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TRANSPORT OF L-METHIONINE IN HUMAN DIPLOID FIBROBLAST STRAIN WI38

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Summary

The transport of L-methionine in human diploid fibroblast strain WI38 was investigated. The uptake of L-methionine was measured in sparse cell cultures in a simple balanced salt solution buffered with either Tris · HCl of *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid (HEPES). Similar results were obtained with these two buffers. Cultures were allowed to equilibrate with the buffered saline before transport was measured. The presence of glucose in the buffered saline results in a slight reduction in the initial rate of transport for the first 2 h of equilibration in buffered saline. L-Methionine is actively transported in WI38 by saturable, chemically specific mechanisms which are temperature, pH and, in part, Na^+ dependent, and are reactive with both L- and D-stereoisomers. Kinetic analysis of initial rates of transport at substrate concentrations from 0.0005 to 100 mM indicated the presence of two saturable transport systems. System 1 has an apparent K_M of 21.7 μM and an apparent V of 3.57 nmol/mg per min. System 2 has an apparent K_M of 547 μM and an apparent V of 22.6 nmol/mg per min. Kinetic analysis of initial rates of transport in Na^+-free media or after treatment with ouabain suggested that system 1 is Na^+ independent and that system 2 is Na^+ dependent. Preloading of cells with unlabeled L-methionine greatly increases the initial rate of uptake. Efflux of transported methionine is temperature dependent, and is greatly increased in the presence of unlabeled L- or D-methionine or L-phenylalanine, but not in the presence of L-arginine. L-Methionine transport is strongly inhibited by other neutral amino acids, and is very weakly inhibited by dibasic amino acids, dicarboxylic amino acids, proline or glycine.

Abbreviation: HEPES, *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid.

Introduction

It has been suggested that alterations in transport activities may be of central importance in the mechanisms of neoplastic transformation [1], cellular aging [2], and performance of the cell cycle [3,4]. Biological transport has been recently reviewed [5].

The transport properties of cultured human diploid fibroblasts have not so far been well characterized, in spite of their importance for the study of human genetics and biochemistry. There have been few investigations of amino acid transport in human diploid fibroblasts.

Groth and Rosenberg [6] investigated the transport of arginine, lysine, cystine, and tryptophan in cultured human fibroblasts from normal subjects, from patients with cystinuria and from patients with Hartnup disease, but were unable to demonstrate any disease-related transport defects. They observed that lysine and arginine share two distinct transport systems, neither of which is reactive with cystine. Tryptophan was also transported by two systems which were not further characterized as to specificities for other amino acids. Na^+ was not required for the transport of any of the amino acids studied.

One of the few other published studies of amino acid transport in cultured human fibroblast is that of Hillman and Otto [7,8]. These workers used the method of Foster and Pardee [9] in which transport is measured in confluent monolayers attached to cover-slips. They report that cells from a patient with α-methyl acetoacetyl-CoA β-ketothiolase deficiency display an apparent defect in the uptake of isoleucine. They suggest, but do not conclude, that these cells have an alteration in the isoleucine transport system. The patient's cells lacked Na^+-dependent, ouabain-sensitive transport, but did not display this phenotype until they had undergone more than 18 population doublings in vitro. In normal cells they report the presence of three saturable systems for the uptake of isoleucine, two Na^+-dependent systems and one Na^+-independent system.

We have characterized the transport systems for a neutral amino acid in the human diploid fibroblast strain WI38. L-Methionine was used as a protype substrate because of its relevance to other investigations in progress in this laboratory.

Experimental

Cells. Human diploid fibroblast strain WI38 was the cell type used in this study. WI38 was derived by Hayflick [10] from female human embryonic lung. Cells for these experiments were obtained from Dr. Hayflick under an N.I.H. contract (N01-HD-2828) or from Flow Laboratories. Cells were used either directly upon receipt from the supplier or after 2—10 passages in this laboratory. All results reported are from experiments done with cells before the 32nd passage. Cells from the two sources displayed no discernable differences in their transport properties.

