BBA 26001

HISTIDINE TRANSPORT INTO ISOLATED ANIMAL CELLS

HALVOR N. CHRISTENSEN

Department of Biological Chemistry, The University of Michigan, Ann Arbor, Mich. (U.S.A.)

(Received April 8th, 1968)

SUMMARY

At pH values where it is predominantly without net charge, histidine in dilute solution divided its uptake by the Ehrlich cell between the Na^+-independent L system and the Na^+-requiring A system. As in every other case studied the L component was partially inhibitable by lysine, partially not. At pH 5, where it is chiefly a cation, an additional component became perceptible, one which could be inhibited by lysine but not by neutral amino acids, which is assigned to the Ly^+ system.

These observations extend generalizations suggesting that the L and Ly^+ systems are associated so that partially competitive inhibition occurs between their respective substrates. An apparent heterogeneity in the interaction between histidine and phenylalanine in the constant-ratio test is probably explained by the role of the Ly^+ system in histidine uptake expected for the cationic form of histidine even at pH 7.4. In various erythrocytes histidine uptake was simpler because of the absence of an A system and the minimal reactivity of histidine with any other Na^+-requiring system. Although it reacted here also both as a cation and a neutral amino acid, interaction between the corresponding transport systems was inconspicuous.

INTRODUCTION

Because its charge changes with pH within a range suitable for the study of transport, histidine presents a special opportunity for testing whether the small list of transport systems described so far for amino acids[1] is incomplete, complete or redundant. Our first observations emphasized its transport by the Ehrlich cell in the form of a neutral amino acid, because the extent of its uptake (like that of glycine or alanine) was increased rather than decreased by the presence of lysine or arginine[2]. Furthermore the extent of histidine accumulation declined as the pH was lowered, much as that of glycine did[2]. A component of uptake in cationic form should become more conspicuous below pH 6, especially since the uptake of lysine is nearly as fast at pH 5 as it is at pH 7.4 (ref. 3). Although the presence of histidine has been observed to inhibit the uptake of lysine, that action was nearly uniform from pH 5 to 7.4, as observed for methionine and other neutral amino acids[3].

On the discovery of the parallel operation of 2 or more distinct systems for the transport of neutral amino acids, histidine was tentatively placed, along with methionine and 1-aminocyclopentane carboxylic acid, among those that divide their uptake more or less evenly between the first 2 systems described, the A and the L

system[4]. Little if any part of the uptake was identified with a second Na^+-requiring system (ASC) described subsequently[5]. In the pigeon red blood cell a similar situation was indicated, which implies that because of the absence of any system corresponding to A, the Na^+-independent system of the L type should account for almost all uptake of neutral histidine[6]. In the mature human, erythrocyte reactivity of histidine with the L type system is relatively weak[7], although we now find it quite characteristic.

We anticipated further that histidine might be especially useful in testing for the reality of a hypothetical transport system ($L^{\circ,+}$) effective for both neutral and cationic amino acids, a system proposed to account for the ability of amino acids of one of these classes to inhibit only a circumscribed portion of the uptake of members of the other class[8]. That behavior could possibly arise from partially competitive inhibition between a system serving for the neutral and one serving for the cationic amino acids, rather than the existence of a distinct system reactive with both classes (see p. 480, ref. 1).

The preliminary assignments listed above have been confirmed and evaluated, but decisive evidence could not be obtained for the distinct existence of the $L^{\circ,+}$ system by the study of the interaction between histidine, lysine and phenylalanine for transport.

METHODS

Uniformly ^{14}C-labeled L-histidine, L-phenylalanine, L-lysine and other labeled natural amino acids were obtained from Schwarz Bioresearch. Corresponding, specifically labeled products from Calbiochem were also used, as previously.

The methods of obtaining cells and of studying uptake by the three cell types have been described elsewhere[5,6]. The human red blood cell was incubated in Krebs–Ringer phosphate medium[6] rather than in the medium of Raker used in earlier work[7]. All optically active amino acids were used in their L forms. Concentrations of Na^+ were set at the same value within each experiment, except where indicated.

