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Tryptophan Transport in Human Fibroblast Cells—A Functional Characterization

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Abstract: There are indications that serotonergic neurotransmission is disturbed in several psychiatric disorders. One explanation may be disturbed transport of tryptophan (precursor for serotonin synthesis) across cell membranes. Human fibroblast cells offer an advantageous model to study the transport of amino acids across cell membranes, since they are easy to propagate and the environmental factors can be controlled. The aim of this study was to functionally characterize tryptophan transport and to identify the main transporters of tryptophan in fibroblast cell lines from healthy controls.

Tryptophan kinetic parameters (V_{max} and K_m) at low and high concentrations were measured in fibroblasts using the cluster tray method. Uptake of ^{3}H (5)-L-tryptophan at different concentrations in the presence and absence of excess concentrations of inhibitors or combinations of inhibitors of amino acid transporters were also measured. Tryptophan transport at high concentration (0.5 mM) had low affinity and high V_{max} and the LAT1 isoform of system-L was responsible for approximately 40% of the total uptake of tryptophan. In comparison, tryptophan transport at low concentration (50 nM) had higher affinity, lower V_{max} and approximately 80% of tryptophan uptake was transported by system-L with LAT1 as the major isoform. The uptake of tryptophan at the low concentration was mainly sodium (Na^+) dependent, while uptake at high substrate concentration was mainly Na^+ independent. A series of different transporter inhibitors had varying inhibitory effects on tryptophan uptake.

This study indicates that tryptophan is transported by multiple transporters that are active at different substrate concentrations in human fibroblast cells. The tryptophan transport trough system-L was mainly facilitated by the LAT1 isoform, at both low and high substrate concentrations of tryptophan.

Keywords: fibroblasts, tryptophan, serotonin, LAT1, amino acid transporters

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Introduction

Serotonin, an important neurotransmitter in the brain, is known to play an important role in functions subserving mood, anxiety, aggression, sleep, appetite and sexual behaviour.[1] Disturbed serotonergic neurotransmission is implicated in the pathogenesis of schizophrenia,[2] bipolar disorder,[3] depression,[3] obsessive-compulsive disorder,[4] and generalized anxiety disorder.[5]

Evidence for disturbed serotonergic neurotransmission in different neuropsychiatric patients was obtained from observations that patients with affective disorders and suicide attempters have lower levels of the serotonin metabolite (5 hydroxyindolacetic acid, 5HIAA) in the cerebrospinal fluid (CSF) compared to controls.[3,6–10] Since 5HIAA is believed to reflect serotonin turnover in the brain,[7] the findings of low levels of this metabolite could implicate a reduction in central serotonergic transmission in both mania and depression. Serotonin re-uptake inhibitors has shown to be effective in the treatment of depression and other psychiatric disorders.[11] Tryptophan is the precursor for serotonin synthesis and reduced availability of tryptophan to the brain has been shown to result in mood changes of healthy volunteers in experimental studies using tryptophan depletion.[12]

The rate of serotonin synthesis in the brain depends on different factors: free plasma tryptophan concentrations, the rate at which tryptophan crosses the blood-brain barrier (BBB), the affinity of tryptophan to the transport protein, the enzymatic activity of tryptophan hydroxylase and the kynurenine pathway that metabolizes more than 90% of tryptophan. Any interference with any or all of the above factors could influence the availability of serotonin.[13–15]

Disturbed transport of tryptophan across the erythrocyte membranes in depressed patients[16–18] and aberrant transport of amino acids such as tyrosine and alanine across the membranes of fibroblasts obtained from patients with schizophrenia and autism is a repeated finding in many studies.[19–25] The reason for aberrant transport of amino acids across the plasma membranes in psychiatric patients has not been identified; some studies have shown abnormal expression and dysfunctional amino acid transporters resulting in disturbed neurotransmission.[26]

Our group has functionally characterized tyrosine transport in fibroblasts and shown that tyrosine is transported into human fibroblasts mainly via the sodium (Na^+) independent system-L and the Na^+ dependent system-A.[27] System-L consists of four isoforms LAT1,[28] LAT2,[29] LAT3[30] and LAT4[31] and these transporters are widely expressed in the body and are present in both BBB and fibroblasts.[32] A recent study by our group has shown that tyrosine is mainly transported through the LAT1 isoform of system-L[33] that is present in the BBB.[34,35] In the same study it was shown that both tyrosine and alanine have affinity for the LAT1 and LAT2 isoforms of system-L. Tyrosine is also transported via ATA2, one of the isoforms of system-A,[33] that is present at the BBB[36] and in fibroblasts.[27,33]

