Biochimica et Biophysica Acta 856 (1986) 413–420
Elsevier

BBA 73046

The transport of glutamine into rat mesenteric lymphocytes

M. Salleh M. Ardawi * and Eric A. Newsholme

Department of Biochemistry, University of Oxford, South Parks Road, Oxford, OX1 3QU (U.K.)

(Received December 5th, 1985)

Key words: Amino-acid transport; Glutamine; Serine; (Rat mesenteric lymphocyte)

(1) The transport of glutamine into isolated rat mesenteric lymphocytes was studied. (2) This transport appears to be dependent upon the Na^+ gradient. The K_m for glutamine transport was about 1.0 mM. (3) A large number of amino acids were shown to inhibit the rate of transport of both serine and glutamine into lymphocytes. The transport of glutamine was competitively inhibited by serine and that for serine was similarly inhibited by glutamine. In contrast, histidine and 2-(methylamino)isobutyrate inhibited the transport of both serine and glutamine noncompetitively. (4) It is concluded that glutamine is transported into rat mesenteric lymphocytes by a process similar to System ASC described for other cells.

Introduction

Most mammalian cells have at least three distinct transport systems responsible for the entry of neutral amino acids. These are designated as Systems A, ASC and L. In addition, System N, which preferentially transports the amido amino acids and histidine, is present in hepatocytes. These transport systems have been characterized on the basis of their substrate specificity and properties (for review, see Refs. 1–3).

Systems ASC and L have both been shown to be primarily responsible for the transport of neutral amino acids in lymphocytes [4–6]. In resting lymphocytes, System A is either absent or inactive [4,7], but it is present after cellular activation [4–5]. One particular amino acid, glutamine, has been shown to be of importance as an energy source for lymphocytes [8–10] and it must be present for in vitro proliferation to occur. Indeed, the acute stimulation of proliferation by concanavalin A increases the rate of glutamine utilisation by lymphocytes. Furthermore, the transport of glutamine across the cell membrane of lymphocytes is probably one of the regulatory steps involved in the control of glutamine metabolism [10–14]. hence the specificity and properties of the transport of glutamine into rat mesenteric lymphocytes have been studied and the results compared with those for glutamine transport in hepatocytes and enterocytes obtained in other work [3,15]; the effect of concanavalin A stimulation on glutamine transport has also been investigated and the results are presented and discussed.

Materials and Methods

Animals. Male Wistar albino rats (160–180 g) were obtained from Batin and Kingman, Grimston, Hull, U.K.

Chemicals and enzymes. All chemiclas and enzymes were obtained from Boehringer Corp. (London), London, U.K., except for the following: glycine, scintillants and all inorganic reagents were obtained from Fisons Scientific Apparatus,

* To whom correspondence should be addressed at (present address): Department of Biochemistry, College of Medicine and Allied Sciences, P.O. Box 9029, Jeddah, Saudi Arabia 21413.

Loughborough, U.K.; concanavalin A, nigrosine, ouabain, 2,4-dinitrophenol, iodoacetate, rotenone, cycloserine, choline salts, serum albumin (Fraction V) and all amino acids were obtained from Sigma (London) Chemical Co., Kingston-upon-Thames, U.K.; Repelcote, Silicone Fluid MS 500 and dinonylphthalate were obtained from Hopkins & Williams, Chadwell Heath, Essex, U.K.; foetal bovine serum was obtained from Flow Laboratories, Irvine, U.K.; the serum was dialyzed as described previously [9]; and ion-exchange chromatography resins were obtained from Bio-Rad, Watford, U.K. All radiochemical compounds were obtained from the Radiochemical Centre, Amersham, U.K.

Preparation of lymphocytes. Lymphocytes were prepared from rat mesenteric lymph nodes as described by Ardawi and Newsholme [8]. Cells were suspended in phosphate-buffered saline [16] that had been oxygenated for 30 min, under 100% O_2 at $2 \cdot 10^8$ cells/ml until use.

