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ELECTRONEUTRAL Na^+/DICARBOXYLIC AMINO ACID COTRANSPORT IN RAT INTESTINAL BRUSH BORDER MEMBRANE VESICLES

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L-Glutamate and L-aspartate transport into osmotically active intestinal brush border membrane vesicles is specifically increased by Na^+ gradient (extravesicular>intravesicular) which in addition energizes the transient accumulation (overshoot) of the two amino acids against their concentration gradients. The 'overshoot' is observed at minimal external Na^+ concentration of 100 mM for L-glutamate and 60 mM for L-aspartate; saturation with respect to [Na^+] was observed at a concentration near 100 mM for both amino acids. Increasing amino acid concentration, saturation of the uptake rate was observed for L-glutamate and L-aspartate in the concentration range between 1 and 2 mM. Experiments showing mutual inhibition and transtimulation of the two amino acids indicate that the same Na^+-dependent transport system is shared by the two acidic amino acids. The imposition of diffusion potentials across the membrane vesicles artificially induced by addition of valinomycin in the presence of a K^+ gradient supports the conclusion that the cotransport Na^+/dicarboxylic amino acid in rat brush border membrane vesicles is electroneutral.

Introduction

So far, the permeation of L-glutamate and L-aspartate through the small intestinal mucosa, in contrast to that of many other amino acids, has been poorly characterized. The use of 'in vivo' and 'in vitro' methods have up to now failed to show transport against a concentration gradient or intracellular accumulation [1–4] of the two amino acids because they are rapidly transaminated [3–6]. However, indications in favour of a specific transport system for the two anionic amino acids have been reported.

Gibson and Wiseman [7] observed that the L-stereoisomers of the two amino acids are absorbed 'in vivo' more rapidly than the D-stereoisomers. Schultz and Zalusky [8] found that the addition of L-glutamate to the mucosal solution of rabbit ileum gave rise to an increase of the transepithelial short circuit current value. Stronger evidence came from Schultz et al. [9] who, using a short incubation time, measured unidirectional influxes of L-glutamic and L-aspartic acid across the brush border membrane of rabbit ileum without the influence of the tissue metabolism. Under these experimental conditions the authors found that L-glutamate and L-aspartate influxes were saturable processes, subject to competitive inhibition and markedly influenced by the Na^+ concentration.

The introduction of the isolated plasma membrane as a tool for the study of intestinal absorption [10] has made it possible to completely avoid the interference due to metabolism. In the last years, using this technique, a number of Na^+-dependent transport systems for sugar, amino acids and other metabolites located in the intestinal brush border membrane have been characterized [10–16]. In addition the fundamental role of the

sodium gradient and of the membrane potential in energizing the substrate translocation against their electrochemical gradient has received strong evidence [17,11,12].

In the present study, using the technique of the isolated brush border plasma membranes, we provide evidence for a specific sodium-dependent non-electrogenic transport system for L-glutamate and L-aspartate located in the intestinal brush border membrane.

An abstract of the communication of some preliminary data of this work will be published [18].

Materials and Methods

Chemicals. L-[2,3-^{3}H]Glutamic acid, L-[2,3-^{3}H]aspartic acid and D-[1-^{3}H(N)]glucose were obtained from New England Nuclear, Boston, MA, U.S.A. All the chemicals used through the study were of analytical grade purity.

Preparation of membrane vesicles. Brush border membrane vesicles were isolated by a $CaCl_2$ precipitation method previously described by Schmitz et al. [19] as modified by Kessler et al. [20]. The membrane vesicles for the uptake, unless stated otherwise, were equilibrated with a solution containing 300 mM mannitol, 20 mM Hepes-Tris (pH 7.5). The protein concentration was about 10 mg/ml in the final suspension.

Uptake studies. Uptakes of L-[^{3}H]glutamate, L-[^{3}H]aspartate and D-[^{3}H]glucose were carried out by a Millipore filtration technique as described by Hopfer et al. [10]. Unless stated otherwise, 20 μl of membrane suspension were added to 80 μl of incubation medium kept at 25°C. The composition of the incubation media will be indicated in the legends of figures and tables. The uptake was stopped by directly pipetting, at selected time intervals, 20 μl of the incubation mixture on a membrane filter (0.45 μm pore size, Millipore, Bedford, MA, U.S.A.). The filters were immediately rinsed with 5 ml of ice-cold stop solution containing 100 mM mannitol, 20 mM Hepes-Tris (pH 7.5), 100 mM NaCl and 0.5 mM $HgCl_2$ which prevented the outflux of the taken up amino acids (unpublished data). When D-glucose uptake was measured 1 mM phlorizin was present in addition. Uptakes of the L-glutamate and L-aspartate were carried out in parallel using for the same kind of experiment the same membrane preparation. All experiments presented in this paper were repeated at least three times and were always performed in triplicates. Using different membrane preparations, qualitatively similar results were obtained for the same experiments but, in view of the significant variation in the equilibrium uptake values observed, only results of typical experiments are shown. As blanks, membrane free incubation media were handled in an identical manner.

