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Sodium-Dependent and -Independent Transport of L-Glutamate in the Rat Intestinal Crypt-like Cell Line IEC-17

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Mechanisms of L-glutamate transport in intestinal crypts were investigated using the rat intestinal crypt-like cell line IEC-17. Kinetic analysis and competition experiments run in the presence or in the absence of extracellular sodium indicate that L-glutamate uptake occurs through three different transport components: (1) a high affinity Na^+-independent component also carrying cystine, similar to system x_C^-; (2) a high affinity Na^+-dependent component inhibited by D- and L-aspartate corresponding to the ubiquitous system $X_{A,G}^-$; and (3) a low affinity Na^+-dependent system resembling the neutral amino acid transport system ASC. The simultaneous presence of these three components suggest that crypt cells are ready to face potential high variations of L-glutamate concentration in the intestinal villus environment.

Nutrient absorption is usually assumed to occur in fully differentiated enterocytes of the upper half of the intestinal villi. This assumption is supported by the intestinal distribution of the H^+-peptide cotransporter [1] and Na^+-glucose cotransporter [2,3], both expressed in the villus cells and absent in the proliferating cells of the crypts. In contrast, data concerning amino acid transport during epithelial cells differentiation are conflicting, so far. Results from transport measurement in isolated intestinal sheets suggest that the initial expression of leucine and lysine transport occurs in the upper third of the villi in 32 h old cells [4] whereas recent studies in the Caco-2 cell line have shown a decrease in the transport rate of dipolar and cationic amino acids through system B^0 and y^+ as cells stop to proliferate and undergo differentiation [5,6]. Therefore, it seems necessary to improve our knowledge concerning the amino acid transport systems expressed in undifferentiated crypt cells in order to clarify these points. IEC-17 cells resemble to immature crypt cells since they have been shown to express intestinal-specific antigens and to exhibit scarce microvilli, but lack the villus markers of differentiated enterocytes [7]. We have recently reported that proline transport in these cells occurs through the system A whereas the epithelial-specific systems B^0 and IMINO are not involved in that process [8]. The present study has been designed to characterize the mechanisms involved in the transport of the anionic amino acid L-glutamate in the IEC-17 cell line.

MATERIALS AND METHODS

Cell culture. The IEC-17 cell line was kindly provided by Dr. Kedinger (INSERM, Strasbourg, France) and used between passage 25 and 45. Cells were maintained routinely in 75 cm^2 flask at 37°C in humidified atmosphere with 5 % CO_2 and were subcultured every 5 days. The culture medium consisted in Dulbecco's modified Eagle's medium (Gibco BRL, France) containing 4.5 $g.l^{-1}$ glucose, 200 $\mu g.ml^{-1}$ gentamycin, 4 $\mu g.ml^{-1}$ insulin and supplemented with 5 % heat decomplemented fetal calf serum. For transport experiments, 2×10^4 $cells.cm^{-2}$ were seeded in 24-well plates (Falcon, France) and grown until confluence.

Transport measurements. The uptake of L-glutamate was measured for 2 minutes at 37°C using a cluster-tray technique [9]. Briefly, cell monolayers were preincubated for 15 min in the following transport medium without substrate : 137 mM NaCl, 5.4 mM KCl, 2.8 mM $CaCl_2$, 1 mM $MgSO_4$, 0.3 mM NaH_2PO_4, 0.3 mM KH_2PO_4, 10 mM glucose and 10 mM HEPES, adjusted to 7.4 with Tris. This buffer was then discarded and transport was initiated by adding 1 ml of transport medium containing 0.01 to 2.5 mM L-glutamate trace-labeled by 9.25 kBq L-[G-^{3}H]-glutamate (1.8 $TBq.mmol^{-1}$, Amersham, Les Ulis, France). The uptake was terminated by removing the transport medium and washing the cells by 3×1 ml of ice-cold transport medium containing an excess of unlabeled L-glutamate. The cells were harvested in 500 μl of 0.1 N NaOH and the cell-associated radioactivity determined by liquid scintillation counting. In selected experiments to measure the sodium-independent L-glutamate transport, NaCl was replaced by choline chloride and NaH_2PO_4 was omitted in the transport medium. The Na^+-dependent transport of L-glutamate was then determinated as the difference between

