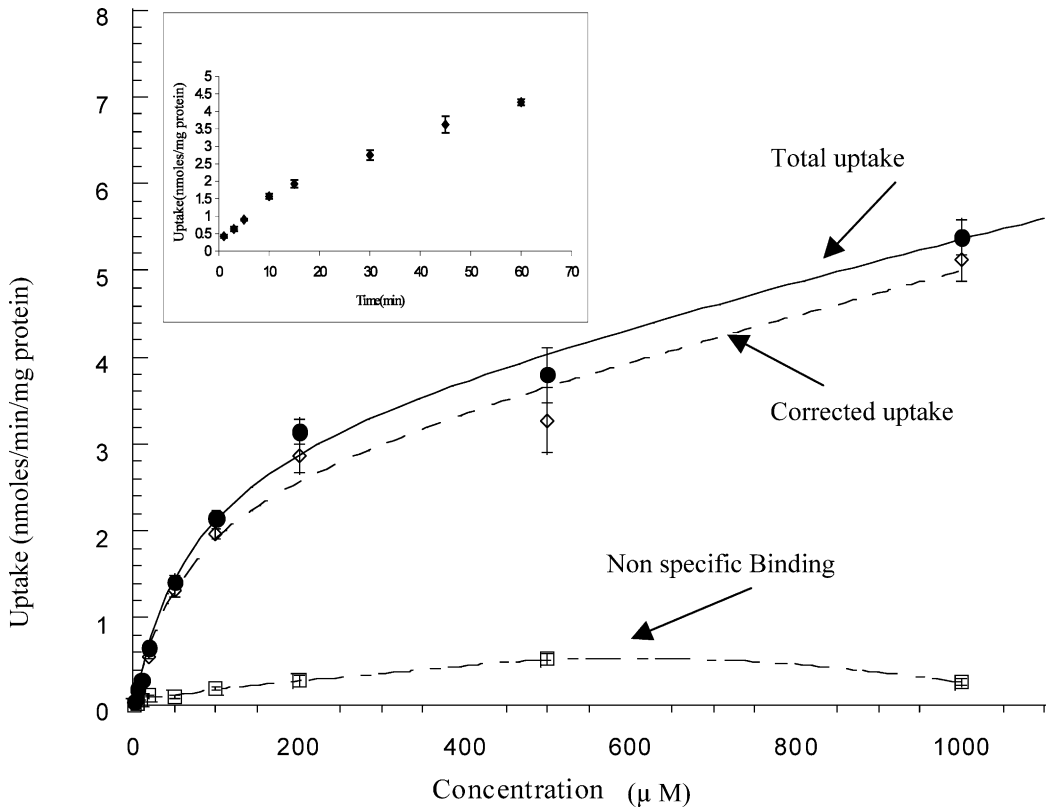

Fig. 2. Concentration dependent L-tyrosine uptake in SIRC cells at 37 °C, pH 7.4. ●, Total uptake; □, non-specific binding (zero time uptake at 4 °C); ◇, corrected uptake. Inset shows time dependent uptake of ^{3}H-L-tyrosine (0.25 μCi/ml) by SIRC cells. Each value represents the mean ± standard deviation ($n = 3$–4).

Additionally the carrier was found to be stereoselective as seen by the variable inhibition by L- and D- isomers of phenylalanine, tryptophan and tyrosine (Fig. 3). As there are reports of overlapping substrate specificities of LNAA transporter and peptide transporter, the uptake of L-tyrosine was determined in the presence of dipeptides glycyl-sarcosine and tyrosine-glycine (Surendran et al., 1999). As shown in Table 2, the dipeptides had no significant inhibition on the uptake of L-tyrosine.

We further investigated the possibility of involvement of multiple transport systems in the uptake process. The effect of 1 mM *N*-methylaminoisobutyric acid (NMAIB) (a specific inhibitor of alanine preferring neutral amino acid transport system) and 2-aminobicyclo (2.2.1) heptane-2-carboxylic acid (BCH) (a specific inhibitor of the leucine preferring amino acid transport system) was determined. As shown in Fig. 4, uptake of L-tyrosine was sensitive to the presence of BCH as compared to NMAIB which did not have any

Table 1
Effect of metabolic inhibitors and sodium free buffer on L-tyrosine uptake by SIRC cells at 37 °C

Inhibitor	Concentration (mM)	Relative uptake (percentage of control)
Ouabain	1	106.6 ± 10.7
Sodium azide	1	108.9 ± 5.9
2,4-Dinitrophenol	1	112.3 ± 9.4
Sodium free buffer	–	102.2 ± 6.8

Each value represents the mean ± standard deviation ($n = 3$–4).