Incubation procedures. Incubations were performed at 37°C in 10 ml silicone-treated Erlenmeyer flasks in a total volume of 1.0 ml of phosphate-buffered saline (which had been oxygenated by 100% O_2 for 30 min) supplemented with 1% (v/v) dialyzed foetal bovine serum at pH 7.2 [16]. The incubation medium also contianed [U-^{14}C]glutamine or [U-^{14}C]serine (0.05 μCi) together with [^{3}H]sucrose (0.2 μCi); the latter acted as a marker for the extracellular space.

To study the effect of concanavalin A on the transport of glutamine, lymphocytes were incubated in phosphate-buffered saline with 5 mM glucose for 60 min in the presence or absence of concanavalin A, after which cells were separated by centrifugation ($400 \times g$ for 5 min) and washed twice with phosphate-buffered saline, and concanavalin A-activated lymphocytes were then used for the measurement of glutamine transport.

In some experiments in which various metabolic and other inhibitors were used, lymphocytes were incubated for 15 min in the presence or absence of an inhibitor, after which cells were separated by centrifugation, resuspended and used for the measurement of glutamine transport.

Measurement of transport. In the present work, the initial rate of glutamine or serine transport was determined by the silicone-oil-layer technique (for review, see Ref. 17). A series of 1.5 ml Eppendorf polyethylene tubes were prepared by layering 0.15 ml of silicone-oil mixture (a mixture of 1 : 1 (v/v) silicone fluid MS 550 and dinonylphthalate) on top of 0.1 ml of 12% (w/v) $HClO_4$. Glutamine or serine transport was initiated by the addition of 0.1 ml of lymphocyte suspension ($2 \cdot 10^8$ cells/ml) to the incubation medium which contained [^{3}H]sucrose to act as a marker of the extracellular space together with [U-^{14}C]glutamine or [U-^{14}C]serine to give the required final concentration. Samples (0.5 ml) were removed at appropriate time intervals and the transport was terminated by centrifugation of cells through the silicone-oil-layer into $HClO_4$ layer at $8500 \times g$ for 40 s in an Eppendorf microcentrifuge. Preliminary experiments established that, during the incubation, the lymphocytes maintained their biochemical viability as indicated by ATP content and the nigrosine exclusion test. In addition, no mixing of the layers occurred and approx. 95% of the cells were recovered in the $HClO_4$ layer after only 10 s of centrifugation.

At the completion of a transport experiment, the radioactivity in the $HClO_4$ layer was measured using a scintillant mixture which contained 750 ml Triton X-100, 6.0 g 2,5-diphenyloxazole and 0.15 g 1,4-bis(phenyloxazolyl)benzene in 1.5 litres toluene. Radioactivity was measured in a Beckman liquid scintillation counter (Model LS7500).

In some experiments in which glutamine uptake was determined by enzymatic methods, the procedure was modified. Samples were withdrawn at appropriate time intervlas and cells were immediately separated from the incubation medium by centrifugation for 5 s at $8500 \times g$ in an Eppendorf microcentrifuge. The lymphocyte pellet was acidified with $HClO_4$ and neutralized as described previously [9] and three or four pooled neutralized extracts were used for the assay of glutamine. There were no significant differences in glutamine determinations between radioactive and enzymatic methods.

Metabolism of glutamine or serine during the cours eof the transport studies. In order that the uptake of a labelled amino acid should provide an accurate estimate of the rate of transport, intracellular accumulation of the amino acid must not restrict transport. This restriction is unlikely, since

rat lymphocytes have a high capacity to metabolise glutamine [9,10]. Nonetheless, preliminary experiments were performed to assess the amount of glutamine utilised and its fate; during the first 5 min of an incubation, lymphocytes utilised only 9% of glutamine in the incubation medium (i.e., in the presence of a 0.5 mM glutamine) almost all of which was converted to glutamate and ammonia (Table I). Furthermore, the initial rate of [U-^{14}C]glutamine transport was linear with time for 2 min (Fig. 1). This was confirmed by further experiments in which lymphocytes were incubated in the presence of [U-^{14}C]glutamine (0.5 mM) for periods up to 15 min. Incubations were terminated as described previously [9]. Glutamine and the glutamate formed were separated by the use of ion-exchange chromatography (Dowex AG-1 X8, acetate form) as described previously [18]. This separation indicates that if measurements are restricted to about 2 min, the uptake of [U-^{14}C]glutamine should represent transport. Similar conclusions were made for glutamine-transport measurements in enterocytes [15].

