BBA 75434

HISTIDINE UPTAKE AND EXCHANGE IN S37 ASCITES TUMOR CELLS

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(Received November 21st, 1969)

SUMMARY

1. Two distinguishable sites participate in the uptake of histidine by S37 and Ehrlich ascites tumor cells.

2. The two sites have affinities for histidine which differ by more than an order of magnitude. This enables their contributions to histidine transport to be differentiated in a conventional Lineweaver–Burk plot.

3. Histidine apparently utilizes only one of the sites to leave the cell during exchange.

4. Both sites appear to participate in the uptake of histidine in the absence of sodium.

INTRODUCTION

In the course of studies which led them to propose the involvement of two sites (the 'A' and 'L' sites) in the uptake of neutral amino acids by ascites tumor cells, OXENDER AND CHRISTENSEN[1] suggested that methionine utilized both sites. On the basis of recent studies of methionine uptake in ascites tumor cells, BELKHODE AND SCHOLEFIELD[2] suggested that the uptake is mediated by two systems whose K_m values differ by a factor of 5 but could find no appreciable deviation from linearity in a LINEWEAVER–BURK plot[3] of methionine uptake. However, CHRISTENSEN[4] had shown that two transport systems acting on a solute would require K_m values differing by at least an order of magnitude for the Lineweaver–Burk plot of uptake data to show a clear break. Studies were therefore undertaken to identify an amino acid which is transported into ascitic tumor cells by two distinct systems and where the K_m values and v_{max} values differ sufficiently to enable one to distinguish the two systems in a simple Lineweaver–Burk plot. Recent results by CHRISTENSEN[5] suggested that histidine might be a suitable amino acid for such studies. NAKAMURA[6] studied histidine uptake in brain slices and demonstrated not only good accumulation of this amino acid but also rapid exchange diffusion. The techniques employed in the recent study[2] of methionine uptake were therefore applicable and preliminary experiments indicated that histidine did in fact give rise to broken Lineweaver–Burk plots. Anomalies were encountered in studies of the sensitivities of these two systems to withdrawal of Na^+ and the present report deals with an analysis of the two systems, their participation in the exchange process and their sensitivities to other amino acids and to Na^+.

MATERIALS AND METHODS

Amino acids

All optically active amino acids used were of the L form. L-[*ring*-2-^{14}C]Histidine was obtained from the Radiochemical Centre (Amersham). Unlabelled amino acids were purchased from Nutritional Biochemicals Corp., Cleveland, Ohio.

S37 cells

Male Swiss white mice weighing 20–25 g were used. Cells were propagated by intraperitoneal injection of the fluid tumor and were harvested 6–8 days later. Mice were sacrificed by cervical dislocation, an incision made in the abdomen to withdraw the ascitic fluid and the cells washed and isolated as previously described[7].

Incubation medium

The incubation medium contained 131 mM NaCl, 5.2 mM KCl, 1.3 mM $MgSO_4$ and 1.0 mM $CaCl_2$ *plus* 10 mM sodium phosphate buffer (pH 7.4). In contrast to previous studies, Ca^{2+} has been included in the medium since it was found to increase amino acid uptake in these cells[8]. The total volume of the incubation medium was approx. 30–50 times the packed volume of the cells present.

Determination of intracellular histidine

Incubations were terminated by mixing the sample with 8 ml chilled buffered salts medium and centrifuging for 1 min at 800 $\times$ *g*. The supernatants were removed and the cells washed with 8 ml chilled buffered salts to remove extracellular histidine entrained with the cell pellet. The quantity of cells present was assayed by determination of the wet weight (intracellular space was determined using labelled sulfate), the cells were extracted with 3 ml 95 % ethanol for 30 min and a 0.5-ml aliquot was removed for liquid-scintillation counting.

