Biochimica et Biophysica Acta, 978 (1989) 51–55
Elsevier

BBA 74259

Transport of glutamine in rat intestinal brush-border membrane vesicles

Kent Van Voorhis, Hamid M. Said, Fayez K. Ghishan and Naji N. Abumrad

Departments of Pediatrics, Molecular Physiology / Biophysics and Surgery Vanderbilt University Medical School, Nashville, TN (U.S.A.)

(Received 20 September 1988)

Key words: Glutamine transport; Brush-border membrane; (Rat jejunum)

Transport of glutamine across the brush-border membrane of the rat intestine was examined using brush-border membrane vesicle (BBMV) technique. Osmolarity and temperature studies indicated that the uptake of glutamine by BBMV is mostly the result of transport of the substrate into the intravesicular space. Transport of glutamine was Na^+-gradient dependent (out > in) with a distinct 'overshoot' phenomenon. Initial rate of transport of glutamine as a function of concentration was saturable both in the presence and absence of a Na^+ gradient (out > in). Apparent K_m of 3.50 and 3.34 mM and V_{max} of 707 and 282 pmol/mg protein per 7 s, were calculated for the Na^+-dependent and the Na^+-independent transport processes of glutamine. The transport of [^{3}H]glutamine by the Na^+-dependent and the Na^+-independent processes was severely inhibited by the addition to the incubation medium of other amino acids and unlabelled glutamine. Inducing a relatively negative intravesicular compartment with the use of valinomycin and an outwardly directed K^+ gradient stimulated glutamine transport. This indicates that transport of the substrate by the Na^+-dependent process is electrogenic in nature. Transport of glutamine by the Na^+-independent process, however, appeared to be electroneutral in nature. These results demonstrate the existence of two carrier-mediated transport processes for glutamine in the rat intestinal BBMV, one is Na^+-dependent and the other is Na^+-independent. Furthermore, the results suggest that glutamine transport by the Na^+-dependent process probably occurs by a glutamine/Na^+ cotransport mechanism.

Introduction

Glutamine is an important amino acid that is involved in the synthesis of numerous biologically active compounds in mammalian cells and is a carrier form of ammonia [1]. Glutamine in the body is derived from both dietary and endogenous sources (glutamine is produced endogenously as an end product of amino acid catabolism by different tissues, particularly the skeletal muscles).

The absorptive epithelial cells of the small intestine maintain a high level of metabolic activity, thus, they require a substantial amount of energy. Recent studies have demonstrated that glutamine, not glucose, is the major substrate for energy production in the intestinal epithelial cells [2–4]. In these cells glutamine is obtained from both luminal (i.e., dietary) and arterial sources [2–4]. Limited studies are available describing glutamine transport into the intestinal epithelial cells [5,6]. In this study, we examined the characteristics of glutamine transport across the brush-border membrane (BBM) of the rat intestine using a brush-border membrane vesicles (BBMV) technique.

Materials and Methods

Materials

L-[G-^{3}H]Glutamine (sp. act. 39 mCi/mmol) and scintillation fluid (ACS) were purchased from Amersham/Searle, Des Plaine, IL; unlabelled glutamine and other amino acids were purchased from Sigma Chemical Company, St. Louis, MO. All other chemicals and reagents were obtained commercially and were of analytical quality.

Methods

Preparation of intestinal BBMV and transport studies. Male Sprague-Dawley rats (180–220 g) (Sasco, Omaha, NE) were used in this study. Rats were killed by an overdose of ether. The jejunum (the 50 cm of the intestine that followed the ligament of Tritz) was removed, washed, everted and the mucosa was scraped

Correspondence: H.M. Said, University of California, Department of Medicine, Division of Gastroenterology, Irvine, CA 92717, U.S.A.

with a glass slide. Intestinal BBMV were isolated by a modification of Kessler's divalent cations (Mg^{2+}) precipitation technique [7] as described in detail by us previously [8–15]. All preparation steps were conducted at 4°C. By use of a Waring blender-type homogenizer at maximum speed, the mucosal scraping from one tube was homogenized for 3 min in 60 ml of 300 mM mannitol, 5 mM ethyleneglycol bis(β-aminoethyl ether)-*N,N'*-tetraacetic acid (EGTA), and 12 mM tris(hydroxymethyl)aminomethane (Tris)-HCl (pH 7.1); 240 ml of ice-cold distilled water was then added. The homogenate was treated with 3 ml of 1 M $MgCl_2$ and centrifuged at $3000 \times g$ for 15 min (centrifuge model J2-21, Beckman Instruments, Fullerton, CA). The supernatant was then centrifuged at $27000 \times g$ for 30 min. The resulting pellet was resuspended in 60 ml of 60 mM mannitol, 5 mM EGTA, and 12 mM Tris-HCl (pH 7.1), and homogenized in a Potter-Elvehjem tube for 10 strokes at the highest speed. The homogenate was treated with 0.6 ml of 1 M $MgCl_2$ and centrifuged at $3000 \times g$ for 15 min. The pellet was resuspended in 30 ml of 250 mM mannitol and 20 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (Hepes)-Tris buffer and centrifuged at $50000 \times g$ for 30 min. With the use of a tuberculin syringe with a 25-gauge needle, the pellet was resuspended in the desired volume of the transport (intravesicular) buffer (280 mM mannitol and 20 mM Hepes-Tris (pH 7.4)). The suspension was then preincubated at room temperature for 2 h to load the inside of the vesicles.