The uptake of the labeled histidine showed no measurable "instantaneous" phase to suggest surface fixation, nor was the uptake by thick suspensions of cells disproportionately small, as may occur when adsorbable radioactive impurities are present in the preparation. A correction for instantly fixed ^{14}C, required for a previous lysine preparation, was inconspicuous with present samples.

RESULTS

The Ehrlich cell

Fig. 1 shows that about one third of the uptake of histidine at 1 mM could be inhibited by α-(methylamino)isobutyric acid, and is therefore attributed to the A system[5]. Fig. 2 shows the determination of the kinetic parameters for the portion of histidine uptake abolished by α-(methylamino)isobutyric acid, and for two components of histidine uptake retained in the presence of α-(methylamino)isobutyric acid, one inhibited by lysine, one not. The further addition of phenylalanine in the presence of excess α-(methylamino)isobutyric acid reduced the uptake to a rate of about 0.1 min^{-1} (Fig. 1), which approximates the rate of the so-called non-saturable uptake when the period of observation is 1 min (refs. 4, 9, 10). When phenylalanine

was tested as an inhibitor at various levels, with α-(methylamino)isobutyric acid present at 25 mM, a rectangular hyperbola was described without clear evidence of a more difficultly inhibited component, as would be expected if any substantial uptake of histidine occurred by the ASC system[5]. Fig. 3 provides another demonstration that such a component must be small: The removal of Na^+ from the medium containing α-(methylamino)isobutyric acid had only a small effect on the rate of histidine uptake.

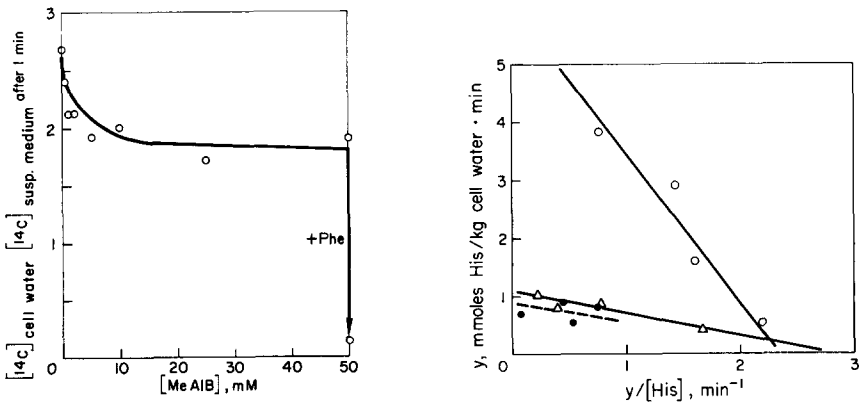


Fig. 1. Component of histidine uptake by the Ehrlich cell sensitive to α-(methylamino)isobutyric acid. 1 mM L-[^{14}C]histidine in Krebs–Ringer bicarbonate medium at pH 7.4 and 37°. Time, 1 min. The vertical arrow shows the degree to which the residual uptake was inhibited by phenylalanine (10 mM).

Fig. 2. Augustinsson plot of kinetics of histidine uptake at pH 7.4. Uptake rate measured for a single lot of cells during 1 min from Krebs–Ringer bicarbonate medium at 37°, $[Na^+]$ = 103 mM, either in the presence of 10 mM α-(methylamino)isobutyric acid (I) or of 50 mM lysine (II) or both (III). The upper line shows result II *minus* result III, the uptake being ascribed to the A system. The middle line and the triangles show result III *minus* a nonsaturable contribution. The lowest line and the solid circles show result I *minus* result III, and represents the $L^{\circ,+}$ component. The histidine concentration is measured in mM. Although essentially the same value for the v_{max} of the A system of 6 mmoles/kg cell water was obtained in other experiments, a K_m value of 4 mM was more typical than the value of 2.6 mM indicated here.

A significant rate of uptake can in fact be excluded more reliably on the basis of the first test[5].