Cultivation of cells. Procedures for maintenance and preservation of these cell strains generally followed the protocol of Hayflick (unpublished). All cells were maintained in Dulbecco's modification of Eagle's minimal essential medium [11], with 10% (v/v) active fetal bovine serum, 100 units penicillin G and

100 μg streptomycin per ml, and 15 mmol HEPES buffer per l. Cultures were generally subcultivated at a 1 : 4 split ratio. Medium was replaced every 2—3 days until confluency was reached. At the time of subcultivation cells were removed from flasks with 0.05% trypsin in HEPES-buffered Hanks' balanced salt solution with 100 units penicillin G and 100 μg streptomycin per ml. Cultures were routinely maintained in 75-cm^2 plastic Falcon tissue culture flasks. Cultures of WI38 from both sources were checked for mycoplasma contamination by the method of Schneider et al. [12] and were found to be uninfected.

Materials. L-Methionine was obtained in radiolabeled form from New England Nuclear Corp. as L-[*Me*-^{14}C]methionine or as L-[*Me*-^{3}H]methionine. L-[*Me*-^{14}C]Methionine was checked for purity by paper chromatography and was found to be substantially free from contamination with other compounds. L-[*Me*-^{3}H]Methionine was purified by paper chromatography before use to eliminate a small amount of radiolabelled methionine sulfoxide.

Fetal bovine serum, growth medium, and antibiotics were obtained from Grand Island Biological Co. Twice crystallized type II bovine pancreatic trypsin was obtained from Sigma Chemical Co. Other reagents were in general the highest grade available from Sigma Chemical Co. or from California Corporation for Biochemical Research.

Paper chromatography. Ascending paper chromatography in one dimension of radiolabeled material was performed with Whatman No. 1 chromatography paper in *N*-butanol/acetic acid/water (25 : 4 : 10, v/v). For material extracted from cells with 5% trichloroacetic acid, standards were treated similarly before application to the paper. After development, the paper was cut into 1-cm strips and counted for radioactivity.

Measurement of amino acid transport. Uptake was measured by a modification of methods used by a number of workers in other cell culture systems [13,14]. The following protocol was used with minor variations. Cells are seeded into 6-cm Falcon dishes to give a desired cell density and allowed to incubate 1—2 days in complete growth medium at 37°C. The dishes are then rinsed with 5 × 2 ml of buffered saline at pH 7.4 and allowed to equilibrate with the buffered saline usually for 60—90 min. The equilibration time was always held constant for any particular experiment. Transport is initiated by removing the buffered saline and adding radioisotope in 1 ml buffered saline. The reaction is stopped a few seconds after removal of most of the radioisotope-containing buffered saline by rinsing with 5 × 2 ml buffered saline at 0°C. The amount of radiolabel taken up by the cells can then be determined in one of several ways. For initial rate determination, in which very little of the radiolabel in the cells is incorporated into protein, the following procedure has proved useful. The washed cells are extracted with 1 ml and then with 2 × 0.5 ml ice cold 5% trichloroacetic acid. Radioactivity in the combined extracts is then measured in a liquid scintillation counter using internal standardization. The extracted cells, which remain attached to the dish, are then dissolved in 0.5 ml 1 M NaOH. Cell protein is measured by the method of Lowry et al. [15] against a bovine serum albumin standard. Background for both the protein and radioactivity measurements is taken, respectively, as the amount of protein bound to dishes incubated with growth medium and serum but no cells, and the radioactivity extracted from these dishes after they are processed in the

same manner as those with cells. After the appropriate conversion, uptake is expressed in nmol/mg per min. For determination of radioactivity remaining in the trichloroacetic acid-precipitated material, cells are dissolved in 1 ml 1 M NaOH. Exactly half of this is taken for determination of radioactivity, again using an internal standard, using the other half for the Lowry reaction. In kinetic experiments a uniform specific activity was used at all substrate concentrations in a given experiment. For measurement of efflux, cells were typically incubated 30 min with radioisotope in buffered saline without preincubation; radioactivity remaining with the cells at intervals of time after rinsing with non-radioactive buffered saline was then determined. The buffered saline contained, per l: 116 mequiv. Na^+, 2.7 mequiv. K^+, 2 mequiv. Ca^{2+}, and 2 mequiv. Mg^{2+} (all cations added with Cl^-) buffered at pH 7.4 with either 20 mM Tris · HCl or 15 mM HEPES. For substrate concentrations greater than 10 mM the buffered saline was diluted appropriately for the maintenance of uniform osmolality. Integrity of the cells was maintained under all above-stated conditions. All initial rate determinations reported here were derived from 1-min accumulations of radiolabel. Departure from linearity with time occurs between 1 and 2 min of uptake.

