REACTIONS OF NEUTRAL AMINO ACIDS PLUS Na^+ WITH A CATIONIC AMINO ACID TRANSPORT SYSTEM *

Halvor N. CHRISTENSEN and Mary E. HANDLOGTEN

Department of Biological Chemistry, The University of Michigan, Ann Arbor, Michigan, USA

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1. Introduction

Before we could understand the transport interactions between the neutral and the basic amino acids, we needed to answer four questions:

1. *To what extent do diamino acids actually react with transport systems in their neutral forms?*

First, α,β-diamino propionic acid, $pK'_2 = 6.7$, was shown to behave in transport almost entirely as a neutral amino acid, an analogue to serine and alanine in one neutral form, and to isoserine and β-alanine in the other [1,2]. Next, we found that α,γ-diaminobutyric acid [3], although largely cationic in neutral solution, was also conspicuously reactive for transport as a neutral acid [4]. Finally it became clear that all the diamino acids, including lysine, were sufficiently reactive as neutral amino acids so that at high concentrations this reactivity could predominate over that with the cationic amino acid ($L\overset{+}{y}$) transport system [6]. Accordingly, we were persuaded to use homoarginine as a test substrate for that system, taking advantage of (a) the exceedingly small extent to which the guanidinium group exists in its depronated form in neutral solutions, and (b) a high transport activity arising from the presence of a large apolar section in the sidechain.

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2. *Is there a special transport system largely indifferent to the presence or absence of a cationic charge on the sidechain?*

When overwhelmingly cationic test substrates are used, we find exceedingly little inhibition of their transport by specific substrates of the A and the L systems, respectively, and also very little inhibition in the reverse direction. The ability of neutral amino acids to inhibit the uptake of cationic amino acids is totally unrelated to their reactivity with system ASC in the Ehrlich cell.

3. *To what extent, if any, do cationic amino acids of appropriate structure react with Na^+-requiring transport systems for neutral amino acids, the second amino group perhaps occupying the site otherwise taken by Na^+?*

This phenomenon exists, we believe. The uptake by the Ehrlich cell of α,γ-diaminobutyric acid at high concentrations appears not as totally dependent on the presence of Na^+ as is the case for corresponding neutral amino acid substrates [4]. Furthermore the Na^+ flux generated during the uptake of this amino acid appears smaller than that seen for typical neutral amino acids. Although lysine and homoarginine appear not to behave in this manner in the Ehrlich cell, preliminary evidence suggests that they inhibit transport by the ASC system in the pigeon red blood cell *.

4. *To what extent, if any, do neutral amino acids in association with Na^+ or another small cation react with site $L\overset{+}{y}$, the small cation taking the position other-*

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wise occupied by the distal amino group of the diamino acid?

This phenomenon proves to be the principal basis of the transport interaction between neutral and cationic amino acids in the Ehrlich cell [6], as well as in the rabbit reticulocyte [6,7] and the pigeon erythrocyte *. Some results with the first of these cells will be reported in this paper.

2. Methods

Uptake of isotopically labelled amino acids by the Ehrlich cell was studied, usually during 1 min at 37° from Krebs-Ringer bicarbonate medium (KRB), modified by partial or complete replacement of Na^+ by choline. Both the cellular and the extracellular compartments were analyzed to observe changes in the distribution of the amino acid. Exodus from the cell was observed also during 1 min, after a 15-min interval of uptake of the labelled amino acid [8]. Each substrate was shown to be largely unmodified during the experimental period. Details of handling the cells and of the conduct of experiments as well as the sources of substrates, are described in prior publications [8,9].