Fig. 4 shows the nearly complete inhibitory action of phenylalanine alone on histidine uptake. This amino acid is known to be able to suppress uptake by the following systems: easily, by the L and the hypothetical $L^{\circ,+}$; more difficultly, by the A and ASC. The effect of lysine inhibition superimposed on that by phenylalanine is shown by the lower curve. It corresponds to rather little entry by any lysine-inhibited system inaccessible to phenylalanine, hence, by definition, by the Ly^+ system. The result does not, however, exclude histidine as a substrate of that system, because at pH 7.4 only about 5 % of the amino acid is present in the cationic form.

Figs. 1, 3 and 4 have shown that a major part of the uptake of histidine in dilute solutions at pH 7.4 corresponds in properties to the L system as originally described. Subsequently a portion of the uptake of various amino acids corresponding to that description of the L system was shown to be inhibited about as readily by lysine as by phenylalanine[3]. This component can be considered either intrinsic to, or separate from, the L system, depending on which model one accepts to account

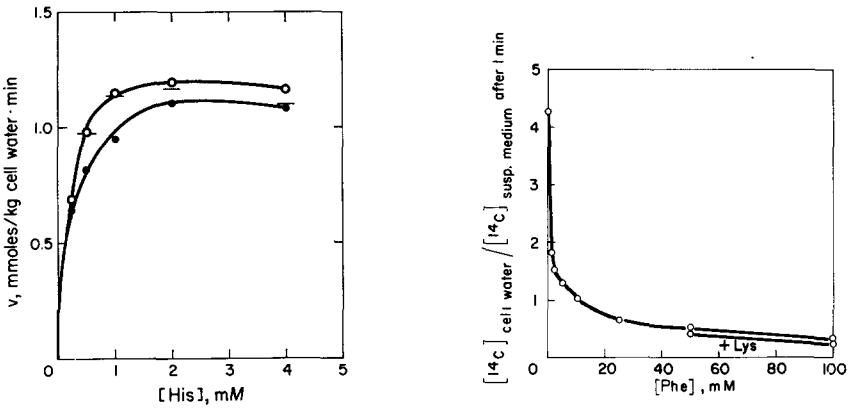


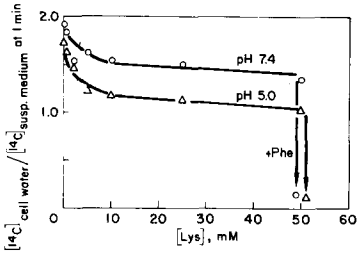
Fig. 3. Has histidine an ACS component of uptake by the Ehrlich cell? Uptake during 1 min at pH 7.4 and 37° in the presence of 25 mM α-(methylamino)isobutyric acid from (upper curve) ordinary Krebs–Ringer bicarbonate and from (lower curve) the same with choline replacing Na^+. The lower curve is corrected for cell shrinkage, so that both rates can be related to 200 g dry wt. or 1 kg initial cellular water. The horizontal bars represent the calculated rate in the presence of Na^+, had we been able to use an infinite concentration of α-(methylamino)isobutyric acid and thus to suppress totally uptake by the A system. Accordingly, residual operation of the incompletely inhibited A system does not fully account for the small discrepancy between the 2 curves.

Fig. 4. Phenylalanine-sensitive component of histidine uptake by the Ehrlich cell at pH 7.4. 1 mM L-[^{14}C]histidine in Krebs–Ringer bicarbonate medium at 37°; uptake during 1 min; [Na^+] = 143 mM—[Phe]/2, in mM. The lower line shows that the component sensitive to lysine under near-maximal phenylalanine inhibition is very small.

for it[3,10]. The upper curve of Fig. 5 shows the dimensions of this component of histidine uptake at pH 7.4; the disappearance of lysine sensitivity on the addition of excess phenylalanine (Fig. 4) identifies the component.

To measure the lysine-insensitive L component separately from this lysine-inhibitable component more accurately than we did in Fig. 2, histidine uptake was observed at 7 concentrations in the presence of excesses both of α-(methylamino)-isobutyric acid and lysine (Fig. 6). The results correspond to a K_m of 0.4 mM and a v_{max} of 1.7 mmoles/kg cell water · min. The corresponding parameters for the lysine-inhibitable component could be obtained only with poor accuracy (Fig. 2), the maximal velocity being about 1 mmole/kg cell water · min. The action of histidine, also at pH 7.4, in accelerating the exodus of previously accumulated methionine from the cells was far greater than that of lysine and similar to that of valine and methionine (Table I), again supporting its principal role as a substrate of the L rather than the Ly^+ system.