Unmediated solute transfer

It was considered impractical to attempt to measure non-saturable uptake directly in this system, because the higher of the two K_m values seen for histidine uptake appears to be about 10 mM. As a result the velocity would only be half maximal even at a substrate concentration of 10 mM. Although simple efflux may not be due solely to unmediated diffusion, a study of efflux of histidine at high concentrations was used to obtain an upper limit for an unmediated permeability coefficient. Each of three determinations yielded a value for k_F at 37° of 0.02 min^{-1} and CHRISTENSEN AND LIANG[9], working with Ehrlich ascites cells at 37°, found 0.01 min^{-1} as the upper limit for k_F. The effect of this component on uptake in experiments of 1 or 2 min duration would therefore be negligible. Estimates were made of the potential contribution due to passive diffusion in one set of data for 5-min uptakes. It was found that only one data point out of ten would be altered to the extent of more than 5 % and the general effect was to raise slightly the intercepts of both line segments seen in a double-reciprocal plot, *i.e.* to decrease slightly the values of all four kinetic constants (both K_m and both v_{max} values). There was no straightening effect on the biphasic plot. In view of its limited quantitative significance, no corrections were made for unmediated solute transfer.

Measurement of uptake

Uptake over a wide concentration range for short periods of time was first studied at 37°. Intracellular labelled histidine was measured after 1 and 5 min in similar experiments. To compare the low K_m value for uptake with the K_m value for exchange efflux, uptake experiments were also conducted at 20° since it is much more convenient to follow exchange efflux at this temperature.

Measurement of efflux during exchange

The cells were first incubated with labelled histidine at 37° for 20–30 min, washed in chilled buffered salts medium and samples of the cells pipetted into media containing various concentrations of unlabelled amino acid at 20°. One sample of cells was taken before the second incubation, and another sample of cells was incubated with no extracellular unlabelled amino acid present. Incubations were terminated by adding the second incubation media (or an aliquot) to 8 ml chilled buffered salts medium contained in an ice-cold centrifuge tube. Efflux due to exchange was calculated as described previously[2] as the difference between the efflux (decrease of intracellular histidine concentration) found with unlabelled extracellular amino acid present and the efflux observed in the absence of added extracellular amino acid.

RESULTS

Uptake over a broad concentration range

Fig. 1 shows the two intersecting lines obtained when the 5-min uptake data were plotted according to the method of LINEWEAVER AND BURK[3]. In plotting the reciprocal of the intracellular concentration *versus* the reciprocal of the extracellular concentration, it was assumed that the intracellular concentration found after a brief exposure to an external amino acid was a measure of the initial velocity of uptake. This is not strictly true[10] but presumably the briefer the interval of exposure to extracellular amino acid, the more precisely the uptake will represent the initial velocity of uptake. When samples were also removed after 1 min, the lower of the two apparent K_m values decreased somewhat, a break in the plot was still apparent and the scatter of data points was greater. The 1-min uptake data will be used later for purposes of comparison of the K_m of the low concentration uptake system with that of the system operating for efflux during exchange. Two duplicate experiments gave similar results to those described above, the conspicuous feature of the Lineweaver–Burk plot being its biphasic nature. This clearly indicates that the uptake of histidine is not mediated by a single agency and it will be assumed that there are only two sites involved in the uptake of histidine until such time as it appears necessary to postulate more. CHRISTENSEN[5] gave values of 0.4 and 4 mM for the K_m values of histidine uptake by two sites into Ehrlich cells. The corresponding values for uptake into S37 cells are 0.12 and about 10 mM.

Ehrlich ascites cells

As may be seen from Fig. 2, the Ehrlich ascites cells in use in this laboratory exhibit a similar response although they do not give rise to quite so sharp a biphasic plot as do S37 cells (*cf.* Fig. 1).