3. Results

Table 1 provides an illustrative protocol showing the role of Na^+ in the inhibition of basic amino acid uptake into the Ehrlich cell by neutral amino acids. Fig. 1 shows that the inhibition of homoarginine uptake by phenylalanine is very extensive and appears to include essentially all the mediated uptake of the cationic amino acid, given that enough Na^+ and phenylalanine are provided. An earlier observation that the inhibition of lysine uptake by phenylalanine is a circumscribed one, and that the uptake of cationic amino acids must therefore be divided between two or more agencies, is now taken to be an artefact of the low Na^+ concentrations inherent to that test [6]. Similar results have been obtained for several other neutral amino acids, including homoserine, inhibition by which is quite small in the absence of Na^+ (table 1). In the rabbit reticulocyte no inhibition whatever of lysine uptake by homoserine could be observed in the absence of Na^+ [7].

A second aspect of the interaction under study in the stimulation of cationic amino acid exodus by the

* Unpublished results, doctoral research of E.L.Thomas in this laboratory.

Table 1

Na^+ participation in the inhibition by neutral amino acids of lysine uptake by the Ehrlich cell. Uptake of lysine-^{14}C in mmoles/kg cell water during 1 min at 37° from a 0.2 mM solution in Krebs-Ringer bicarbonate medium, containing either no Na^+ or Na^+ at 0.113 N. Choline replaced Na^+ in an iso-osmotic manner. Inhibitor present at 0.02 M. Illustrative protocol.

Inhibitory amino acid	No Na^+	$[Na^+]$ = 0.113 N
none	0.61	0.47
cysteine	0.45	0.33
homoserine	0.54	0.23
phenylalanine	0.43	0.25
cysteine and phenylalanine	0.33	0.20
homoserine and phenylalanine	0.37	0.20

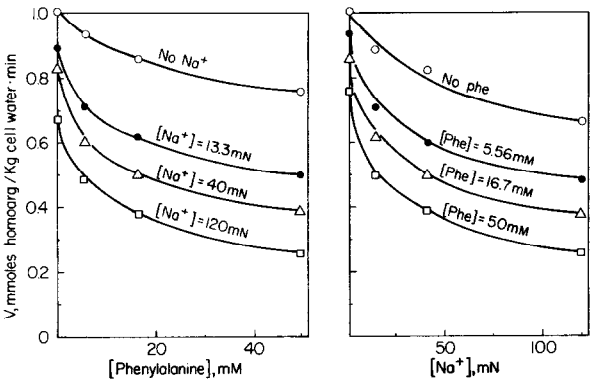

Fig. 1. *Increasing inhibition of homoarginine uptake as the phenylalanine concentration is increased at various concentrations of Na^+ (left); increasing inhibition as the Na^+ concentration is increased at various phenylalanine levels (right).* Same data, *left* and *right*, obtained as indicated in table 1. The curves have been drawn by inspection. Precision was not high enough in the presence of only one of the inhibitors to justify calculation of K_i values for that condition. The results suggest that a larger and larger proportion of the uptake of homoarginine becomes subject to inhibition by one component as the concentration of the other component is increased.

Table 2

Na^+ participation with neutral amino acids in stimulating homoarginine exodus from the Ehrlich cell. Homoarginine-^{14}C was first permitted to be accumulated to about 3 mM by incubation for 15 min in KRB containing the amino acid at 1 mM. After washing twice with KRB, or the same medium in which choline replaced Na^+, exodus was observed by the decrease in cellular ^{14}C during one min at 37° into over 100 volumes of KRB or choline-KRB, containing the indicated amino acid at 10 mM. The results are exodus coefficients, $v/[\text{homoarg}]_{\text{internal}}$, calculated from the apparent mid-time cellular level of homoarginine-^{14}C [8]. Illustrative protocol.

Exp. No.	External amino acid	Relative exodus rate		Factor of increase by Na^+
		No Na^+	$[Na^+]$ = 138 mN	
1	none	0.007	0.041	5.9
	leucine	0.21	0.64	3.5
	phenylalanine	0.11	0.18	1.6
2	none	0.044	0.14	3.2
	methionine	0.23	0.67	3.4
	homoserine	0.092	0.74	8.0
	serine	0.006	0.23	3.8

