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The L-3,4-dihydroxyphenylalanine transporter in human and rat epithelial intestinal cells is a type 2 hetero amino acid exchanger

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Abstract

Information on the intestinal transport of L-3,4-dihydroxyphenylalanine (L-DOPA) is scarce. We present here the functional characteristics and regulation of the apical inward L-DOPA transport in two intestinal epithelial cell lines (human Caco-2 and rat IEC-6). The inward transfer of L-DOPA and L-leucine was promoted through an energy-driven system but with different sensitivity to extracellular Na^+ concentration: a minor component of L-leucine uptake (~ 25%) was found to require extracellular Na^+ in comparison with L-DOPA transport which was Na^+-independent. L-DOPA and L-leucine uptake was insensitive to *N*-(methylamino)-isobutyric acid, but competitively inhibited by 2-aminobicyclo(2,2,1)-heptane-2-carboxylic acid (BCH). L- and D-neutral amino acids, but not acidic and basic amino acids, markedly inhibited L-DOPA and [^{14}C]L-leucine accumulation in both cell lines. The [^{14}C]L-DOPA and [^{14}C]L-leucine outward were markedly increased by L-leucine and BCH present in extracellular medium, but not by L-arginine. In both cell lines, L-DOPA transport was stimulated by acidic pH in comparison with [^{14}C]L-leucine inward which was pH-independent. In conclusion, it is likely that system B^0 might be responsible for the Na^+-dependent uptake of L-leucine in Caco-2 and IEC-6 cells, whereas sodium-independent uptake of L-leucine and L-DOPA may include system type 1 and type 2 L-amino acid transporter (LAT1 and LAT2), the activation of which results in *trans*-stimulation of substrates outward transfer.

Keywords: L system; L-DOPA (L-3,4-dihydroxyphenylalanine)-leucine; Caco-2 cell; IEC-6 cell; Amino acid exchanger

1. Introduction

Dopamine-induced inhibition of Na^+–K^+-ATPase activity has been suggested to play a role in the regulation of Na^+ absorption at the intestinal level (Finkel et al., 1994; Vieira-Coelho et al., 1998). This appears to be particularly important during high salt intake (Finkel et al., 1994) and when renal function has not reached maturation (Vieira-Coelho and Soares-da-Silva, 2000) or has been compromised (Vieira-Coelho et al., 2000). The effects of dopamine were shown to be mediated through the activation of dopamine D_1-like receptors and these occur predominantly at low concentrations of the amine (Vieira-Coelho and Soares-da-Silva, 2000). Inhibition of jejunal Na^+–K^+-ATPase activity by dopamine through dopamine D_1-like dopamine receptors involves the activation of a cholera toxin-sensitive G protein of the G_s class (Vieira-Coelho and Soares-da-Silva, 2000). Saturation experiments with [*N*-methyl-^{3}H]R[+]-7-chloro-2,3,4,5-tetrahydro-3-methyl-1-phenyl-1*H*-3benzazepine-8-ol ([^{3}H]Sch 23390), the selective dopamine D_1-like receptor antagonist performed in membranes from jejunal epithelial cells revealed the presence of single class of receptor, with an apparent K_D in the low nmol/l range (Lucas-Teixeira et al., 2000b). The relative importance of dopamine in controlling intestinal Na^+ absorption assumes particular relevance in view of the findings that dopamine D_1-like receptor agonists fail to inhibit jejunal Na^+–K^+-ATPase activity in spontaneous hypertensive rats (SHR), which contrast with that in their normotensive controls (Wistar–Kyoto rats) (Lucas-Teixeira et al., 2000c). Most of dopamine responsible for these effects has its origin in epithelial cells of intestinal mucosa rich in aromatic L-amino acid decarboxylase (AADC) activity and using circulating or luminal L-DOPA as a source for dopamine (Vieira-Coelho and Soares-da-Silva, 1993). A high salt intake has been found to constitute an important stimulus for the production of dopamine in rat jejunal epithelial cells (Finkel et al., 1994; Lucas-Teixeira et al., 2000a), this being accompanied by a decrease in Na^+ intestinal absorption (Finkel et al., 1994) and Na^+–K^+-ATPase activity (Vieira-Coelho et al., 1998).