Cell volume estimation. For determination of cellular volume, trypsinized cells were washed by centrifugation twice with Tris-buffered saline, and once with a small volume of Tris-buffered saline containing 1 μCi [^{14}C]inulin per ml. The cells were then resuspended in Tris-buffered saline with 1 μCi [^{14}C]inulin per ml, incubated 10 min, and pelleted into centrifuge tubes with graduated chambers. The volumes of the pellets were recorded. The pellets were then dissolved in 1 ml (total volume) of 0.01% sodium dodecyl sulfate/0.001 M ethylenediaminetetracetic acid. Protein and radioactivity were measured in aliquots of this homogenate, using an internal standard for radioactivity determination. Inulin-labeled water was considered to be extracellular. A value of 0.617 μl/mg cellular protein was obtained. This value was used as a conservative estimate of cellular water in the calculation of distribution ratios.

Results

Effect of cell density

Transport rates were measured exclusively in sparse cell cultures. It was found that the initial rates of transport of L-methionine calculated per mg cellular protein does not vary significantly as a function of cell density over the range used in this study, up to 0.25 mg per 6-cm dish.

Effect of pH

Initial rate of transport of L-methionine in WI38 is pH dependent. The optimal pH for uptake is 7.4 within the 6.8—8.5 range examined. This correlates well with the optimal pH reported for growth in WI38 [16].

Metabolic conversion

Paper chromatography of L-[*Me*-^{14}C]methionine extracted from cells labeled for 2 min was used to estimate the degree to which methionine is metabolized. After 1 min; virtually all of the radiolabel is recovered as material which co-

chromatographs with authentic L-[*Me*-^{14}C]methionine. Radiolabeled methionine sulfoxide, an oxidation product, present as a radiolabeled trace contaminant does not appear in the cell extract. Contaminated methionine was purified by paper chromatography prior to kinetic experiments, however. After 1 h of incubation, approx. 18% of the radiolabel is transformed to material that does not migrate with either methionine or methionine sulfoxide. Unless otherwise stated, uptake rates have not been corrected for metabolic conversion.

Effect of preincubation in buffered saline

Initial rate of transport declines as a function of time of preincubation in buffered saline. After 4 h of preincubation the initial rate of transport is 80—85% of the rate without preincubation. However, during the first 30 min of preincubation, the initial rate is significantly elevated. To measure the rate of uptake reproducibly in a simple salt solution, it is therefore necessary to allow the cells a period of at least 30 min for equilibration. The decrease in initial rate with time in buffered saline is completely prevented by addition of excess methionine to the buffered saline used for preincubation.

Time course of uptake

The time course of methionine uptake is shown in Fig. 1. Velocity of uptake is maximal during the first 2 min. Thereafter, the velocity of accumulation of trichloroacetic acid-soluble methionine slows and finally reaches a plateau after about 40 min with 4.8 nmol/mg cellular protein at a substrate concentration of 0.01 mM. This corresponds to an equilibrium distribution ration of 78. After 2 min of uptake, less than 2% of the total radiolabel taken up by the cells was