For the transport of [U-^{14}C]serine into lymphocytes, preliminary experiments indicated that the initial rate of uptake was linear for at least 2 min (results not shown) and no significant changes in rate were observed when 10 mM cycloserine was included in the incubation medium. Furthermore, when lymphocytes were incubated in the presence of [U-^{14}C]serine (0.5 mM) for periods up to 10 min, all the radioactivity was recovered as

TABLE I

TIME-COURSE OF GLUTAMINE METABOLISM BY RAT MESENTERIC LYMPHOCYTES

Lymphocytes were incubated ($2 \cdot 10^7$ cells/ml) for the times indicated in the presence of 0.5 mM glutamine. Incubations were terminated as described in Materials and Methods. Rates of utilisation (indicated by a minus sign) or production are given as means ± S.E. for four separate experiments (each experiment consists of two cellular incubations).

Time of incubation (min)	Rates of utilization or production (nmol/mg protein)			
Metabolites:	glutamine	glutamate	aspartate	ammonia
5	−3.5 ± 0.1	0.3 ± 0.1	-	0.6 ± 0.1
10	−7.2 ± 0.3	4.5 ± 0.2	1.8 ± 0.1	2.6 ± 0.2
60	−58.5 ± 4.4	33.4 ± 2.6	14.1 ± 1.9	36.3 ± 2.9

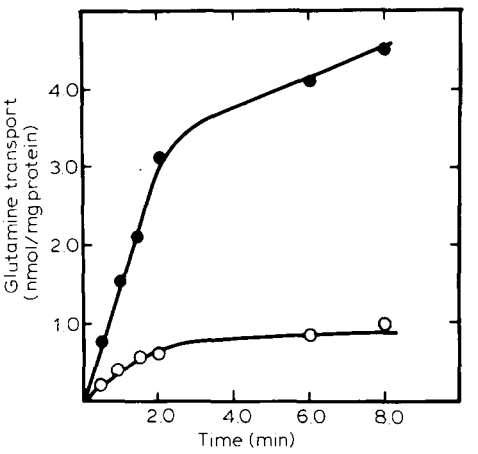

Fig. 1. Time-course of the transport of glutamine by rat mesenteric lymphocytes in the presence (●) or absence (○) of added Na^+. The initial concentration of glutamine was 0.5 mM. For experiments performed in the absence of added Na^+, choline replaced Na^+.

serine which was separated by the use of ion-exchange chromatography (Dowex AG 1-X8, formate form) [19]. It is considered that [U-^{14}C]serine uptake measured over the first 2 min should therefore represent the uptake of serine under the conditions used here.

Intracellular water of mesenteric lymphocytes was determined as the difference in volume available to [U-^{14}C]sucrose and that available to ^3H_2O. The value was found to be 2.37 ± 0.08 μl per mg lymphocyte protein (mean of six cell preparations).

Analytical methods. Glutamine and other metabolites in neutralized extracts of lymphocytes were determined by the same methods as described previously [9]. Cell protein was determined by the biuret method [20].

Expression of results. Two to four replicate determinations were made for each value presented in the results section. The initial rate of glutamine or serine uptake is expressed as nmol amino acid taken up per min per mg protein of cells, calculated on the basis that $1 \cdot 10^7$ lymphocytes are equivalent to 0.851 ± 0.05 mg prtoein.