Efflux during exchange

Fig. 3 displays the time-course of the intracellular concentration of histidine (c_i) in S37 cells which had been loaded with labelled histidine and then incubated in either a buffered salts medium alone or one containing various concentrations of unlabelled histidine. These data are from a single experiment which was replicated twice. It shows clearly that exchange efflux occurs. In the absence of unlabelled amino acid the efflux is obviously quite slow, whereas in the presence of unlabelled amino acid it is many times faster, continues at a diminishing rate during the 15-min

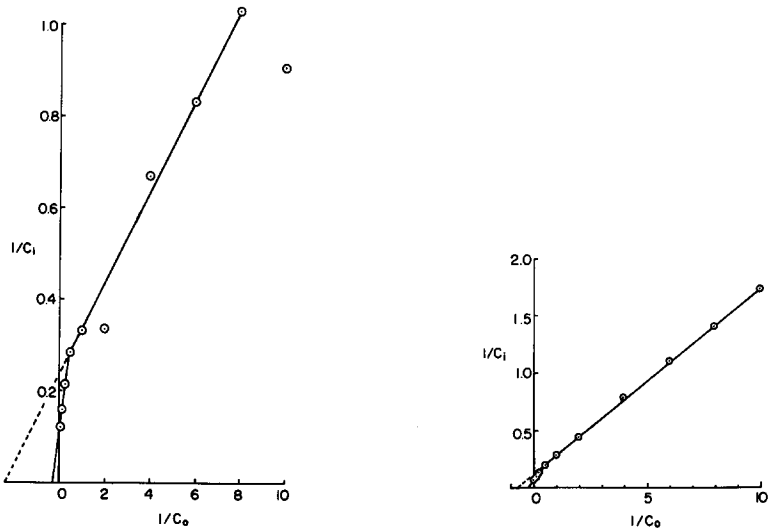

Fig. 1. Uptake of histidine by S37 ascites cells. Incubations were carried out for 5 min at 37°. The reciprocal of the mM concentration of intracellular labelled histidine (c_i) is plotted against the extracellular concentration (c_0).

Fig. 2. Uptake of histidine by Ehrlich ascites cells. Experimental details as described in the egend to Fig. 1.

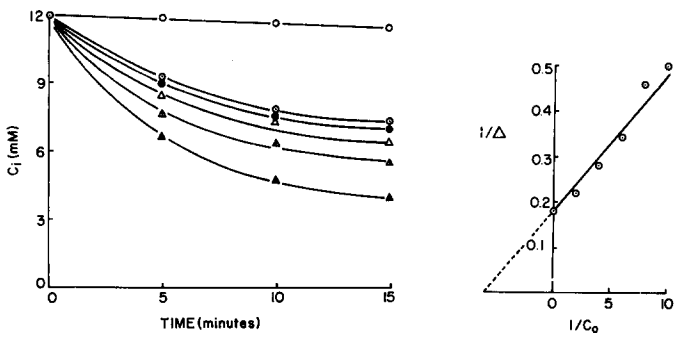

Fig. 3. Time-course of efflux of histidine. The intracellular concentration of labelled histidine remaining after incubation of prepacked cells in the presence of 0 (○), 0.1 (⊙), 0.125 (●), 0.167 (△), 0.25 (△) and 0.5 mM (▲) extracellular non-radioactive histidine was measured after 5, 10 and 15 min incubation at 20°.

Fig. 4. Exchange efflux of histidine. The relation between the increased efflux of histidine (△) and the extracellular concentration (mM) of histidine (c_0) is plotted according to the method of LINEWEAVER AND BURK[8]. The incubations were for 2 min at 20°.

incubation period and is progressively greater as the extracellular concentration is increased over the range 0.1–0.5 mM. The K_m value of extracellular unlabelled histidine for the mediator involved in the exchange efflux of labelled intracellular histidine was determined in three separate experiments and the mean value was 0.12 ± 0.03 mM (mean and S. E.). Data obtained from experiments carried out for 5 min yielded slightly higher values. The results obtained in a typical experiment were plotted according to LINEWEAVER AND BURK[3] and are shown in Fig. 4. The point corresponding to a high concentration (10 mM) falls directly on the straight line and no evidence could be obtained for the presence of a second mediator acting for exchange purposes as seen for uptake (Fig. 1).