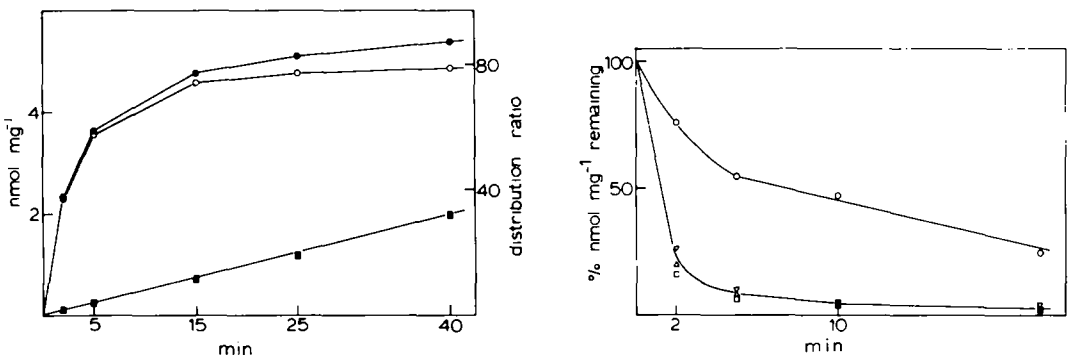

Fig. 1. Accumulation of L-methionine at 37°C and at 5°C. Uptake of L-[^{3}H]methionine (0.01 mM) in WI38-23 was measured in HEPES-buffered saline after an initial 30-min preincubation in HEPES-buffered saline. Each point is the mean of triplicate measurements. Distribution ratio is internal concentration of trichloroacetic acid-soluble methionine divided by external concentration. ●, total nmol/mg (at 37°C); ○, trichloroacetic acid-soluble nmol/mg (at 37°C); □, total nmol/mg (at 5°C); △, trichloroacetic acid-soluble nmol/mg (at 5°C).

Fig. 2. Efflux of L-methionine into buffered saline or into buffered saline containing L-methionine, D-methionine, or L-ethionine. Efflux of L-[^{14}C]methionine (labeling concentration = 0.01 mM) was measured in WI38-16 at 37°C in HEPES-buffered saline, in HEPES-buffered saline with L-ethionine at 0.1 mM, in HEPES-buffered saline with L-methionine at 0.1 mM, or in HEPES-buffered saline with D-methionine at 0.1 mM. Cells were labeled 30 min before efflux was initiated. Each point is the mean of triplicate determinations. ○, HEPES-buffered saline; □, HEPES-buffered saline + L-ethionine; △, HEPES-buffered saline + L-methionine; ▽, HEPES-buffered saline + D-methionine.

incorporated into trichloroacetic acid-soluble material. Uptake of methionine is temperature dependent. At 5°C uptake is linear with time and much slower than at 37°C.

Efflux

The rate of exit of exogenously supplied methionine is similar in magnitude to the rate of accumulation (Fig. 2). Cells lose most of the methionine accumulated in 30 min in the first 20 min of exodus. The efflux system displays the phenomenon of accelerative exchange diffusion [17], i.e. the presence of L-methionine in the external medium greatly enhances the rate of exit. D-Methionine, L-ethionine, and L-phenylalanine are also active in accelerating efflux (Fig. 2). L-Arginine, putrescine, spermine, D-glucose, 3-*O*-methylglucose (at 0.1 mM or ten times labeling concentration), or ouabain (at 1 mM), however, have no significant effect on efflux. Efflux is temperature dependent, being neglibible at 0°C. Efflux in the presence of external methionine is also reduced at 0°C, but not so much as is efflux at 0°C into buffered saline alone.

Initial rate kinetics

Initial velocities of transport as a function of substrate concentration were measured in WI38 over a low (less than 1 mM) and a high (greater than 1 mM) range of substrate concentration. Earlier work on phenylalanine transport in other strains suggested that at least two systems were active in mediating transport. Two widely separated ranges of concentration were studied since kinetic analysis over a concentration range in which both systems have substantial activity is unproductive, i.e. no reliable constants can be derived from a biphasic double reciprocal plot if only the curvilinear part of the curve is known.