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Although the intestinal epithelium is endowed with one of the highest levels of AADC in the body and common foodstuffs are a major source of L-DOPA (Cuche, 1988), the rate-limiting step for the synthesis of dopamine in the intestinal epithelium is still a matter of debate. In fact, the K_m values for L-DOPA uptake are 10 times lower than the K_m values for decarboxylation of L-DOPA, suggesting that L-DOPA uptake, rather than its decarboxylation, may rate limit the formation of dopamine (Vieira-Coelho et al., 1997; Vieira-Coelho and Soares-da-Silva, 1998). On the other hand, recent studies from our laboratory have shown that L-DOPA uptake in renal epithelial cells and at the level of brain capillary endothelium may be promoted through the L-type amino acid transporter (Gomes and Soares-da-Silva, 1999; Sampaio-Maia et al., 2001; Soares-da-Silva et al., 1994; Soares-da-Silva and Serrao, 2000). The high cellular heterogeneity of the intestinal epithelium and its complex structural characteristics, however, constitute major difficulties in the study of intestinal physiology. For these reasons, the present work was aimed at evaluate the activities of the L-type, for leucine preferring, amino acid transporter and the L-DOPA transporter in two intestinal epithelial cell lines, Caco-2 and IEC-6 cells. Caco-2 cells are an established epithelial cell line derived from a human colon adenocarcinoma which undergoes enterocyte differentiation in culture (Pinto et al., 1983). This cell line has been also suggested to possess attributes that make it a suitable in vitro model for the investigation of transport across the small intestinal epithelium (Hidalgo et al., 1989; Meunier et al., 1995). IEC-6 cells are a rat epithelial cell line that in culture have features of small intestinal crypt cells (Quaroni et al., 1979).

2. Methods

2.1. Cell culture

Caco-2 cells (ATCC 37-HTB; passages 39–49) were obtained from the American Type Culture Collection (Rockville, MD) and maintained in a humidified atmosphere of 5% CO_2–95% air at 37 °C. Cells were grown in Minimal Essential Medium (Sigma, St. Louis, MO, USA) supplemented with 100 U/ml penicillin G, 0.25 μg/ml amphotericin B, 100 μg/ml streptomycin (Sigma), 20% foetal bovine serum (Sigma) and 25 mM *N*-2-hydroxyethylpiperazine-*N*′-2-ethanosulfonic acid (HEPES; Sigma). IEC-6 cells were obtained from the "Deutsche Sammlung von Mikroorganismen und Zellkulturen" (ACC-111; passages 3–14) and maintained in a humidified atmosphere of 5% CO_2–95% air at 37 °C. Cells were grown in Dulbecco's Modified Eagle's Medium (45%) and RPMI 1640 (45%) supplemented with 10% foetal bovine serum, 0.1 U/ml insulin, 100 U/ml penicillin G, 0.25 μg/ml amphotericin B and 100 μg/ml streptomycin. For subculturing, the cells were dissociated with 0.05% trypsin–EDTA, split 1:3 (Caco-2) or 1:4 (IEC-6) and subcultured in Costar Petri dishes with 21 cm^2 growth area (Costar, Badhoevedorp, The Netherlands). For uptake studies, the cells were seeded in collagen-treated 24-well plastic culture clusters (internal diameter: 16 mm, Costar) at a density of 40,000 cells/well (2.0×10^4 cells/cm^2). The cell medium was changed every 2 days, and the cells reached confluence after 4 (IEC-6) or 7 (Caco-2) days of initial seeding. For 24 h prior to each experiment, the cell medium was free of foetal bovine serum. Experiments with IEC-6 and Caco-2 cells were generally performed 2 and 5 days after cells reached confluency, usually 6 and 12 days after the initial seeding, respectively; each cm^2 contained about 20 and 100 μg of cell protein.

