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The transport of glutamine and alanine into rat colonocytes

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1. The transport of glutamine and alanine into isolated rat colonocytes was studied. 2. The transport of both amino acids appears to be dependent on a Na^+ gradient. 3. The apparent K_m values for the transport of glutamine and alanine were 2.56 ± 0.84 and 5.35 ± 1.20 mM respectively, but with similar $V_{max.}$ values. 4. Glutamine and alanine transport were mutually competitive, and the transport of both amino acids was competitively inhibited by 2-methylaminoisobutyrate. In contrast, histidine inhibited the transport of both glutamine and alanine non-competitively. 5. It is concluded that glutamine and alanine are transported into rat colonocytes by a common carrier system similar to System A of other cells. 6. It is suggested that the metabolic function of this transport system in rat colonocytes might be the partial exchange of extracellular glutamine for intracellular alanine.

INTRODUCTION

Glutamine is considered to be a key amino acid in rapidly dividing cells since its oxidation provides energy and its carbon and nitrogen provide precursors for several biosynthetic processes (for reviews see Kovacevic & McGivan, 1983; Ardawi & Newsholme, 1984, 1985*b*; Newsholme *et al.*, 1985). Glutamine is a major source of energy for the epithelial cells of the small intestine (for review, see Windmueller, 1984) and provides an important source of energy in lymphocytes (for reviews, see Ardawi & Newsholme, 1985*b*; Newsholme *et al.*, 1985) and thymocytes (Brand *et al.*, 1984). In a similar manner to other dividing cells, colonocytes are characterized by a high rate of glutamine utilization with partial oxidation to glutamate, aspartate and alanine (Roediger, 1982; Ardawi & Newsholme, 1985*a*).

Both glutamine and alanine have been shown to be primarily transported into rat enterocytes by a common Na^+-dependent carrier, which was identified as System A of other cells (Bradford & McGivan, 1982). In hepatocytes, glutamine is transported by a different carrier designated as System N, which preferentically transports amido amino acids and histidine (Kilberg *et al.*, 1980), whereas in lymphocytes glutamine transport is achieved by a carrier similar to that of System ASC of other cells (Ardawi & Newsholme, 1986). However, the metabolic significance of glutamine in rat colonocytes is different from that of the liver cells, whereby glutamine is not considered to be a major energy substrate for hepatic metabolism (for review see Lund, 1979).

By using the same approach that was used in rat enterocytes (see Bradford & McGivan, 1982), it was decided to identify in colonocytes the transporting system(s) for glutamine and alanine, as the latter is a major end-product of glutamine metabolism in these cells (Ardawi & Newsholme, 1985*a*). Furthermore, as suggested for rat lymphocytes (see Newsholme *et al.*, 1985; Ardawi & Newsholme, 1985*b*), it is possible that the transport of glutamine into rat colonocytes may be one of the regulatory steps involved in the control of glutamine metabolism. Thus the main object of the present work was to provide information on the properties of the transporting system(s) for glutamine and alanine into isolated colonocytes of the rat.

MATERIALS AND METHODS

Animals

Male Wistar albino rats (230–280 g) were obtained from King Fahd Medical Research Center (KFMRC), College of Medicine and Allied Sciences, King Abdulaziz University, Jeddah, Saudi Arabia. Animals were maintained on a standard laboratory diet [commercial rat cubes containing (w/w) approx. 18% of protein, 3% of fat, 77% of carbohydrate and 2% of an inorganic-salt mixture with a vitamin supplement] (Grain Silos and Flour Mills Organization, Jeddah, Saudi Arabia) and water *ad libitum*.

Chemicals and enzymes

All chemicals and enzymes were obtained from the same sources described previously (Ardawi & Newsholme, 1985*a*, 1986).

Preparation and incubation of colonocytes

Colonocytes were prepared as described previously by Ardawi & Newsholme (1985*a*). Cells were incubated in an incubation medium consisting of phosphate-buffered saline [that had been oxygenated by 100% O_2 for 30 min (Culvenor & Weidemann, 1976)] plus 5 mM-dithiothreitol and 2.5% (w/v) dialysed bovine albumin (fraction V) at pH 7.2. Incubations were performed at 37 °C in 25 ml plastic flasks at 5–10 mg of cell protein/ml in a total volume of 1 ml of medium under 100% O_2. For the measurement of alanine transport, 0.5 mM-aminooxyacetate was included in the incubation medium before the initiation of the transport studies.

