NORMAL AMINO ACID UPTAKE BY CULTURED HUMAN FIBROBLASTS DOES NOT REQUIRE GAMMA-GLUTAMYL TRANSPEPTIDASE

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Summary

The uptake kinetics for four amino acids (cystine, glutamine, methionine, and alanine) which are among the best gamma-glutamyl acceptors have been determined for normal human fibroblasts and for a cell line containing undetectable quantities (<0.5 % normal mean) of gamma-glutamyl transpeptidase activity. Apparent Km and Vmax for uptake for each of the four amino acids were normal in the mutant fibroblasts. Insulin increased the uptake of α-aminoisobutyrate as in control cells. Intracellular levels of 16 amino acids were also normal in this cell strain; the intracellular concentrations of phenylalanine, cystine, and cysteine were increased. In human fibroblasts, amino acid transport appears to proceed normally in the absence of active gamma-glutamyl transpeptidase.

Introduction

The enzyme gamma-glutamyl transpeptidase (E.C. 2.3.2.2.) has a widespread distribution in human and other mammalian tissues (1,2,3). It acts as a glutathionase and catalyzes the transfer of the glutamyl moiety of glutathione (L-γ-glutamyl-L-cysteinylglycine) to a variety of acceptor molecules including certain L-amino acids (4,5). Data have been published compatible with the important hypothesis that this enzyme, in conjunction with the gamma-glutamyl (or glutathione) cycle, plays a crucial role in cellular amino acid transport (5,6), and might be especially important in the translocation of cystine from extracellular to intracellular sites (7). We have tested this hypothesis by examining the transport kinetics for several amino acids, including cystine, in a human fibroblast cell line grossly deficient in gamma-glutamyl transpeptidase activity.

Materials and Methods

The source of the mutant cell line deficient in gamma-glutamyl transpeptidase has been described (8,9). Normal and mutant fibroblasts were propogated by standard methods in Eagle's Minimal Essential Medium with added non-essential amino acids (Grand Island Biological, catalog numbers 109G and 114). Gamma-glutamyl transpeptidase was assayed with gamma-glutamyl-p-nitroanilide (9). Intracellular amino acid pools were determined on fibroblast lines by previously published methods (10) on an automated Beckman model 121M micro-column amino acid analyzer. Intracellular cystine, cysteine, and glutathione concentrations were determined using the cystine-binding protein assay of Schneider et al. (11) and by radiolabelling and high voltage electrophoresis after reaction with N-ethylemaleimide (12).

Amino acid uptake studies were performed with ^{14}C-amino acids of high specific activity and purity using the variant of published methods described in the legend of Table 1 (13). This technique was more time-consuming, but in our hands yielded more reproducible data, than attempts to perform transport studies using cells attached to coverslips. Insulin stimulation of α-aminoisobutyrate uptake was measured as previously described (14).

Results

Gamma-glutamyl transpeptidase activity was undetectable in the mutant cell line (9); by assaying relatively large quantities of crude fibroblast extract we were able to determine that gamma-glutamyl transpeptidase activity was less than 0.5% of the normal mean in the presence of glycylglycine acceptor.

The apparent Vmax and Km values for uptake of radioactive cystine, methionine, glutamine, and alanine are shown in Table 1. Each result is based on at least three determinations on the gamma-glutamyl transpeptidase deficient cell line, and at least two determinations on each of three or more normal cell lines. The data for uptake of all amino acids studied at 5 or more different concentrations over the range of 0.01-1.0 mM were compatible with apparent Michaelis-Menten kinetics, and were so computed. Uptake by the gamma-glutamyl transpeptidase -deficient cell line appeared normal ($p>0.2$) for all four amino acids by these kinetic criteria.

Insulin stimulated uptake of α-aminoisobutyrate in mutant and

Table 1. Apparent kinetic parameters for amino acid uptake by normal and gamma-glutamyl transpeptidase deficient cultured human fibroblasts.

Amino Acid	Vmax* (± S.E.M.)		Km# (± S.E.M.)	
	Mutant	Controls	Mutant	Controls
Cystine	0.268 (.047)	0.334 (.050)	0.107 (.042)	0.116 (.028)
Methionine	0.193 (.039)	0.226 (.025)	0.055 (.009)	0.051 (.010)
Glutamine	0.412 (.027)	0.352 (.039)	0.011 (.003)	0.011 (.002)
Alanine	0.442 (.135)	0.472 (.099)	0.044 (.014)	0.055 (.012)

*Nanomoles/min./mg. cell protein # millimolar

Cells were plated into 60 mm. diameter sterile plastic dishes (Falcon Plastics). 3-4 days later, prior to confluency, the medium was removed, cells were washed 3 times with Dulbecco's phosphate buffered saline, pH 7.4 (Grand Island Biologicals, catalog number K-13), and were then covered by 2 ml. of the same medium with added 0.1% glucose. After 30 minutes at 37°C., this was replaced by the same solution containing a single radioactive amino acid at concentrations of 0.01 - 1.0 mM. Incubation time was 5 minutes, since preliminary experiments had indicated that this yielded approximations of initial uptake rates. After incubation, the medium was removed, the cells rapidly rinsed 2 times with Dulbecco's phosphate buffered saline (3 ml.), detached into 1.0 ml. 0.25% trypsin by scraping with a rubber spatula, and intracellular radioactivity determined as described (13) on the washed cell pellets from quadruplicate aliquots (0.2 ml.) of the cell suspension. Uptake is expressed per unit cell protein, determined by the method of Lowry (20).

normal lines approximately 1.5 fold at an insulin concentration of 200 ng/ml.; the insulin dose response curve in the mutant line was also normal (14,15).