2.2. Transport of [^{14}C]L-DOPA and [^{14}C]L-leucine

On the day of the experiment, the growth medium was aspirated and the cell monolayers were preincubated for 30 min in Hanks' medium at 37 °C. The Hanks' medium had the following composition (mM): NaCl 137, KCl 5, $MgSO_4$ 0.8, Na_2HPO_4 0.33, KH_2PO_4 0.44, $CaCl_2$ 0.25, $MgCl_2$ 1.0, Tris–HCl 0.15 and sodium butyrate 1.0, pH = 7.4. The incubation medium also contained benserazide (30 μM), tolcapone (1 μM) and pargyline 100 μM in order to inhibit the enzymes AADC, catechol-*O*-methyltransferase and monoamine oxidase, respectively. Apical uptake was initiated by the addition of 1 ml Hanks' medium with a given concentration of the substrate. Time course studies were performed in experiments in which cells were incubated with 2.5 μM L-DOPA or 0.25 μM [^{14}C]L-leucine for 1, 3, 6, 12, 30 and 60 min. Saturation experiments were performed in cells incubated for 6 min with 0.25 μM [^{14}C]L-DOPA or 0.25 μM [^{14}C]L-Leucine in the absence and in the presence of increasing concentrations of the unlabeled substrate. In experiments performed in the presence of different concentrations of sodium, sodium chloride was replaced by an equimolar concentration of choline chloride. In inhibition studies, test substances were applied from the apical side, and were present during the incubation period only. During preincubation and incubation, the cells were continuously shaken and maintained at 37 °C. Uptake was terminated by the rapid removal of uptake solution by means of a vacuum pump connected to a Pasteur pipette followed by a rapid wash with cold Hanks' medium and the addition of 500 μl of 0.1% v/v Triton X-100 (dissolved in 5 mM Tris–HCl, pH 7.4). Radioactivity was measured by liquid scintillation counting.

Fractional outflow of intracellular [^{14}C]L-DOPA and [^{14}C]L-leucine was evaluated in cells loaded with 2.5 μM [^{14}C]L-DOPA or 0.25 μM [^{14}C]L-leucine for 6 min and then the corresponding efflux monitored over 12 min, in the absence and the presence of different amino acids. Fractional outflow was calculated using the expression

$$[^{14}\mathrm{C}]\mathrm{L} - \mathrm{a.a}^{\mathrm{fluid}} / ([^{14}\mathrm{C}]\mathrm{L} - \mathrm{a.a}^{\mathrm{fluid}} + [^{14}\mathrm{C}]\mathrm{L} - \mathrm{a.a}^{\mathrm{cell}})$$

where [^{14}C]L-a.a$^{\mathrm{fluid}}$ indicates the amount of radiolabeled amino acid (in pmol/mg protein) that reached the fluid