Comparison of K_m values for low concentration uptake and exchange efflux

Six experiments were carried out to determine the K_m for histidine uptake in the lower concentration range. Incubations for this purpose were carried out for either 1 or 2 min and the K_m value obtained was 0.12 ± 0.03 mM (mean and S.E.). The results obtained in one experiment are presented in Fig. 5 where it has again been assumed that the initial velocity of uptake is much greater than the velocity of diffusion processes. The K_m value characterizing uptake under these conditions is the same as that found for interaction of extracellular histidine with the mediator active in exchange efflux (see above).

Effects of other amino acids on histidine uptake and histidine exchange efflux

Table I contains data comparing the effects of a variety of amino acids on uptake of 0.1 mM histidine with their ability to elicit histidine efflux by hetero-exchange. In measurements of uptake, incubations were for 3 min at 20°. The third column presents data on the stimulatory effect of 0.1 mM amino acid on histidine efflux expressed as a percentage of the stimulation observed with 0.1 mM extracellular histidine. Again, incubations were for 3 min at 20°. The amino acids employed may be divided into four classes with each showing similar effects on both uptake of a low concentration of histidine (0.1 mM), and in eliciting exchange efflux.

Effect of withdrawal of Na^+ from the medium

Data for 5-min uptakes of histidine into S37 cells from an isotonic sucrose medium are presented in Fig. 6. It is evident from the persistence of the biphasic

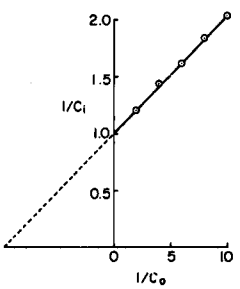

Fig. 5. Uptake of low concentrations of histidine by S37 ascites cells. Incubations were for 1 min at 20°, and the results (mM) are plotted according to the method of LINEWEAVER AND BURK[3].

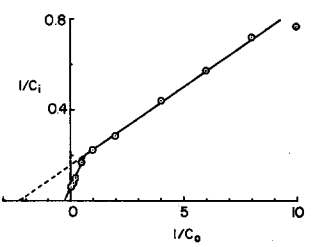

Fig. 6. Uptake of histidine by S37 ascites cells in the absence of Na^+. Incubations were for 5 min at 37° in isotonic sucrose medium, and the data are presented in units of mM^{-1} by the method of LINEWEAVER AND BURK[3].

TABLE I

COMPARISON OF THE EFFECTS OF OTHER AMINO ACIDS UPON HISTIDINE UPTAKE AND EXCHANGE EFFLUX IN S37 ASCITES TUMOR CELLS

The inhibitory effects of the various amino acids were assessed by determining the decrease in uptake of 0.1 mM radioactive histidine caused by the presence of 3 mM amino acid in incubations carried out for 3 min at 20°. Their influence on efflux was assessed by first pre-packing the S37 cells with radioactive histidine through incubating with this amino acid at a concentration of 5 mM for 30 min at 37°. The cells were then washed and the washed cells incubated alone or in the presence of the amino acids indicated (0.4 mM) for 3 min at 20°. Increased efflux of the radioactive histidine is recorded as a percentage of the increased efflux observed in the presence of added histidine in the incubation medium.

Amino acid added	*Inhibition of uptake* (%)	*Stimulation of efflux* (%)
Leucine	74	104
Methionine	63	99
Tryptophan	84	72
Phenylalanine	81	76
1-Aminocyclopentanecarboxylic acid	45	36
Valine	57	46
Lysine	6	10
Alanine	7	8
Aspartic acid	−5	−1
α-Aminoisobutyric acid	2	−3
Proline	−1	−1
Glycine	4	−2

plot that both uptake mediators are operating under this condition. Similar results were obtained when a choline-chloride medium was used instead of sucrose and when uptake of histidine from a choline medium was studied with Ehrlich ascites cells. It is concluded that at least two systems operate in the Ehrlich ascites cells even in the absence of extracellular Na^+.