Radioactivity retained by the filters was analyzed by standard liquid scintillation procedures. Radioactivity of the filters containing membranes was at least 3-times that of the blanks.

Enzyme assays. Membrane purification was routinely checked by measuring alkaline phosphatase (EC 3.1.3.1) and sucrase (EC 3.2.1.48) as marker enzymes for the brush border membrane. Sucrase was measured as described by Dalqvist [21]. Alkaline phosphatase was measured as described by Berner et al. [16]. The enrichment factor of this enzymatic activity in the brush border membrane with respect to the homogenate was at least 20. Protein was determined by the method of Lowry et al. [21] after precipitation with ice-cold 10% trichloroacetic acid using bovine serum albumin as a standard.

Results

Fig. 1 illustrates the uptake of L-glutamate and L-aspartate at 0.1 mM concentration by rat intestinal brush border membrane vesicles as a function of time. In the presence of 100 mM Na^+ in the extravesicular medium and no Na^+ in the intravesicular medium (i.e., inwardly directed sodium gradient), both L-glutamate and L-aspartate enter the vesicular space much more rapidly than in the presence of an equivalent gradient of choline.

L-Aspartate shows a faster initial uptake rate than L-glutamate; in addition, after 2 min both amino acids reach an intravesicular concentration which exceeds their final equilibrium value. The 'overshoot', which is more pronounced for L-aspartate, has been already observed for a number of Na^+-dependent transport systems [10–17] and represents direct evidence for the ability of the brush border membrane to accumulate these sub-

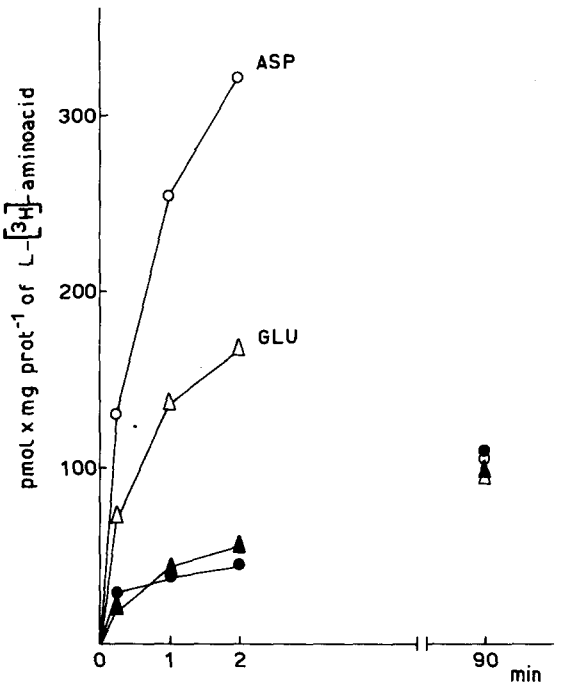

Fig. 1. Uptake of L-[^{3}H]aspartate (circles) and L-[^{3}H]glutamate (triangles) in the presence of NaCl (○, △) or choline chloride (●, ▲) gradient. Brush border membranes were prepared in a solution containing 300 mM mannitol, 20 mM Hepes-Tris (pH 7.5); the uptake of amino acids was measured by incubating an aliquot of membranes in a medium containing a final concentration of 100 mM mannitol, 100 mM NaCl or choline chloride, 20 mM Hepes-Tris (pH 7.5), 0.1 mM ^{3}H-labelled L-amino acid.

strates into the vesicular space. Of importance is that after 90 min the same equilibrium values are obtained either in the presence of sodium or in the presence of choline salts.