Transport of tryptophan across the plasma membranes differ between cell types in different cell lines.[37] Presence of two tryptophan transport systems in human fibroblasts were reported and that the kinetic parameters V_{max} and K_m differed between them.[38] System-1 was shown to have high affinity and low V_{max} for tryptophan, while system-2 was shown to have lower affinity and higher V_{max} for tryptophan. It was shown that the transport of tryptophan at low substrate concentrations was Na^+ dependent while the transport at high substrate concentration was Na^+ independent.[38] In a recent study, a novel amino acid transporter with high affinity (K_m value in nanomolar range) and an unusual selectivity for tryptophan in human monocyte-derived macrophages was described.[39] It was indicated that tryptophan transport across the brush border of placenta is through the LAT1 isoform of system-L, while tryptophan transport across the basal membrane of the placenta is through LAT2 isoform of system-L and system-y^+L.[37]

The tryptophan transport with respect to the isoforms of system-A and L and the tryptophan affinity to other transporters have not been fully characterized in human fibroblasts. The specific aims of the present study were to functionally characterize the tryptophan transport and to identify the main transporters of tryptophan in fibroblast cell lines from healthy controls.

Materials and Methods

Materials

Fibroblast cell lines obtained from healthy controls were used. Three randomly selected fibroblast cell lines (n = 3) obtained from a Biobank[33] were used to study

Na^+ dependency of tryptophan transport, to determine tryptophan transport at low and high concentrations in the presence and absence of different inhibitors and to determine tryptophan kinetic parameters at low and high concentration ranges. One cell line (n = 1) was used to study the tryptophan transport in the presence of tryptophan itself as a competitive inhibitor. The ethical committee at Karolinska Institute, Stockholm, Sweden approved the study.

Cell culture growth media, antibiotics, growth media supplements and fetal bovine serum (FBS) were obtained from Gibco Invitrogen cell culture (Sweden). ^3H (5)-L-tryptophan with specific activity 30 Ci/mmol was obtained from Larodan Fine Chemicals AB (Malmö, Sweden). D-Glucose was obtained from Ambresco (Ohio, USA) and phosphate buffered saline (PBS) was from The National Veterinary Institute (SVA) (Uppsala, Sweden). All chemicals, inhibitors and amino acids were purchased from Sigma-Aldrich (Stockholm, Sweden). Cell culturing flasks and 24 multi-well trays were purchased from Costar Europe Ltd. Micro-well plates used for protein determination were purchased from Nunc (Roskilde, Denmark). Liquid scintillation cocktail (Optiphase Hi Safe-3) and the liquid scintillation counter (Winspectral 1414) were purchased from PerkinElmer Life Sciences, USA. All amino acid solutions and inhibitor solutions were made with phosphate buffer saline solution (PBS) or with sodium free choline HEPES buffer and the pH was maintained between 7.35 and 7.4.

Methods

Cell culturing

Fibroblast cells were cultured in minimal essential medium containing 10% FBS, L-glutamine (2 mM/L), penicillin (100 mg/ml), streptomycin (100 mg/ml) and Amnio-Max™. Cells were maintained in a humidified atmosphere at a temperature of 37 °C with 5% CO_2 in air. Cells were grown in tissue culture flasks for confluence and were seeded into 2 cm^2 multiwell plates to carry out the measurements. Cells in the multiplates were grown for approximately 5 days to attain confluence. Cell lines between the 4th and the 21st passage (number of splitting) were used for the experiments.

Uptake studies

To functionally characterize the tryptophan transport at low concentration, fibroblasts cultured in multiwell plates were washed twice with PBS containing calcium and magnesium. The cells were then pre-incubated for one hour at 37°C in PBS with 1% D-glucose to deplete the cells of endogenous amino acids. The preincubation medium was removed and the fibroblast cells were incubated with uptake solution by using the cluster tray method[23,40] for 5 minutes at 37°C. The uptake solution contained 50 nM of ^3H (5)-L-tryptophan in combination with excess concentrations (50 µM) of different inhibitors (Table 1). The reaction was terminated by rapidly washing the cells with ice cold PBS twice. The cells were then lysed for 60 minutes by using

Table 1. Inhibitors and their selectivity's to the isoforms of both system-L and -A and other amino acid transporters and their effect on tryptophan uptake at low concentration (50 nM) in fibroblast cell lines from healthy controls (n = 3).

