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L-TRYPTOPHAN TRANSPORT IN HUMAN RED BLOOD CELLS

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Summary

1. The initial rate of L-tryptophan uptake into human red cells as a function of the concentration in the medium was studied at 25 and 37°C.

2. Uptake was resolved into saturable and linear components. Kinetic constants at 37°C were, apparent K_m 1.55 mM, V 0.145 mmol/l cell water per min and apparent K_D 0.0103 min^{-1}.

3. Inhibitor studies showed that L-tryptophan transport via the saturable component represents uptake by a previously unidentified transport system, designated the T-system. The linear component represents L-tryptophan transport via the L-system.

4. The substrate specificity of the T-system is apparently limited to the aromatic amino acids, L- and D-tryptophan, L-tyrosine and L-phenylalanine. The main route of L-phenylalanine transport is, however, via the L-system. L-Tyrosine is partly transported via the T-system, partly via the L-system.

Introduction

So far, at least three discrete transport systems for amino acids have been described in human red cells. The major pathway for large neutral amino acids is designated the L-system [1], and leucine transport by this route has been characterized kinetically in some detail [2—4]. There is also a system for dibasic amino acids (the Ly^+-system) whose principal substrates are ornithine, lysine and arginine [4,5]. Finally, there is a low-capacity, high-affinity Na^+-

Abbreviations: Hepes, *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulphonic acid; Tes, *N*-tris[hydroxymethyl]methyl-2-aminoethanesulphonic acid.

dependent system specific for small neutral amino acids [6,7]. Although these broad definitions of amino acid transport systems have been made, there is still a lack of detailed information on the transport of particular amino acids by the red cell (e.g., see Ref. 8). In particular, data on tryptophan transport are limited, even though Christensen [9] made the suggestion that this amino acid might behave anomalously as a substrate for the L-system. Since tryptophan is an important precursor for serotonin biosynthesis in the central nervous sytem, its transport across the blood-brain barrier has received considerable attention (for a review see Ref. 10). It therefore seemed worthwhile to characterize in detail the mechanism(s) by which this amino acid is transported into red cells.

Preliminary kinetic studies from our two independent laboratories indicated a major, selective tryptophan pathway distinct from the L-system in human red cells [11,12]. In the present paper we combine and extend these observations to define this new transport system whose specificity is directed towards the aromatic amino acids tryptophan, tyrosine and phenylalanine.

Materials and Methods

Materials. Amino acids were purchased from Sigma Chemical Co., St. Louis, MO. All amino acids are L-amino acids unless otherwise stated. Phloretin was obtained from K and K Biochemicals, Plainview, NY. ^{14}C-labelled amino acids were obtained from The Radiochemical Centre, Amersham, U.K. as L-[*methylene*-^{14}C]tryptophan, L-[*carboxyl*-^{14}C]tyrosine, L-[U-^{14}C]leucine and L-[U-^{14}C]phenylalanine. Radiochemical purity was assessed by thin-layer chromatography in butanol/water/acetic acid.

Uptake experiments. Heparinized blood was taken from healthy subjects and washed three times in incubation medium. The buffy coat was discarded and cell suspension were used within 24 h.

Transport experiments in Copenhagen and Cambridge were performed by different methods. In Copenhagen, amino acid uptake was measured at 25°C by a dibutyl phthalate separation method [11] using an incubation medium composed of 138 mM NaCl, 5 mM KCl, 1 mM $MgCl_2$, 1 mM Na_2HPO_4, 10 mM Hepes and 7.5 mM Tes (pH 7.4 at 25°C). Experiments in Cambridge were performed at 37°C by an isotonic $MgCl_2$ wash procedure [13] with an incubation medium containing 140 mM NaCl, 5 mM KCl, 2 mM $MgCl_2$ and 15 mM Tris (pH 7.4 at 37°C). Isoosmolality at various amino acid concentrations was maintained by adjusting the concentration of NaCl in the medium. All values refer to initial uptake rates (maximum intracellular concentrations did not exceed 5% of extracellular levels) [11,13].