Results

General properties of glutamine transport

Ion and energy dependence. The concentration ratio of intra- to extracellular glutamine achieved

after 2 min of incubation at 0.5 mM glutamine, was 2.43 ± 0.3 (radioactively determined). These results suggest that the transport of glutamine is energy-dependent. This was supported by the finding that treatment of rat mesenteric lymphocytes with various concentrations of 2,4-dinitrophenol, rotenone or iodoacetate decreased the rate of glutamine transport (Table II). The decrease in rate was, however, small, suggesting either a low rate of entry of these inhibitors or an ability to maintain an Na^+ gradient for a long period of time despite a decrease in the rate of ATP formation. Many active transport processes utilise the energy of the Na^+ gradient and this was investigated by studying the effect of ouabain and the dependence of transport on Na^+. Incubation of rat lymphocytes for 15 min with 1 mM ouabain decreased the rate of transport of glutamine by 60% (Fig. 2). The Na^+ in the incubation medium (NaCl and $NaHCO_3$) was replaced by equivalent concentrations of choline chloride and bicarbonate. In the absence of added Na^+, the rate of glutamine transport was low (Fig. 1) (about 20% of that in the presence of Na^+). The effect of increasing the Na^+ concentration is shown in Fig. 3. The concentration required to produce the half-maximal effect was about 10 mM. It was also observed that Li^+ could not substitute for Na^+ (Table III). These results are in contrast to those found for the System A in ascites tumour cells [21] and for System ASC or System N in hepatocytes [3,22], but are similar to that found for the System ASC in pig erythrocytes and rabbit reticulocytes [23].

TABLE II

EFFECTS OF VARIOUS METABOLIC INHIBITORS ON THE INITIAL RATES OF GLUTAMINE TRANSPORT IN MESENTERIC LYMPHOCYTES

For details of experimental procedures see Mateirals and Methods. The rates of transport were measured after 1 min incubation. The results are presented as mean ± S.E. with the numbers of separate cell preparations given in parenthesis. Statistical significance of differences between control rates and rates in the presence of an inhibitor is indicated by * ($P < 0.01$), ** ($P < 0.001$).

Additions to incubation	Concentration (mM)	Initial rate of glutamine transport (nmol/min per mg protein)
None (control)		1.60 ± 0.05 (4)
2,4-Dinitrophenol	0.1	1.39 ± 0.02 (3)
	0.5	0.92 ± 0.07 (3) *
Iodoacetate	2.0	1.26 ± 0.07 (4)
	5.0	1.17 ± 0.06 (4) *
Rotenone	5 [a]	1.10 ± 0.09 (4) **

[a] μg/ml.

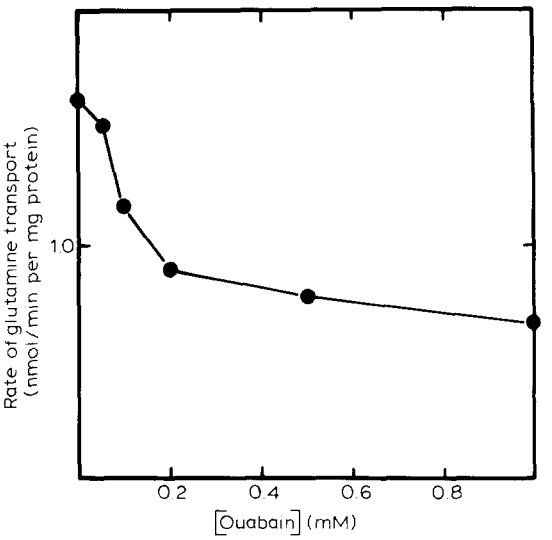

Fig. 2. Effect of ouabain concentration on the initial rate of glutamine trnasport by rat mesenteric lymphocytes. Lymphocytes were preincubated for 15 min with concentrations of ouabain indicated. The initial concentration of glutamine was 0.5 mM.