Measurement of transport

The initial rate of glutamine or alanine transport was determined by the silicone-oil-layer technique as described previously (Halestrap & McGivan, 1979; Ardawi & Newsholme, 1986). The transport of amino acids was initiated by the addition of cells (2–4 mg/ml) to the

incubation medium, which contained [^{3}H]inulin (0.2 μCi) to act as a marker of the extracellular space together with ^{14}C-labelled amino acid (0.05 μCi) to give the required final concentration. Samples (0.3 ml) were withdrawn at appropriate time intervals, and the transport was terminated by centrifugation of cells through the silicone-oil layer into the $HClO_4$ layer at 13800 *g* for 40 s in a Beckman micro-centrifuge. Preliminary experiments established that, during the incubation, colonocytes maintained their biochemical viability, as indicated by ATP content and the linearity of lactate production from 10 mM-glucose; the findings were similar to those reported previously for the same cells (see Ardawi & Newsholme, 1985*a*). At the completion of a transport experiment, the entire contents of the $HClO_4$ layer were mixed well in a scintillation mixture [750 ml of Triton X-100, 6.0 g of 2,5-diphenyloxazole plus 0.15 g of 1,4-bis-(phenyloxazol-2-yl)benzene in 1.5 litres of toluene] and assayed for ^{3}H and ^{14}C by dual-channel liquid scintillation counting.

In some experiments in which amino acid uptake was determined by enzymic methods, the procedure was modified. Colonocytes were incubated as described above and the transport was initiated by the addition of cells to the incubation medium containing glutamine or alanine plus ^3H_2O and [^{14}C]inulin. Samples were withdrawn at appropriate time intervals and cells were immediately separated from the incubation medium by centrifugation for 5 s at 13800 *g* in a Beckman micro-centrifuge. The colonocyte pellet and supernatant were acidified with $HClO_4$ and neutralized as described previously (Ardawi & Newsholme 1985*a*), and two to four pooled neutralized extracts were used for enzymic assays of amino acids. There was no significant difference between enzymic and radioactive determinations of the rates of amino acid transport. Intracellular water of colonocytes was determined as the difference in the volume available to [^{14}C]inulin and that available to ^3H_2O. The value was found to be 3.81 ± 0.50 μl/mg of colonocyte protein (mean $\pm$ S.E.M. for eight cell preparations).

Metabolism of glutamine or alanine during the course of the transport studies

In order that the uptake of a labelled amino acid should provide an accurate estimate of the rate of transport, it is necessary to ensure that no metabolism of the amino acid occurs or that it is inhibited as far as possible, and that the amount of the labelled amino acid corresponds accurately to the intracellular accumulation of this amino acid. However, rat colonocytes have a high capacity to metabolize glutamine (Roediger, 1982; Ardawi & Newsholme, 1985*a*), and it is not possible to inhibit this metabolism completely. Nonetheless, preliminary experiments were performed to assess the extent of glutamine metabolism; during the first 5 min of incubation colonocytes utilized 5–7% of glutamine in the incubation medium (i.e. in the presence of 0.5 mM-glutamine), almost all of which was recovered as glutamate and ammonia (results not shown). Furthermore, the initial rate of [U-^{14}C]glutamine transport was linear with time for 2 min (Fig. 1). This was further confirmed in other experiments in which colonocytes were incubated in the presence of [U-^{14}C]glutamine (0.5 mM) for periods up to 10 min. Incubations were terminated as described previously (Ardawi & Newsholme, 1983). Glutamine and glutamate formed were separated by ion-exchange chromatography (Dowex AG-1; X8, acetate form) as described previously (Pishak & Phillips, 1979). Therefore, initial rates of [U-^{14}C]-glutamine uptake measured over the first 2 min should represent the uptake of glutamine in colonocytes incubated under the conditions described in the present work. In addition, it was found that the proportion of cellular [U-^{14}C]glutamine that was unchanged after 1 min of incubation of colonocytes (at 0.5 mM-[U-^{14}C]glutamine) accounted for approx. 75% of the recovered radioactivity.