Analysis of concentration-dependence curves. Uptake data were fitted for a three-parameter model (i.e., a saturable component in parallel with a linear uptake component):

$$v = VS/(K_m + S) + K_D S$$

where v is the unidirectional influx (mmol/l cell water per min), V the maximum velocity (mmol/l cell water per min), K_m the concentration at half-maximum velocity (mM), K_D (min^{-1}) the rate constant for the linear compo-

nent and S (mM) the concentration of the substrate. Values (± S.D. (n)) for the various parameters were obtained by a non-linear least-square regression analysis using a Gauss-Newton algorithm [14], where n represents the number of individual substrate concentrations used in each experiment. Calculations were performed by IBM 370/165 computer.

Estimates of apparent inhibitor constants K_i (mM) were obtained assuming simple competitive inhibition:

$$v = V \Big/ \left(1 + \frac{K_m}{S}\left(1 + \frac{I}{K_i}\right)\right)$$

where I (mM) is the concentration of the inhibitor. Apparent permeability coefficients (P in cm/s) for tryptophan uptake by human red cells were calculated from the equation:

v (mmol/cm^2 per s) = $P \cdot S$ (mmol/cm^3)

assuming values of $1.42 \cdot 10^{-6}$ cm^2 and $5.3 \cdot 10^{-11}$ cm^3 for the cell surface area and intracellular water volume, respectively.

Results and Discussion

Previous kinetic studies have characterized a major saturable pathway for the transport of large neutral amino acids in the human red cell (the L-system) [1—4,8,15]. Amino acids known to be transported by this route include leucine, phenylalanine, valine, methionine and to a lesser extent alanine and cysteine. Besides its substrate specificity, the L-system is characterized by a high-exchange capacity, lack of sodium dependence and a weak pH dependence. Similar amino acid transport systems have been defined in a variety of cell types and tissues (e.g., Ehrlich ascites [16] and other tissue culture cells [17], placenta [18], kidney [19] and blood-brain barrier [20]). These systems have a broad substrate specificity including leucine, phenylalanine, tyrosine and tryptophan [20]. Although varying between tissues, the kinetic constants for these amino acids are similar for each type of cell. This contrasts with the situation in red cells, where tyrosine and tryptophan are transported considerably less readily than leucine and phenylalanine, and suggests that the red cell L-system is unusually selective [9]. The present aim was to establish the route(s) by which aromatic amino acids, and in particular tryptophan, are transported into human red cells. The results presented in this paper represent data obtained in two independent studies (R.R. in Copenhagen and J.D.Y. and J.C.E. in Cambridge).

Concentration dependence of tryptophan uptake

Tryptophan uptake experiments from the two laboratories over the concentration range 0.005—50 mM are shown in Fig. 1a—f. The experiments at 25° C were performed by a dibutylphthalate method in Copenhagen, whilst the 37° C experiments were carried out in Cambridge by a $MgCl_2$ wash technique. Both sets of data are consistent with two components being present, and were resolved into linear and saturable uptake routes according to the equation