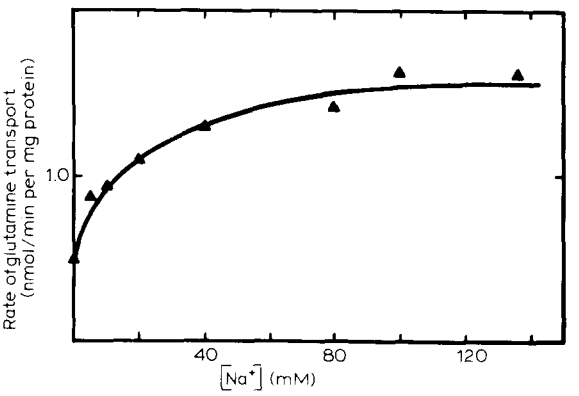

Fig. 3. Effect of Na^+ concentration on the initial rate of glutamine transport by rat mesenteric lymphocytes. The initial concentration of glutamine was 0.5 mM. For experiments in which the Na^+ concentration was below 140 mM, choline replaced Na^+.

TABLE III

EFFECTS OF Na^+ OR Li^+ ON THE INITIAL RATE OF GLUTAMINE TRANSPORT BY MESENTERIC LYMPHOCYTES

For details of experimental procedures, see Materials and Methods. The rate of transport was measured after 1 min incubation. In the absence of Na^+, we replaced NaCl and $NaHCO_3$ with equal concentrations of choline chloride and choline bicarbonate, respectively, and in the presence of Li^+, NaCl and $NaHCO_3$ were replaced by equal concentrations of LiCl and choline bicarbonate, respectively. The results are presented as means ± S.E. for six separate experiments.

Concentration of ion (mM)		Rate of glutamine transport (nmol/min per mg protein)
Na^+	Li^+	
100	0	1.65 ± 0.04
0	0	0.40 ± 0.02
0	10	0.36 ± 0.03
0	50	0.51 ± 0.02
0	100	0.56 ± 0.05

Concentration dependence. The K_m for glutamine transport (obtained from a double-reciprocal plot (Fig. 4)) was about 1.0 mM. This value is an order of magnitude higher than values for System ASC operation with other amino acids in several cell types (see Ref. 1). Similar values were obtained for glutamine transport in rat hepatocytes and enterocytes [3,15], respectively.

Inhibition of glutamine transport by other amino acids

The study of the inhibitory effect of one amino acid on the transport of another amino acid has proved useful for the characterisation of amino acid-transporting systems and such studies have indicated the existence of a number of amino-acid transport systems in mammalian cells [1]. In order to obtain information about the substrate specificity of the transport system for glutamine in rat mesenteric lymphocytes, the effect of various amino acids on this process together with their effects on the transport of serine were investigated. From a Dixon plot (Fig. 5), serine appears to be a competitive inhibitor of glutamine transport, with a K_i of approx. 4.0 mM. The inhibition of the transport of serine by glutamine was also competitive ($K_i = 3.3$ mM) (results not shown). On the other hand, histidine, which is known to use System N in rat hepatocytes [3], was found to inhibit the transport of glutamine in lymphocytes in a

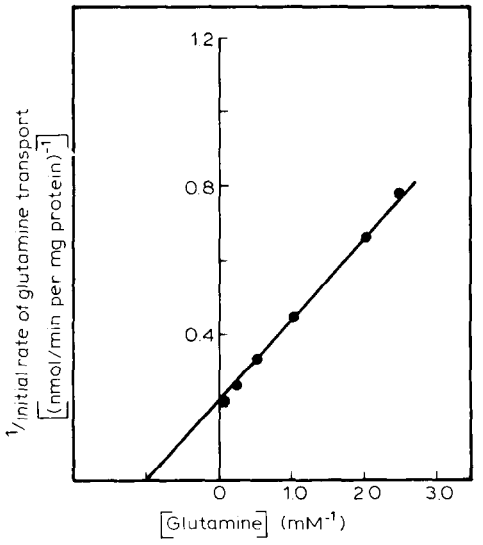

Fig. 4. Double-reciprocal plot showing how the initial rate of glutamine transport varied with the concentration of the amino acid. The initial rates of glutamine transport were measured over a 1 min time intervals. Results are the menas of two separate experiments (each consists of two separate cellular preparations).

