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Cysteine as a System-Specific Substrate for Transport System ASC in Rat Hepatocytes

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Summary

The rapid transport of L-cysteine into isolated rat hepatocytes escapes detectable inhibition by 2-(methylamino)-isobutyric acid at levels up to 50 mM. The system transporting cysteine instead is convincingly similar to the ASC system described for the Ehrlich cell in structural and steric specificity and in pH sensitivity. The Na^+-dependent uptake of 2-aminoisobutyric acid is almost evenly divided between Systems A and ASC, showing better accommodation of its two α-methyl groups by ASC than in the Ehrlich cell. The hepatocyte ASC system tolerates Li^+-for-Na^+ substitution better than does System A, although the tolerance depends on amino acid structure. Adaptive regulation and insulin and glucagon stimulation were not seen under conditions producing these effects for System A.

Introduction

We have described elsewhere a series of unsuccessful efforts to identify a structural feature that will restrict the uptake of an amino acid to transport System ASC in the Ehrlich ascites tumor cell (1). It has not been hard to identify several features any of which restrict uptake to the parallel Na^+-dependent transport System A. The results have allowed wide use of 2-(methylamino)-isobutyric acid (MeAIB*) and related N-methylamino acids as model substrates for that system (2). The absence of a model substrate for ASC has, however, limited researchers to negative criteria for transport by that system, leading to some equivocal assignments of amino acid fluxes for hepatocytes (3), the optic lens (4) and other cells and tissues, namely to assign to that system all Na^+-dependent migration inhibitable by N-methyl amino acids. In other cases all Na^+-dependent uptake has been provisionally assigned to System A even though part or all of that uptake is relatively insensitive to inhibition by glycine,

*Abbreviations: AIB = 2-aminoisobutyric acid; MeAIB = its N-methyl derivative.

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AIB or proline, and much more sensitive to such typical ASC substrates as serine, alanine, threonine, homoserine and 2-amino-n-butyric acid. We have illustrated elsewhere that the principal Na^+-dependent system serving for uptake of amino acids by the intestinal mucosa of the rabbit has features that resemble more closely corresponding features seen for System ASC than for System A (Fig. 5 in ref. 5). These two systems differ sharply in the response to Na^+ occasioned by the position taken by a hydroxyl group on the amino acid side-chain. We take the recent results of Sepûlveda and Smith (6) as adding to the evidence that the principal Na^+-dependent system of the intestine may be ASC and is not A.

Edmondson *et al*. (7) recently described a tolerance of the substitution of Li^+ for Na^+ that might be useful for identifying uptake by System ASC of the rat hepatocyte. This tolerance is reversed from that applying for the Ehrlich cell. In our hands the difference in tolerance in the hepatocyte is not great enough to promise a sharp discrimination of the two systems.

We describe here an extended characterization of this transport system in rat hepatocytes. We find that L-cysteine can serve in the hepatocyte as a specific substrate for System ASC, and hope that it may serve for the discrimination of System ASC fluxes in other tissues, including the intestinal epithelium.

Materials and Methods

Hepatocytes were isolated by the method of Potter and his associates (8) with minor modifications. Cell viability as measured by ability to exclude trypan blue was always greater than 85%. For cell culture experiments 2 to 3 x 10^6 cells were plated on 60-mm plastic culture dishes previously coated with rat tail collagen (8). The cells were incubated at 37°C in Waymouth's medium containing insulin (60 µU/ml) for the first 4 hours. At this time the medium was changed to Waymouth's medium lacking insulin and the cells were incubated an additional 20 hours. In some cases Krebs-Ringer bicarbonate buffer was used instead to provide an amino-acid-free medium. Amino acid transport was measured by adding 3 ml of the uptake medium to each dish for the desired interval of time, after which they were aspirated and rinsed with cold phosphate-buffered saline. The cells were removed with the aid of a rubber "policeman" into 0.2 N NaOH, and aliquots taken for determination of radioactivity and protein (9). Uptake by suspended cells was measured in a manner similar to that used for Ehrlich cells (10). In all experiments with cysteine, dithiothreitol was present at 5 or 10 mM.