Inhibitors	Selectivity of inhibitors (reference number)	Functional transport systems after inhibition	Tryptophan uptake[a]
MeAIB	System-A[42]	System-L + other[b]	95.8 (7.1)[NS]
BCH	System-L[30]	System-A + other[b]	23.4 (5.1)**
NEM	LAT2, LAT3, LAT4[30,31]	System-A and LAT1 + other[b]	97.9 (12.7)[NS]
Tyrosine	ATA2, System-L[39]	y^+L + other[b]	18.2 (2.9)**
Phenylalanine	System-L, b^{0+AT}[39]	System-A, y^+L + other[b]	16.5 (2.3)**
Tryptophan	Tryptophan transporters[39]	Other[b]	10.6 (2.0)**
1MT	Tryptophan transporters[39]	Other[b]	12.0 (1.4)**
D-Methionine	LAT1[44]	System-A, LAT2, LAT3, LAT4 + other[b]	20.6 (3.4)**
Leucine	ATA3, System-L, y^+L[37]	ATA1, ATA2, b^{0+AT} + other[b]	17.7 (2.8)**
Lysine	ATA3, b^{0+AT}, y^+L[37]	ATA1, ATA2, system-L + other[b]	82.3 (27.3)[NS]

Notes: [a]The values of tryptophan uptake are presented as percentages (mean (SD)) of tryptophan uptake, compared to the percentage of tryptophan uptake in the absence of inhibitors set to 100%; [b]Other: Undefined transport system or systems. $^{**}P < 0.005$.
Abbreviations: NS, not significant; LAT1, LAT2, LAT3 and LAT4, isoforms of system-L; ATA2, isoform of system-A; MeAIB, methyl-aminoisobutyric acid; BCH, 2-aminobicyclo heptane-2-carboxylic acid; NEM, N-ethyl maleimide; 1MT, 1-methyl-L-tryptophan.

0.5 M sodium hydroxide (NaOH). An aliquot from the cell lysate was mixed with scintillation cocktail and the radioactivity was assayed by liquid scintillation counting. All assays were performed in triplicates. The uptake of tryptophan was correlated to total amount of protein in each well, determined by Bradford multi-well method[41] using bovine serum albumin as standard.

To functionally characterize the tryptophan transport at high concentration, fibroblasts were incubated with 0.5 mM of unlabelled L-tryptophan, ^{3}H (5)-L-tryptophan in the presence or absence of excess concentrations (2.5 mM) of different inhibitors or combinations of inhibitors (Table 2) using the same procedure as mentioned above.

Competitive inhibitors (transporter selective substrates) such as methyl-aminoisobutyric acid (MeAIB),[42] 2-aminobicyclo heptane-2-carboxylic acid (BCH),[43] D-methionine,[44] tryptophan, tyrosine, 1-methyl-L-tryptophan (1MT),[39] leucine,[37] lysine,[37] *N*-ethylmaleimide (NEM)[31,43] or combinations of the above in excess concentration (50 μM or 2.5 mM) were used to inhibit system A and/or L and/or their selective isoforms or other transporters in order to study the tryptophan transport ability of uninhibited amino acid transporters. An overview of single and combinations of inhibitors and their selectivities for different transport systems are described in Tables 1 and 2.

Sodium dependency

To test the sodium dependency of tryptophan transport at high (0.5 mM) and low (50 nM) concentrations, confluent fibroblasts were incubated for 5 min with 0.5 mM of unlabelled L-tryptophan and ^{3}H (5)-L-tryptophan or with 50 nM of ^{3}H (5)-L-tryptophan. The uptake solutions were prepared either with PBS or with sodium free choline HEPES buffer. Termination of uptake assay and analysis was performed as mentioned above.

Tryptophan Kinetics

Tryptophan kinetic parameters (V_{max} and K_m) at low and high concentrations were determined by incubating the fibroblasts in the multi well plate for 1 minute at 37 °C together with high concentration range (0.75 mM-2 mM) and low concentration range (5 μM–500 μM) of ^{3}H (5)-L-tryptophan. Termination of uptake assay and analysis was performed as mentioned above.