The role of sodium ion in the transport of the anionic amino acids was further investigated measuring the uptake of the two amino acids in the absence of Na^+-gradient. This was obtained by suspending the vesicles in a medium containing NaCl, so that equal concentrations of NaCl were present in the internal and external vesicular medium at the start of uptake. Under these conditions (Table I) the overshoot was abolished; the initial uptake rate of the two amino acids was lower than in the presence of the sodium gradient, but it was still significantly higher than that measured in the presence of choline salt. The results of these experiments indicate that the sodium gradient plays a fundamental role in energizing the uptake rate and the transient accumulation of L-aspartate and L-glutamate into the brush border membrane vesicles. On the other hand, the faster uptake rates of L-aspartate and L-glutamate in the presence of equilibrated NaCl (i.e., in the absence of any driving force) with respect to the values measured when choline is present suggest that there is a direct coupling of sodium ion and L-aspartate (or glutamate) in the translocation mechanism.

With regard to the specificity of the sodium ion in stimulating the uptake of the two dicarboxylic amino acids, Table II shows the uptake rate of L-aspartate measured at 30 s in the presence of NaCl or other chloride salt gradients. It can be noted that the uptake rate in the presence of the Na^+ gradient was at least 4-times that with other cation gradients. Similar results were obtained for L-glutamate when its uptake rate was measured in

TABLE I

Na^+-DEPENDENT DICARBOXYLIC AMINO ACID UPTAKE IN THE ABSENCE OF AN Na^+ GRADIENT

Brush border membrane vesicles were prepared in a medium containing 100 mM mannitol, 20 mM Hepes-Tris (pH 7.5), 100 mM choline chloride (CholCl) or alternatively 100 mM NaCl. The uptake was initiated by adding an aliquot of membrane suspension to an incubation medium to give a final concentration of 100 mM mannitol, 100 mM choline chloride or NaCl, 20 mM Hepes-Tris (pH 7.5), 0.1 mM L-[^{3}H]aspartate or L-[^{3}H]glutamate. Values are expressed as pmol/mg protein and are means of triplicates±S.D.

out 100 mM	in 100 mM	L-Aspartate			L-Glutamate		
		20 s	60 s	90 min	20 s	60 s	90 min
CholCl	CholCl	37± 6	40±10	138± 8	15± 3	28± 7	120±17
NaCl	NaCl	84± 8	118±15	120±10	23± 2	53±10	121±13
NaCl	CholCl	133±17	255±27	140±14	73±10	168±15	131±20

TABLE II

SPECIFICITY OF THE Na^+-STIMULATION ON L-ASPARTATE UPTAKE IN INTESTINAL BRUSH BORDER MEMBRANE VESICLES

Membranes were prepared in 100 mM mannitol, 50 mM choline chloride, 20 mM Hepes-Tris (pH 7.5); 20 μl of this membrane suspension were mixed to 80 μl of incubation medium to obtain at the start of the uptake a solution of final composition: 100 mM mannitol, 20 mM Hepes-Tris (pH 7.5), 0.1 mM L-[^{3}H]aspartate, 50 mM choline chloride (or other chloride salts). Values are expressed in pmol/mg protein and are means of triplicates ± S.D.

min	Li^+	Na^+	K^+	Rb^+	Cs^+	$Choline^+$
0.5	42 ± 8	122 ± 12	39 ± 5	28 ± 6	30 ± 8	37 ± 7
90	132 ± 15	132 ± 7	141 ± 17	138 ± 9	128 ± 13	135 ± 10