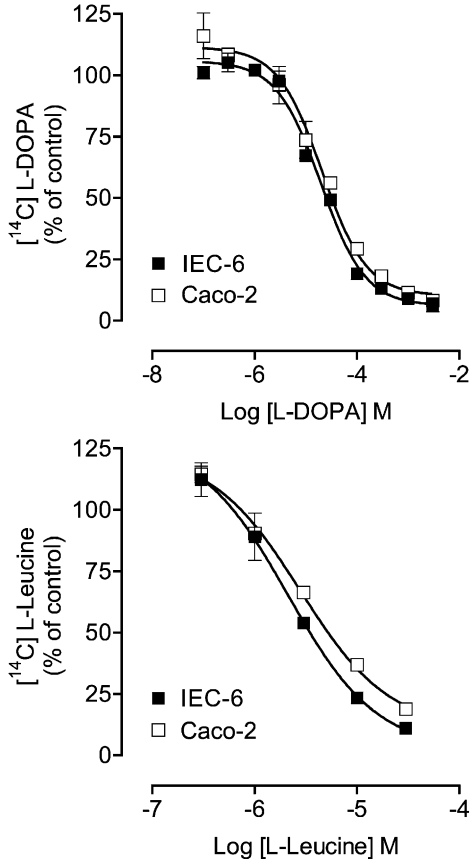

Fig. 1. Effect of increasing concentrations of L-DOPA (3, 10, 30, 100 and 300 μM) on the uptake of [^{14}C]L-DOPA (0.25 μM) in Caco-2 and IEC-6 cells and of L-leucine (0.3, 1, 3, 10 and 30 μM) on the uptake of [^{14}C]L-leucine (0.25 μM) in Caco-2 and IEC-6 cells. Symbols represent the mean of four to eight experiments per group; vertical lines show S.E.M.

bathing the apical cell side and [^{14}C]L-a.a^{cell} (in pmol/mg protein) indicates the amount of radiolabeled amino acid accumulated in the cell monolayer.

2.3. Assay of L-DOPA

L-DOPA was quantified by means of high-pressure liquid chromatography (HPLC) with electrochemical detection, as previously described (Soares-da-Silva et al., 1994). The HPLCsystem consisted of a pump (Gilson model 302; Gilson Medical Electronics, Villiers le Bel, France) connected to a manometric module (Gilson model 802 C) and a stainless-steel 5 μm ODS column (Biophase; Bioanalytical Systems, West Lafayette, IN) of 25-cm length; samples were injected by means of an automatic sample injector (Gilson model 231) connected to a Gilson dilutor (model 401). The mobile phase was a degassed solution of citric acid (0.1 mM), sodium octylsulphate (0.5 mM), sodium acetate (0.1 M), EDTA (0.17 mM), dibutylamine (1 mM) and methanol (8% v/v), adjusted to pH 3.5 with perchloric acid (2 M) and pumped at a rate of 1.0 ml/min. The detection was carried out electrochemically with a glassy carbon electrode, an Ag/AgCl reference electrode and an amperometric detector (Gilson model 141); the detector cell was operated at 0.75 V. The current produced was monitored using the Gilson 712 HPLC software. The lower limits for detection of L-DOPA ranged from 350 to 500 fmol.

2.4. Protein assay

The protein content of cell monolayers was determined by the method of Bradford (1976), with human serum albumin as a standard.

2.5. Cell viability

Cells were preincubated for 30 min at 37 °C and then incubated in the absence or the presence of L-DOPA and test compounds for further 6 min. Subsequently the cells were incubated at 37 °C for 2 min with Trypan blue (0.2% w/v) in phosphate buffer. Incubation was stopped by rinsing the cells twice with Hanks' medium and the cells were examined using a Leica microscope. Under these conditions, more than 95% of the cells excluded the dye.

2.6. Data analysis

K_m and V_{max} values for the uptake of [^{14}C]L-DOPA and [^{14}C]L-leucine, as determined from a competitive uptake inhibition protocol (DeBlasi et al., 1989), were calculated from non-linear regression analysis using the GraphPad Prism statistics software package (Motulsky, 1999). Arithmetic means are given with S.E.M. Statistical analysis was performed by one-way analysis of variance (ANOVA) followed by Newman–Keuls test for multiple comparisons. A P value of less than 0.05 was assumed to denote a significant difference.