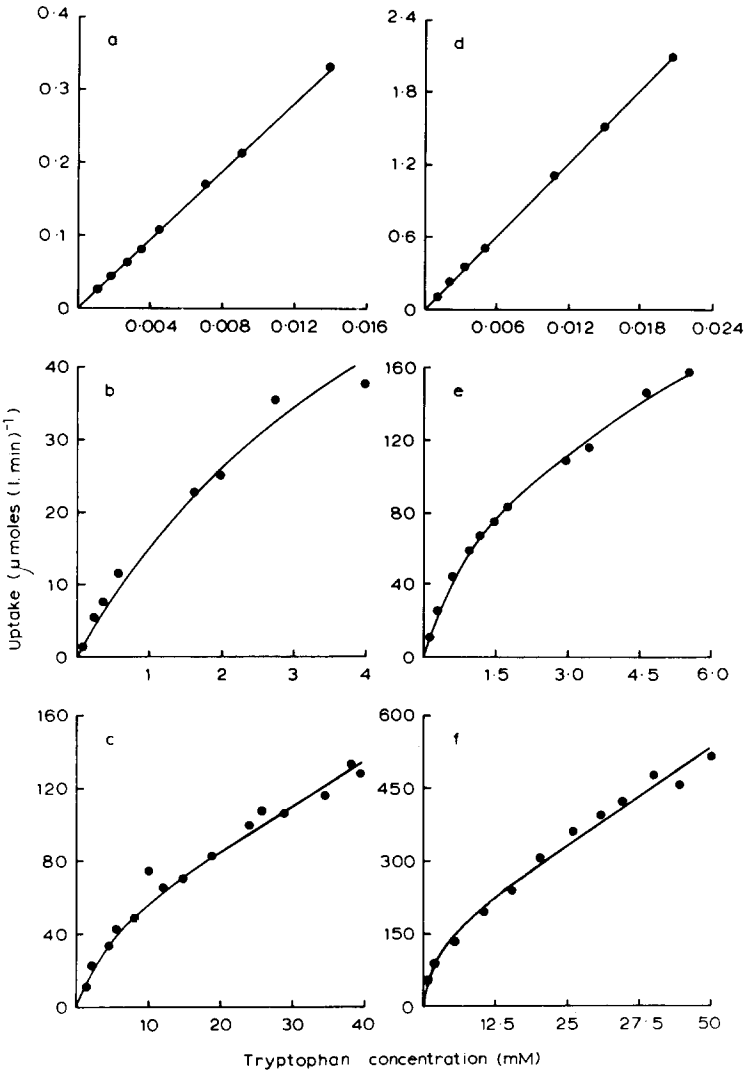

Fig. 1. Concentration dependence of tryptophan uptake by human red cells. Initial rates of L-[*methylene*-^{14}C]tryptophan uptake at 25°C (a—c) and 37°C (d and e) were measured as previously described (25°C, Ref. 11; 37°C, Ref. 13).

$$v = VS/(K_m + S) + K_D S$$

Fitted parameters for these and other experiments are summarized in Table I. A control experiment performed in Copenhagen by the dibutylphthalate method at 37°C gave apparent K_m and V values (± S.D.) of 4.38 ± 2.70 mM and 0.268 ± 0.108 mmol/l cell water per min, respectively, with an apparent K_D of 0.0110 ± 0.0034 min^{-1} (n = 14). There is, therefore, good agreement between the two laboratories. Although V and apparent K_D showed a marked temperature dependence (3- and 5.5-fold higher, respectively, at 37°C), the apparent K_m values at 25 and 37°C were not significantly different. The relatively large

TABLE I

KINETIC CONSTANTS FOR TRYPTOPHAN UPTAKE BY HUMAN RED CELLS

Kinetic constants were obtained from non-linear least-square regression analysis of initial uptake rate vs. extracellular tryptophan concentration. Values are ±S.D. (n) where n represents the number of individual substrate concentrations used in each experiment. Other experimental details are given in the text. A—C, different donors. The arithmetic mean values (±S.E.) for the three 37°C experiments are V 0.145 ± 0.022 mmol/l cell water per min, apparent K_m 1.55 ± 0.44 mM, apparent K_D 0.0103 ± 0.0015 min^{-1}.

	V mmol/l cell water per min	K_m mM	K_D min^{-1}	n
25°C	0.059 ± 0.011	3.53 ± 1.31	0.0019 ± 0.0003	20
37°C				
A	0.179 ± 0.023	1.51 ± 0.52	0.0102 ± 0.0009	12
B	0.152 ± 0.019	2.34 ± 0.63	0.0078 ± 0.0005	24
C	0.105 ± 0.050	0.81 ± 0.11	0.0129 ± 0.0003	12

errors associated with apparent K_m and V estimates arise because these parameters are sensitive to small changes in apparent K_D. At physiological substrate concentrations (less than 0.1 mM), tryptophan is transported predominantly by the saturable route and has an apparent membrane permeability coefficient (P) of $1.03 \cdot 10^{-8}$ cm/s at 25°C or $5.80 \cdot 10^{-8}$ cm/s at 37°C.

