Biochimica et Biophysica Acta, 455 (1976) 144–151

BBA 77481

HETEROGENEITY OF HISTIDINE TRANSPORT IN THE EHRLICH CELL

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(Received March 30th, 1976)

SUMMARY

We have reexamined the heterogeneity shown by histidine in its uptake by the Ehrlich ascites tumor cell, in the face of a contradiction of our earlier interpretation. We again find the fraction of histidine uptake at neutral pH inhibitable by the model substrate for System A, 2-(methylamino)-isobutyric acid, to be fully dependent on the presence of Na^+ or Li^+. The small Na^+-independent component not attributable to System L can be identified with System Ly^+ through its inhibitability by homoarginine. This component increases as the pH is lowered with an apparent pK'_a of 6.1. The simultaneous decrease in the uptake by the neutral systems could be identified, for System L, with the same titration of histidine to its cationic form, but for System A the sharp decrease is identified with the protonation of a structure on the membrane rather than one on the substrate. The action of H^+ in the latter case proved approximately non-competitive with Na^+ when tested with ordinary substrates.

histidine transport with a more detailed analysis than previously because of the availability of model substrates more specific to each system, and because a convenient buffer, ε-aminocaproic acid, $pK'_a = 4.3$, has in the meantime been identified for use at low pH values.

METHODS

Uniformly ^{14}C-labeled L-histidine was obtained from International Chemical and Nuclear Corp. *N*-[1-^{14}C]Methyl-2-aminoisobutyric acid and 2-amino[^{14}C] norbornane-2-carboxylic acid were obtained from New England Nuclear. L-[*Guanidine*-^{14}C]homoarginine was obtained from Calbiochem.

The methods of obtaining cells, of studying uptake by and exodus from Ehrlich cell have been described elsewhere [4, 5]. All suspending solutions were prepared to have nearly identical osmolarities. We will designate in this paper as sodium media solutions with pH about 7.4, buffered with phosphate and containing the usual ions of Krebs-Ringer solution. Where choline replaces Na^+ in this medium, we will use the term choline medium. The concentrations of the principal cation were kept constant within each experiment, as selected amino acids were added to replace NaCl or choline chloride. The rate of uptake during 30 s was measured unless specified otherwise. To measure the rates of uptake as a function of pH, the cells were first washed in an unbuffered isotonic salt solution. Sodium phosphate or choline phosphate, each 25 mM, was used for establishing pH values of the incubation media from 5.4 to 7.4, and 25 mM ε-aminocaproic acid for pH values from 4.0 to 5.4. The pH values observed at the end of incubation were selected for plotting. For incubation periods of over 5 min, 100 % oxygen was passed through the flasks.

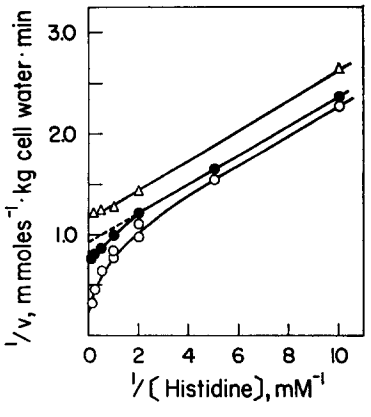

Fig. 1. Lineweaver-Burk plots of the rates of histidine uptake by Ehrlich cell during 30 s as a function of external concentrations of histidine: the raw data (○), after correction for the non-saturable component (●); and the uptake measured in the presence of 10 mM homoarginine and corrected for the non-saturable component (△). Measurements were carried out in choline medium, pH 7.1, as described under Methods.

RESULTS

Fig. 1 shows the effect of histidine concentration from 0.1 to 10 mM on the rate of its uptake in choline medium at pH 7.1. The total rate observed (lowest curve) indicates heterogeneity in the mode of uptake. Correction for a non-saturable component estimated at 0.18 min^{-1} by the equation of Inui and Christensen [4] still left a biphasic plot (middle curve of Fig. 1). When uptake was measured in the presence of excess homoarginine, however, a straight line corresponding to a K_m of about 0.13 mM was obtained. This basic amino acid was added to eliminate any uptake of histidine by the cationic amino acid system [6]. The residual component of uptake was identified with System L by its ready inhibition by 2-aminonorbornane-2-carboxylic acid [7], superimposed on the action of homoarginine (Fig. 3). From the separation between the upper lines of Fig. 1, one can estimate that a progressively larger fraction of histidine uptake is inhibitable by homoarginine, as the histidine level is raised from 0.1 mM (8 % is inhibited) to 10 mM (almost 40 % is inhibited). From this plot, we can estimate that the component inhibited by homoarginine, hence ascribed to System Ly^+, has a K_m greater than 0.3 mM.