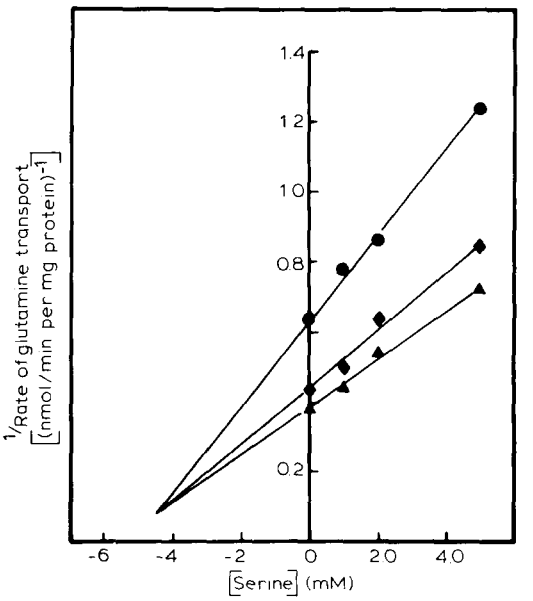

Fig. 5. Dixon plot of the effect of serine on the initial rate of glutamine transport by rat mesenteric lymphocytes. Rates of glutamine transport were measured over 1 min intervlas at various concentrations (mM) of glutamine: ●, 0.5; ◆, 1; ▲, 2. Lines were drawn according to the method of least squares.

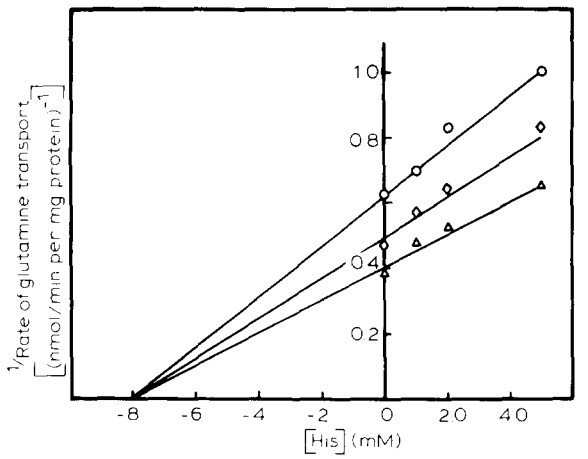

Fig. 6. Dixon plot of the effect of histidine concentration on the initial rate of glutamine transport by rat mesenteric lymphocytes. Rates of glutamine transport were measured over 1 min time intervals at various concentrations (mM) of glutamine: ○, 0.5; ◇, 1; △, 2. Lines were drawn according to the method of least squares.

noncompetitive manner (Fig. 6) with a K_i for histidine of approx. 8.0 mM. Similarly, the inhibition of serine transport by histidine was non-competitive, with a K_i of approximately 8.3 mM (results not shown). 2-Methylaminoisobutyrate, a nonmetabolisable amino acid analogue that is transported only by the Na^+-dependent System A in most cells [1], caused only about 20% inhibition of both glutamine and serine transport in

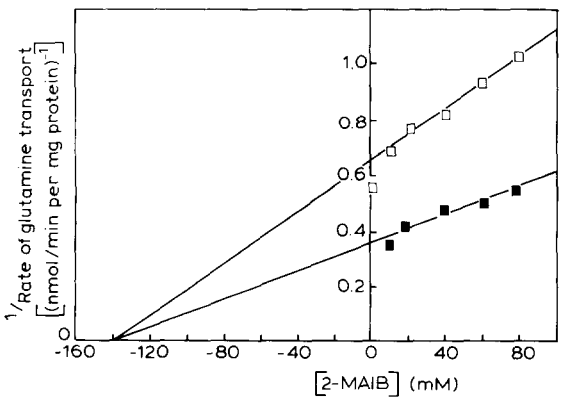

Fig. 7. Dixon plot of the effect of 2-(methylamino)isobutyrate (2-MAIB) concentration on the initial rate of glutamine transport by rat mesenteric lymphocytes. Rates of glutamine transport were measured over 1 min time intervals at concentrations (mM) of glutamine: □, 0.5; ■, 2. Lines were drawn according to the method of least squares.

lymphocytes, and this inhibition was found to be noncompetitive with respect to glutamine, with a K_i of approx. 140 mM (Fig. 7). Similarly, the inhibition of serine transport by 2-(methylamino)-isobutyrate was noncompetitive (K_i = 90 mM) (results not shown).