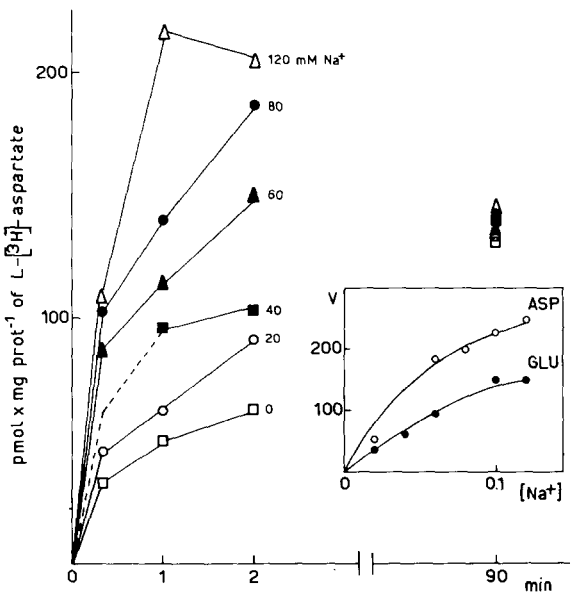

Fig. 2. Sodium concentration dependence of L-aspartate and L-glutamate uptake in rat intestinal brush border membrane vesicles. Membranes were suspended in a solution containing 100 mM mannitol, 100 mM choline chloride, 20 mM Hepes-Tris (pH 7.5). The uptake was measured by incubating an aliquot of membranes in a medium containing a final concentration of 20 mM Hepes-Tris (pH 7.5), 0.1 mM L-[^{3}H]aspartate and NaCl as indicated. In addition for each sample the concentrations of mannitol and choline chloride were adjusted to keep the external osmolarity constant (340 mosM). With the same membrane preparation the dependence of L-glutamate uptake on sodium concentration was also studied (not shown). The curves in the inset result from data of the experiment shown in the figure (L-aspartate) and from the one not shown (L-glutamate) when the 20 s uptake values are plotted against the extravesicular sodium concentration (see text). The rates are expressed as $pmol \cdot min^{-1} \cdot (mg\ protein)^{-1}$.

the presence of different chloride salts (unpublished data).

The uptake of anionic amino acids in the presence of a sodium gradient strongly depends on the external Na^+ concentration. Fig. 2 shows the time-course of L-aspartate uptake in the presence of different sodium concentrations (ranging from 0 to 120 mM). It can be observed that the uptake rate increases with increasing the external concentration of sodium. Interestingly the 'overshoot' appears only at sodium concentrations higher than 60 mM for L-aspartate (Fig. 2) and 100 mM for L-glutamate (unpublished data). The 20-s uptake values in the presence of Na^+ subtracted of the values in the absence of Na^+, have been used to calculate apparent initial uptake rates. In the inset of Fig. 2 these rates are plotted against Na^+ concentration, showing saturation kinetics for both amino acids, although the initial rates are not correctly defined because of the long incubation time.

Fig. 3 shows the dependence of the initial uptake rate (10 s incubation) of the two amino acids on substrate concentration in the presence and in the absence of an Na^+ gradient. In the presence of Na^+, a non linear relationship is observed, providing evidence for saturation of the system; on the other hand, in the absence of Na^+ the rates of L-glutamate and L-aspartate uptake increase linearly with the substrate concentration up to 5 mM. These results suggest that the sodium-independent component is due to simple diffusion; the possibility that at much higher substrate concentrations a

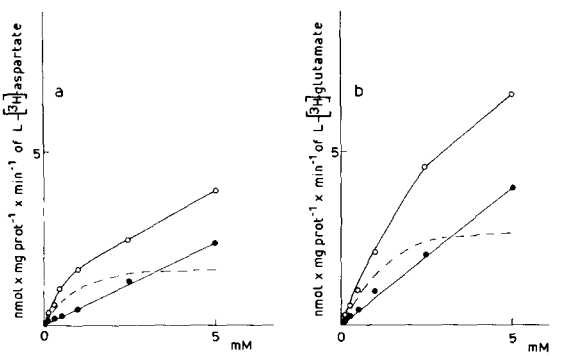

Fig. 3. Concentration dependence of L-aspartate (a) and L-glutamate (b) uptake into rat intestinal brush border membrane vesicles. Membrane vesicles were suspended in 300 mM mannitol, 20 mM Hepes-Tris (pH 7.5); 20 μl of this membrane suspension were added to 40 μl of incubation medium, to obtain a final composition of the extravesicular medium of 100 mM mannitol, 100 mM NaCl (○) (or alternatively choline chloride (●)), 20 mM Hepes-Tris (pH 7.5), plus ^{3}H-labelled L-amino acid at the concentrations indicated in the figure; the incubation time was 10 s.