When tryptophan uptake (25°C, haematocrit 0.5%) was measured at two concentrations (0.2 and 25 mM) in the presence of phloretin (0.16 and 0.21 mM), the uptake was inhibited 95 and 90%, respectively, indicating that phloretin is a strong inhibitor of both components of tryptophan transport.

To test whether either of these tryptophan transport components was Na^+ dependent, tryptophan uptake was measured at 4 mM (25°C) and 100 μM and 10 mM (37°C) in normal and Na^+-free (choline-replaced) media. No difference between the uptake rates in the two solutions was found, indicating no Na^+-dependent flux.

Amino acid inhibition studies

Saturable component. The effectiveness of various amino acids as inhibitors of tryptophan uptake was determined at both 25 and 37°C. In the first series of experiments (25°C) the concentrations used were: 0.1 mM tryptophan; 30 mM inhibitor (except 2 mM tyrosine, its maximum solubility). At the higher temperature the tryptophan concentration was 0.2 mM, inhibitor 10 mM. At these substrate levels 85—90% of the tryptophan uptake is via the saturable component. The results are summarized in Table II. Two amino acids, phenylalanine and D-tryptophan, gave marked inhibition of L-tryptophan uptake. Tyrosine inhibited tryptophan influx by 33% despite being present at a 15-fold lower concentration than the other amino acids. In contrast, leucine and valine proved relatively ineffective inhibitors.

These experiments suggest that the saturable component of tryptophan uptake does not represent transport by the L-system. Participation of other known human red cell amino acid transport systems (Ly^+ and ASC) is ruled out by the lack of inhibition of tryptophan uptake by preferred substrates for these systems (lysine and alanine, respectively). Thus, we may conclude that the satu-

TABLE II

EFFECTS OF DIFFERENT AMINO ACIDS ON L-TRYPTOPHAN UPTAKE BY HUMAN RED CELLS

	% Inhibition	
	25°C (0.1 mM tryptophan; 30 mM inhibitor)	37°C (0.2 mM tryptophan; 10 mM inhibitor)
Glycine	0	0
Proline	30	4
Alanine	0	0
α-Amino-*n*-butyrate	—	4
Valine	23	12
Norleucine	—	18
Leucine	24	18
Phenylalanine	92	62
Histidine	12	4
Lysine	0	4
5-Hydroxytryptamine	—	21
Methionine	30	—
D-Tryptophan	85	—
Aspartate	0	—
Tyrosine (2 mM)	33	—
α-Amino-isobutyrate	0	—

Results are the means of two to four separate experiments with cells from a single donor. S.E. in these and subsequent experiments (Tables II—IV) were less than or equal to 10%. See legend to Fig. 1 and Materials and Methods for other experimental details. At these tryptophan concentrations 80—85% of the uptake occurs by the saturable transport route.

rable component of tryptophan uptake represents transport by a previously unidentified system apparently specific for aromatic amino acids (designated the T-system).

Linear component. When tryptophan uptake was measured at various concentrations in the presence of 50 mM leucine (Fig. 2), a typical substrate of the L-system, there was an almost complete suppression of the linear uptake component, whilst the saturable component was virtually unaffected (see also Table III). Further experiments at lower leucine concentrations indicated that the