Fig. 2 shows similar analysis with Na^+ at 64 mM. Now a third saturable component can be seen inhibited neither by homoarginine nor by 2-aminonorbornane-2-carboxylic acid, with a K_m of about 3 mM. To block histidine uptake by 95 % in the Na^+-containing medium required the presence of 2-(methylamino)-isobutyric acid [8] in addition to homoarginine and the norbornane amino acid (Fig. 4). The results are consistent with our previous division of the uptake of histidine between System L and System Ly^+ in Na^+-free medium, and including additionally System A when Na^+ is present [1].

Fig. 3 shows pH profiles of the rate of histidine uptake in choline medium in the absence of inhibitor or in the presence of excess homoarginine or 2-aminonorbor-

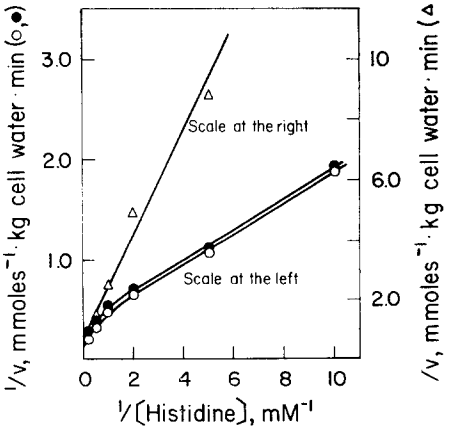

Fig. 2. Lineweaver-Burk plots of the rates of histidine uptake in sodium medium (64 mM = Na^+) as a function of external concentrations of histidine: the uptake before (○), and after (●) correction for the non-saturable component (scale at the left), and the uptake measured in the presence of 10 mM homoarginine and 10 mM 2-aminonorbornane-2-carboxylic acid and corrected for the non-saturable component (△, scale at the right). Other conditions are the same as described in Fig. 1.

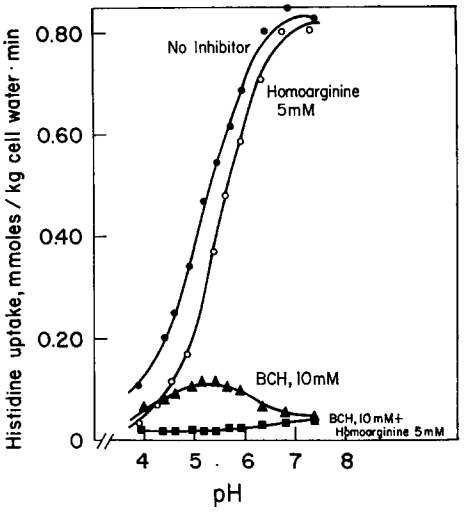

Fig. 3. pH profiles of histidine uptake during the initial 30 s from choline medium with 0.1 mM histidine: in the presence of no inhibitor (●), 5 mM homoarginine (○), 10 mM 2-aminonorbornane-2-carboxylic acid (▲), and both the norbornane amino acid and homoarginine (■). ε-Amino-caproic acid buffers, 25 mM, were used for pH values from 4.0 to 5.4, and choline phosphate buffers, 25 mM, for pH values from 5.4 to 7.3. The pH values at the end of incubation were used for plotting.

nane-2-carboxylic acid or both. The System L component of histidine uptake, insensitive to inhibition by homoarginine, accounts for almost 95 % of the total rate at pH 7.4, but gradually decreases as the pH of the medium is lowered. For example, histidine uptake by System L at pH 4.0 is less than 10 % of that at pH 7.4, and accounts for only 30 % of the total histidine uptake at pH 4.0. By contrast, the System Ly^+ component, insensitive to 2-aminonorbornane-2-carboxylic acid inhibition, shows a bell-shaped response with the maximum activity at pH 5.4, as the pH of the medium is changed from 3.9 to 7.4. Histidine uptake by System Ly^+, however, is a small component at 0.1 mM histidine concentration. Overall, no less than 95 % of the rate of histidine uptake from Na^+-free media could be inhibited by addition of excess 2-aminonorbornane-2-carboxylic acid and homoarginine together at most of pH values examined. The residual transport activity, which could not be further reduced even by the additional presence of excess 2-(methylamino)-isobutyric acid, rises from 0.15 min^{-1} at pH 4.0 to 0.40 min^{-1} at pH 7.4. These values should represent the non-saturable component if we have succeeded in blocking all mediated components; they tended to be a little higher than the value of 0.18 min^{-1} obtained in this study at pH 7.1 by the method of Inui and Christensen [4].