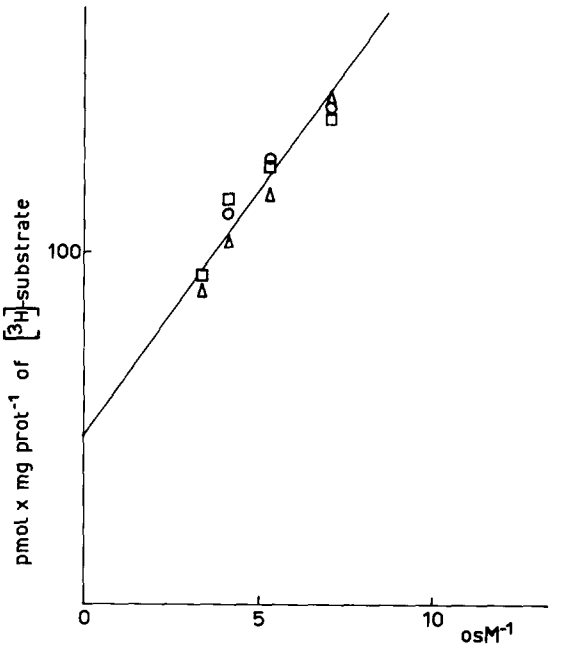

Fig. 4. Effect of medium osmolarity on the uptake of L-aspartate (□), L-glutamate (○) and D-glucose (△) in rat intestinal brush border membrane vesicles. Membranes were prepared in 100 mM cellobiose, 20 mM NaCl, 20 mM Hepes-Tris (pH 7.5); an aliquot of this membrane suspension was added to a solution to give a final incubation medium composition of 20 mM Hepes-Tris (pH 7.5), 20 mM NaCl, 0.1 mM ^{3}H-labelled substrate and cellobiose at such a concentration to reach the osmolarity indicated in the figure.

saturable component for the sodium-independent uptake exists was not investigated. When, at each substrate concentration, the sodium-independent uptake values were subtracted from the uptake values obtained in the presence of sodium, a curve which shows saturability of the Na^+-dependent L-glutamate and L-aspartate transport system was obtained (dashed lines). A Lineweaver-Burk transformation of the data of this experiment (Fig. 3) was compatible with an apparent K_m for L-aspartate of 1.0 mM and for L-glutamate of 1.5 mM and, respectively, V_{max} values of 1.8 and 2.7 nmol/mg protein per min.

The question as to whether L-glutamate and L-aspartate uptake by brush border membranes represents transport into the membrane vesicles or binding to the membrane was studied by measuring equilibrium uptake values at increasing medium osmolarities obtained by the impermeant sugar cellobiose (Fig. 4). In the case of intravesicular transport the amount of amino acid taken up after 90 min should be dependent only on the intravesicular volume. In agreement with this prediction, uptake of L-glutamate and L-aspartate is inversely proportional to the medium osmolarity; when osmolarity is extrapolated to infinite (i.e., zero abscissa), the uptake is reduced to values still significantly different from zero. The same behaviour is observed also when D-glucose is used (Fig. 4) and it has also been reported for other substrates [15,20,23]. As previously discussed by Hildmann et al. [23] it can be attributed either to a small amount of binding or to a transport into a compartment that is not available to further osmotic shrinkage.

Further support for the idea that uptake of L-glutamate and L-aspartate represents transport into the membrane vesicles was obtained by studying the effect of preloading brush border membrane vesicles with unlabelled L-glutamate or L-aspartate on L-[^{3}H]glutamate or L-[^{3}H]aspartate uptake. Fig. 5 shows transtimulation effect on L-glutamate and L-aspartate uptake, no matter which of the two L-amino acids is present into the vesicle space at the start of the uptake. These results strongly suggest that L-glutamate and L-aspartate