It has been shown previously that preincubation of hepatocytes (Edmondson *et al.*, 1977; Joseph *et al.*, 1978) or enterocytes (Bradford & McGivan, 1982) with amino-oxyacetate completely inhibits the metabolism of alanine in transport studies. In the present work, the initial rate of uptake of [U-^{14}C]alanine into colonocytes was linear for at least 4 min (Fig. 1). However, when colonocytes were incubated in the presence of [U-^{14}C]alanine (0.5 mM) plus 0.5 mM-amino-oxyacetate for periods up to 10 min, there were no significant changes in the rate of alanine transport under such conditions. It is therefore considered that [U-^{14}C]alanine uptake measured over the first 2 min should be an accurate estimate of the uptake of this amino acid under the conditions used in the present work.

Determination of metabolites

Glutamine and other metabolites in neutralized extracts were determined by the same methods as described previously (Ardawi & Newsholme, 1983, 1985*a*). Cell protein was measured by the biuret method (Gornall *et al.*, 1949).

Expression of results

Two to three replicate determinations were made for each value presented (unless otherwise indicated) in the Results section. The initial rate of glutamine or alanine transport is expressed as nmol of amino acid taken up/min per mg of cell protein.

RESULTS

Ion- and energy-dependence of glutamine and alanine transport in colonocytes

The time course of the transport of glutamine and alanine is shown in Fig. 1 when amino acids were added at 0.5 mM. The rates of glutamine or alanine transport were linear with time for 2 min or 4 min respectively. The concentration ratios of intra- to extra-cellular glutamine or alanine achieved after 2 min of incubation at 0.5 mM amino acid were 4.20 ± 0.41 or 1.71 ± 0.15 respectively. These results suggest that the transport of both amino acids is an energy-dependent process. This was supported by the observation that treatment of colonocytes with 2,4-dinitrophenol (0.1–2.0 mM) (an uncoupler of oxidative phosphorylation) or ouabain (0.1–2.0 mM) [an inhibitor of ($Na^+ + K^+$)-dependent ATPase] decreased the accumulation ratios to the same extent for both glutamine (16–75%) and alanine (14–73%). Furthermore, the Na^+-dependence of the transport of both amino acids in colonocytes is shown in Fig. 2. The Na^+ in the incubation medium (NaCl) was replaced by equivalent concentrations of choline chloride. In the absence of added Na^+, the rates of glutamine or alanine transport

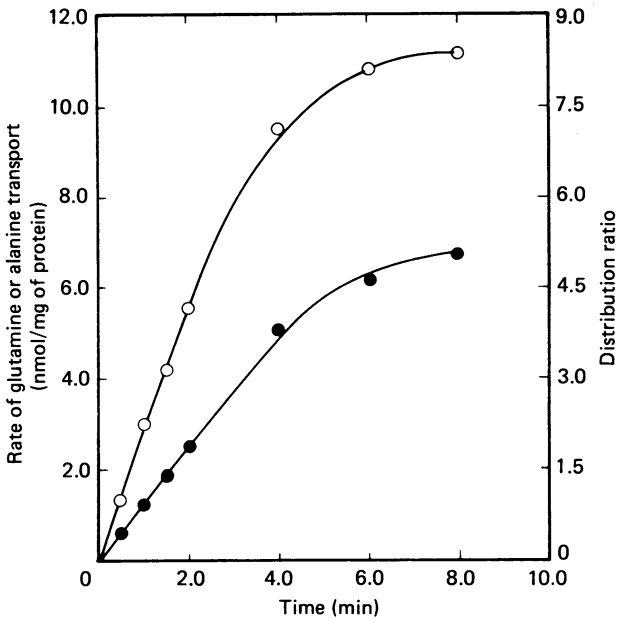

Fig. 1. Time course of the transport of glutamine (○) and alanine (●) by rat colonocytes

The initial concentration of glutamine or alanine was 0.5 mM. Results are presented as means of six separate experiments with separate colonocyte preparations, and the S.E.M. was always less than 8%.