At substrate concentrations up to 0.1 mM, kinetic analysis revealed a trans-

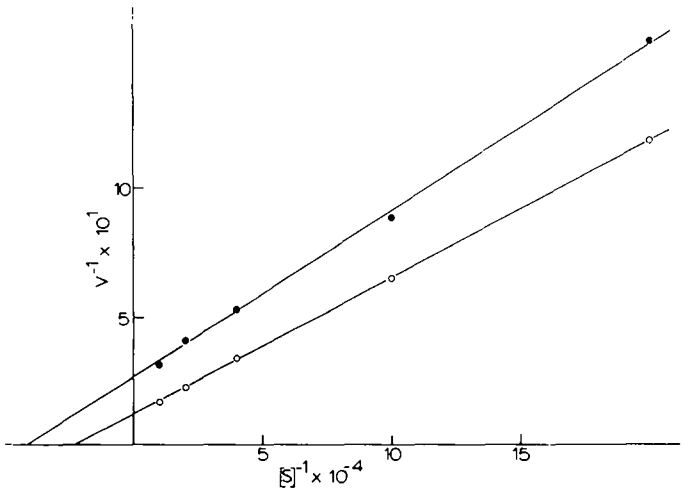

Fig. 3. Double reciprocal plot of initial rate of L-methionine transport against substrate concentration (less than or equal to 0.1 mM) in buffered saline or in Na^+-free buffered saline. Initial rates of transport of L-[^{14}C]methionine in WI38-17 were measured at 37°C after 90 min preincubation in Tris-buffered saline or in Tris-buffered saline with NaCl replaced by choline chloride. Lines shown were fitted by linear regression. Each point is the mean of triplicate measurements. ○, Tris-buffered saline; ●, Na^+-free Tris-buffered saline.

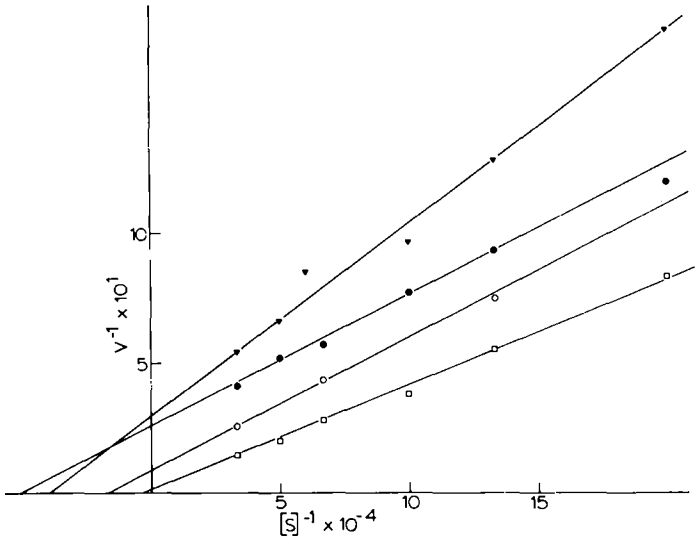

Fig. 4. Double reciprocal plot of initial rate of L-methionine transport against substrate concentration (less than or equal to 0.1 mM) in buffered saline, in Na^+-free buffered saline, in buffered saline after preincubation with ouabain, or in buffered saline after preincubation with excess non-radioactive L-methionine. Initial rates of transportation of L-[^{14}C]methionine in WI38-23 were measured at 37°C after 150 min preincubation. Cells were rinsed with 2 ml HEPES-buffered saline immediately before uptake. Lines shown were fitted by linear regression. Each point is the mean of triplicate measurements. V is in nmol/mg per min; S is in M. ○, preincubation and uptake in HEPES-buffered saline; ●, preincubation and uptake in HEPES-buffered saline, with Na^+ replaced by Tris-buffered saline; ▼, preincubation in HEPES-buffered saline, with ouabain at 1 mM; uptake, in HEPES-buffered saline; □, preincubation in HEPES-buffered saline, with L-methionine at 10 mM; uptake, in HEPES-buffered saline.

port process for methionine which conforms to the Michaelis-Menten equations (Figs. 3 and 4).

Analysis of the data in Fig. 3 gives an apparent K_m of 44.4 ± 7.3 μM and an apparent V of 7.26 ± 0.89 nmol/mg per min. If NaCl in the buffered saline is replaced with either choline chloride or Tris · HCl, or if the cells are precincubated in the presence of ouabain, both the K_m and the V are lowered (Figs. 3 and 4). Analysis of these data give similar estimates of the reduced values of the kinetic constants. The means of the three estimates are an apparent K_m of 21.7 ± 2.0 μM and an apparent V of 3.7 ± 0.12 nmol/mg per min.

Preincubation in 10 mM non-radioactive L-methionine results in much higher transport rates of all concentrations (Fig. 4). This phenomenon has been called trans-stimulation [18]. The proximity to the origin of the ordinate intercept of the fitted line for these data in the double reciprocal plot suggests that uptake after preincubation with excess substrate is not a saturable process.