Calculations

Uptake of tryptophan in nmol/min/mg protein or pmol/min/mg protein was obtained from the average

Table 2. Inhibitors, combinations of inhibitors, their selectivities' for the isoforms of system-L and -A amino acid transporters and their effect on tryptophan uptake at high concentration (0.5 mM) in fibroblast cell lines from healthy controls (n = 3).

Inhibitors	Selectivity of inhibitors (reference number)	Functional transport systems after inhibition	Tryptophan uptake[a]
MeAIB	System-A[42]	System-L + other[b]	98.0 (3.2)[NS]
BCH	System-L[30]	System-A + other[b]	63.4 (6.9)*
NEM	LAT2, LAT3, LAT4[30,31]	System-A and LAT1 + other[b]	78.9 (16.6)[NS]
D-methionine	LAT1[44]	System-A, LAT2, LAT3, LAT4 + other[b]	60.8 (7.6)*
Tryptophan	Tryptophan transporters[39]	Other[b]	47.6[1]
1MT	Tryptophan transporters[39]	Other[b]	48.6 (7.3)*
MeAIB + BCH	ATA2 + system-L	Other[b]	54.6 (7.9)*
MLT + MeAIB	Tryptophan transporters + system-A	Other[b]	47.4 (4.7)**
MLT + BCH	Tryptophan transporters + system-L	Other[b]	47.4 (5.6)**
MLT + D-Methionine	Tryptophan transporters + LAT1	Other[b]	45.9 (6.3)**
MLT + NEM	Tryptophan transporters + LAT2, LAT3, LAT4	Other[b]	29.2 (5.1)**

Notes: [a]The values of tryptophan uptake in the presence of inhibitors are presented as percentage (mean (SD)) of tryptophan uptake, compared to the percentage of tryptophan uptake in the absence of inhibitors set to 100%; [b]Other: Undefined transport system or systems. [1]One cell line (n = 1) was used to carry out the experiment. *$P < 0.02$, **$P < 0.005$.

Abbreviations: NS, not significant; LAT1, LAT2, LAT3 and LAT4, isoforms of system-L; ATA2, isoform of system-A; MeAIB, methyl-aminoisobutyric acid; BCH, 2-aminobicyclo heptane-2-carboxylic acid; NEM, N-ethyl maleimide; 1MT, 1-methyl-L-tryptophan.

of counts per minute correlated to the amount of protein. The uptake values of tryptophan are presented as percentages (%) of tryptophan uptake, relative to tryptophan uptake in the absence of inhibitors set to 100%. For uptake assays, each experiment was performed in triplicates.

Kinetic parameters (V_{max} and K_m) of tryptophan uptake were determined according to Michaelis-Menten's equation and the double reciprocal method of Lineweaver and Burk. In short, the initial rates of uptake are plotted against the low and high concentration ranges to obtain the diffusion constant (K_d) as the slope of regression. The inverse values of uptake, corrected for K_d, are then fitted to a straight line against the inverted values of the concentrations. The straight line intercepting at the Y-axis gives the $1/V_{max}$ value and the X-axis intercept gives the $-1/K_m$ value. V_{max} is the maximal transport capacity of the carrier-mediated process (nmol/min/mg protein) and K_m is the affinity constant (the concentration at half-saturation; µmol/l). Each experiment was performed in duplicates.

Statistical analysis

Kinetic parameters are presented as means with standard deviations (SD). The values of tryptophan uptake and inhibition in the presence and absence of inhibitors and sodium are presented as percentage (%) of tryptophan uptake. The percentage uptake values of tryptophan in the presence or absence of inhibitors, sodium and kinetic parameters of tryptophan at low and high concentration ranges were normally distributed when tested with the Shapiro-Wilk test. Paired samples *t*-test was used to compare the percentage uptake values of tryptophan at different conditions of inhibition to uptake of tryptophan without inhibition, to compare uptake values of tryptophan in the presence and absence of sodium and to compare the kinetic parameters of tryptophan at low and high concentration ranges. A *P*-value below 0.05 was considered to denote statistical significance.