Fig. 5 shows that the maximal inhibitory action of lysine on the uptake of histidine concerned about 30% of the component insensitive to α-(methylamino)-isobutyric acid at 7.4, and about 40% at pH 5.0. In each case excess phenylalanine largely suppressed residual histidine uptake. The quantitative similarity of the lysine-inhibitable components at these 2 pH values does not, however, imply that they represent the same transport agency. Whereas the component inhibited by lysine at pH 7.4 was almost entirely phenylalanine-inhibitable (compare Figs. 4 and 5), and hence identified with the $L^{\circ,+}$ component, Fig. 7 shows that at pH 5 a considerable



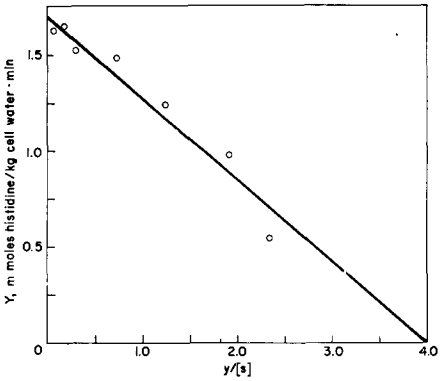


Fig. 5. Lysine-sensitive component of histidine uptake by the Ehrlich cell at pH 7.4 and 5.0. Uptake observed at 37° during 1 min for 1 mM L-[^{14}C]histidine in Krebs–Ringer phosphate medium, containing 10 mM α-(methylamino)isobutyric acid to inhibit the A system. The arrows at the right show that, under near-maximal inhibition by lysine, the residual uptake of histidine is largely sensitive to the inhibitory action of L-phenylalanine (10 mM). Lowering the pH from 7.4 to 5.0 has increased the lysine-sensitive component from about 30% to about 40% of the total rate of uptake.

Fig. 6. Augustinsson plot of rate of uptake of [^{14}C]histidine (1 mM) in the presence of 10 mM α-(methylamino)isobutyric acid and 50 mM lysine. Krebs–Ringer bicarbonate medium at pH 7.4, 37°; interval, 1 min. The rate, y, has been corrected for an apparently non-saturable component measured as 0.12 min^{-1}. The line corresponds to a v_{max} of 1.7 mmoles/kg cell water·min and a K_m of 0.4 mM. The uptake represents the L system, as originally described[4], less the L$^{\circ,+}$ component, which may in fact not be a distinct entity. The middle curve of Fig. 2 yielded a similar K_m but a somewhat lower v_{max} than the present result.

TABLE I

ACCELERATION OF METHIONINE EXODUS FROM THE EHRLICH CELL BY EACH OF SEVERAL AMINO ACIDS

[^{14}C]Methionine accumulated to an apparent concentration of 3.4 mM (Expt. 1) or 1.90 mM during 5 min in Krebs–Ringer bicarbonate medium at 37°, pH 7.4. Decrease of cellular radioactivity then observed during 1 min at 37° in 25 vol. of a similar medium containing one of the following amino acids at 20 mM. Similar results were obtained at 40 mM external levels of valine, lysine or both.

Amino acid in external medium	*Loss of ^{14}C* (%)
Expt. 1	
None	31
Valine	86
Methionine	83
Histidine	79
1-Aminocyclopentane carboxylic acid	85
Serine	55
Lysine	50
Expt. 2	
None	30
Valine	82
Methionine	79
Lysine	74
Valine + lysine	89
Histidine	87

portion of the lysine-inhibitable component was phenylalanine insensitive, *i.e.* it could still be observed in the presence of 50–100 mM phenylalanine. This component, inaccessible to neutral amino acids but accessible to lysine, is by definition attributed to the Ly^+ system. This result was anticipated, because lowering the pH from 7.4 to 5.0 will make histidine cation the dominant species, increasing the concentration of this likely substrate for the Ly^+ system by nearly 19-fold. This change can well account for the obviously greater separation of the parallel curves in the lower right

TABLE II

INHIBITION OF LYSINE UPTAKE BY THE EHRLICH CELL AT pH 5 BY HISTIDINE AND OTHER AMINO ACIDS

Uptake from 1 mM [^{14}C]lysine solutions in Krebs–Ringer phosphate medium during 1 min at 37°.