Transport studies were performed by a rapid-filtration technique [16]. All incubations were done at room temperature to decrease possible metabolism of glutamine. The reaction was initiated by adding a 20-μl aliquot of membrane vesicle suspension to 80 μl of incubation buffer (final concentrations: 100 mM NaCl or KCl, 80 mM mannitol, and 20 mM Mes-Tris (pH 6.5), changes in this buffer are mentioned in the figure legends) containing various amounts of radiolabelled and unlabelled substrate plus other constituents. The preincubation and incubation media, unless otherwise stated, were always isosmotic. After incubation for the desired period of time, the reaction was terminated by the addition of 1 ml of ice-cold stop solution (200 mM NaCl, 100 mM mannitol, 10 mM K_2HPO_4 (pH 7.4)). The cold, diluted reaction mixture was immediately pipetted onto a prewetted filter and kept under suction. The filter was rinsed with 5 ml ice-cold stop solution and then dissolved in 5 ml of ACS scintillation cocktail.

Radioactivity was counted in a scintillation counter (model LS 3801, Beckman Instruments, Irvine, CA). Nonspecific binding of the substrate to the filter (background) was determined by filtering a reaction mixture that contained an identical solution, but no vesicles, and was subtracted from the transport data. Transport data are the result of three to six separate determinations and are expressed as the mean ± S.E. in picomoles per milligram protein per unit time.

Protein concentrations were measured by the method of Lowry et al. [17] using bovine serum albumin as a standard.

The purity and suitability for transport studies of intestinal BBMV preparations has been demonstrated in previous studies in our laboratory [8–15].

Results

Transport versus binding

To determine whether the uptake of glutamine by jejunal BBMV is due to transport of the substrate into the intravesicular space or binding to membrane surfaces, we examined the uptake of 0.02 mM glutamine (5 min incubation, i.e., equilibrium uptake, see below) as a function of incubation medium osmolarity. Osmolarity was adjusted by changing the mannitol concentration in the incubation medium. Fig. 1 shows the relationship between glutamine uptake and 1/osmolarity. The relationship appeared to be linear with a correlation coefficient (r) of 0.87. Extrapolating the line to infinite osmolarity showed no uptake. These results indicate that uptake of glutamine by jejunal BBMV at equilibrium and under isotonic conditions is the result of transport of the substrate into the intravesicular space with no binding to membrane surfaces.

To further examine the issue of transport versus binding, we examined the uptake of 0.02 mM glutamine at room temperature and at 4°C. The results showed that the uptake of glutamine is 8.3-fold higher ($P < 0.005$) at room temperature than at 4°C (uptake of 9.15 ± 1.1 and 1.1 ± 0.5 pmol/mg protein per 15 s, respectively). This finding further indicates that gluta-

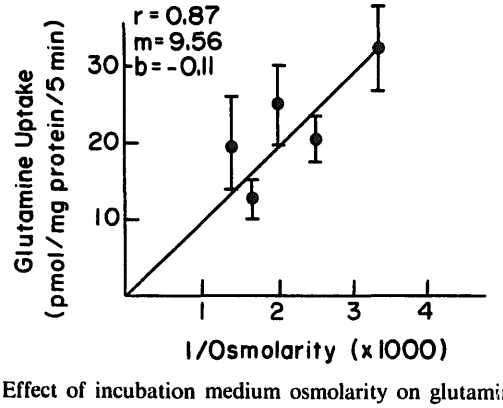

Fig. 1. Effect of incubation medium osmolarity on glutamine transport. Jejunal BBMV were preloaded with a buffer of 280 mM mannitol and 20 mM Hepes-Tris (pH 7.4). Incubation was performed for 5 min (equilibrium uptake) at room temperature in an incubation medium of 100 mM NaCl, 20 mM Mes-Tris (pH 6.5) and sufficient amounts of mannitol to give the indicated osmolarity. Glutamine (0.02 mM) was added to the incubation medium at the onset of experiment. $Y = mx + b$, where m = slope, $b = y$ intercept. r represents the correlation coefficient.

mine uptake by BBMV is mostly the result of transport of the substrate into the intravesicular space.