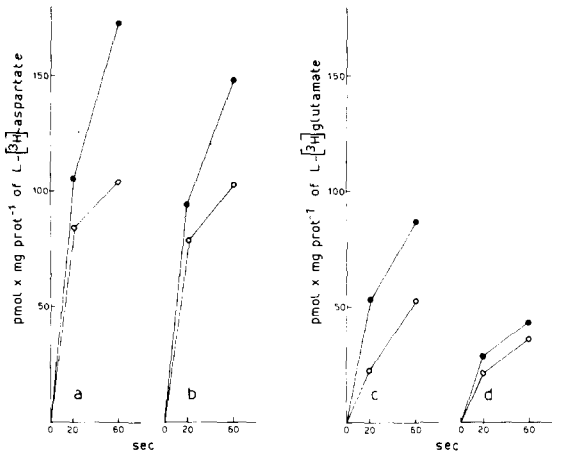

Fig. 5. Transtimulation by L-aspartate and L-glutamate of L-[^{3}H]aspartate (a, b) or L-[^{3}H]glutamate (c, d) uptake into rat brush border membrane vesicles. The membrane vesicles were suspended in a solution containing 100 mM mannitol, 100 mM NaCl, 20 mM Hepes-Tris (pH 7.5). In the control experiments (open circles) 10 μl of membrane suspension were mixed with 70 μl of incubation medium to give a final external concentration of 100 mM mannitol, 100 mM NaCl, 20 mM Hepes-Tris (pH 7.5) and 0.1 mM L-[^{3}H]aspartate (a) or 0.1 mM L-[^{3}H]aspartate + 0.1 mM L-glutamate (b); 0.1 mM L-[^{3}H]glutamate (c) or 0.1 mM L-[^{3}H]glutamate+0.1 mM L-aspartate (d). In the transtimulation assay (closed circles) membranes were preloaded with 0.8 mM L-aspartate (a, d) or L-glutamate (b, c) so that at the start of the incubation the amino acid concentrations were respectively (a): 0.8 mM L-aspartate inside/0.1 mM L-aspartate outside the vesicles; (b): 0.8 mM L-glutamate in/0.1 mM L-glutamate+0.1 mM L-aspartate out; (c): 0.8 mM L-glutamate in/0.1 mM L-glutamate out; (d): 0.8 mM L-aspartate in/0.1 mM L-aspartate+0.1 mM L-glutamate out. In addition 100 mM mannitol, 100 mM NaCl, 20 mM Hepes-Tris (pH 7.5) were present in the incubation medium. The uptake values are expressed in pmol/mg protein; at equilibrium they ranged between 103 and 111.

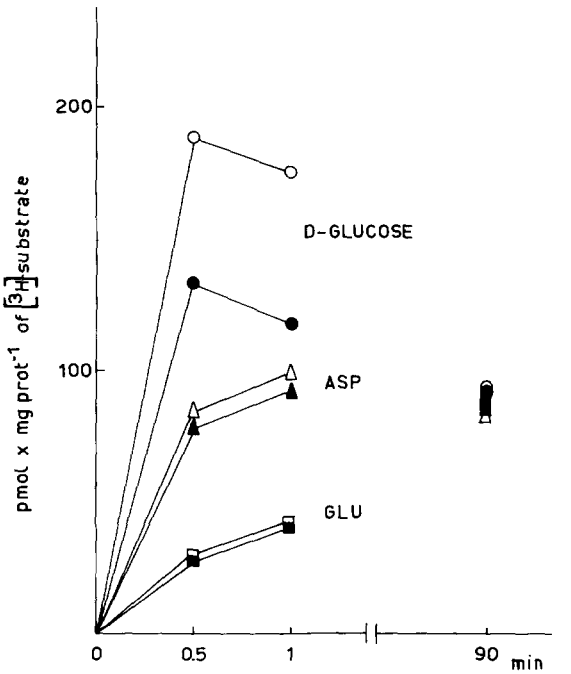

Fig. 6. The effect of a K^+ diffusion potential (interior positive) generated by valinomycin on the Na^+ gradient-dependent uptake of L-aspartate (△), L-glutamate (□) and D-glucose (○). Membrane vesicles were preloaded with 300 mM mannitol, 20 mM Hepes-Tris (pH 7.5); an aliquot of this membrane suspension was mixed with an incubation medium to give a final composition of 75 mM mannitol, 56.25 mM KCl, 56.25 mM NaCl, 20 mM Hepes-Tris (pH 7.5), 0.1 mM ^{3}H-labelled substrate and 1.25% ethanol with (closed symbols) or without (open symbols) valinomycin (8 μg/mg protein).

are translocated into the vesicle space by the same carrier mediated mechanism.

Table III gives information on the specificity of the sodium-dependent L-glutamate and L-aspartate transport system. In this table the inhibition of labelled L-glutamate or L-aspartate uptake in the presence of unlabelled analogues is reported. It is evident that either L-aspartate or D-aspartate are able to inhibit the uptake of both amino acids, while for glutamate, only the L-stereoisomer is inhibitory. Similar results on dicarboxylic amino acid transport systems have been found in liver and other cells as reported by Christensen [24].

The strong inhibition observed by the use of L-cysteate suggests a strict requirement for the second negative charge.

The importance of the side chain length extending from the α-carbon can be noted comparing the inhibitory effect of DL-α-aminoadipate with those of L-aspartate and L-glutamate. The increase of the chain length clearly decreases the inhibition on the L-aspartate uptake and, to a lesser extent, that on the L-glutamate uptake. Most of these characteristics were observed by Schneider et al. [25] for the L-glutamate transport system in rabbit kidney brush border membrane vesicles.