Inhibitors	*Velocity (mmoles/kg cell water·min)*
None	0.256
50 mM phenylalanine	0.116
25 mM histidine	0.015
Both of the above	0.013
25 mM alanine	0.094
25 mM histidine + 25 mM alanine	0.015

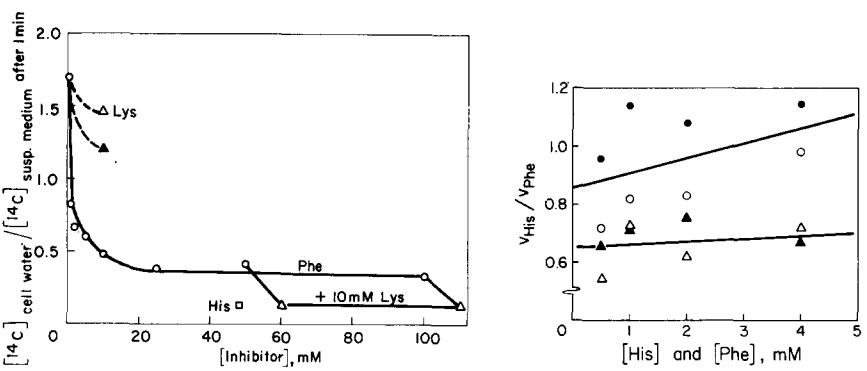


Fig. 7. Inhibition of the uptake of histidine (1 mM) at pH 5 by phenylalanine and lysine. Uptake during 1 min from Krebs–Ringer phosphate medium under O_2, 37°. Open symbols identify results obtained in a single experiment. The solid triangle is the mean of 3 subsequent observations. The line marked by circles represents the inhibition by phenylalanine. A definite residual component, sensitive to histidine itself (single point indicated by a square), remained. The superimposed action of lysine eliminated this component (lowest line, △). (The dotted lines have been drawn arbitrarily.)

Fig. 8. Test for heterogeneity in the interaction for transport into the Ehrlich cell between histidine and lysine by the method of INUI AND CHRISTENSEN[11]. Effect of lysine in excess. Identical concentrations of histidine and phenylalanine were present together in Na^+-free, choline-containing Krebs–Ringer bicarbonate medium, pH 7.4, 37°. Uptake of each during 1 min was observed in paired observations, one being histidine-labeled, the other being phenylalanine-labeled. The open symbols represent one experiment, the closed another. Upper line and circles, no lysine added; lower line and triangles, [lysine] = 50 mM in the suspending fluid; both lines drawn by the method of least squares.

of Fig. 7, compared with the same feature of Fig. 4, the two ordinate scales being numerically equivalent.

To support that assignment excess histidine was shown as effective alone at pH 5 as in conjunction with neutral amino acids in inhibiting lysine uptake (Table II). At the same time a distinct loss in the size of the component sensitive to lysine at pH 5 did occur when excess phenylalanine was added (Fig. 5). We therefore conclude that histidine cation (the predominant species at pH 5) has a component of uptake sensitive to both inhibitors, as well as one sensitive to lysine only. Had we found instead that the uptake of histidine cation, or of any other amino acid, is sensitive exclusively to lysine, we should have established that the Ly^+ system can function separately from the $L^{\circ,+}$ system. Na^+ dependence of histidine uptake at pH 5 was inappreciable, as anticipated by the suppression of System A at this pH (ref. 4).

Another use of histidine to test the nature of the L–$L^{\circ,+}$ heterogeneity of the Na^+-independent uptake of neutral amino acids is shown in Fig. 8. The constant-ratio test of INUI AND CHRISTENSEN[11] here suggests (upper curve) that the interaction of phenylalanine and histidine for uptake in the absence of Na^+ was not limited to a single transport system. The test shows that at pH 7.4 increasing the concentration of each amino acid simultaneously by steps from 0.5 to 4 mM appeared to favor the rate for histidine uptake more than that for phenylalanine. Within the precision we could obtain, this advantage for histidine was, however, eliminated by adding 50 mM lysine (lower line, Fig. 8).