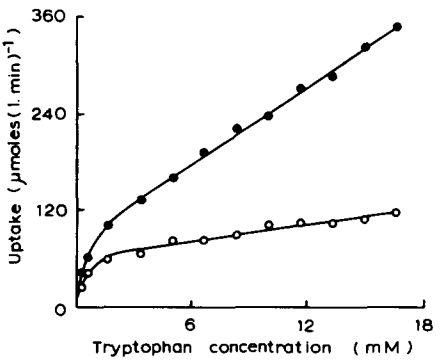

Fig. 2. Concentration dependence of tryptophan uptake by human red cells in the presence and absence of leucine. Initial tryptophan uptake rates at 37°C in the presence (○) and absence (●) of 50 mM leucine were determined as described in Materials and Methods.

apparent K_i for leucine inhibition of the linear component was around 3—6 mM at 37°C, comparable with the apparent K_m for leucine uptake by the L-system at this temperature (5 mM at 37°C, 2 mM at 25°C) [4,8,15]. Additional competition experiments were carried out at 25°C, when (a) L-tryptophan uptake from three different concentrations (0.1, 10 and 25 mM) was measured in the presence of 22.5 mM leucine, methionine or valine, and (b) 0.1, 1, 6 and 15 mM L-tryptophan influx was measured in the presence of 25 mM D-tryptophan. From the results summarized in Table III it is clear that leucine, methionine, and valine mainly inhibit the linear component of L-tryptophan uptake, whereas D-tryptophan inhibits the saturable route. The relatively low inhibition by D-tryptophan at high L-tryptophan concentrations is to be expected assuming competition between the two isomers for the saturable uptake system.

The data suggest that a substantial fraction (greater than 80%) of the linear component of tryptophan transport occurs via the L-system, but with a low affinity. This conclusion was tested further by investigating the ability of tryptophan (2.5—20 mM) to inhibit leucine uptake (2 mM, 37°C). There was a small progressive inhibition with increasing tryptophan concentration, giving 21% inhibition at 20 mM. A further experiment using 0.1 mM leucine and 40 mM tryptophan at 25°C gave 50% inhibition of leucine uptake. These results are consistent with tryptophan having a significant but low affinity for the L-system, the estimated apparent K_i values being approx. 40 and 60 mM at 25 and 37°C, respectively.

Tyrosine and phenylalanine uptake

Since tyrosine seemed to be acting as an inhibitor of the saturable component of tryptophan uptake, its transport and interactions with the T- and L-systems were characterized in more detail. The concentration dependence of tyrosine uptake at 25°C was measured over the range 0—2 mM, its solubility limit. The uptake was linear over the whole concentration range, the absolute transport rate (42 μmol/l cell water per min at 2 mM) being slightly higher than that of tryptophan, and lower than those of typical L-system substrates such as leucine. To attempt to resolve the route(s) responsible for tyrosine uptake,

TABLE III

INHIBITION OF L-TRYPTOPHAN UPTAKE BY LEUCINE, METHIONINE, VALINE AND D-TRYPTOPHAN

Results are the means of duplicate independent experiments at 25°C using cells from one donor. Experimental details are given in the text.

L-Tryptophan concentration (mM)	% inhibition				% of flux via the linear
	Leucine (22.5 mM)	Methionine (22.5 mM)	Valine (22.5 mM)	D-Tryptophan (25 mM)	
0.1	24	30	23	95	10
1.0	—	—	—	88	13
6.0	—	—	—	44	23
10.0	49	51	57	—	30
15.0	—	—	—	26	37
25.0	70	61	64	—	48

measurements were made at 25°C in the presence of leucine and/or tryptophan (Table IV). Tyrosine uptake was markedly inhibited by both leucine and tryptophan, and the effects of the two amino acids were partly additive. Since leucine and tryptophan were both at concentrations above their apparent K_m values for the L- and T-systems, respectively, it is likely that tyrosine uptake at this concentration (0.02 mM) occurs by both systems. When tyrosine (2 mM) was tested on tryptophan uptake (0.02 mM) at 25°C, there was a 61% inhibition and in the complementary experiment tyrosine (2 mM) inhibited leucine uptake (0.02 mM) by 23% (see also Table IV). If these data are analyzed for simple competitive inhibition, we can estimate apparent K_i values for tyrosine as an inhibitor of the L- and T-systems, arriving at values of approx. 7 and 2 mM, respectively, at 25°C.