A very important feature of the Na^+-dependent dicarboxylic amino acid transport system is to establish whether it is electrogenic or electroneutral. The use of an intravesicular to extravesic-

TABLE III

EFFECT OF STEREOISOMERS AND ANALOGUES OF DICARBOXYLIC AMINO ACIDS ON THE TRANSPORT OF L-[^{3}H]ASPARTATE AND L-[^{3}H]GLUTAMATE

Intestinal brush border membrane vesicles were suspended in 300 mM mannitol, 20 mM Hepes-Tris (pH 7.5); 20 μl of this membrane suspension were added to 40 μl of solution to give a final incubation medium of composition: 100 mM mannitol, 100 mM NaCl, 20 mM Hepes-Tris (pH 7.5), 2 mM unlabelled test compound, 0.1 mM ^{3}H-labelled L-amino acid. Uptake was measured at 10 s. Values are expressed in pmol/mg protein and are means of triplicates ± S.D. In addition relative values with respect to the uptake in the absence of added competitor (taken as 100%) are given.

Test compound	Uptake of			
	L-[^{3}H]Asp	% of control	L-[^{3}H]Glu	% of control
None	98 ± 11	100	90 ± 16	100
L-Glutamate	63 ± 5	64	66 ± 5	73
D-Glutamate	96 ± 7	98	81 ± 7	90
L-Aspartate	55 ± 4	56	48 ± 7	53
D-Aspartate	65 ± 5	66	53 ± 2	58
L-Cysteate	34 ± 6	37	44 ± 4	57
DL-α-Aminoadipate	82 ± 11	84	65 ± 6	72

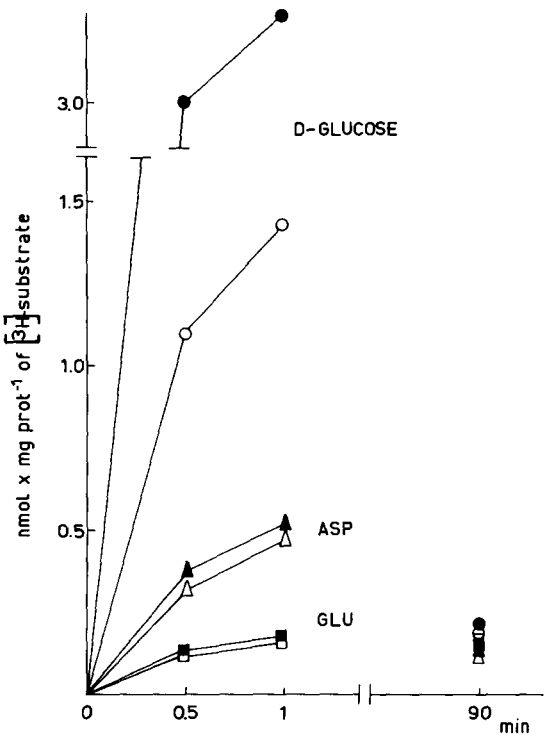

Fig. 7. The effect of a K^+ diffusion potential (interior negative) generated by valinomycin on the Na^+ gradient-dependent uptake of L-glutamate, L-aspartate and D-glucose. Membrane vesicles were prepared in a medium containing 100 mM mannitol, 100 mM KCl, 20 mM Hepes-Tris (pH 7.5); an aliquot of the membrane suspension was mixed with incubation medium to obtain a final composition of 100 mM mannitol, 12.5 mM KCl, 87.5 mM NaCl, 20 mM Hepes-Tris (pH 7.5), 0.1 mM ^{3}H-labelled substrate and 1.25% ethanol outside the vesicles with (closed symbols) or without (open symbols) valinomycin (8 μg/mg protein). L-Glutamate (□); L-aspartate (△); D-glucose (○).

ular directed K^+ gradient in the presence of valinomycin induces a diffusion potential which should affect the uptake rate of L-glutamate and L-aspartate if the cotransport results in a transfer of a net charge.

This has been established for D-glucose and neutral amino acids [17,14]. In the experiments reported in Figs. 6 and 7 we have studied the effect of the membrane potential on the Na^+-dependent uptake of L-glutamate and L-aspartate. Fig. 6 shows that, in the presence of an inwardly directed K^+ gradient, the addition of valinomycin significantly reduces the D-glucose uptake without affecting that of the dicarboxylic amino acids. On the other hand (Fig. 7), the addition of valinomycin when the K^+ gradient is outwardly directed dramatically enhances the D-glucose uptake with no effect on L-glutamate and L-aspartate transport. Valinomycin specifically induces the flux of K^+ down its electrochemical gradient, generating a transient membrane potential which accelerates the uptake of D-glucose when the interior becomes negatively charged and reduces its uptake when

the interior becomes positively charged. The lack of effect on dicarboxylic amino acid uptake strongly suggests that the cotransport of L-glutamate (or L-aspartate) and sodium ion is not accompanied by the transfer of a net charge as it happens to be for D-glucose.