The pigeon red blood cell

Here also histidine uptake occurs mainly by a system of the L type. The uptake of 0.05 mM [^{14}C]phenylalanine by this cell during 5 min from the Na^+-free Krebs–Ringer phosphate medium at pH 7.4 was inhibited about as strongly by 1 mM histidine as it was by leucine (see legend to Table III in ref. 6). Conversely, the uptake of histidine from Na^+-free medium was readily inhibited by phenylalanine and tryptophan. Histidine uptake was not increased by introducing Na^+ into the medium, and sensitivity to serine addition was small and equivocal (Table III). In the converse test, the addition of 5 mM histidine inhibited the uptake of 0.05 mM serine from normal Krebs–Ringer phosphate medium by 21 %.

Another route also participates in histidine uptake by pigeon red blood cells.

TABLE III

INHIBITION OF HISTIDINE UPTAKE BY VARIOUS AMINO ACIDS IN THE PIGEON RED BLOOD CELL
Uptake of 0.05 mM histidine during 5 min from Krebs–Ringer phosphate medium, or a corresponding solution in which choline replaced Na^+.

Inhibitory condition	*Na^+ present*	*Inhibition (%)*
1 mM serine	yes	14
Removal of Na^+	—	(−10)
1 mM serine	no	(−15)
0.32 mM phenylalanine	no	56
0.32 mM methionine	no	40
1 mM tryptophan	no	58
1 mM histidine	no	57

The addition of 5 mM lysine slowed the uptake of histidine from Krebs–Ringer phosphate medium by 12–22 %, an effect that could still be observed in the presence of 5 mM phenylalanine. Histidine was in turn able largely to inhibit lysine uptake. These results identify the uptake of histidine by the pigeon erythrocyte mainly with the Na^+-independent system designated Lp (ref. 6), and a smaller component with a separate system for cationic amino acids.

The human erythrocyte

In a prior study[7] we were impressed by the slowness of the uptake of histidine and tryptophan by the human red blood cell, relative to leucine or phenylalanine, and the corresponding weakness of counter transport; hence we tended to regard the L system as particularly selective in that cell. In the present experiments this slowness was confirmed, the apparent concentration in the cell water reaching only 80 % of extracellular level (0.05 mM) in 60 min, with no indication that a steady state had yet been approximated. Nevertheless a characteristic uptake occurs with properties corresponding to the L system. A K_m value of about 10 mM, $v_{max} = 0.4$ mmole/kg cell water, could be estimated, approximating from the data a non-saturable component with a rate of 0.04 min^{-1}. Mediated processes with high K_m values probably contributed to the latter component here, and also in the cases of leucine, methionine, phenylalanine and valine, because these apparent rates of non-saturable entry are much higher than that shown by glycine (see Table III in ref. 7). The uptake of 0.05 mM histidine was 55–62 % inhibited by 5 mM phenylalanine, for which a K_m of 4.3 mM was previously estimated. Table IV shows no evidence for a Na^+-dependent component of histidine uptake. A corresponding demonstration made for the rabbit reticulocyte is more significant, since Na^+-dependent systems are easily demonstrated in that cell, whereas only a trace of such dependency can be shown in the adult mammalian red cell[7,12].

Although the uptake of 1 mM phenylalanine was insignificantly affected by the presence of 20 mM lysine, the uptake of histidine showed a distinct sensitivity to lysine in excess, indicating that a system for cationic amino acids probably makes a contribution to histidine uptake by the human erythrocyte, as in the pigeon red blood cell. Fig. 9 shows the time course and the concentration dependence of lysine uptake. Also recorded are the inhibitory action of histidine and phenylalanine on lysine uptake. The action of phenylalanine here indicates that probably only one aspect of the interaction between the transport systems serving characteristically for lysine and phenylalanine of the kind seen in the Ehrlich cell and elsewhere is present in the human red blood cell.