Results

Results obtained in studies of reciprocal inhibition between MeAIB and L-cysteine are shown in Table I. MeAIB clearly had no inhibitory action on 100 μM cysteine uptake at concentrations up to 50 mM. Previous experiments (11) have shown that MeAIB retains the sharp specificity for System A first shown for the Ehrlich cell (2), allowing the use of inhibition by this amino acid to measure System A mediation. In contrast to the lack of MeAIB inhibition of cysteine transport, the uptake of MeAIB was decreased significantly by the addition of unlabeled cysteine. This action on System A is reminiscent of the inhibition of System ASC in the Ehrlich cell by non-substrate analogs, and means that although cysteine can serve as a model substrate for System ASC, its inhibitory action is not that sharply limited. To determine if the absence of MeAIB inhibition arose from an unusually high K_m for L-cysteine uptake by System A, the transport of cysteine was tested at a concentration of 2 mM. Even at this higher substrate level, no System A component was detected (data not shown). Inhibition of cysteine uptake by the model System L substrate, 2-aminonorbornane-2-carboxylic acid, was less than 10% (from 355 ± 29, to 322 ± 10 nmoles·g cellular water^{-1}·min^{-1}), part of which inhibitory action was retained in the absence of Na^+. Other typical ASC substrates (e.g., alanine, serine) produced major decreases in cysteine uptake. Cysteate and cysteine failed to show competition for transport; hence cysteine is not taken up appreciably by the system serving for anionic amino acid such as glutamate (Table I in ref. 1).

When compared to System A, System ASC of the Ehrlich cell shows a higher stereospecificity and a lower sensitivity to H^+ (13). In hepatocytes, inhibition of L-cysteine uptake by unlabeled D- or L-cysteine showed so much less activity for the D isomer as to suggest stereospecificity as high as that of the Ehrlich cell (data not shown). When the external pH was lowered from 7.4 to 6.5, the Na^+-dependent uptake of MeAIB was abolished, while that of L-cysteine was only suppressed by about 30% (78.5 to 51.7 nmoles·g ICW^{-1}·min^{-1}).

Table I

Reciprocal inhibition of MeAIB and L-cysteine in isolated rat hepatocytes in suspension.

Test amino acid 100 μM →	L-cysteine	MeAIB
Inhibitor mM → ↓	MeAIB	L-cysteine
0 (Na^+)	88.1 ± 2.3	20.6 ± 0.5
0 ($Chol^+$)	20.3 ± 0.8	9.8 ± 0.7
2	89.3 ± 5.0	19.4 ± 2.3
10	91.2 ± 1.3	15.9 ± 0.5
25	87.1 ± 3.3	12.8 ± 0.3
50	89.4 ± 2.4	12.9 ± 1.0

Uptake of the test amino acid during 1 min. was measured at 37°C and pH 7.4. Differences in osmotic strength were compensated by isoosmotic addition of choline chloride. The data, expressed as nmoles·g $ICW^{-1}\cdot min^{-1}$, are the averages ± S.D. of three determinations.

Several natural and model amino acids were surveyed to determine the role that System ASC plays in their Na^+-dependent transport (Table II). The results of lines 1 and 7 point to a lack of specificity of AIB , whose transport (along with that of its natural analogs, alanine and glycine) was equally divided between the two systems. As expected from earlier work (13), the presence of a sidechain hydroxyl or sulfhydryl group resulted in a greater specificity for System ASC. The thio ether group of S-methyl cysteine had a similar effect.

During these studies, results of Edmondson et al. (7) suggested that its ability to tolerate Li^+-for-Na^+ substitution could be used to measure hepatic ASC transport. In partial agreement we find that System ASC retains in this cell rather more of its activity in the presence of Li^+ than does System A (11). On the other hand we find that the uptake of amino acids in response to Li^+ depends on their structure (Table III). For example, although half of Na^+-dependent AIB uptake was mediated by System ASC (Table II), very little AIB accumulation occurred when Li^+ was substituted for Na^+ (Table III). Furthermore, the portion of alanine uptake mediated by System A surviving in the presence of Li^+ rose from 4% to almost 20% when the hepatocyte donors, normally fed *ad libitum*, were

Table II

Relative contribution of Systems A and ASC to the total Na^+-dependent transport by isolated hepatocytes in suspension.

Test amino acid 100 μM	Per cent of total Na^+-dependent transport		Comparative Na^+-dependent rates (nmoles·g ICW^{-1}·min^{-1})
	A	ASC	
2-(methylamino)-isobutyric acid	100	0	11
L-Methionine	70	30	5
L-Valine	67	33	2
L-Proline	64	36	8
L-Serine	60	40	28
L-Alanine	56	44	17
2-Aminoisobutyric acid	55	45	3
Glycine	47	53	12
L-Leucine	30	70	1
S-Methyl-L-cysteine	19	81	16
L-Threonine	16	84	28
L-Homoserine	14	86	82
L-4-Hydroxylproline	9	91	9
L-Phenylalanine	Approx. 0	100	3
L-Cysteine	0	100	190

The Na^+-dependent transport of the test amino acids listed (100 μM) was differentiated into the A and ASC components by inhibition with 25 mM MeAIB. Only approximate comparisons are provided by the rates in the final column because they derive from several different lots of cells. The rates are all averages of at least three determinations.

