STRUCTURAL REQUIREMENTS FOR AMINO ACID INHIBITION OF SYNAPTOSOMAL AMINO ACID UPTAKE

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1. Introduction

In a previous study [1] we have shown that amino acids have inhibitory effects on the synaptosomal accumulations of one another. The present study deals with the structural requirements of amino acid inhibition of Na^+-non-dependent synaptosomal amino acid transport systems and evaluates the relative roles of the length of the side chain, the α-amino group and the –COOH group in such inhibition. It is shown that while all of the above three are important in regulating the inhibitory activity of an amino acid, the α-amino group of a neutral side chain is indispensable in the interaction of an amino acid with the transport site.

2. Materials and methods

Uniformly labeled L-[^{14}C]amino acids were purchased from New England Nuclear Corp. (Boston, MA) and had the following specific radioactivities (mCi/mmol): phenylalanine 410; leucine 298; valine 225. *N*-acetyl leucine and leucine ethyl ester were products of Research Plus Labs (Denville, NJ). *N*-methyl leucine, *N*-acetyl phenylalanine, phenylalanine amide, phenylalanine ethyl ester, *N*-acetyl phenylalanine ethyl ester, α-ketoisocaproic acid, α-ketoisovaleric acid and isovaleric acid were obtained from Sigma Chemical Co. (St. Louis, MO). All other compounds were purchased from Calbiochem (San Diego, CA).

Cerebral cortices obtained from adult Sprague-Dawley rats were homogenized in 10 vol. ice-cold 0.32 M sucrose solution. Synaptosomal fractions were prepared from the homogenates by the method of Kurokawa et al. [2] and suspended in a buffer medium containing 10 mM Tris–HCl, pH 7.4, 15 mM $MgCl_2$ and 300 mM sucrose. Protein concentrations of the suspensions were determined by the method of Lowry et al. [3]. Portions of the suspensions containing 0.1–0.2 mg of synaptosomal protein were incubated (25°C, 3 min) with a labeled amino acid (0.1 μCi) and various concentrations of unlabeled amino acids/amino acid derivatives, in a final volume of 1 ml. At the end of the incubation period the particles were collected on Millipore filters and assayed for radioactivity as described elsewhere [1].

3. Results

The inhibitory effects of several amino acids on the Na^+-non-dependent synaptosomal uptakes of leucine and phenylalanine are summarized in table 1. The data show a progressive decrease in the I_{50} values with an increase in the length of the side chain, with glycine being the least inhibitory amino acid.

Several amino acid derivatives that had variations in the carboxyl group and in the α-NH_2 position were tested for their ability to inhibit the influx of ^{14}C-labeled phenylalanine, leucine and valine. Results obtained with compounds involving alterations of the carboxyl group are summarized in table 2. Phenylalanine amide, phenylalanine ethyl ester and leucine ethyl ester at concentrations of 0.5 and 1 mM, substantially inhibited the uptakes of the three substrate amino acids. The I_{50} values for these compounds,

Table 1
Inhibition of synaptosomal amino acid uptake by amino acids

Inhibitor Amino acid	Uptake I_{50} (μM)			
	[^{14}C]Phenylalanine		[^{14}C]Leucine	
Glycine	1000 ± 300	(2)	2000	(1)
Alanine	90 ± 25	(3)	235 ± 42	(8)
Valine	10 ± 2	(10)	30 ± 5	(7)
Isoleucine	9 ± 1	(6)	17 ± 3	(8)
Leucine	7 ± 2	(25)	[9 ± 2]	(11)
Phenylalanine	[5 ± 0.5]	(7)	9 ± 2	(7)

Synaptosomal fractions obtained from adult rat brain cortices were incubated with ^{14}C-labeled phenylalanine and leucine under conditions described in the text. The values for I_{50} were derived from Hill type plots in which log $[(v_0-v)/v]$ (v_0 is the uptake rate in the absence of inhibitor and v the rate in the presence of inhibitor at a concentration $[I]$) was plotted against log $[I]$. Values represent means ± SD and values in parentheses are the number of experiments.

however, were considerably higher than those for the corresponding free amino acids. No significant differences could be seen between the inhibitory effects of the amide and of the ethyl ester. In contrast, when the α-amino group was blocked by a methyl or an acetyl group, the inhibitory activity was totally lost. Thus, *N*-acetyl phenylalanine, *N*-acetyl leucine and *N*-methyl leucine failed to inhibit the uptakes of phenylalanine, leucine and valine, even at concentrations as high as 1 mM (table 3). Replacement of the α-NH_2 group with a hydrogen, a keto or a hydroxyl group resulted in elimination of inhibitory activity. Thus, amino acid uptake was not inhibited by the following compounds: phenylpyruvic acid, phenylacetic acid, phenyllactic acid, α-ketoisocaproic acid, α-ketoisovaleric acid and isovaleric acid. As expected from the above results, *N*-acetyl phenylalanine ethyl ester, a compound which has both the carboxyl and the α-amino groups blocked, did not inhibit synaptosomal amino acid uptake (table 3).