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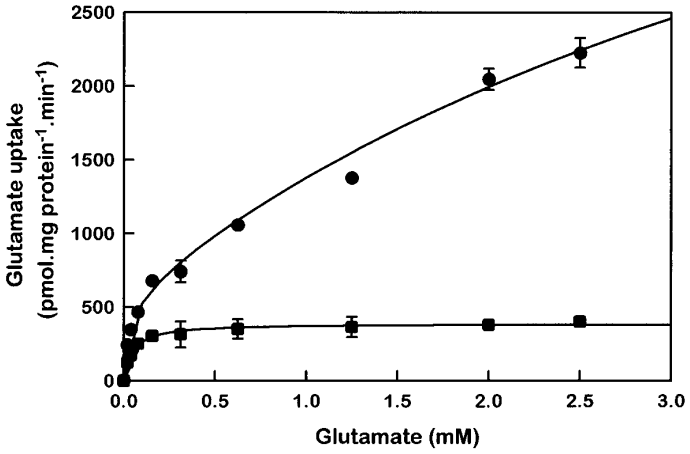

FIG. 1. Concentration dependence of L-glutamate transport in IEC-17 cells. The uptake was measured for 2 min over a range of 0.02 to 2.5 mM L-glutamate in the presence (●) and the absence (■) of sodium. Points are means ± SEM of n = 6 determinations.

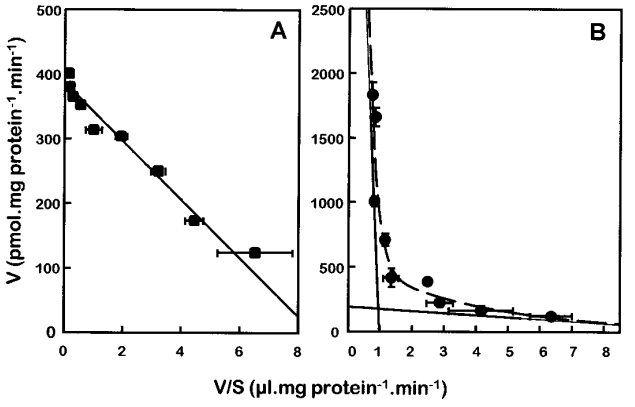

FIG. 2. Eadie-Hofstee representation of the Na^+-independent (A) and the Na^+-dependent (B) transport of L-glutamate. Transport was measured for 2 min over a range of 0.02 to 2.5 mM L-glutamate, either in a Na^+-containing or in a Na^+-free medium. In (B), the Na^+-dependent fraction of L-glutamate uptake was calculated by substracting Na^+-independent from total uptake (i.e. measured in the presence of sodium). Points are means ± SEM of n = 6 determinations.

the uptakes in NaCl and choline chloride transport medium. For competition experiments, amino acids were added to the transport medium in a 2.5 to 10 fold excess. All results were corrected for the zero time value for the accumulation of L-glutamate, as previously described [8]. The protein contents of the wells were determined by the method of Smith et al. [10] and results were expressed as pmol L-glutamate transported per mg of cell proteins.

Calculations. Results were expressed as means ± SEM and statistical comparisons were done using the Tukey's studentized range test (GLM procedure, SAS 6.03, SAS Institute, Cary (NC), USA). Transport kinetic parameters were obtained by fitting data to Michaelis-Menten equation or to a linear model (NLIN and REG procedures, SAS 6.03, SAS Institute, Cary (NC), USA).