Results

Effect of sodium on uptake of tryptophan

The uptake of tryptophan at high concentration (0.5 mM) was lowered by 26.2% ($P = 0.026$) in the absence of Na^+ ions in the uptake medium. Tryptophan uptake at low concentrations (50 nM) was lowered by 71.0% ($P = 0.035$) in the absence of Na^+ ions in the uptake medium (Fig. 1).

Tryptophan kinetics at low and high concentration ranges

The kinetic parameters for tryptophan transport in the low concentration range (5 µM–500 µM) resulted in a V_{max} of 6.5 (1.9) nmol/min/mg protein and a K_m of 17.1 (8.5) µmol/L. The kinetic parameters for tryptophan transport in the high concentration range (0.75 mM–2 mM) was for V_{max} 8.8 (2.2) nmol/min/mg protein and for K_m 459.3 (138.7) µmol/L. Thus, the transport of tryptophan at low concentrations had a lower V_{max} ($P = 0.030$) and around 25 fold higher affinity ($P = 0.029$) (Fig. 2) in comparison to tryptophan transport at high concentration range (Fig. 2 insert).

Effect of different inhibitors on uptake of low concentration of tryptophan (50 nM)

Uptake of tryptophan at the low concentration (50 nM) was studied in the presence of excess concentration (50 µM) of different inhibitors. Uptake of tryptophan in the presence of MeAIB was inhibited by only 4.2% (Table 1).

Uptake of tryptophan in the presence of BCH was 23.4% and NEM inhibited around 2% of tryptophan uptake, which demonstrates a less specific role of the isoforms LAT2, LAT3, and LAT4 in the uptake of tryptophan at low concentrations (Table 1).

Tyrosine, a substrate for System-A (ATA2) and system-L (LAT1, LAT2) inhibited around 82% of

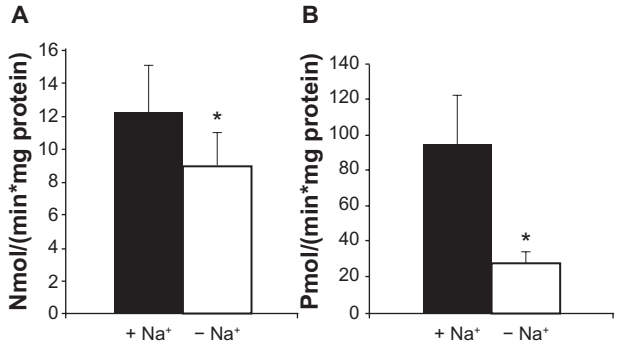

Figure 1. Uptake of tryptophan into fibroblast cell lines from three (n = 3) healthy controls in the presence and absence of sodium (Na^+) ions. Absence of Na^+ ions in the uptake medium resulted in a 26.2% decrease in uptake of tryptophan (0.5 mM) ($P = 0.026$) (A) and a 71.0% decrease in the uptake of tryptophan (50 nM) ($P = 0.035$) (B), when compared to uptake of tryptophan in the presence of Na^+ ions in the uptake medium.

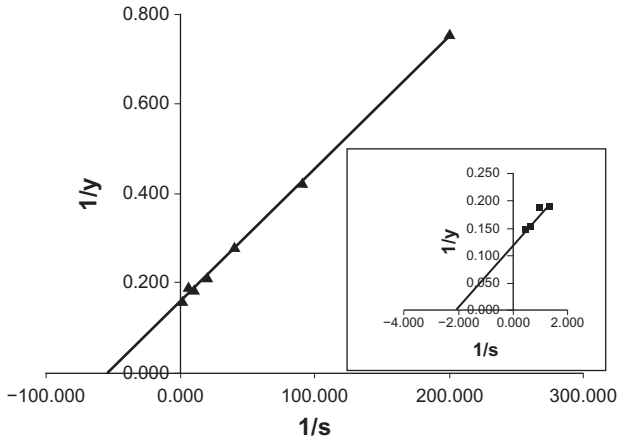

Figure 2. Division of kinetic analysis of the tryptophan uptake at high (0.75–2 mM) and low (5–500 μM) concentration ranges. Initial rates of uptake corrected for the diffusion constant Y (nmol/min/mg protein) is plotted against low and high (insert) substrate concentration S (mM) according to the double reciprocal method of Lineweaver and Burk. Each point represents the means of six determinations of three cell lines.

tryptophan uptake. Phenylalanine a substrate for LAT1, LAT2 and system-b^{0+AT} inhibited around 83.5% of tryptophan uptake (Table 1).