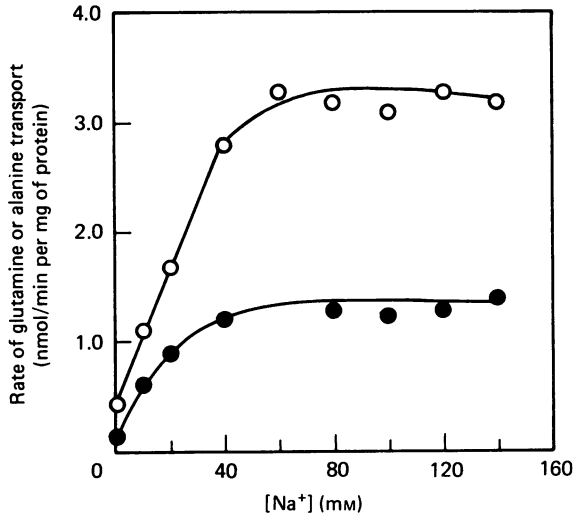

Fig. 2. Effect of Na^+ concentration on the initial rates of glutamine (○) or alanine (●) transport by rat colonocytes

The initial rates of transport into rat colonocytes were measured in an incubation medium in which NaCl was replaced with equivalent concentrations of choline chloride. Colonocytes were suspended in isolation media in which the concentration of NaCl ranged from 0 to 140 mM, and the osmolarity of solutions was kept constant by the addition of an appropriate concentration of choline chloride. Glutamine and alanine were added at a final concentration of 0.5 mM and transport was measured after 1 min. Results are presented as means of three separate experiments with separate colonocyte preparations, and the S.E.M. was less than 10%.

were inhibited by approx. 85 and 89% respectively. Na^+ had a half-maximal effect on the rate of transport of glutamine or alanine when present at approx. 15 or 11 mM respectively.

Concentration-dependence of glutamine and alanine transport in colonocytes

Saturation kinetics have been observed for the transport of both glutamine and alanine (results not shown). Half-maximal rates of transport were calculated to be 2.56 ± 0.84 mM for glutamine or about 5.35 ± 1.96 mM for alanine (obtained from Lineweaver–Burk plots). Those values are similar to that obtained for glutamine or alanine transport in other cells (Joseph *et al.*, 1978; Bradford & McGivan, 1982). However, $V_{max.}$ values for the transport of glutamine (18.05 ± 2.66 nmol/min per mg) and alanine (16.42 ± 3.62 nmol/min per mg) were of similar magnitude.

Inhibition of glutamine and alanine transport in colonocytes by other amino acids

In order to obtain information about the substrate specificity of the transport system(s) for glutamine or alanine in rat colonocytes, the effect of various amino acids on the rate of transport of both amino acids was investigated. The results are shown in Table 1. All amino acids tested were found to inhibit the initial rate of transport of glutamine to the same extent as that of alanine. For instance, serine, leucine and threonine produced similar inhibitory effects on the transport of both glutamine and alanine. These results are in contrast with those found in hepatocytes (Joseph *et al.*, 1978), but similar to the findings in rat enterocytes (Bradford & McGivan, 1982). However, inhibitory effects on transport of one amino acid by the addition of another amino acid

Table 1. Effect of various amino acids on the initial rate of transport of glutamine or alanine by rat colonocytes

The initial concentration of glutamine or alanine was 0.5 mM. The rates of transport of glutamine or alanine were measured after 1 min in the presence or absence of various amino acids added to a final concentration of 5.0 mM in the incubation medium. The results are expressed as percentage inhibition of the control rate (glutamine or alanine only), and are presented as means ± S.E.M. for five experiments with separate cell preparations. Statistical significance of difference (Student's *t* test) between rates for glutamine (or alanine) only and for glutamine or alanine plus various amino acids is indicated by * $P < 0.05$, ** $P < 0.001$.