Discussion

The results here reported on the specificity of Na^+ stimulation (Fig. 1, Table II), saturability of the sodium-dependent component (Fig. 3), inhibition by stereoisomers and analogues (Table III), transtimulation (Fig. 5), obtained using isolated brush border membrane vesicles, provide definitive evidence that a specific sodium dependent dicarboxylic amino acid transport system is located in the microvilli membrane of rat enterocytes. Moreover, the experiments performed in the presence of equal transmembrane Na^+ concentration suggest that the translocation of L-glutamate or L-aspartate is directly coupled to the translocation of the sodium ion by a cotransport mechanism.

The fundamental role of the sodium ion has been ascertained in a number of transport systems [11,17]. It is the general opinion that the sodium-dependence of these transport systems associated with the asymmetric distribution of Na^+ across the brush border membrane maintained by the $(Na^+ + K^+)$-dependent ATPases, which is located on the basal-lateral membrane, plays a fundamental role in the translocation and the accumulation of different molecules. With regard to L-lactate, inorganic phosphate, sulphate transport systems [14–16] the Na^+ gradient seems to provide the overall energy to accumulate these substrates inside the cells; on the other hand for other substrates as D-glucose and neutral amino acids also the transmembrane electrical potential seems to play an important role [12,13,17].

We have presented results (Fig. 1) that a transient accumulation inside the vesicles over the equilibrium value of both L-glutamate and L-aspartate can be obtained in the presence of an inwardly directed sodium gradient. These results give for the first time evidence that in the absence of metabolism the transport system for acidic amino acids present in the intestinal brush border is able to accumulate L-glutamate and L-aspartate against their electrochemical gradient.

Of importance is the question as to whether the inward sodium gradient represents the only driving force in energizing the L-glutamate (or L-aspartate) accumulation inside the cell, or other driving forces, such as the transmembrane electrical potential or an outward K^+ gradient, are utilized by the transport system. This problem has been extensively studied in renal proximal tubule and has led to conflicting results in the different laboratories [26,27].

Schneider and Sacktor [25] reported evidence for a sodium-dependent L-glutamate transport in rabbit renal brush border membrane vesicles. This sodium-dependent uptake was specifically enhanced by the presence of K^+ inside the vesicles and, when sodium had the same concentration inside and outside the vesicles, the outwardly directed K^+ gradient provided the driving force for the uphill transport of L-glutamate [26]. The authors found that this transport system was completely unaffected by change in transmembrane potential either in the presence or in the absence of K^+ inside the vesicles [26].

Similar sodium-gradient dependence and intravesicular K^+ stimulation effect has been reported in rat renal brush border membrane vesicles by Burckhardt et al. [27]. However, at variance, when K^+ was present inside the vesicles the L-glutamate uptake resulted to be sensitive to changes in the transmembrane electrical potential difference. These results are in agreement with those reported by Samarzija and Frömter [28] using an electrophysiological approach.

A rheogenic Na^+-dependent L-glutamate transport mechanism which strictly requires an outward K^+ gradient has been described also in synaptosomal rat brain membrane vesicles by Kanner and Sharon [29].

The lack of any effect obtained in our laboratory (Figs. 6 and 7) on the influence of membrane potential changes on the uptake of L-aspartate and L-glutamate is in favour of the idea that in the brush border membrane vesicles of rat enterocytes this sodium-dependent uptake is mediated by an electroneutral mechanism.

The possibility that also in the intestinal brush border membrane vesicles a K^+ gradient (inside > outside) can play a role in stimulating the Na^+-

dependent L-glutamate (L-aspartate) transport as well as in rendering this transport sensitive to changes in the transmembrane electrical potential difference has not yet been examined. To shed light on this question experiments are in progress in our laboratory.

On the basis of the reported results the only driving force utilized by the glutamate-aspartate carrier system located in the rat intestinal brush border membrane appears to be the sodium gradient.

Thus the simplest stoichiometry in the translocation mechanism would imply the cotransport of one sodium cation and one L-glutamate (or L-aspartate) anion in an electroneutral manner.

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