Around 90% of tryptophan uptake was inhibited by both tryptophan and 1MT, a tryptophan analogue. D-methionine inhibited tryptophan uptake by 80%, which indicates that the LAT1 isoform of system-L is the major (approximately 80%) transporter of tryptophan at low concentrations. Leucine, a substrate for ATA3, system-L and system-y^+L inhibited around 82.3% of tryptophan uptake (Table 1). When lysine, a substrate for ATA3, System-b^{0+AT} and system- y^+L, was used as an inhibitor it resulted in around 17.7% inhibition of tryptophan uptake. These results indicate that system-L and other undefined transporters together transport the major part of tryptophan at low concentrations.

Effect of different inhibitors on uptake of high concentration of tryptophan (0.5 mM)

Uptake of tryptophan at the high concentration (0.5 mM) was studied in the presence of excess concentrations (2.5 mM) of different inhibitors or combinations of inhibitors (Table 2).

MeAIB, a system-A inhibitor, did not result in any significant inhibition of tryptophan uptake. In the presence of BCH, a system-L inhibitor, 36.6% of tryptophan uptake was inhibited, indicating that 63.4% of tryptophan at high concentration is transported by transport systems other than system-L. When LAT1, one of the isoforms of system-L, was inhibited by D-methionine it resulted in 39.2% inhibition of tryptophan uptake. When LAT2, LAT3 and LAT4, isoforms of systems-L, where inhibited by NEM, it resulted in around 21.1% inhibition of tryptophan uptake (Table 2).

When using 1MT as an inhibitor it resulted in an inhibition of tryptophan uptake by 51.4%. To check if 1MT has the same inhibitory effect as tryptophan, tryptophan was used as an inhibitor at a concentration of 2.5 mM to inhibit ^3H (5)-L-tryptophan uptake (0.5 mM) and it resulted in an inhibition of 52.4% of tryptophan uptake (Table 2).

Effect of combinations of inhibitors on uptake of high concentration of tryptophan (0.5 mM)

MeAIB and BCH together inhibited the tryptophan uptake by 45.4%, indicating that 54.6% of tryptophan is transported through a system other than system-A and system-L (Table 2).

When using 1MT and MeAIB in combination it resulted in 52.6% inhibition of tryptophan uptake and when 1MT and BCH were used in combination 52.6% of tryptophan uptake was inhibited. This also indicates that approximately 50% of tryptophan is transported through a system other than system-A and system-L (Table 2).

1MT and D-methionine together inhibited around 54.1% of tryptophan uptake, indicating that LAT2, LAT3, LAT4 and a system other than system-A and system-L (LAT1) is responsible for around 45.9% of tryptophan uptake (Table 2).

1MT and NEM together inhibited around 70.8% of tryptophan uptake, indicating that the system other than system-A and system-L and LAT1 accounts for 29.2% of tryptophan uptake. These results also show that NEM slightly inhibits other tryptophan specific systems than system-A and system-L (Table 2).

Discussion

Functional characterization of tryptophan transport at both low and high concentrations was carried out in the present study. It was demonstrated that the transport of tryptophan differed at low vs. high substrate concentrations and that low and high tryptophan concentrations differed regarding Na^+ dependency,

kinetic parameters, and effects on tryptophan uptake of different transporter inhibitors.

Uptake of tryptophan at high concentration (0.5 mM) is mostly Na^+ independent, while tryptophan uptake at low concentration (50 nM) is mainly Na^+ dependent. Absence of Na^+ ions in the uptake solution resulted in around 26% inhibition of tryptophan uptake at the high concentration (0.5 mM) and a 71% inhibition of tryptophan uptake at the low concentration (50 nM). These results are in accordance with the study by Groth et al, 1972,[38] which demonstrated that tryptophan uptake in the absence of Na^+ is inhibited at low concentrations but not at high concentrations.

The kinetic parameters of tryptophan differed between low and high concentrations.

Tryptophan transport at the low concentration range (5 µM–500 µM) has a low V_{max} and higher affinity indicated by a low K_m value, while tryptophan transport at the high concentration range (0.75 mM–2 mM) has a higher V_{max} and lower affinity indicated by a higher K_m value. This observation is also in accordance with Groth et al, 1972,[38] and this indicates the involvement of more than one transporter in the uptake of tryptophan.