Additional amino acid	Inhibition (%) of initial rate of transport of	
	Glutamine	Alanine
Alanine	59.9 ± 8.9**	–
Asparagine	47.0 ± 9.2**	41.1 ± 8.4**
Glutamine	–	55.9 ± 14.5**
Histidine	40.1 ± 6.9**	41.0 ± 12.2*
Leucine	70.8 ± 12.1**	62.9 ± 12.8**
Proline	43.6 ± 6.4**	39.6 ± 9.5*
Serine	78.8 ± 6.2**	75.9 ± 8.0**
Threonine	14.2 ± 4.0	14.8 ± 2.6
2-Methylamino-isobutyrate	19.5 ± 3.9	16.7 ± 3.5

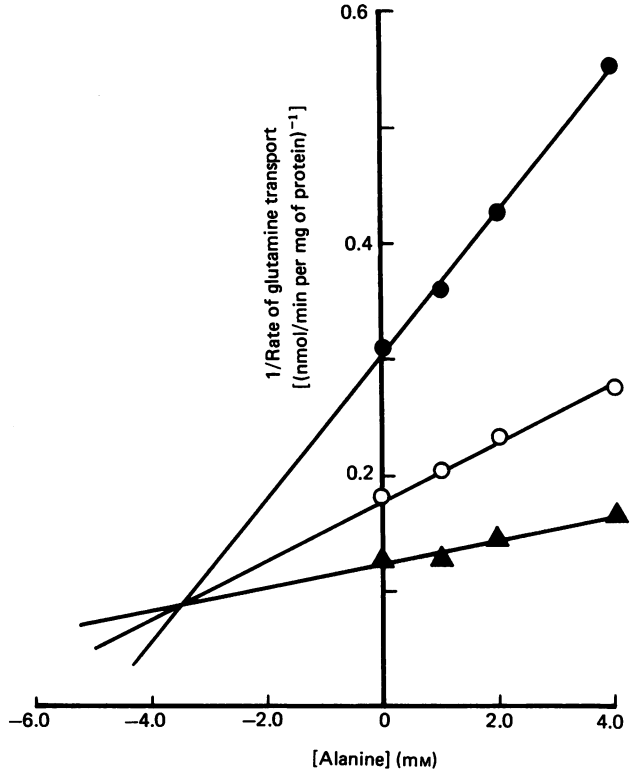

Fig. 3. Dixon plot of the effect of alanine on the initial rate of glutamine transport by rat colonocytes

Rates of glutamine transport were measured over 1 min intervals at various concentrations of glutamine: (●) 0.5 mM, (○) 1 mM, (▲) 2 mM. Lines were drawn by the method of least squares. Results are presented as means of five separate experiments with separate colonocyte preparations, with S.E.M. 8–12%.

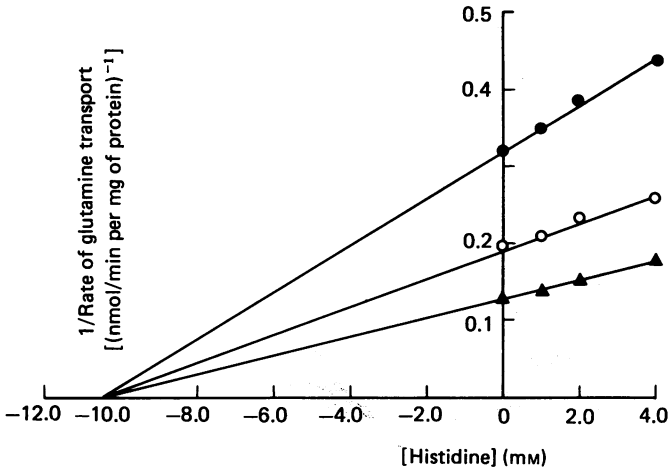

Fig. 4. Dixon plot of the effect of histidine concentration on the initial rate of glutamine transport by rat colonocytes

Rates of glutamine transport were measured over 1 min intervals at various concentrations of glutamine: (●) 0.5 mM, (○) 1 mM, (▲) 2 mM. Lines were drawn by the method of least squares. Results are presented as means of five separate experiments with separate colonocyte preparations; S.E.M. was less than 10%.

do not always imply that the transport of both amino acids is mediated by a common transporting system(s).