2.7. Drugs

The L- and D-amino acids, 2-aminobicyclo (2,2,1)-heptane-2-carboxylic acid (BCH), *N*-(methylamino)-isobutyric acid and Trypan blue were purchased from Sigma. Tolcapone was kindly donated by late Professor Mosé Da Prada (Hoffman La Roche, Basel, Switzerland). [^{14}C]L-leucine, specific activity 303 mCi/mmol and [^{14}C]L-DOPA specific

Table 1
K_m (μM) and V_{max} (pmol mg protein^{-1} 6 min^{-1}) values for the saturable component of [^{14}C]L-DOPA and [^{14}C]L-leucine uptake in cultured Caco-2 and IEC-6 cells

	[^{14}C]L-DOPA		[^{14}C]L-leucine	
	K_m	V_{max}	K_m	V_{max}
Caco-2	19.2 ± 3.1	1867 ± 201	2.6 ± 0.3	299 ± 49
IEC-6	17.2 ± 0.2	3020 ± 31[a]	1.9 ± 0.2	464 ± 116[a]

Values are means ± S.E.M. of six experiments per group.
[a] Significantly different from Caco-2 cells ($P < 0.05$).

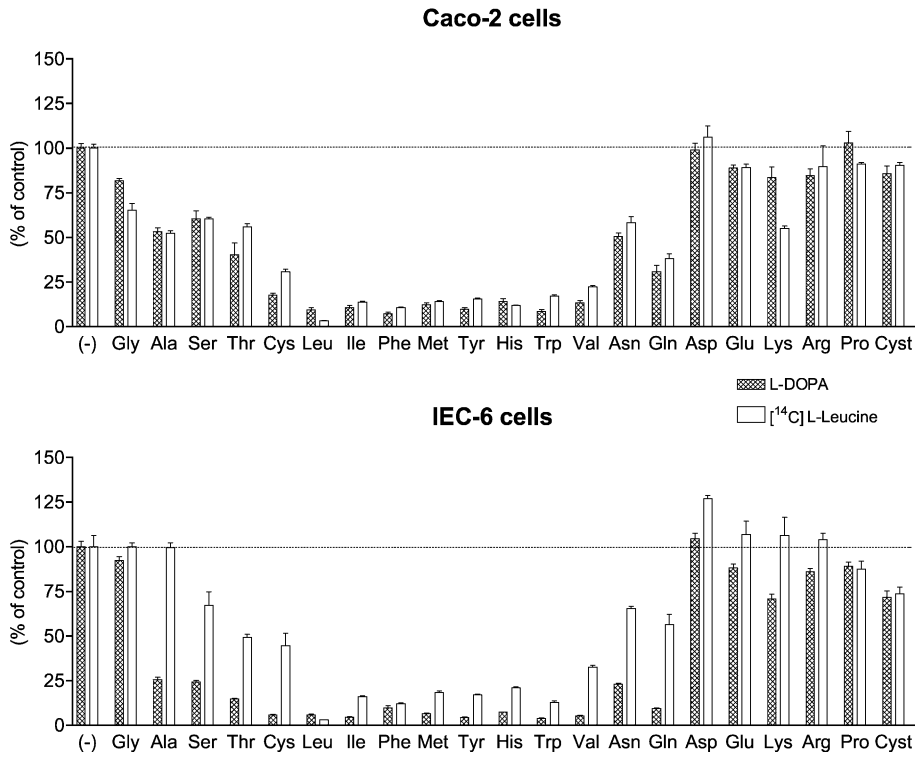

Fig. 2. Effect of Glycine (1 mM) and the indicated L-amino acids (1 mM) on the uptake of L-DOPA (2.5 μM) and [^{14}C]L-leucine (0.25 μM) in Caco-2 and IEC-6 cells. Columns represent the mean of four experiments per group; vertical lines show S.E.M.