RESULTS AND DISCUSSION

The uptake of L-glutamate by IEC-17 cells was measured following incubation with L-glutamate concentrations ranging from 0 to 2.5 mM (Fig 1). The analysis of uptake in the presence and in the absence of extracellular sodium revealed that L-glutamate uptake exhibited both Na^+-independent and Na^+-dependent saturable components. When 1 mM L-glutamate was added to IEC-17 cells, the Na^+-independent transport accounted for one fourth of the total L-glutamate transport. The Eadie-Hofstee plot of the Na^+-independent transport of L-glutamate indicated the presence of a single high affinity component with K_t = 0.045 ± 0.004 μM and V_{max} = 386 ± 11 pmol.mg protein^{-1}.min^{-1} (Fig 2A). In contrast, the Na^+-dependent L-glutamate transport involved both a high affinity (K_t = 0.015 ± 0.003 μM and V_{max} = 188 ± 26 pmol.mg protein^{-1}.min^{-1}) and a low affinity component (K_t = 5.58 ± 1.20 mM and V_{max} = 5413 ± 715 pmol.mg protein^{-1}.min^{-1}) (Fig 2B). In fact, for 10 μM L-glutamate, approximately 95 % of the transport occurs through both Na^+-independent and Na^+-dependent high affinity pathways whereas for 2 mM L-glutamate, 70 % of the uptake occurs through the low affinity Na^+-dependent pathway.

The two high affinity pathways were further characterized using 10 μM L-glutamate and 100 μM amino acid competitors (Table 1). In the presence or absence of extracellular Na^+, the rate of L-glutamate uptake was not affected by representative neutral amino acids (L-leucine and L-alanine) or by the cationic L-lysine amino acid. Addition of L-cystine resulted in a significant reduction of L-glutamate transport in Na^+-free condition (−53 %, $P < 0.05$) without affecting the Na^+-dependent uptake. Conversely, 100 μM L- or D-aspartate reduced the Na^+-dependent L-glutamate uptake by 74 % and 76 %, respectively, whereas the Na^+-inde-

TABLE 1

Effect of Amino Acid Competitors on L-Glutamate Uptake in IEC-17 Cells

	10 μM L-glutamate uptake (pmol.mg protein^{-1}.min^{-1})		
	Total	Na^+-independent	Na^+-dependent
L-glutamate	244 ± 15	100 ± 8	144 ± 15
+ L-lysine	253 ± 3	86 ± 2	167 ± 3
+ L-leucine	255 ± 2	88 ± 2	167 ± 2
+ L-alanine	249 ± 1	89 ± 1	160 ± 1
+ L-cystine	211 ± 4	47 ± 1*	164 ± 4
+ D-glutamate	232 ± 10	107 ± 7	125 ± 10
+ L-aspartate	143 ± 3*	106 ± 4	37 ± 3*
+ D-aspartate	135 ± 4*	101 ± 4	34 ± 4*

Note. The transport of 10 μM L-glutamate was measured for 2 min in the presence and the absence of sodium and a 10 fold excess of competitor. Points are means ± SEM of n = 6 determinations. * denotes a significant inhibition at $P < 0.05$.

pendent transport was unaffected. No competition was observed between L- and D-glutamate. Taken together, these results suggest that the Na^+-independent uptake of L-glutamate in the IEC-17 cell line occurs through the system x_c^- and that the main Na^+-dependent transport system involved in L-glutamate uptake at low concentrations is the system $X_{A,G}^-$. The system x_c^- is known to exhibit reactivity toward L-glutamate and L-cystine in its anionic form, and to exclude dipolar amino acids [9]. This carrier has been shown to be expressed in fibroblasts, hepatocytes and myotubes [9,11,12] but this is the first report of its involvement in the L-glutamate transport in epithelial cells. Conversely, the presence of the high affinity Na^+-dependent system $X_{A,G}^-$ in the intestinal epithelium is now completely established [13-17]. This system is characterized by a high affinity for L-glutamate (K_t ranging from 15 to 90 μM) and by a striking stereoselectivity with both L- and D-isomers of aspartate being transported, whereas D-glutamate is excluded [15,17]. Our results clearly suggest that the system $X_{A,G}^-$ is also expressed in undifferentiated crypt epithelial cells and plays a major role in the L-glutamate uptake at low concentrations.