Table 2
Effect of various amino acids and dipeptides on L-tyrosine uptake by SIRC cells at 37 °C

		Concentration (mM)	Relative uptake (percentage of control)
Large neutral aromatic amino acids	L-Tyrosine	1	3.1±0.23*
	L-Phenylalanine	1	8.0±0.57*
	L-Tryptophan	1	8.3±0.17*
	L-DOPA	1	16.7±0.54*
Large neutral aliphatic amino acids	L-Leucine	1	15.6±3.48*
	L-Isoleucine	1	12.8±0.46*
Small neutral amino acids	L-Alanine	1	73.5±4.8*
	L-Cysteine	1	60.2±3.16*
Acidic amino acids	L-Glutamic acid	1	89.7±4.3
Basic amino acids	L-Lysine	1	94.3±0.46
	L-Asparagine	1	90.3±2.36
	α-Methyl L-tyrosine ester	1	57.5±10.13*
Dipeptides	Tyrosine-glycine	1	133.5±2.84*
	Glycyl sarcosine	1	95.0±2.78

Cells were exposed simultaneously to the inhibitor and substrate. Each value represents the mean±standard deviation ($n = 3$–4).
* Represents significant difference from control ($P < 0.05$).

significant effect on the uptake, indicating that uptake is predominantly mediated by the leucine preferring neutral amino acid transport system.

Inhibition of uptake was observed with NEM treated cells while dithiothreitol had no effect. As the cells treated with these compounds were used for the uptake after washing with buffer, direct interaction of L-tyrosine with these reagents is unlikely. Thus the inhibitory effects are probably due to specific interactions with the carrier protein responsible for the uptake of L-tyrosine.

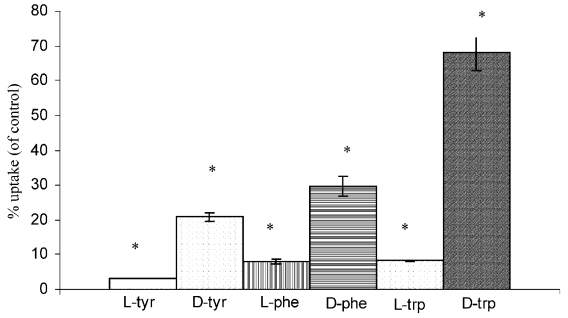

Fig. 3. Effect of D- and L- isomers of different amino acids (1 mM) on the uptake of ^{3}H-L-tyrosine in SIRC cells, indicating the stereoselectivity of the transport system. Each value represents the mean±standard deviation ($n = 3$–4).

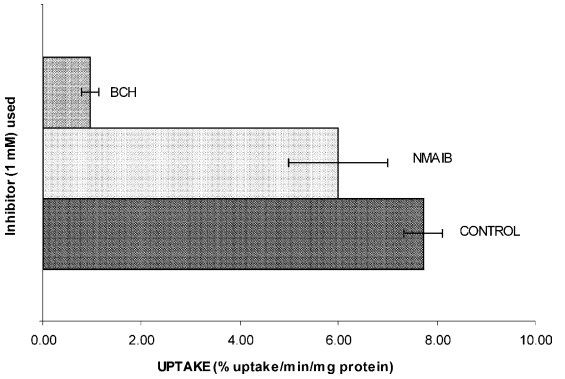

Fig. 4. Effect of 1 mM concentrations of BCH and NMAIB on the uptake of ^{3}H-L-tyrosine in SIRC cells. Each value represents the mean±standard deviation ($n = 3$–4). * Represents significant difference from control ($P < 0.05$).

3.4. Competitive inhibition with model L-tyrosine conjugates

Inhibition studies with model conjugates indicated that the conjugates of L-tyrosine inhibited the uptake of L-tyrosine in a concentration dependent fashion. To study the mechanism of inhibition by the conjugates the inhibition effect was analyzed kinetically. The data was fitted into a Lineweaver–Burk transform (Eq. (2)). As shown

in Figs. 5 and 6, a change in concentration of the model conjugates increased the slope and x-intercept of the plot while the y-intercept remained constant indicating that the inhibition of L-tyrosine uptake is competitive in nature. The K_i values calculated using Eq. (3) were found to be 39.6 ± 9.0 and 38.8 ± 3.9 μM (mean ± SD) for the p-chlorobenzyl and p-nitrobenzyl ether conjugates, respectively. The low K_i values indicate that the conjugates have a high affinity for the transporter.