At high substrate concentrations, uptake is non-saturable (Fig. 5). Subtraction of the non-saturable flux reveals that presence of a saturable system which operates preferentially at high substrate concentrations. The contribution of the non-saturable flux to the observed rates of transport was estimated by three methods which gave essentially identical results. With the method of Akedo and Christensen [19] the apparent permeability constant, K_p, is taken from the ordinate intercept of a plot of distribution ratio per min against reciprocal substrate concentration. The value of the intercept is equal to the value of the

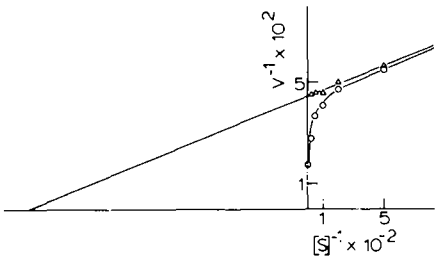

Fig. 5. Double reciprocal plot of initial rate of L-methionine transport against substrate concentration (greater than or equal to 2.0 mM), corrected for diffusion. Initial rates of transport of L-[^{14}C]methionine in WI38-20 were measured at 37°C after 90 min preincubation in HEPES-buffered saline. Observed velocities were corrected for diffusion by methods described in the text. Each point is the mean of triplicate measurements. V is in nmol/mg per min; S is in M. ○, observed flux; △, saturable flux.

term, $1 - e^{-k_p t}$, in the equation:

$$D_m = \frac{K_p}{1 - e^{-k_p t}} D_0 - (1 - e^{-k_p t})$$

(D_m = distribution ratio resulting from a mediated process, D_0 = Distribution ratio observed). For these data the value of K_p is 0.0478.

The other two methods of correction used the following equation:

$$v_m = v_0 - K_D [S]$$

i.e. mediated velocity, v_m, equals observed velocity, v_0, with the velocity increasing linearly as a function of substrate concentrations, K_D [S], subtracted. The apparent diffusion constant, K_D, was determined in two ways. The ordinate intercept of a plot of velocity divided by substrate concentration against reciprocal substrate concentration has been used to estimate the apparent diffusion constant [7]. The K_D from the plot of these data is 0.288/min. The other method for estimating K_D was in iterative procedure which sought a value for K_D which gave the least error of fit to a straight line for derived v_m values in a double reciprocal plot. This procedure yielded a best K_D of 0.275/min. The means of the constants derived for the high substrate concentration data (Fig. 5) are an apparent K_m of 547 ± 19 μM and an apparent V of 22.6 ± 0.11 nmol/mg per min.

A plot of the averaged apparent kinetic constants for the rates at high substrate concentrations, at low substrate concentrations and at low substrate concentrations in the absence of Na^+ or in the presence of ouabain are shown in Fig. 6. Line d in the plot was derived by addition of the low substrate concentration values indicated by extrapolation of the line for the system most active at high substrate concentration to the ouabain-insensitive, Na^+-independent values at substrate concentrations less than 0.05 mM. It is clear that the observed rates at substrate concentrations less than 0.05 mM can be accounted for as the sum of rates for two kinetically distinguishable modes of entry, suggesting that there are two saturable systems for the uptake of methionine in WI38: a high K_m, Na^+-dependent system, and a low K_m, Na^+-independent system.