The importance of a neutral side chain in the inhibition is dramatically illustrated by the contrasting effects of aspartic acid, glutamic acid and lysine and their uncharged derivatives on amino acid uptake.

Table 2
Effect of modifications of the carboxyl group

Compound	Concentration (mM)	Percent Inhibition of uptake		
		[^{14}C]Phenylalanine	[^{14}C]Leucine	[^{14}C]Valine
Phenylalanine amide	0.5	39	40	33
	1.0	55	50	42
Phenylalanine ethyl ester	0.5	47	37	37
	1.0	63	57	51
Leucine ethyl ester	0.5	54	35	27
	1.0	66	54	36

Synaptosomal fractions were incubated with the ^{14}C-labeled amino acid and the various compounds as described in the text.

Table 3
Effect of modifications in the α-amino position

Compound	Concentration (mM)	Percent Inhibition of uptake		
		[^{14}C]Phenylalanine	[^{14}C]Leucine	[^{14}C]Valine
N-Acetyl phenylalanine	0.5	11	0	7
	1.0	11	0	8
N-Acetyl phenylalanine ethyl ester	0.5	0	8	6
	1.0	0	11	6
N-Acetyl leucine	0.5	3	4	0
	1.0	6	6	0
N-Methyl leucine	0.5	0	3	0
	1.0	0	8	0
Phenylacetic acid	1.0	0	2	3
Phenylpyruvic acid	1.0	4	2	0
Phenyllactic acid	1.0	8	1	2
α-Ketoisocaproic acid	1.0	2	1	2
α-Ketoisovaleric acid	1.0	1	2	0
Isovaleric acid	1.0	0	0	2

Synaptosomal fractions were incubated with the ^{14}C-labeled amino acids and the various compounds as described in the text.

Table 4
Neutral side chain requirement for inhibition of amino acid uptake

Compound	Concentration (mM)	Percent Inhibition of uptake	
		[^{14}C]Leucine	[^{14}C]Phenylalanine
Lysine	1.0	0	0
Norleucine[a]	0.02	61	63
	0.1	78	82
Aspartic acid	1.0	2	4
Asparagine	0.1	44	68
	0.5	71	85
Glutamic acid	1.0	0	6
Glutamine	0.1	51	68
	0.5	77	86

[a] Used in the DL-form

Synaptosomal fractions were incubated with the ^{14}C-labeled amino acids and with the various compounds under conditions described in the text.

Lysine did not inhibit the uptakes of leucine and phenylalanine even at concentrations as high as 1 mM. But its uncharged analog, norleucine (in which the ϵ-NH_2 group is replaced by hydrogen) was highly inhibitory (table 4). Similarly, neither aspartic acid nor glutamic acid inhibited the uptakes of leucine and phenylalanine. However, when the negative charges in their side chains were eliminated by introduction of amide groups in the γ and δ positions, the resulting compounds, asparagine and glutamine, proved to be strongly inhibitory. Total or near total inhibition was observed at 0.5 mM (table 4) and the I_{50} values for inhibition of phenylalanine uptake were in the 30 μM range.

4. Discussion

The results of the present study clearly indicate that while the α-amino group, the carboxyl group and the length of the side chain all influence the inhibitory activity of an amino acid, the α-amino group plays the most significant role in the inhibition. The extent of inhibition increased with the length of the side chain. Blocking of the -COOH group reduced the affinity of the amino acid for the binding site, but did not abolish it. However, replacement of the α-amino group by hydrogen or by a keto group totally abolished the inhibition. Introduction of a methyl group or an acetyl group into the amino group also eliminated the inhibitory ability of the amino acid suggesting that the charge in the α-amino group is not a primary requisite for the inhibition. Thus it appears that in the interaction of the $-NH_3^+$ group with the transport site groups larger than hydrogen cannot be accommodated.

The requirement of a neutral side chain in the inhibition of synaptosomal amino acid uptake is clearly illustrated by the results obtained with the derivatives of aspartic and glutamic acids and of lysine. These results show that compounds with net positive or negative charge in their side chains had no inhibitory effect on synaptosomal amino acid uptake while elimination of the net charge in their molecules resulted in strong inhibition. The present studies lead to a picture of the amino acid binding site in the synaptosomal transport system that is qualitatively similar to that suggested by Lin et al. [4] and by Hajjar and Curran [5] for the intestinal and the brush border transport systems, respectively. They clearly show that the α-amino group of the neutral side chain is a 'sine qua non' in the interaction of a molecule with the transport site and that the length of the side chain contributes to the binding energy.

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