In another study we examined the degree of metabolism of the transported glutamine following incubation of BBMV with 0.02 mM [^{3}H]glutamine for 5 min at room temperature. In this study BBMV were immediately filtered following incubation, washed with ice-cold stop solution and resuspended in 0.5 ml of boiling distilled water. The samples were then placed in a boiling water bath for 10 min. Following that, the supernatant was applied to a cellulose precoated TLC plate and run using a solvent system of *n*-butanol/pyridine/water/acetic acid (30:30:20:20, v/v) [18] (stock [^{3}H]glutamine was ran simultaneously as a standard). The results showed that 94.7 percent of the incubated glutamine to be transported into BBMV in the intact form.

Effect of Na^+

In this experiment we examined the transport of 0.02 mM glutamine with time in the presence and absence of a Na^+ gradient (outside = 100 mM, inside = 0 mM) (in the absence of a Na^+ gradient, a K^+ gradient was used). The results (Fig. 2) showed that in the presence of a Na^+ gradient the transport of glutamine was rapid and linear for approximately 10 s of incubation with a distinct 'overshoot' phenomenon. Equilibrium was reached after 5 min of incubation. In the absence of a Na^+ gradient, glutamine transport was slower, no 'overshoot' was observed and equilibrium was reached after 5 min of incubation. These results indicate that glutamine transport is Na^+ dependent.

Transport as a function of concentration

Transport of glutamine as a function of concentration (0.1–10 mM) was examined in the presence and absence of a Na^+ gradient (outside = 100 mM, inside = 0 mM) (in the absence of a Na^+ gradient, a K^+ gradient was used). Incubation was performed for 7 s (i.e., initial rate). The results showed that glutamine transport is saturable both in the presence and in the absence of a Na^+ gradient (Fig. 3). These findings indicate the existence of a Na^+-dependent and a Na^+-independent carrier-mediated processes for glutamine transport in the rat intestinal BBMV. Transport kinetic parameters i.e., apparent K_m and V_{max}, of the Na^+-dependent and the Na^+-independent transport processes of glutamine were calculated using a computerized model of the Michaelis-Menten equation as described by Wilkinson [19]. Apparent K_m of 3.50 and 3.34 mM and V_{max} of 707 and 281 pmol/mg protein per 7 s were calculated for the Na^+-dependent and the Na^+-independent transport process, respectively.

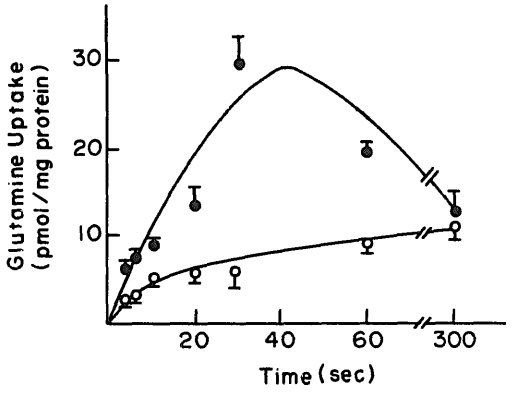

Fig. 2. Transport of glutamine in the presence and absence of a Na^+ gradient as a function of time. Jejunal BBMV were preloaded with a buffer of 280 mM mannitol and 20 mM Hepes-Tris (pH 7.4). Incubation was performed in an incubation buffer of 100 mM NaCl (●) or KCl (○), 80 mM mannitol and 20 mM Mes-Tris (pH 6.5). 0.02 mM glutamine was added to the incubation medium at the onset of incubation.

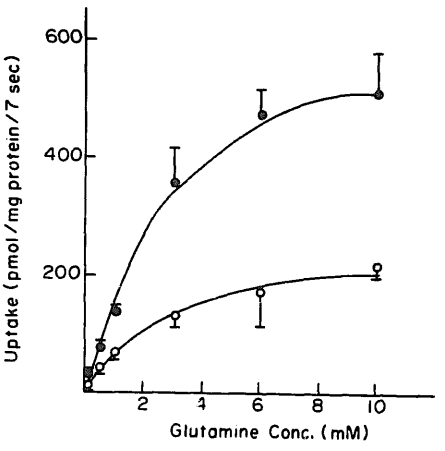

Fig. 3. Transport of glutamine in jejunal BBMV as a function of concentration in the presence and absence of a Na^+ gradient. Jejunal BBMV were preloaded with a buffer of 280 mM mannitol and 20 mM Hepes-Tris (pH 7.4). Incubation was performed for 7 s at room temperature in an incubation buffer of 100 mM NaCl (●) or KCl (○), 80 mM mannitol and 20 mM Mes-Tris (pH 6.5). Different concentrations of glutamine were added to the incubation medium at the onset of experiments.