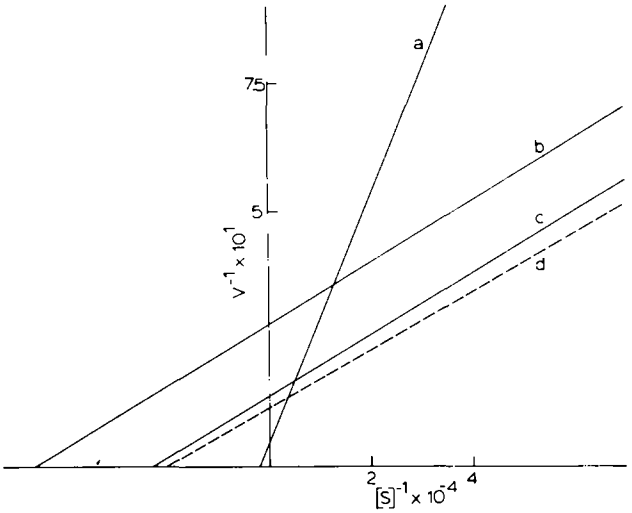

Fig. 6. Double reciprocal plot of kinetic constants for L-methionine transport derived from analysis of averaged data for high and low substrate concentrations. a, Constants were derived from corrected rates at high substrate concentrations; b, constants were derived from rates at low substrate concentrations in the absence of Na^+ or after ouabain treatment; c, constants were derived from rates at low substrate concentrations; d, line was drawn through points derived by summing extrapolated rates at substrate concentrations less than 0.05 mM from line a, with those from line b, at substrate concentrations less than 0.05 mM.

Inhibition by other amino acids

The results of inhibition experiments suggest that methionine transport in WI38 is not stereospecific. D-Methionine is an excellent inhibitor of L-methionine uptake (Table I), and also is as effective as L-methionine in accelerating efflux of transported L-methionine when present in the exit medium (Fig. 2).

Inhibition of L-methionine uptake by other amino acids is shown in Table I. Generally, amino acids whose side chains are uncharged at neutral pH are good inhibitors. Amino acids with side chains bearing a charge at neutral pH are generally very poor inhibitors, as are α-amino-isobutyric acid, cystine, glycine, and proline. Serine, threonine, asparagine, and cysteine inhibit to a degree intermediate between these two groups.

The effects of ouabain on transport rate are shown in Fig. 7. The effect of ouabain on the kinetics of methionine transport is similar to the effect of removing Na^+ from the buffered saline. The trans-stimulation effect (the increase in transport rate in cells preincubated in excess substrate) is not reduced by incubation with ouabain, that is, the reduction in rate observed with ouabain in cells preincubated in excess methionine is less than the reduction in rate observed with ouabain in cells preincubated in buffered saline alone. This is consistent with the observation that the transport rate becomes more sensitive to ouabain inhibition as cells are preincubated in buffered saline (Fig. 7). At a substrate concentration of 0.2 mM, the maximum distribution ratio achieved in an accumulation experiment is reduced 48% by ouabain at 0.5 mM. Simultaneous inhibition of initial transport rate by ouabain and either L-alanine, L-serine, or L-asparagine suggests that these amino acids do not preferentially inhibit either ouabain-sensitive or ouabain-insensitive uptake at a substrate concentration of 0.2 mM. Ouabain has no significant effect on efflux.

TABLE I

INHIBITION OF L-METHIONINE UPTAKE BY OTHER AMINO ACIDS

Initial rates of uptake in the presence of the inhibiting amino acid are expressed as a percentage of the initial rate observed without an inhibitor. The substrate concentration is 5 μM, except where indicated.

Inhibitor	Ratio of [I] to [S]					
	1	2.5	10	25	50	100
L-Ethionine	82.0 *	—	38.0 *	—	—	10.0 *
D-Methionine	90.3 *	—	38.2 *	—	—	10.7 *
L-Isoleucine	—	—	—	—	—	16.6
L-Histidine	—	—	—	—	—	19.0
L-Tryptophan	—	—	—	—	—	19.5
L-Tyrosine	—	—	—	—	—	19.7 **
L-Valine	—	—	—	—	—	21.6
L-Leucine	—	—	—	—	—	23.0
L-Phenylalanine	59.0 *	57.6	29.1 *	31.3	28.5	24.0 *
L-Alanine	—	—	27.0 ***	—	—	59.7
L-Serine	—	—	43.2 ***	—	—	57.9
L-Asparagine	—	—	52.4 ***	—	—	56.9
L-Threonine	—	—	—	—	—	62.2
L-Cysteine	—	98.8	—	100.4	64.8	—
L-Arginine	84.7 *	—	84.7 *	—	—	78.9 *
α-Amino-iso-butyric acid	—	100.6	—	89.7	84.0	—
L-Proline	—	—	—	—	—	84.7
L-Lysine	—	—	—	—	—	87.9
Glycine	—	98.8	—	96.5	92.3	—
L-Cysteine	101	97.3	101	97.3	95.6	—
L-Aspartic acid	—	—	—	—	—	96.8
L-Glutamic acid	—	101.7	—	101.2	100.3	—

* S = 10 μM.
** I to S ratio = 95.
*** S = 200 μM.