DISCUSSION

OXENDER AND CHRISTENSEN[1] defined two distinct sites for uptake of neutral amino acids into Ehrlich ascites cells and termed the two sites 'A' and 'L' in reference to their specificities, *i.e.* 'alanine-preferring' and 'leucine-preferring'. These authors also suggested that the A system is dependent on alkali metal cations, and that some neutral amino acids utilize both A and L systems to an appreciable extent. Their data indicated that histidine is a moderately good inhibitor of both alanine and leucine uptake and the recent results of CHRISTENSEN[5] are further evidence in favour of the involvement of two sites for histidine uptake. The present data confirm that there are two mediators for uptake of histidine into Ehrlich ascites tumor cells and show that a similar phenomenon occurs in S37 cells. They also indicate that the corresponding K_m values are more than an order of magnitude apart and that consequently the Lineweaver–Burk plots over a wide concentration range yield clearly broken lines.

Various authors (see review, ref. 11) have found Na^+ and K^+ to be of importance

in the transport of sugars and some amino acids, but in the definition of the A and L systems it has been held that the A system is Na^+-dependent and the L is not. The present results, indicating that both sites involved in histidine uptake appear to function sufficiently well in the absence of Na^+ and K^+ to yield the obvious break in the reciprocal plot were therefore somewhat surprising. However, the result was confirmed after changing from a sucrose medium to a choline medium, and it was found with Ehrlich ascites cells as well as with S37 ascites cells. These results are at variance with the comment by CHRISTENSEN[5] that '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'. However, the only data presented in which a Na^+-free medium was employed by this author were complicated by the fact that 2-(methylamino)isobutyric acid was also present. Inasmuch as the system for histidine uptake which shows the lower K_m value also participates in exchange, and shows a specificity for such amino acids as leucine, methionine, tryptophan and phenylalanine, it would seem to correspond very well with the L system described by OXENDER AND CHRISTENSEN[1]. However, the system showing the higher K_m value for uptake fits the description of the A system only partially since it is apparently not active in exchange but is active in a Na^+-free medium. It seems probable that histidine is taken up by two systems one of which has properties which correspond well with the L system and one of which has properties in common with but not identical to the A system as previously defined. Attempts are currently being made to further characterize this second system.

ACKNOWLEDGEMENTS

The financial support of the National Cancer Institute of Canada and the Medical Research Council of Canada is gratefully acknowledged.

REFERENCES

1 D. L. OXENDER AND H. N. CHRISTENSEN, *J. Biol. Chem.*, 238 (1963) 3686.
2 M. L. BELKHODE AND P. G. SCHOLEFIELD, *Biochim. Biophys. Acta*, 173 (1969) 290.
3 H. LINEWEAVER AND D. BURK, *J. Am. Chem. Soc.*, 56 (1934) 658.
4 H. N. CHRISTENSEN, *Federation Proc.*, 25 (1966) 850.
5 H. N. CHRISTENSEN, *Biochim. Biophys. Acta*, 165 (1968) 251.
6 R. NAKAMURA, *J. Biochem. Tokyo*, 53 (1963) 314.
7 R. M. JOHNSTONE AND P. G. SCHOLEFIELD, *Cancer Res.*, 19 (1959) 1140.
8 C. A. LESLIE, R. H. MATTHEWS AND P. G. SCHOLEFIELD, in preparation.
9 H. N. CHRISTENSEN AND M. LIANG, *Biochim. Biophys. Acta*, 112 (1966) 524.
10 E. HEINZ, *J. Biol. Chem.*, 211 (1954) 781.
11 R. K. CRANE, *Federation Proc.*, 24 (1965) 1000.