Uptake of tryptophan at low concentration (50 nM) was mainly through the system-L, since the system-L inhibitor BCH inhibited around 76.6% of tryptophan uptake. Confirmation of this finding was demonstrated by obtaining approximately the same percentage of inhibition of tryptophan uptake in the presence of tyrosine, phenylalanine and leucine, which also are inhibitors for system-L. The LAT1 isoform of system-L is the major transporter of tryptophan at low concentration, as demonstrated when using D-methionine as an inhibitor, which resulted in around 80% inhibition of tryptophan uptake. In the presence of MeAIB, the inhibitor for system-A, and NEM, the inhibitor for LAT2, LAT3 and LAT4, almost no inhibition of the uptake of tryptophan at low concentration was shown, which indicates minor role of these transporters in the uptake of tryptophan at low concentrations. Tyrosine, which is mainly transported by the LAT1 isoform and to a smaller extent by the ATA2 isoform,[33] when used as an inhibitor it resulted in around 81% inhibition of tryptophan uptake showing that tryptophan transport at low concentrations may be in similar fashion as tyrosine transport.

The uptake of tryptophan at low concentration is with high affinity, is mainly Na^+ dependent and has different inhibitory effects of the inhibitors, when compared to tryptophan uptake at high concentration. This may indicate the presence of a hitherto unidentified transporter functioning at low concentrations of tryptophan. Alternatively, it could be a variant of a known transporter that has different functional properties due to an alteration in the structure of the transporter protein. As an example, the functional properties of system-L are known to alter due to different light-chain subunits.[39,45]

Tryptophan uptake at high concentration (0.5 mM) through system-L was around 36.6%. This is demonstrated by using BCH as system-L selective inhibitor. System-A has no specific role in the uptake of tryptophan at high concentration, which is demonstrated by using MeAIB as a system-A inhibitor. The role of the isoforms (LAT1, LAT2, LAT3, and LAT4) of system-L in uptake of tryptophan was also studied in the present study by using the inhibitors of the isoforms. D-methionine used as an inhibitor for LAT1 isoform showed that the uptake of tryptophan (at high concentration) through LAT1 was around 40%. The role of LAT2, LAT3, and LAT4 in the uptake of tryptophan (at high concentration) was around 21%, which was demonstrated by using NEM as an inhibitor for LAT2, LAT3, and LAT4 isoforms. The percentage of tryptophan uptake by individual isoforms of system-L are obtained when each isoform is inhibited by a inhibitor, but when system-L is working in the absence of inhibitors the isoforms of system-L could be working in parallel with different transport capacities.

When using either 1MT, which is a close structural analogue of tryptophan, or tryptophan as inhibitors at a concentration of 2.5 mM, it resulted in only about 48.6% uptake of tryptophan (at high concentration). This shows that the rest of tryptophan uptake is probably through a transport system that transports higher concentrations of tryptophan ie, more than 2.5 mM. This transport system seems to be different from system-A and system-L as it was not inhibited completely by either MeAIB or BCH. This transport system is probably a Na^+ independent system since uptake of tryptophan at high concentration was found to be mainly Na^+ independent. The reason for tryptophan and 1MT not being able to completely

inhibit the uptake of tryptophan could be that the transport system that transports tryptophan at high concentrations is not totally saturated at a competitive inhibitor concentration of 2.5 mM.

The results of the present study indicate that the uptake of tryptophan into human fibroblasts is subserved by different transport systems at different substrate concentrations. Tryptophan uptake at different concentrations is different in terms of Na^+ dependency, kinetic parameters, substrate selectivity, and inhibitory effects of different competitive inhibitors or transporter selective inhibitors. Moreover, the present findings show that tryptophan transport through system-L is mainly facilitated through the LAT1 isoform, at both low and high concentrations of tryptophan. However, these observations are only based on functional activity of the transporters and not based on data at the protein level. Hence, further biochemical studies with more selective or specific transporter inhibitors are necessary to understand the transport of tryptophan across cell membranes, but such inhibitors are presently not available.

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Disclosures

This manuscript has been read and approved by all authors. This paper is unique and is not under consideration by any other publication and has not been published elsewhere. The authors and peer reviewers report no conflicts of interest. The authors confirm that they have permission to reproduce any copyrighted material.

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