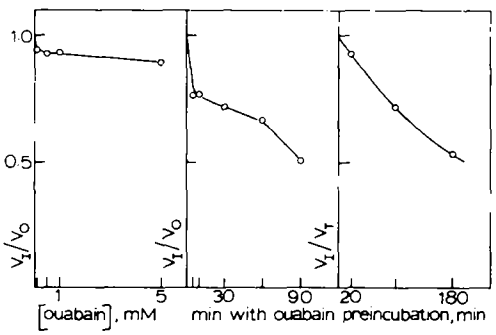

Fig. 7. Effects of ouabain on initial rate of L-methionine transport. Inital rates of transport of L-[^{14}C]-methionine (0.2 mM) were measured in WI38 at 37°C in HEPES-buffered saline after preincubation in HEPES-buffered saline or in HEPES-buffered saline with ouabain. In the first panel, ouabain concentration was varied; all cells were preincubated 20 min. In the second panel, time of exposure to ouabain (0.5 mM) was varied; all cells were preincubated 90 min, with ouabain added at various times before uptake, except for the first point; uptake rate is expressed as a fraction of the rate in cells preincubated for the indicated times without ouabain. V_I is the rate with ouabain treatment; V_0 and V_T are the rates without ouabain treatment; all V values are in nmol/mg per min. Each point is the mean of 2—3 measurements.

TABLE II

SUMMARY OF RESULTS ON NEUTRAL AMINO ACID TRANSPORT IN CULTURED HUMAN FIBROBLASTS

Substrate	[S] < 1 mM		Requires Na^+	[S] > 1 mM		Requires Na^+	Preincubated	Cell density	Range of [S] (mM)	Ref.
	K_M *	V *		K_M *	V *					
Met	21.7	3.57	no	547	22.6	yes	yes	low	0.0005—100	this study
Trp	20.0	12.35	no	12 500	61.7	no	no	— **	0.04—10	11
Ile	150.0	35.0	yes	8 330	103	yes	no	high	0.05—150	12
Ile	—	—	—	510	37	no	no	high	0.05—150	12
Cystine	43	1.11	—	—	—	—	yes	high	0.04—0.125	15
α-AIB ***	—	—	—	1 000	8.2	—	yes	high	0.1—2.0	18
α-AIB ***	—	—	—	1 520	4.41	—	yes	low	0.1—4.0	17
α-AIB ***	—	—	—	1 500	3.03	—	yes	high	0.1—4.0	17

* K_M in μM, V in nmol/mg per min (V converted from other units where necessary, using a value of 0.162 mg cellular protein per μl cellular water).

** Transport activities measured in suspensions of trypsinized cells.

*** α-Amino-isobutyric acid.

Discussion

There are at least three kinetically distinguishable modes of entry of L-methionine in WI38: (1) A saturable system with a low apparent K_m (21.7) and a low apparent V (3.57 mmol/mg per min); does not require Na^+ and is insensitive to ouabain (system 1). (2) A saturable system with a high apparent K_m (547 μM) and a high apparent V (22.6 mmol/mg per min); requires Na^+ and is sensitive to ouabain (system 2). (3) A non-saturable mode of entry which is discernable only at high substrate concentration (physical diffusion).

The results of transport studies by other investigators with other neutral amino acids in normal cultured human diploid fibroblasts are summarized in Table II. One important source of variation is the contribution of trans-stimulated flux to the rates observed in some studies. When cells are removed from growth medium and placed in a simple buffered saline lacking substrate and substrate analogues, they require at least 30 min to achieve stable transport activity. The results of efflux experiments suggest that this is the approximate amount of time required to lose most of the free internal pool. Apparent transport activity is significantly elevated during this period. A narrow range of substrate concentrations has been used by earlier workers. In one case the lowest concentration used exceeds the K_m of our system 1 by a factor of 2—5.

At typical plasma concentrations of L-methionine, uptake is principally mediated by system 1. The apparent K_m for system 1 (21.7) μM) correlates quite well with reported plasma concentrations (3—29 μM with a mean of 21 μM [20]). The physiological significance of system 2 is uncertain. It could be of value to cells when plasma concentrations are increased in the non-fasted state.

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