Table III

Effect of overnight fasting of the donor rat on hepatocyte transport by Systems A and ASC in the presence of Na^+, Li^+, and choline.

Nutritional status of donor rat	Test amino acid 100 μM	nmoles·g ICW^{-1}·min^{-1}		
		Na^+	Li^+	$Chol^+$
Fed	L-Alanine	32.1 ± 2.8	21.1 ± 0.4	12.3 ± 0.2
	Alanine + 25 mM MeAIB	28.1 ± 2.5	20.7 ± 0.7	13.3 ± 0.6
Fasted	L-Alanine	51.1 ± 2.5	32.9 ± 1.4	23.6 ± 2.0
	Alanine + 25 mM MeAIB	40.5 ± 2.3	31.2 ± 1.0	25.1 ± 2.3
	AIB	16.5 ± 1.4	9.0 ± 1.0	8.1 ± 0.3

Uncultured hepatocytes in suspension were used to measure 1-min uptake of the indicated amino acid at 37°C and pH 7.4. All of the media used contained 25 mM choline bicarbonate and the chlorides of Na^+, Li^+, or choline (117 mM) as indicated. The results are averages ± S.D. of three determinations.

Table IV

Lack of adaptive regulation of System ASC in cultures hepatocytes.

Inhibitor	Cation	100 μM MeAIB		100 μM L-cysteine	
		Amino-acid-rich medium	Amino-acid-free medium	Amino-acid-rich medium	Amino-acid-free medium
None	Choline	5.9 ± 3.9	6.1 ± 2.4	48.7(47.7-49.7)	43.2(38.7-47.7)
None	Na^+	11.9 ± 0.6	85.1 ± 1.9	478 ± 30	517 ± 41
MeAIB	Na^+	7.5 ± 0.9	9.0 ± 1.0	477 ± 21	511 ± 19
L-Cysteine	Na^+	7.0 ± 1.9	10.2 ± 3.2	74.6(70.7-78.5)	98.5(96.7-100.3)

Cells were incubated in the presence (in Waymouth's medium) or the absence (in Krebs-Ringer bicarbonate medium) of extracellular amino acids for 6 h at 37°C prior to assay. Experiments with inhibitors used a decreased concentration of NaCl to compensate for the osmolarity of the added inhibitor (25 mM). In some cases the data, expressed as pmoles·mg $protein^{-1}$·min^{-1}, are the average of three determinations, while other values are averages of the numbers shown in parentheses.

subjected to an overnight fast (Table III). This fast of about 18 h also resulted in an increase in the Na^+-dependent uptake of alanine (19.8 compared to 27.5) most of which could be accounted for by an increase in System A activity (Table III, lines 1 and 3).

In contrast to earlier reports (14), Kelley and Potter (8) and our laboratory as well (15) have shown stimulation of System A uptake after amino acid starvation of cultured hepatocytes. Table IV shows enhanced MeAIB transport on amino acid deprivation, and inhibition of both basal and stimulated activities by the non-substrate L-cysteine. System ASC was not significantly stimulated by amino acid starvation (Table IV).

Discussion

The present results show that the activity of System ASC in rat hepatocytes can be tested directly with L-cysteine. The hepatic ASC system resembles that characterized in the Ehrlich cell with two notable exceptions. First, in contrast to the situation in the Ehrlich cell, it tolerates Li^+-for-Na^+ substitution better than System A. In this connection, the rate of Li^+-dependent uptake by System A increased when the total Na^+-dependent rate was increased by fasting. Secondly, as we want to emphasize here, AIB does not serve reliably as a speci-

fic test substrate for System _A_ in hepatocytes. Other investigators have also reported heterogeneity in AIB uptake (8,16,17). Further characterization of System _ASC_ may result in a reassessment of some previous studies made with AIB. Interestingly, the small Na^+-dependent component of phenylalanine uptake was not inhibitable by MeAIB. Goldenberg _et al._ (18) have found that a putative _ASC_ system in LPC-1 plasmacytoma cells shows a strong affinity for phenylalanine.

Our tests for adaptive regulation (19), as well as preliminary tests of hormone action, suggest that System _ASC_ may largely escape stimulation in these ways in which System _A_ is stimulated. In spite of this lack of regulation, its high transport rates for metabolically active amino acids makes attention to System _ASC_ important in biological studies.

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