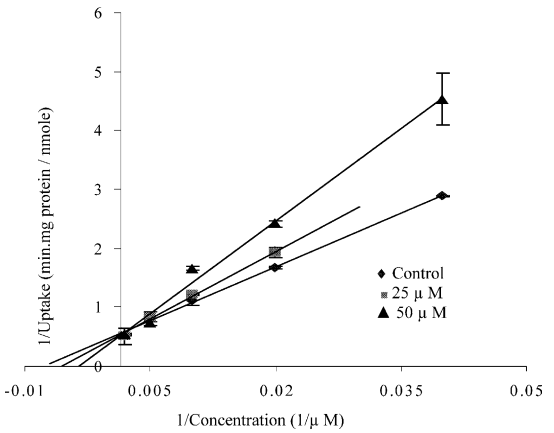

Fig. 5. Lineweaver–Burk plot exhibiting competitive inhibition of ^{3}H L-tyrosine uptake by p-nitrobenzyl ether conjugate of tyrosine in SIRC cells at 37 °C. Each value represents the mean ± standard deviation ($n = 3$–4).

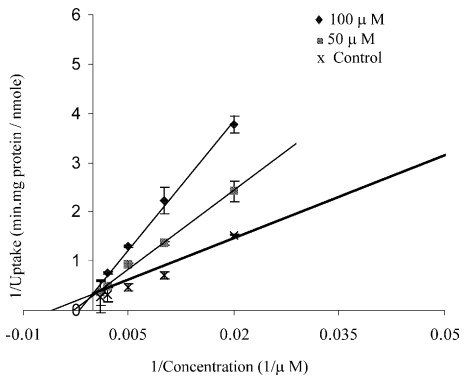

Fig. 6. Lineweaver–Burk plot exhibiting competitive inhibition of ^{3}H L-tyrosine uptake by p-chlorobenzyl ether conjugate of tyrosine in SIRC cells at 37 °C. Each value represents the mean ± standard deviation ($n = 3$–4).

3.5. Corneal transport studies

As seen in Fig. 7, the transport of 200 μM ^{14}C-L-phenylalanine across freshly excised rabbit cornea was inhibited markedly in the presence of 2 mM L-tyrosine indicating the utilization of the same transport systems by the two aromatic amino acids L-tyrosine and L-phenylalanine. The transport of ^{3}H-L-Tyr was also reduced significantly in the presence of the chlorobenzyl ether conjugate (data not shown). Thus, it appears that the chlorobenzyl ether conjugate of tyrosine utilizes the LNAA transporter present on the rabbit corneal epithelium to be transported across the cornea.

4. Discussion

Current knowledge of drug permeation across the cornea indicates passive diffusion to be the primary mechanism of drug transport.

In the present study we used L-tyrosine uptake as a marker for the amino acid transport systems and investigated whether any carrier mediated system exists for the transport of amino acids on the corneal epithelium.

Uptake of L-tyrosine by the SIRC cells was saturable at higher concentrations and was not affected by the presence of any metabolic inhibi-

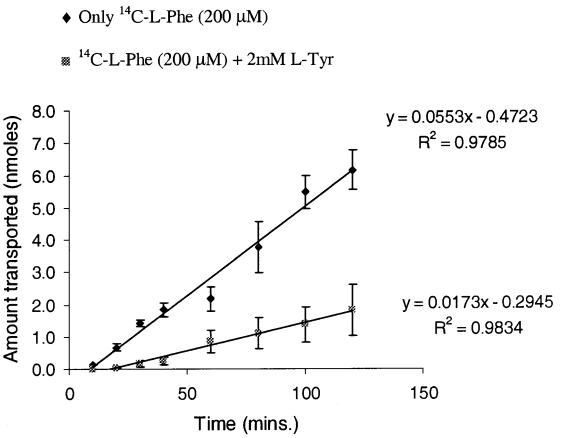

Fig. 7. Transport of ^{14}C-L-Phe (200 μM) across freshly excised rabbit cornea in the absence (◆) and presence (■) of 2 mM L-tyrosine. Each value represents the mean ± standard deviation ($n = 3$–4).

tors, which is suggestive of an energy independent facilitated transport process. The uptake was inhibited significantly by LNAAs like L-phenylalanine, L-leucine and L-isoleucine and moderately by the small amino acids like L-alanine suggesting that the carrier is specific for LNAAs.