Profiles of the rates of histidine uptake in the Na^+-containing medium as a function of pH are shown in Fig. 4. Our intention here was to emphasize the Na^+-dependent component. This component was examined in two ways: the transport activity sensitive to 2-(methylamino)-isobutyric acid and the saturable transport activity insensitive to 2-aminonorbornane-2-carboxylic acid and homoarginine inhibition. In either way we obtained almost the same rate of histidine uptake and almost the same pH profile for this histidine uptake. Note that System A component becomes apparent only at a pH of the medium above 6 and increases as the pH is raised to

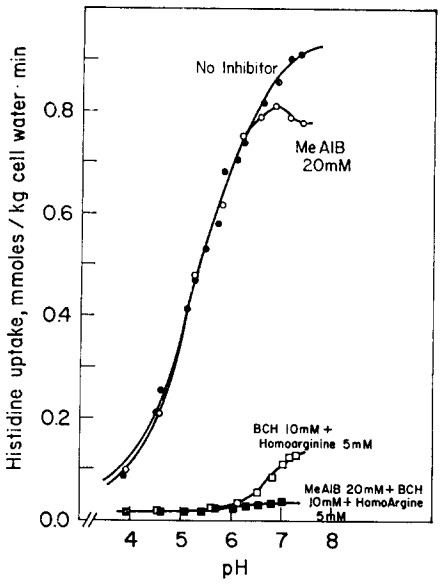

Fig. 4. pH profiles of histidine uptake from sodium medium (64 mM = Na^+) without inhibitor (●), with 20 mM 2-(methylamino)-isobutyric acid (○), with 10 mM 2-aminonorbornane-2-carboxylic acid and 5 mM homoarginine (□), or with all of the three model substrates at the same time (■). Other details were the same as described in Fig. 3.

slightly above pH 7. In addition, the non-inhibitable component of histidine uptake, as measured by uptake in the presence of excess 2-(methylamino)-isobutyric acid, 2-aminonorbornane-2-carboxylic acid and homoarginine in the Na^+-containing medium, shows the same pH profile and almost the same values as those obtained in the choline medium (refer to Fig. 3).

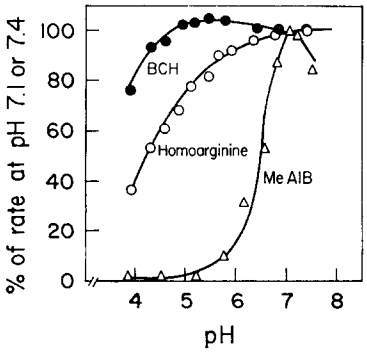

Fig. 5. Normalized pH profiles of the initial rates of entry of the three model substrates: 2-aminonorbornane-2-carboxylic acid (●), homoarginine (○) and 2-(methylamino)-isobutyric acid (△). Uptake of the norbornane amino acid and homoarginine was from choline medium, and 2-(methylamino)-isobutyric acid from sodium medium. Percent of rate was calculated on the basis of the uptake at pH 7.4 for the norbornane amino acid and homoarginine, and the uptake at pH 7.1 for 2-(methylamino)-isobutyric acid. Other details were the same as described in Figs. 3 and 4.

The pH profiles of the individual components of histidine uptake examined so far reflect not only the changes in the state of charge of histidine but also changes in the state of dissociation of titratable groups associated with the transport system of the plasma membrane. As a first step to distinguish between these two effects of pH on the operation of the transport systems, we examined the pH profiles of the initial rate of entry of the three model substrates, 2-aminonorbornane-2-carboxylic acid, homoarginine and 2-(methylamino)-isobutyric acid. Charges of these substrates are not significantly variable over the range of pH studied. The results are shown in Fig. 5. The pronounced decrease in transport activity of System A shown by 2-(methylamino)-isobutyric acid on lowering the pH of the medium contrasts with a remarkably low sensitivity of System L, as illustrated by the norbornane amino acid. An intermediate sensitivity was observed for System Ly^+, as illustrated by homoarginine. Certain other substrates show an enhanced uptake by System L as the pH is lowered from 7.4 to 5.