TABLE IV

Na^+ INDEPENDENCE OF THE UPTAKE OF HISTIDINE BY THE HUMAN RED BLOOD CELL

Total uptake during 5 min at 37° from Krebs–Ringer phosphate medium, or from a corresponding solution in which all Na^+ was replaced by $choline^+$. The rates have been normalized to a 1 mM concentration.

Histidine concn. (mM):	*0.05*	*0.5*	*5*
Uptake with Na^+	0.13	0.10	0.06
Uptake without Na^+	0.12	0.08	0.07

The rabbit reticulocyte

Characteristically this cell has several transport systems that regress on maturation to the erythrocyte stage. Histidine and lysine are amino acids whose extent of uptake by human and rat reticulocytes appears to be Na^+ independent. Both reach higher steady-state distribution ratios at the reticulocyte stage than in the mature erythrocyte[13]. For the rabbit reticulocyte, we find the uptake of 0.2 mM histidine to proceed slowly, approaching a distribution ratio of 1 between the cellular and extracellular water in an hour. The line relating the initial rate to histidine concentration had only a small curvature below 1 mM, and was not subject to easy analysis. About a third of the uptake at a 0.2 mM concentration could be eliminated by lysine in large excess, this proportion not being clearly different at pH 6.2 than at 7.4. The converse relationship was quite different: Histidine at 2 mM became at least 3 times as effective in inhibiting lysine uptake when the pH was lowered from 7.4 to 6.2. Other basic amino acids were very effective inhibitors of lysine uptake, but neutral amino acids had only minor effects (unpublished results of J. A. ANTONIOLI in this laboratory). The agency for lysine transport accounting for this behavior is partially lost during maturation of the reticulocyte[14].

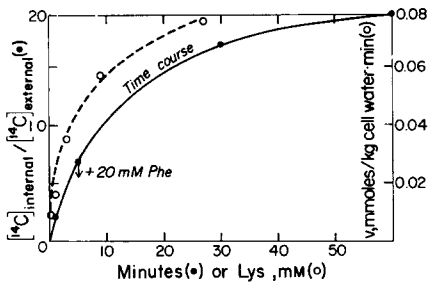


Fig. 9. Time course (———) and concentration dependence (– – – –) of lysine uptake by the human red blood cell. Total uptake from Krebs–Ringer phosphate medium at pH 7.4 and 37° is recorded. The time course was observed for 0.1 mM [^{14}C]lysine. The arrow shows the degree of inhibition produced by adding 20 mM phenylalanine. Concentration dependence was observed during 5 min. At 0.11 mM lysine, adding 20 mM phenylalanine inhibited lysine uptake by 32%; adding 20 mM histidine inhibited lysine uptake by 72%; both these amino acids present together caused 86% inhibition. The dashed curve corresponds to a K_m of about 5.6 mM and a v_{max} of about 0.09 mmole/kg cell water·min.

DISCUSSION

Our assignments for the uptake of histidine by the Ehrlich cell at 1 mM and at pH 7.4 are as follows: by System A, 30%; by System L, according to its original broad description[3], 66%, one third of which uptake can be eliminated by lysine in excess; by System Ly^+, roughly 1%, calculated by the Henderson–Hasselbalch equation from the results at pH 5; by the non-saturable route, 3%. At lower, physiological concentrations, dominance of the L system will be greater.

Uptake by erythrocytes of various types corresponds to a simpler pattern. Because the A system cannot be detected in these cells, and because histidine undergoes little if any transport by the other wide-spectrum Na^+-requiring system (ASC), histidine uptake is dominated by a Na^+-independent route of the L type. An earlier

impression that histidine and tryptophan were largely excluded from that agency in the human red blood cell has now been corrected; the uptake of histidine is slow but otherwise characteristic. A second, smaller component of histidine uptake shows in each case the properties of a transport system for cationic amino acids; but in these several red blood cells the transport interactions between neutral and cationic amino acids with large side chains is either minimal or one-sided.