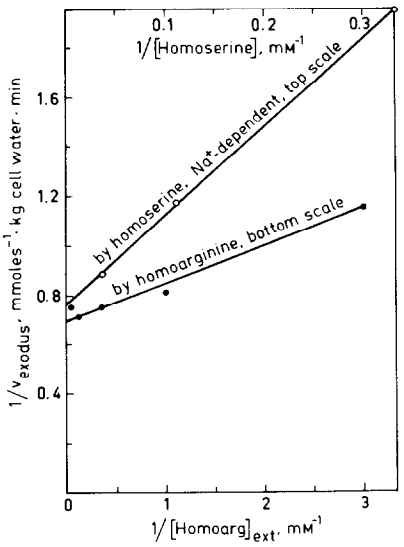

Fig. 2. *Concentration dependence of stimulation of homoarginine exodus from the Ehrlich cell by external homoarginine, and by external homoserine, the latter for the difference between* $[Na^+] = 0$ *and* 116 mN. The cells were loaded to about 3 mM in homoarginine-^{14}C, and then exodus observed during 1 min at 37° into about 100 volumes of Krebs-Ringer bicarbonate medium, pH 7.4, containing 116 mN Na^+ and the indicated amino acid, or an isoosmotic quantity of choline chloride. *Lower line,* effect of external homoarginine concentration, *scale at bottom.* The line corresponds to a V_{max} of 1.3 mmoles per kg cell water·min for the augmentation of homoarginine exodus, a concentration of about 0.1 mM homoarginine producing half that rate. This value corresponds rather well with the K_m of homoarginine for uptake by system L^+_y (0.06 to 0.09 mM). *Upper line,* Na^+-dependent component (shown by difference between exodus at $[Na^+]$ = 116 mN and at initial $[Na^+] = 0$, replacing the NaCl with choline chloride, and $NaHCO_3$ by $KHCO_3$) of the exodus augmentation produced by homoserine, scale at top. This line corresponds to a similar V_{max} for exodus augmentation by homoserine for an increase of $[Na^+]$ from 0 to 116 mN, with 5 mM homoserine sufficing to produce half that augmentation. A direct plot of the same data appears as fig. 9 in ref. [10].

presence of a neutral amino acid in the suspending medium [6]. This effect was likewise found to be greatly enhanced by Na^+. Illustrative protocols are provided in table 2, and fig. 2 compares the dependence of the exodus of homoarginine on the external homoarginine concentration with its dependence on the external homoserine concentration, excluding in the latter case the small Na^+-independent component. Although external Na^+ serves to stimulate cationic amino acid exodus even when no amino acid is present in the medium, its action is weaker than, and competitive with, that of external homoarginine [6]. The K_i value of 5 mM for homoserine in inhibiting homoarginine uptake [6] is indistinguishable from the value for K_e, the concentration producing a half-maximal stimulation in the rate of exodus of homoarginine (fig. 2).

4. Discussion

Study of the structure required for maximal effectiveness of neutral amino acids in influencing the distribution of cationic amino acids shows that a linear hydrocarbon chain is optimal when no external Na^+ is provided, whereas for maximal enhancement of the effects by Na^+, the further attachment of an oxygen or sulfur atom to the sidechain, as in homoserine, homocysteine or glutamine, is optimal [6]. A specific substrate of system L is totally ineffective in both respects. Even when we attempt to stimulate migration

by exchange, we observed in the Ehrlich cell no significant component of neutral amino acid transport sensitive to the presence of a cationic amino acid.

We interpret these results to mean that many neutral amino acids are able to combine with the receptor site for cationic amino acid transport, provided that Na^+ or another suitable cation (not K^+ or choline) is present to take the position normally occupied by the distal amino group of the diamino acid. In this reaction the quasi-substrate, neutral amino acid plus Na^+, appears to be a defective substrate in that the neutral amino acid component did not enter into an exchange for the cationic amino acid. Findings for the movements of the Na^+ component of the two-part substrate are being reported elsewhere [6]. It appears for the second time (see summary of the first occasion in ref. [10]) that neutral amino acids have been shown to generate a transport-related binding site for Na^+; in the present case the position of Na^+ with relation to that of the amino acid seems evident.

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