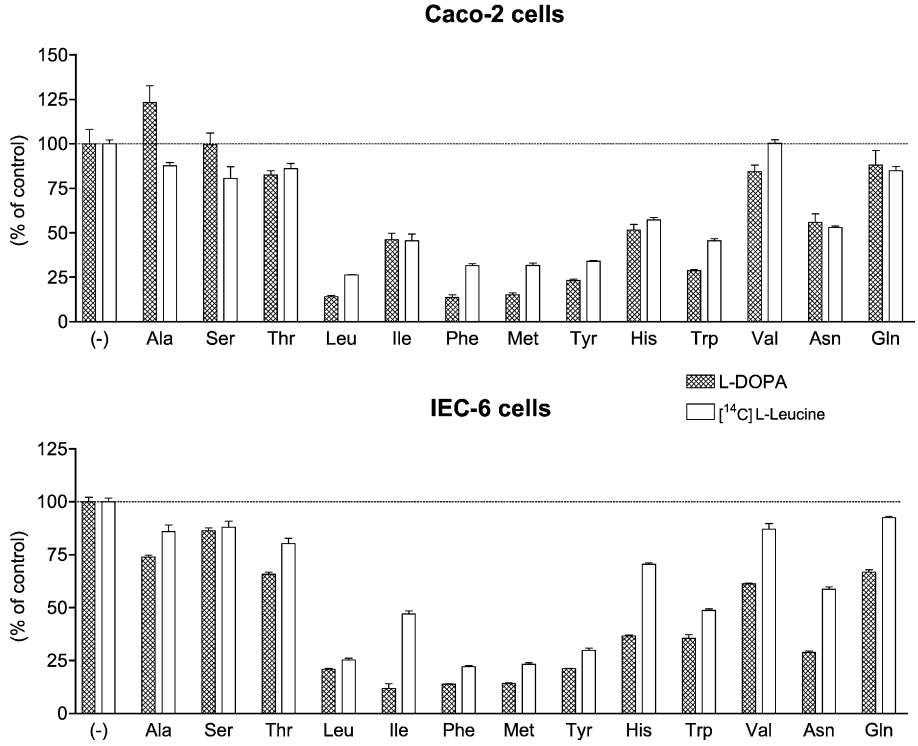

Fig. 3. Effect of the indicated D-amino acids (1 mM) on the uptake of L-DOPA (2.5 μM) and [^{14}C]L-leucine (0.25 μM) in Caco-2 and IEC-6 cells. Columns represent the mean of eight experiments per group; vertical lines show S.E.M.

activity 51 mCi/mmol were purchased from Amersham Pharmacia Biotech (Little Chalfont, UK).

3. Results

In both cell types, uptake of a non-saturating concentration of the substrates (2.5 μM L-DOPA or 0.25 μM [^{14}C]L-leucine) was linear with time for up to 60 min of incubation (data not shown). At 6 min, when uptake was linear and considering intracellular water as 7.0 ± 0.7 μl/mg protein (Vieira-Coelho and Soares-da-Silva, 1998), the cellular L-DOPA and [^{14}C]L-leucine concentration was in Caco-2 cells 27.3 ± 1.6 and 2.3 ± 0.1 μM, respectively. This represented an intracellular concentration of L-DOPA and [^{14}C]L-leucine that was 10.9 ± 0.7 and 9.1 ± 0.5 times higher than the corresponding medium concentration. In IEC-6 cells, L-DOPA and [^{14}C]L-leucine also appear to be transported by a facilitated mechanism with intracellular concentration values of L-DOPA and [^{14}C]L-leucine of 7.0 ± 0.5 and 5.3 ± 0.5 μM, respectively. In fact, these values of intracellular concentration were 2.8 ± 0.2 and 21.2 ± 1.9 times higher than the extracellular concentration of the respective substrate.