Alanine was found to inhibit glutamine transport, and vice versa (Table 1), and from a Dixon plot (Fig. 3) this inhibition appears to be competitive, with a K_i of approx. 3.4 mM. The inhibition of the transport of alanine by glutamine was also competitive (K_i = 4.2 mM) (results not shown). On the other hand, histidine, which is known to use System N in rat hepatocytes (Kilberg *et al.*, 1980), was found to inhibit non-competitively the transport of glutamine in colonocytes (Fig. 4), with a K_i for histidine of 10.2 mM. Similarly, the inhibition of alanine transport by histidine was found to be non-competitive (K_i = 12.6 mM) (results not shown).

2-Methylaminoisobutyrate, a non-metabolizable amino acid analogue that is only transported by the Na^+-dependent System A in most cells (Guidotti *et al.*, 1978), caused about 15–20% inhibition (at a concentration of 5 mM) of both glutamine and alanine transport in rat colonocytes (Table 1). This inhibition was found to be competitive for both glutamine and alanine (results not shown). These results are similar to those found in rat enterocytes (Bradford & McGivan, 1982), but unlike those found for either rat lymphocytes or glutamine transport in rat hepatocytes (Ardawi & Newsholme, 1986; Kilberg *et al.*, 1980) respectively.

DISCUSSION

The results of the present work suggest that both glutamine and alanine are transported mainly by a common Na^+-dependent carrier, which is similar to System A in other cells (Christensen, 1975). Evidence for the existence of such a carrier system in rat colonocytes is provided from the following observations.

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

(ii) The transport of glutamine and alanine is mutually competitive, with a K_m for glutamine transport that is similar to the K_i for the inhibition of alanine transport by glutamine, and vice versa.

(iii) Amino acids that inhibit the uptake of glutamine inhibit the uptake of alanine to the same extent.

(iv) Histidine, a selective substrate for System N in hepatocytes, caused a non-competitive rather than a competitive inhibition of both glutamine and alanine transport.

(v) 2-Methylaminoisobutyrate, a selective substrate for System A in most cells, caused a competitive inhibition of the transport of both glutamine and alanine.

These observations are similar to those found for rat enterocytes, in which System A mainly contributes to the transport of both glutamine and alanine (Bradford & McGivan, 1982), but contrast with those reported for glutamine transport by hepatocytes, where System N predominates (Kilberg *et al.*, 1980), and lymphocytes, where glutamine is mainly transported by System ASC (Ardawi & Newsholme, 1986).

It is therefore concluded that neither System N of rat hepatocytes nor System ASC of rat lymphocytes and other cells makes a major contribution to the transport of glutamine into rat colonocytes, and that both

glutamine and alanine are transported mainly via a common carrier similar to that of System A in other cells.

Isolated incubated rat colonocytes are characterized by high rates of aerobic glycolysis and glutamine utilization, with partial glutamine oxidation to glutamate, aspartate and alanine (Ardawi & Newsholme, 1985*a*). Similarly, glutamine is considered to be a major energy source for isolated incubated rat enterocytes (Watford *et al.*, 1979), and yields glutamate to provide in part for the carbon skeleton of alanine that can be readily metabolized by the liver (Hanson & Parsons, 1977; Watford *et al.*, 1979), thus avoiding the accumulation of glutamate in the portal blood.

It has been suggested that the major metabolic function of the transporting system of glutamine and alanine into rat enterocytes is to provide an exchange process by which extracellular glutamine is exchanged for intracellular alanine to ensure the removal of glutamate (in the form of alanine), which is not readily metabolized by the liver (Bradford & McGivan, 1982). However, unlike enterocytes, glutamine utilization by colonic mucosa produced much less alanine, which accounted for 12% of the rate of glutamine utilization (Ardawi & Newsholme, 1985*a*). It is therefore possible that System A, which contributes to the transport of glutamine and alanine into rat colonocytes, plays a similar role, but not to the same extent as that proposed for the same system in rat enterocytes, and that the partial conversion of glutamate into alanine may be looked upon as a detoxification mechanism which further protects the peripheral circulation from an increase in glutamate as a product of glutamine metabolism by the small- and large-intestinal mucosa. Furthermore, it has to be established how important is the rate of transport of glutamine into rat colonocytes in the overall regulation of the metabolism of this amino acid in these cells.

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