Effect of concanavalin A stimulation on glutamine transport

An increase in the rate of transport of amino acids across the cell membrane is a feature of the response of mammalian cells in general to growth stimuli [24,25]. The incubation of lymphocytes with concanavalin A increased the initial rate of glutamine transport. (1.57 ± 0.08 nmol/min per mg protein for resting cells and 2.44 ± 0.11 nmol/min per mg protein for concanavalin-A-stimulated cells). The V (maximal velocity) of glutamine transport increased from 5.6 to 8.0 nmol/min per mg protein, but there was no change in the value of the K_m.

Discussion

The results of this study on the rate of glutamine transport into mesenteric lymphocytes suggest that it is achieved by a carrier similar to that known as the System ASC [1]. The following points are in support of this suggestion.

(i) The transport of glutamine is energy-dependent and probably uses the free energy of the Na^+ gradient.

(ii) The transport of glutamine was competitively inhibited by serine and vice versa. The K_m for glutamine transport was similar to the K_i for the inhibition of serine transport by glutamine and, similarly, the K_m for serine transport is similar to the K_i for the inhibition of glutamine transport by serine.

(iii) 2-Methylaminoisobutyrate, a selective substrate for the System A, and histidine, a selective substrate for the System N, caused noncompetitive rather than competitive inhibition of glutamine transport.

(iv) Amino acids that inhibit the uptake of glutamine inhibit the uptake of serine to the same extent.

However, if the transport ASC system is reponsible for glutamine transport into rat

mesenteric lymphocytes, it is different from that reported in other cells, since it appears to transport a large number of amino acids (results not shown). In this respect it is similar to System ASC present in pig lymphocytes [5]. Indeed, it was concluded that the System ASC was responsible for the utpake of all neutral amino acids in pig lymphocytes [5]. Consistent with this view, the rate of transport by the System A was found to be low or absent in lymphocytes from rat spleen or the pig [4,5,7]. Similarly, the System A is absent in other blood cells, including erythrocytes and reticulocytes [26—7]. It is concluded, therefore, that neither the System N of rat hepatocytes [3,24] nor the System A of rat enterocytes [15] makes a major contribution to the transport of glutamine into rat mesenteric lymphocytes and that glutamine is transported into these cells mainly via a system resembling the System ASC of other cells.

During mitogenic stimulation of lymphocytes, an increase in the transport of both syntheitc and natural amino acids was reported [24,28,29]. It has been proposed that mitogen-mediated stimulation of amino acids transport in lymphocytes requires de novo synthesis of a protein factor or a component of the transport system [30,31]. The present work indicated a 55% increase in the rate of glutamine transport in concanavalin-A-stimulated lymphocytes. This stimulation resulted from an increase in the V of glutamine transport with no change in the K_m. By analogy with insulin stimulation of glucose transport, it is possible that the rate of glutamine transport is enhanced by increasing the number of carriers in the cellular membrane.

Glutamine is an important fuel for rat mesenteric lymphocytes [9–14]. The marked increase in the rate of glutamine transport upon mitogenic stimulation of lymphocytes provides further support for the importance of this amino acid during proliferation. How far the increase in rate of transport of glutamine into lymphocytes is an important step for control of the rate of glutamine utilisation remains to be established.

Acknowledgements

We acknowledge the interest and encouragement of the late Professor R.R. Porter, F.R.S. M.S.M.A. was a recipient of a Ministry of High Education (Kingdom of Saudi Arabia) Scholarship and an Overseas Research Student's Award (No. 81360).

