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

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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, pK₂ = 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 (Lγ) system [5]. 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 deprotonated form in neutral solutions, and (b) a high transport activity arising from the presence of a large apolar section in the sidechain.

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 Lγ, the small cation taking the position other-
wise occupied by the distal amino group of the di-

This phenomenon proves to be the principal basis
of the transport interaction between neutral and cat-
ionic amino acids in the Ehrlich cell [6], as well as in
the rabbit reticulocyte [6,7] and the pigeon erythro-
cyte *. 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), modi-
ﬁed by partial or complete replacement of Na+ by
choline. Both the cellular and the extracellular com-
partments 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 inter-
val 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 publica-
tions [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 up-
take by phenylalanine is very extensive and appears
to include essentially all the mediated uptake of the
cationic amino acid, given that enough Na+ and phenyl-
alanine are provided. An earlier observation that the
inhibition of lysine uptake by phenylalanine is a cir-
cumscribed 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

* Unpublished results, doctoral research of E.L. Thomas in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

Table 1

<table>
<thead>
<tr>
<th>Inhibitory amino acid</th>
<th>No Na+</th>
<th>[Na+] = 0.113 N</th>
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</thead>
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<tr>
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<td>0.47</td>
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<tr>
<td>cysteine</td>
<td>0.45</td>
<td>0.33</td>
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<tr>
<td>homoserine</td>
<td>0.54</td>
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</tr>
<tr>
<td>phenylalanine</td>
<td>0.43</td>
<td>0.25</td>
</tr>
<tr>
<td>cysteine and phenylalanine</td>
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</tr>
<tr>
<td>homoserine and phenylalanine</td>
<td>0.37</td>
<td>0.20</td>
</tr>
</tbody>
</table>

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this laboratory.

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 ab-
sence of Na+ [7].

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

Fig. 1. Increasing inhibition of homoarginine uptake as the
phenylalanine concentration is increased at various concen-
trations of Na+ (left); increasing inhibition as the Na+ con-
centration is increased at various phenylalanine levels (right).
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 Kt values for that condition. The results
suggest that a larger and larger proportion of the uptake of
homoarginine becomes subject to inhibition by one compo-
nent as the concentration of the other component is increased.
Table 2

<table>
<thead>
<tr>
<th>Exp. No.</th>
<th>External amino acid</th>
<th>Relative exodus rate</th>
<th>Factor of increase by Na⁺</th>
</tr>
</thead>
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<tr>
<td>1</td>
<td>none</td>
<td>0.007</td>
<td>5.9</td>
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<td></td>
<td>leucine</td>
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<td>phenylalanine</td>
<td>0.11</td>
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<tr>
<td>2</td>
<td>none</td>
<td>0.044</td>
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</tr>
<tr>
<td></td>
<td>methionine</td>
<td>0.23</td>
<td>3.4</td>
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<tr>
<td></td>
<td>homoserine</td>
<td>0.092</td>
<td>8.0</td>
</tr>
<tr>
<td></td>
<td>serine</td>
<td>0.006</td>
<td>3.8</td>
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</table>

Na⁺ participation with neutral amino acids in stimulating homoarginine exodus from the Ehrlich cell. Homoarginine-[14C] 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 [14C] 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-[14C] [8]. Illustrative protocol.

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.

References