There are reports of overlapping substrate specificity between the LNAA transporter and the peptide transporter (Surendran et al., 1999). Thus we investigated if the same holds true in the case of SIRC cells as well. However no significant inhibition of L-tyrosine uptake was seen in the presence of dipeptides like tyrosine-glycine as well as glycyl-sarcosine, indicating a high degree of substrate selectivity of the transporter for amino acids and no affinity for dipeptides. A much lesser degree of inhibition in the presence of D-amino acids as compared to L-amino acids indicates that the transporter is stereoselective for L-amino acids. These results cumulatively suggest that the carrier system on the corneal epithelial cells may belong to a class of neutral amino acid carrier system. In general, amino acid transport systems are classified into different systems—L, y^+L, X_c^-, ASC, asc, A, b^{0+}, B^{+0}, Gly, N and T (Christensen et al., 1994; Broer et al., 2001). The A-system is sodium dependent and inhibited by NMAIB, L-system is sodium independent and inhibited by BCH and an ASC-system is sodium dependent but not inhibited by NMAIB (Sershen and Latha, 1979; Shotwell et al., 1981). Based on the fact that the uptake is not inhibited in the absence of sodium, is highly sensitive to inhibition by BCH and is not sensitive to inhibition by NMAIB, it appears that the uptake is primarily occurring through the L-system. The uptake seems to be pH independent indicating that the transporter is not proton coupled which is consistent with the previous reports on the characteristics of LNAA carrier system (Hargreaves and Pardridge, 1988). Results from our studies suggest the involvement of Na^+-independent facilitative transport system in the uptake of L-tyrosine. The physiological role of this amino acid transport system on the corneal epithelium is not known at this point.

Competitive inhibition of the uptake of L-tyrosine by the model conjugates suggests that L-tyrosine and the model conjugates compete for the same substrate-binding site on the LNAA transporter. As the ether conjugates are known to be chemically and enzymatically very stable, the inhibition effect is purely due to the conjugates and not due to the breakdown products. The K_i values for these conjugates are lower compared to the K_m value of L-tyrosine thus indicating that the conjugates have a higher affinity for the transporter. The low K_i values are in good agreement with the previously reported values from our laboratory on the absorption of these conjugates via the amino acid transporter on the nasal mucosa (Yang and Mitra, 2001). This observation suggests that there may be a hydrophobic pocket in the substrate-binding region of the transporter protein, which may result in a stronger binding of the conjugate to the substrate-binding site. Further in our previous study (Yang and Mitra, 2001) it was observed that these conjugates were indeed transported by the amino acid transporter and their transport was inhibited competitively by L-tyrosine.

The L-system of amino acid transport has been extensively studied and has been of particular interest in the transport of amino acid drugs across the intestinal mucosa and the blood brain barrier. Studies conducted with the transport of L-DOPA and L-α-methyl DOPA (phenylalanine derivatives) across the brain microvessel endothelial cells have shown that these molecules are transported via the amino acid transport system (Kageyama et al., 2000). However their metabolites L-dopamine and L-α-methyl dopamine are not transported by this system. Similarly an NMDA antagonist PD 158473, which is a phenylalanine derivative, has recently been shown to be a substrate for the LNAA system (Surendran et al., 1999). L-Tyrosine analogue of phosphonoformate is a substrate for the LNAA transporter in porcine brain microvessel endothelial cells (Walker et al., 1994). Recent studies in our laboratory have shown that the nasal absorption of acyclovir was improved significantly by L-aspartate β-ester prodrug, which was a substrate for the amino acid transporter whereas the amino acid α-ester prodrugs were not transported. Results presented in this study show limited inhibition of uptake by α-methyl ester of L-tyrosine, suggesting that the

presence of an α-amino group and a C-terminal carboxyl group are required for affinity towards the transporter (Yang et al., 2001).

In intestinal transport studies the amino acid transport system has not been found to be a very versatile and robust target as compared to the peptide transporter. However the expression and distribution of amino acid and peptide transport systems vary from tissue to tissue. And thus the amino acid transport systems located at the other sites e.g. nasal and corneal epithelium may be utilized to improve drug absorption.

In conclusion, our results indicate the presence of a LNAA transport system on SIRC cell line as well as freshly excised rabbit cornea. The model L-tyrosine conjugates appear to be substrates for the LNAA transporter. The study provides useful information on the substrate specificity of this carrier system. A full understanding of the molecular and structural features of this transporter will help in the rational design of prodrugs based on the structural features of the transporter, which may be transported via this carrier system. Further studies involving identification of the transporter at the molecular level are currently in progress in our lab.

Acknowledgements

This work was supported by NIH grants EY09171 and EY10659.

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