The involvement of a low affinity Na^+-dependent system in L-glutamate uptake has been already suggested in intestine [18]. The system present in IEC-17 cells has been characterized using competition experiments with 2 mM L-glutamate and a 2.5 to 10 fold excess of amino acid competitors. In these conditions, the low affinity pathway is expected to account for about 85 % of the Na^+-dependent uptake of L-glutamate. A 10 fold excess of the $X_{A,G}^-$-specific substrate D-aspartate induced a 27 % ($P < 0.05$) decrease in the Na^+-dependent L-glutamate uptake consistent with the marginal contribution of this pathway at high L-glutamate concentrations (Fig 3). In contrast, a significant reduction (−45 %, $P < 0.05$) of the Na^+-dependent L-glutamate accumulation was achieved with a 2.5 fold excess of L-alanine and the maximal inhibition (−77%, $P < 0.05$) was measured with 10 mM of this competitor. These observations suggest that the uptake of high L-glutamate concentrations mainly occurs through a Na^+-dependent system which also transports dipolar amino acids. In accordance with previous observations in other cell lines, no competition was observed between L-glutamate and MeAIB ruling out the involvement of system A [9,19]. Despite previous report suggesting that the epithelial-specific system B^0 transports the protonated form of L-glutamate [20], the absence of competition observed between L-glutamate and the system B^0 substrate L-phenylalanine [21] suggests that this system is unlikely to be involved in L-glutamate uptake in IEC-17. This last observation is consistent with our previous studies suggesting that system B^0 was not present in the IEC-17 cell line [8]. Of interest, our competition experiments suggest that the uptake of L-glutamate

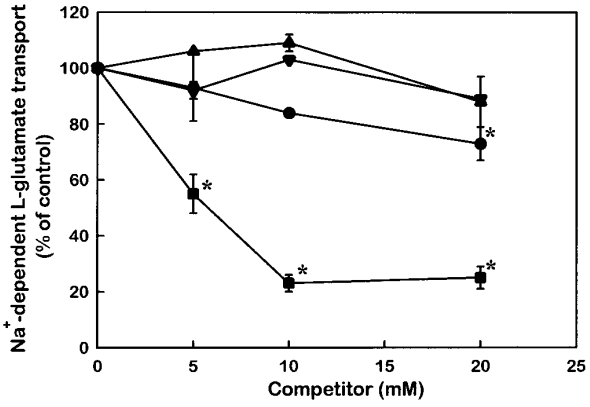

FIG. 3. Inhibition profiles of L-glutamate Na^+-dependent uptake by L-alanine, MeAIB, L-phenylalanine and D-aspartate. Transport assay at 2 mM L-glutamate concentration was performed in the absence and in the presence of increasing concentrations (5 to 20 mM) of L-alanine (■), MeAIB (▲), L-phenylalanine (▼) and D-aspartate (●). Results are means ± SEM of n = 6 determinations and are expressed as the percentage of the transport measured in the absence of competitor, the value control was 1907 ± 169 pmol.mg protein^{-1}.min^{-1}. * denotes a significant inhibition at $P < 0.05$.

would rather occur through an ASC-like transport system. The Na^+-dependent ASC transport system exhibits a much greater affinity for small dipolar amino acids such as L-alanine than for bulky amino acids such as L-phenylalanine, and completely excludes MeAIB. This system is known to be expressed in both the apical and basolateral membrane of intestinal epithelial cells [22] and has been shown to be involved in L-glutamate accumulation in fibroblasts and hepatocytes [9,19]. The recent demonstration that the ASCT-2 protein, which is responsible for the expression of an ASC-like transport system in different tissues including kidney, muscle and the large intestine, also transports L-glutamate further supports our hypothesis [23].

It seems therefore that L-glutamate uptake in the intestinal crypt-like cell line IEC-17 occurs through three different transport systems, namely system $X_{A,G}^-$, x_c^- and ASC, all three contributing to the maintenance of an adequate intracellular concentration of this amino acid. In contrast to differentiated enterocytes, crypt cells are unlikely to face high extracellular concentrations of L-glutamate and therefore, the contribution of system ASC to L-glutamate accumulation is probably marginal at the intestinal crypt level. This is probably no longer true on top of the intestinal villi, where a dramatic increase in L-glutamate concentration may occur promptly following meal ingestion. Our results suggest that the different transport systems needed to ensure an efficient post-prandial absorption of L-glutamate are already present in undifferentiated crypt cells. The impact of enterocytic differentiation on the expression of these transporters remains to be determined.

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