In contrast to tryptophan and tyrosine, the rate of phenylalanine uptake by human red cells was very rapid (0.211 mmol/l cell water per min at 1 mM). Transport was saturable and conformed to simple Michaelis-Menten kinetics giving apparent K_m and V values similar to those for leucine uptake by the L-system (apparent K_m 2.1 mM, V 0.72 mmol/l cell water per min at 25°C) [15]. In the present context, phenylalanine was an effective inhibitor of saturable tryptophan transport, so we tested the ability of tryptophan (10 mM) to inhibit phenylalanine (0.075 mM) uptake. This gave a 20% inhibition at 25°C. The estimated apparent K_i value for tryptophan on phenylalanine uptake is, therefore, 40 mM. This value is the same as that estimated for tryptophan inhibition of leucine uptake, supporting the view that phenylalanine is mainly transported via the L-system. Cross-inhibition and accelerative exchange diffusion studies, presented elsewhere [15], confirm that the L-system is the major route for phenylalanine transport. Although phenylalanine probably does show a significant affinity for the T-system, the high capacity of the L-system effectively masks transport by this path.

TABLE IV

CROSS-INHIBITION STUDIES WITH TRYPTOPHAN, TYROSINE AND LEUCINE

The uptake of 0.02 mM amino acid at 25°C in the presence of competing amino acids was compared to control experiments without inhibitor. Results are the means of four independent experiments using cells from the same donor. See Materials and Methods for other experimental details.

	Uptake (μmol/l cell water per min)	% inhibition
Tyrosine (0.02 mM)		
Control	1.06	—
10 mM leucine	0.35	67
10 mM tryptophan	0.23	78
10 mM leucine and 10 mM tryptophan	0.06	94
Tryptophan (0.02 mM)		
Control	1.64	—
2 mM tyrosine	0.64	61
Leucine (0.02 mM)		
Control	9.60	—
2 mM tyrosine	7.36	23

Species comparison

The concentration dependence of tryptophan uptake was also investigated in red cells from two other species (cat and sheep). In these animals the uptake was linear, with no evidence of a high-affinity uptake component, suggesting that the T-system was absent from these cells. Transport was 3-fold lower than the non-saturable component found in human cells, the rate (25 mM) being 0.075 mmol/l cell water per min at 37°C in both species. This correlates with previous studies which have shown the L-system to be absent in these species [4]. The basal permeability of the sheep and cat red blood cell membrane may represent simple diffusion of tryptophan through the membrane. It remains to be established whether the T- and L-systems are always present together, or whether these two systems can exist independently.

Conclusions

The present results demonstrate that human red cells possess a previously unknown amino acid transport system (T-system) selective for aromatic amino acids, particularly tryptophan. This system does not require Na^+ and is inhibited by phloretin. It is absent from sheep and cat red cells. The substrate specificity of the T-system overlaps with that of the L-system.

The physiological significance of a T-system in the red cell besides an L-system remains to be studied. The availability of tryptophan in the blood is, however, important for serotonin synthesis in the central nervous system. It is well known that plasma protein binding accounts for an appreciable fraction of the total tryptophan in plasma (80—93% bound; total tryptophan approx. 0.04 mM [21]). Intracellular tryptophan in red cells may also represent an important pool and play a significant role in the body's tryptophan metabolism.

Resolution of transport components with overlapping substrate specificities is a difficult task, even in cells like the erythrocyte where flux studies are methodologically simple and accurate. It is, therefore, possible that the T-system is present in other cells and tissues, but has so far been included under the umbrella of the L-system due to the difficulties of detailed kinetic analysis. For instance, the presence of a distinct T-system in the intestine could explain the transport differences between Hartnup's disease [22,23] and blue diaper syndrome [24]. Our results emphasise the importance of detailed kinetic analysis of even apparently well-characterized systems.

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