References

1 Christensen, H.N. (1975) Biological Transport, W.A. Benjamin, Reading, MA
2 Guidotti, G.G., Borghetti, A.F. and Gazzola, G.C. (1978) Biochim. Biophys. Acta 515, 329–366
3 Kilberg, M.S., Handlogten, M.E. and Christensen, H.N. (1980) J. Biol. Chem. 225, 4011–4019
4 Borghetti, A.F., Kay, J.E. and Wheeler, K.P. (1970) Biochem. J. 182, 27–32
5 Borghetti, A.F., Tramacere, M., Ghiringhelli, P., Severini, A. and Kay, J.E. (1981) Biochim. Biophys. Acta 646, 218–230
6 Segel, G.B. and Lichtmen, M.A. (1981) J. Cell Physiol. 106, 303–308
7 Wise, W.C. (1978) J. Cell. Physiol. 97, 161–168
8 Ardawi, M.S.M. and Newsholme, E.A. (1982) Biochem. J. 208, 743–748
9 Ardawi, M.S.M. and Newsholme, E.A. (1983) Biochem. J. 212, 835–842
10 Ardawi, M.S.M. and Newsholme, E.A. (1983) Hoppe-Seyler's Z. Physiol. Chem. 364, 1251–1252
11 Ardawi, M.S.M. and Newsholme, E.A. (1984) in Glutamine Metabolism in Mammalian Tissues (Haussinger, D. and Sies, H., eds.), pp. 235–246, Springer-Verlag, Berlin
12 Ardawi, M.S.M. and Newsholme, E.A. (1985) in Essays in Biochemistry Vol. 21 (Marshall, R.D. and Tipton, K.F., eds.), pp. 1–43, Academic Press, London
13 Newsholme, E.A., Crabtree, B. and Ardawi, M.S.M. (1985) Biosci. Rep. 5, 393–400
14 Newsholme, E.A., Crabtree, B. and Ardawi, M.S.M. (1985) Q. J. Exp. Physiol. 70, 473–489
15 Bradford, N.M. and McGivan, J.D. (1982) Biochim. Biophys. Acta 689, 55–62
16 Culvenor, J. and Weidemann, M.J. (1976) Biochim. Biophys. Acta 37, 354–363
17 Halestrap, A.P. and McGivan, J.D. (1979) Techn. Metab. Res. B206, 1–23
18 Pishak, M.R. and Phillips, A.T. (1979) Anal. Biochem. 94, 88–92
19 McGivan, J.D., Bradford, N.M. and Beavis, A.D. (1977) Biochem. J. 162, 147–15
20 Gornall, H.G., Bardawill, C.J. and Savie, M.M. (1949) J. Biol. Chem. 177, 751–766
21 Christensen, H.N. and Handlogten, M.E. (1977) J. Membrane Biol. 37, 193–211
22 Edmondson, J.W., Lumeng, L. and Li, T.K. (1979) J. Biol. Chem. 254, 1653–1658
23 Thomas, E.L. and Christensen, H.N. (1971) J. Biol. Chem. 246, 1682–1688
24 Mendelsohn, J., Skinner, A. and Kornfeld, S. (1971) J. Clin. Invest. 50, 818–826

25 Pardee, A.B., Dubrow, R., Hamlin, J.L. and Kletzien, R.F. (1978) Annu. Rev. Biochem. 47, 715–750
26 Christensen, H.N. (1969) Adv. Enzymol. 32, 1–20
27 Wise, W.C. (1976) J. Cell. Physiol. 87, 199–212
28 Whitney, R.B. and Sutherland, R.M. (1973) Biochim. Biophys. Acta 298, 790–797
29 Van den Berg, K.J. and Betel, I. (1973) Exp. Cell. Res. 76 63–72
30 Van den Berg, K.J. and Betal, I. (1974) Exp. Cell. Res. 84 412–418
31 Greene, W.C., Parker, C.M. and Parker, C.W. (1976) J Biol. Chem. 215, 4017–4025