Fig. 6 shows changes in the rates of histidine uptake by the Systems L, Ly^+, and A when the charge on the histidine molecule changes as a function of pH. In the course of constructing this figure, the rate observed for histidine uptake by each system at a particular pH as obtained from Figs. 3 and 4 was divided by a normalized rate of model substrate uptake by the corresponding system at the same pH obtained from Fig. 5. This manipulation served to isolate the effect of the changes in the state of charge of the histidine molecule on the operation of its transport systems. The profiles of histidine uptake by Systems L and A obtained in this way resemble the titration curve of neutral species of histidine, showing pK'_{a2} 5.4 and 6.2, respectively, whereas that of System Ly^+ matches very closely with the titrimetric formation of the protonated species with pK'_{a2} 6.1. The displacement of the pH effect on the transport of histidine by System L from the known pK'_{a2} 5.95 may be attributed to stabilization of neutral species by the supposedly hydrophobic environment of the site occupied by the lipophilic side chains of System L substrates, and has been seen with various other substrates [9].

The distinct requirement of System A for Na^+ and its extraordinary sensitivity toward H^+ led us to examine the relation between these two monovalent cations in

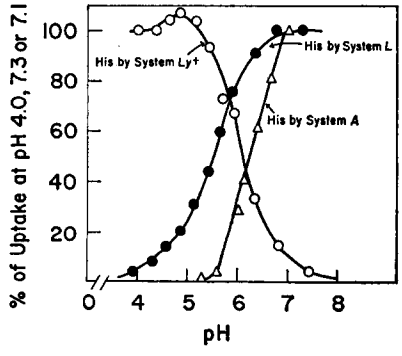

Fig. 6. Plot showing changes in the rates of histidine uptake by the Systems L (●), Ly^+ (○) and A (△) when the charge on the histidine molecule changes as a function of pH. See Results for further details.

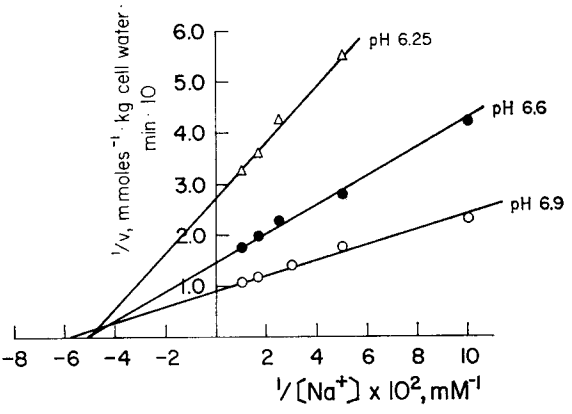

Fig. 7. Lineweaver-Burk plots of the rates of Na^+-dependent glycine uptake as a function of external Na^+ concentrations at pH values of the medium 6.25 (△), 6.6 (●) or 6.9 (○). External level of Na^+ was varied from 10 to 100 mM. Glycine uptake was at 30 mM, 10 times K_m, during the initial 30 s. Na^+-dependent uptake of glycine as obtained by subtracting the uptake observed in choline medium under otherwise the same conditions. When pH of the medium at the end of incubation drifted somewhat from the expected value, the rate at the latter pH was deduced from a plot of the uptake rate as a function of pH at a given concentration of Na^+.

this system. At a fixed excess (10 times K_m) of 2-(methylamino)-isobutyric acid (30 mM) or glycine 30 mM, the initial rates of Na^+-dependent uptake of the amino acids were measured in the presence of 10, 20, 40, 60 and 100 mM Na^+ at pH 6.25, 6.6 and 6.9. Fig. 7 shows Lineweaver-Burk plots of the rate of glycine uptake vs. Na^+ concentration as a function of pH. The principal effect of lowering pH was to decrease V. The calculated V values were 12.3, 6.6 and 3.8 mmol/kg of cell water at pH 6.9, 6.6 and 6.25, respectively. The K_m for Na^+, approx. 20 mM, was not significantly affected. These data are approximately consistent with a non-competitive inhibition by H^+ with respect to Na^+. The value of K_i for H^+ in the same frame of reference is approx. $10^{-6.6}$ M. A closely similar relation between Na^+ and H^+ was found in experiments with 2-(methylamino)-isobutyric acid instead of glycine.

The gradual increase in the non-saturable component of histidine uptake as the pH of the medium is raised from 4 to 7 follows the general pattern of neutral amino acids which do not have any appreciably dissociable groups in that pH range [10]. It should be noted again that the non-saturable uptake probably does not occur by simple diffusion.

ACKNOWLEDGEMENTS

These experiments received support through a grant (HD01233) from The National Institute of Child Health and Human Development, National Institutes of Health, United States Public Health Service.

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