Effect of other amino acids and unlabelled glutamine on the transport of [^{3}H]glutamine

In this study we examined the effect of adding to the incubation medium of 40 mM of the amino acids serine, asparagine, and unlabelled glutamine on the transport of 0.58 mM [^{3}H]glutamine into BBMV in the present of a Na^+ gradient (outside = 100 mM, inside = 0 mM). The results (Table I) showed that all amino acids examined caused significant ($P < 0.01$–0.005) inhibition in glutamine transport.

Similarly, in the absence of a Na^+ gradient (K^+ gradient replaced Na^+ gradient, outside = 100 mM, inside = 0 mM) the amino acids asparagine, serine and unlabelled glutamine added to the incubation medium at 40 mM caused significant ($P < 0.01$–0.005) inhibition in the transport of 5 mM [^{3}H]glutamine into BBMV

TABLE I

Effect of other amino acids and unlabelled glutamine on the transport of [^{3}H]glutamine in rat intestinal BBMV the presence and absence of a Na^+ gradient (out > in)

Jejunal BBMV were preloaded with buffer of 280 mM mannitol and 20 mM Hepes-Tris (pH 7.4). Incubation was performed for 7 s at room temperature in an incubation buffer of 100 mM NaCl or KCl, 40 mM mannitol and 20 mM Mes-Tris (pH 6.5) (adjustments in mannitol concentration were made in order to maintain buffer osmolarity at 300 mosmol). Glutamine (5 mM) and 40 mM of the compound under investigation were added to the incubation medium at the onset of incubation. *P* values were calculated using the Student's *t*-test. Comparison was made relative to simultaneously-performed control.

Compound	Transport (% of control)	
	Na^+ gradient (out > in)	K^+ gradient (out > in)
Control	00.0 ± 4.00	100.0 ± 8.5
Serine	62.7 ± 3.48 ($P < 0.01$)	52.4 ± 9.8 ($P < 0.01$)
Asparagine	44.9 ± 5.44 ($P < 0.005$)	36.0 ± 8.1 ($P < 0.005$)
Unlabelled glutamine	33.6 ± 7.71 ($P < 0.005$)	28.3 ± 4.2 ($P < 0.005$)

(Table I). These results further indicate the existence of a Na^+-dependent and a Na^+-independent carrier-mediated processes for glutamine transport in the rat intestinal BBMV.

Effect of transmembrane electrical potential

The effect of imposing an electrical potential across the BBM on the transport of the neutral amino acid glutamine was examined using previously described methods [8,9,11,20–23]. The electrical nature of the Na^+-dependent transport process of glutamine was examined using a valinomycin-induced K^+ diffusion electrical potential method. In this method the transport of 0.02 mM glutamine was examined in the presence of an outwardly-directed K^+ gradient ($[K_o^-] = 0$ mM, $[K_i^+] = 50$ mM) and the presence of the K^+-ionophore valinomycin (10 g/mg protein) (Expt. I) (incubation buffer was 90 mM NaCl, 100 mM mannitol and 20 mM Mes-Tris (pH 6.5); transport buffer was 50 mM potassium gluconate, 180 mM mannitol and 20 mM Hepes-Tris (pH 7.4)). The result was compared to that of a simultaneously-performed experiment (Expt. II) in the absence of a K^+ gradient ($[K_o^+] = [K_i^+] = 50$ mM) and the presence of valinomycin (10 g/mg protein), i.e., a 'voltage clamp' condition (incubation buffer was 90 mM NaCl, 50 mM potassium gluconate and 20 mM Mes-Tris (pH 6.5); transport buffer was 50 mM potassium gluconate, 180 mM mannitol and 20 mM Hepes-Tris (pH 7.4)). The rapid efflux of K^+ in the first experiment will generate a relatively negative intravesicular space thereby affecting any electrogenic component of glutamine transport. The results showed significantly higher ($P < 0.005$) glutamine transport in Expt. I as compared to Expt. II (6.4 ± 0.21 and 3.44 ± 0.32 pmol/mg protein per 7 s, respectively). These results suggest that the Na^+-dependent transport process of glutamine is electrogenic in nature.