Intracellular amino acid concentrations for the mutant and normal fibroblast cell lines are shown in Table 2. The mean intracellular free amino acid contents of the mutant and normal cells are not significantly different ($p>.05$) for 16 individual amino acids. The phenylalanine, cystine, and cysteine concentrations are moderately but significantly increased in the mutant line.

Table 2. Intracellular free amino acid concentrations in normal and gamma-glutamyl transpeptidase deficient cultured human fibroblasts

Amino acid	Mutant, mean (± S.E.M.)* (nanomoles/mg. protein)	Control, mean (± S.E.M.)# (nanomoles/mg. protein)
Aspartate	10.4 (5.00)	6.16 (1.43)
Threonine	5.36 (1.98)	3.73 (0.75)
Serine	6.43 (2.86)	3.95 (1.00)
Glutamine	2.46 (0.99)	1.66 (0.35)
Proline	7.83 (4.60)	6.81 (1.30)
Glutamate	58.6 (16.5)	35.4 (7.35)
Glycine	16.7 (6.17)	8.65 (2.02)
Alanine	8.34 (1.81)	5.63 (1.14)
Valine	2.78 (0.87)	1.90 (0.31)
Methionine	2.16 (0.62)	2.00 (0.40)
Isoleucine	2.16 (1.08)	1.67 (0.23)
Leucine	3.45 (0.82)	3.03 (0.34)
Tyrosine	1.94 (0.34)	1.76 (0.36)
Phenylalanine	1.89 (0.32)+	1.22 (0.14)
Lysine	1.76 (0.68)	2.86 (0.58)
Histidine	0.60 (0.30)	1.09 (0.28)
Arginine	2.92 (0.19)	4.02 (0.69)
Cystine	0.172 (0.024)+	0.068 (0.007)
Cysteine	17.2 (0.26)+	6.98 (0.42)

* 3 samples

\# 1 sample from each of eight normal lines except for cystine and cysteine (3 normals)

\+ $p<0.05$, mutant vs. control. For the 16 other amino acids, $p>0.05$. Glutathione concentrations were mutant 34.4 (± 3.51) and control 13.8 (± 0.27), $p<0.05$.

Discussion

The hypothesis that gamma-glutamyl transpeptidase is a significant mediator of mammalian amino acid transport finds support in the studies of Meister and associates (5,6) and of others, but evidence to the contrary has been published (16,17). We previously reported that the patient whose fibroblasts have been studied in the present report had normal endogenous renal amino acid clearance, and therefore presumably normal renal resorption (transport) for multiple amino acids (9).

The present report documents that a mutant human fibroblast cell line with undetectable levels of gamma-glutamyl transpeptidase activity

has normal initial uptake kinetics for cystine, glutamine, methionine, and alanine, appropriate enhancement of α-aminoisobutryrate uptake by insulin, and normal intracellular pool sizes of many amino acids. This constitutes a powerful argument against the requirement for gamma-glutamyl transpeptidase in amino acid uptake in these human cells. Furthermore, the extracellular concentrations of amino acids at which uptake proceeds at half maximal rates in both normal and mutant fibroblasts approximate the extracellular concentrations of these amino acids in intact man, but are far lower than the Kms for cystine, methionine, and glutamine acting as glutamyl acceptors in the transpeptidation reaction in several human tissues (2.0 mM cystine, 9.8 mM methionine, and 6.0 mM glutamine) (18). These human kinetic data are compatible with the high Km for methionine (4.5 mM) reported for rat kidney transpeptidase and which has been interpreted by Else & Broxmeyer as evidence against the involvement of the transpeptidase reaction with amino acids in a transport system functioning at physiological extracellular concentrations of these amino acids (17). Our conclusion that gamma-glutamyl transpeptidase does not participate in a significant manner in human fibroblast amino acid uptake is not contradicted by the moderate increases in intracellular phenylalanine, cystine, and cysteine in the mutant line. Even if these increases are not coincidental, they certainly do not suggest deficient uptake; also, phenylalanine is a poor γ-glutamyl acceptor in human tissues (18), and the increased cysteine and cystine pool sizes are most likely related to elevated glutathione concentrations resulting from glutathionase (gamma-glutamyl transpeptidase) deficiency with secondary reduction of cysteine flow through the γ-glutamyl cycle mediated by feedback mechanisms (19).

The gamma-glutamyl cycle, which is present in cultured fibroblasts and many other cell types, appears responsible for the synthesis and degradation

of glutathione but its additional physiological significance remains uncertain.

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