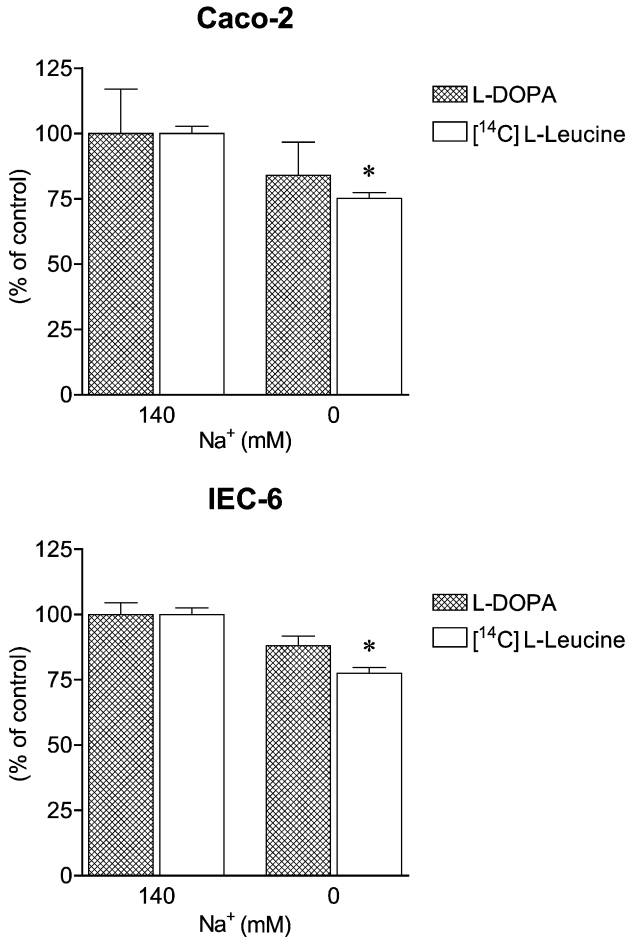

Fig. 4. Effect of Na^+ during incubation on the uptake of L-DOPA (2.5 μM) and [^{14}C]L-leucine (0.25 μM) in Caco-2 and IEC-6 cells. Columns represent the mean of six experiments per group; vertical lines show S.E.M. Significantly different from control value (* $P < 0.05$).

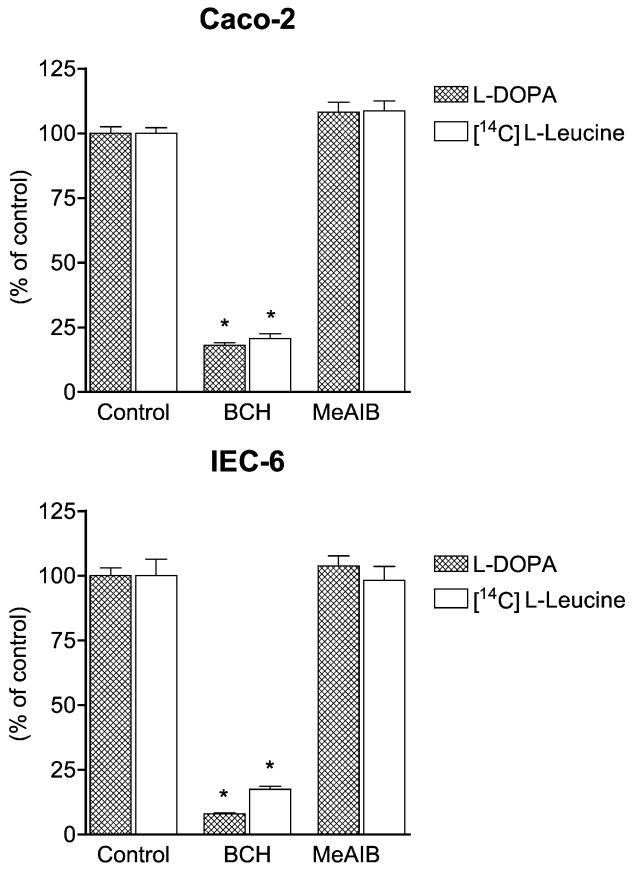

Fig. 5. Effect of BCH (1 mM) and MeAIB (1 mM) on the uptake of L-DOPA (2.5 μM) and [^{14}C]L-leucine (0.25 μM) in Caco-2 and IEC-6 cells. Columns represent the mean of eight experiments per group; vertical lines show S.E.M. Significantly different from control value (* $P < 0.05$).