Turning to the strong interactions between amino acids of these 2 classes in the Ehrlich cell, we find that the 2 predominant forms of histidine behave in agreement with 2 generalizations for which we do not yet have an exception:

1. Neutral amino acids reactive with the L system suffer a significant fractional inhibition of their uptake by lysine, and inhibit a significant fraction of the uptake of lysine.

2. Cationic amino acids reactive with the Ly^+ system inhibit a significant fraction of the uptake of phenylalanine, and have a significant fraction of their uptake inhibited by phenylalanine.

If the $L^{\circ,+}$ site were really distinct from the Ly^+ site in its reaction with basic amino acids, and distinct from the L site in its reaction with neutral amino acids, one would suppose that eventually we should encounter a basic amino acid able to differentiate between the $L^{\circ,+}$ and Ly^+ sites, or a neutral amino acid able to differentiate between the $L^{\circ,+}$ and L sites*. This differentiation might appear as a total unreactivity with one of either pair, or instead as a difference between the kinetic constants K_m or K_i measuring that reactivity. The unusual chemistry of histidine had raised our hopes that it might show the discrimination for which we were looking. Therefore, the heterogeneity in the interaction between histidine and phenylalanine for uptake from Na^+-free medium was of special interest, especially since the presence of lysine in excess eliminated this heterogeneity, as would be expected through its blocking of the $L^{\circ,+}$ component of histidine uptake. Unfortunately, the observed heterogeneity was not large enough for unequivocal measurement, nor was it larger than might arise from the uptake of histidine cation by the Ly^+ system, which would become more conspicuous as the uptake of the α-zwitterion by other routes approaches saturation.

The study of histidine transport has, in short, uncovered no clear inadequacies in the list of transport agencies described so far.

ACKNOWLEDGEMENTS

This work was supported in part by the Institute for Child Health and Human Development (Grant HD 01233), National Institutes of Health, U.S. Public Health Service.

The competent technical assistance of the following persons with one part or another of this research is gratefully acknowledged: MARIE LIANG CHIU, INEZ ANDREWS HAWK, MARY E. HANDLOGTEN, ISABELLE LAM.

* Since completion of this work a new amino acid, 2-aminobicyclo-[2,2,1]heptane-2-carboxylic acid, has been observed to react characteristically with the L system, but not measurably with the component of lysine transport ($L^{\circ,+}$) easily inhibited by most other neutral amino acids (unpublished experiments with M. E. HANDLOGTEN, I. LAM AND R. ZAND).

REFERENCES

1 H. N. CHRISTENSEN, *Perspectives Biol. Med.*, 10 (1967) 471.
2 H. N. CHRISTENSEN AND T. R. RIGGS, *J. Biol. Chem.*, 194 (1952) 57.
3 H. N. CHRISTENSEN, *Proc. Natl. Acad. Sci. U.S.*, 51 (1964) 337.
4 D. L. OXENDER AND H. N. CHRISTENSEN, *J. Biol. Chem.*, 238 (1963) 3686.
5 H. N. CHRISTENSEN, M. LIANG AND E. G. ARCHER, *J. Biol. Chem.*, 242 (1967) 5237.
6 E. EAVENSON AND H. N. CHRISTENSEN, *J. Biol. Chem.*, 242 (1967) 5386.
7 C. G. WINTER AND H. N. CHRISTENSEN, *J. Biol. Chem.*, 239 (1964) 872.
8 H. N. CHRISTENSEN AND M. LIANG, *J. Biol. Chem.*, 241 (1966) 5542.
9 H. AKEDO AND H. N. CHRISTENSEN, *J. Biol. Chem.*, 237 (1962) 113.
10 H. N. CHRISTENSEN AND M. LIANG, *Biochim. Biophys. Acta*, 112 (1966) 524.
11 Y. INUI AND H. N. CHRISTENSEN, *J. Gen. Physiol.*, 50 (1966) 203.
12 K. P. WHEELER AND H. N. CHRISTENSEN, *J. Biol. Chem.*, 242 (1967) 1450.
13 A. A. YUNIS AND G. K. ARIMURA, *J. Lab. Clin. Med.*, 66 (1965) 177.
14 H. N. CHRISTENSEN AND J. A. ANTONIOLI, *Federation Proc.*, 27 (1968) 515.