The electrical nature of the Na^+-independent transport process of glutamine was also examined using the anion substitution method [21–23]. This method is based on the fact that incubation with a relatively lipid-permeable anion (SCN^-) will create a relatively greater negative intravesicular compartment than a poorly permeable anion (Cl^-), thereby affecting any electrogenic component of glutamine transport to a relatively larger extent. In this experiment BBMV were pre-loaded with 280 mM mannitol and 20 mM Hepes-Tris (pH 7.4), and were incubated in 100 mM KSCN or KCl, 80 mM mannitol and 20 mM Mes-Tris (pH 6.5). The results showed similar transport of glutamine in the presence of SCN^- and Cl^- (transport of 7.94 ± 1.9 and 8.79 ± 1.1 pmol/mg protein per 7 s, respectively). This finding suggests that glutamine transport by the Na^+-independent process is electroneutral in nature.

Discussion

The present study examined glutamine transport across the BBM of rat intestine using a well-validated BBMV technique [8–15]. First we determined whether glutamine uptake by BBMV is the result of transport of the substrate into the intravesicular space or is due to binding to membrane surfaces. This was accomplished by examining the effect of changing the osmolarity of the incubation medium and of the temperature of incubation on the uptake of glutamine by BBMV. The results of both studies have indicated that glutamine uptake by intestinal BBMV is mostly the result of transport of the substrate into the intravesicular space with less binding to membrane surfaces. In another study we found that glutamine transport into BBMV following 5 min incubation to occur without metabolic alterations.

In the presence of a Na^+ gradient (out > in) we found glutamine transport to be rapid with a distinct 'overshoot' phenomenon. The 'overshoot' phenomenon indicates that the substrate is transiently accumulated (i.e., uphill movement) against a concentration gradient in the intravesicular space. In the absence of a Na^+ gradient, glutamine transport was slower and no 'overshoot' was observed. The transport of glutamine in intestinal BBMV was saturable as a function of concentration both in the presence and in the absence of a Na^+ gradient. This observation indicates the existence of a Na^+-dependent and a Na^+-independent carrier-mediated transport processes for glutamine. This conclusion was further supported by the finding that other amino acids and unlabelled glutamine significantly inhibit the transport of [^{3}H]glutamine both in the presence and in the absence of a Na^+ gradient. It is not

clear at this stage whether there are actually two independent carrier systems for glutamine transport in the rat intestinal BBMV or whether there is only one carrier which behaves differently in the presence or the absence of a Na^+ gradient. In this paper we referred to these processes as Na^+-dependent and Na^+-independent transport processes. The existence of a Na^+-dependent and a Na^+-independent carrier-mediated transport processes for glutamine have also been noticed in other systems such as liver plasma membrane [24] and renal brush border membrane [25,26]. Under normal physiological conditions an inwardly directed Na^+ gradient is maintained across the brush-border membrane of the enterocyte by the activity of the Na^+/K^+-ATPase at the basolateral membrane. Thus, the transport of glutamine by the Na^+-dependent process could easily function in vivo. The existence and significance of having a Na^+-independent transport process for glutamine in the intact intestine, however, is not clear. It should be stated here that while glutamine transport in rat renal BBMV have been shown to involve a Na^+-dependent and a Na^+-independent transport process, only the Na^+-dependent processes was recently shown to exist in situ in the kidney [18].

The electrical nature of the transport process of the neutral glutamine by the Na^+-dependent and the Na^+-independent processes was also examined in this study using established methodologies. Inducing a relatively negative intravesicular compartment with the use of valinomycin and an outwardly directed K^+ gradient caused significant stimulation in glutamine transport by the Na^+-dependent process. This finding indicate that the transport of glutamine by the Na^+-dependent process is electrogenic in nature and is associated with translocation of a positive charge. This is probably occurring through a glutamine/Na^+ cotransport process. The transport of glutamine by the Na^+-independent process, on the other hand, appeared to be electroneutral in nature as shown by the study with anions of different lipid permeability indicating that glutamine is crossing the BBM alone.

In summary, the present study demonstrates that glutamine transport in rat intestinal BBMV is mediated by a Na^+-dependent and a Na^+-independent carrier-mediated processes. Furthermore, the Na^+-dependent process appears to be electrogenic in nature and most probably occurs through a glutamine/Na^+ cotransport mechanism. On the other hand, the Na^+-independent process appears to be electroneutral in nature.

Acknowledgments

This study was supported by NIH grants AM 26657 and DK 39501. One of us, K.V.V., a second year medical student, is a recipient of an NIH summer training grant AMO 7383-87.

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