In order to determine the kinetics of the transporters, cells were incubated for 6 min with [^{14}C]L-DOPA (0.25 μM) or [^{14}C]L-leucine (0.25 μM) in the absence or in presence of increasing concentrations of unlabeled substrate, respectively L-DOPA (3–300 μM) and L-leucine (0.3–30 μM) (Fig. 1). The uptake of both substrates was reduced in the presence of the respective unlabeled compound in a concentration-dependent manner. Kinetic parameters of [^{14}C]L-DOPA and [^{14}C]L-leucine uptake (K_m and V_{max}) were determined by non-linear analysis of the specific analysis of inhibition curve for L-DOPA and L-leucine and are given in Table 1. As shown in the table, the affinity of the transporter for L-leucine as well as for L-DOPA was similar in both cell lines as evidenced by K_m values, but was greater for L-leucine than for L-DOPA. Substrate selectivity of L-DOPA and L-leucine uptake was investigated by inhibition experiments in which 2.5 μM L-DOPA or 0.25 μM [^{14}C]L-leucine uptake was measured in the presence of 1 mM of unlabeled amino acids (Fig. 2). As shown in figure, in Caco-2 cells the uptake of the substrate was inhibited by glycine

and the L-isomers of neutral amino acids and histidine and lysine. Proline, the acidic amino acids aspartate and glutamate and the basic amino acid arginine and cystine did not inhibit L-DOPA and [^{14}C]L-leucine uptake in Caco-2 cells. The profile of inhibition in IEC-6 cells was similar to that observed in Caco-2 cells, with the exception that glycine, L-alanine and L-lysine were ineffective in reducing [^{14}C]L-leucine, but not L-DOPA accumulation. In both Caco-2 and IEC-6 cells, L-aspartate produced a slight increase in [^{14}C]L-leucine accumulation. The inhibitory effect of D-amino acids on L-DOPA and [^{14}C]L-leucine uptake was less marked than that obtained with L-isomers, as shown in Fig. 3. Irrespective of their optical conformation, the most effective neutral amino acids in reducing the uptake of L-DOPA and [^{14}C]L-leucine in both Caco-2 and IEC-6 cells were isoleucine, phenylalanine, methionine, tyrosine and tryptophan.

All the experiments mentioned above were performed in the presence of 140 mM Na^+ in the uptake solution. Because amino acid transport across plasma membranes can be mediated by both Na^+-dependent and Na^+-independent transporters, NaCl was replaced by an equimolar concentration of choline chloride in order to determine a potential Na^+ dependency in L-DOPA and [^{14}C]L-leucine apical inward. As show in Fig. 4, the effect of removing Na^+ from the uptake solution produced a slight, but statistically significant ($P < 0.05$), reduction of [^{14}C]L-leucine uptake in both Caco-2 and IEC-6 cells, but did not alter L-DOPA transport. Altogether, these results indicate that transport of L-DOPA and L-leucine in both types of cells may be promoted through the L-type amino acid transporter. In order to confirm this hypothesis, the next series of experiments were addressed to study the effect of the amino acid analogues *N*-(methylamino)-isobutyric acid (MeAIB) and 2-aminobicyclo(2,2,1)-heptane-2-carboxylic acid (BCH), inhibitors of the A- and L-type amino acid transporters, respectively. As depicted in Fig. 5, BCH, but not MeAIB, produced a marked decrease in substrate accumulation. These results suggest that the inward transfer of L-DOPA and [^{14}C]L-leucine in both Caco-2 and IEC-6 cells may be largely promoted through the BCH-sensitive and Na^+-independent L-type amino acid transporter.

The effect of pH on L-DOPA and [^{14}C]L-leucine influx was examined by changing the pH of the uptake solution. As shown in Fig. 6, [^{14}C]L-leucine accumulation in Caco-2 and IEC-6 cells was not affected by changing pH of the

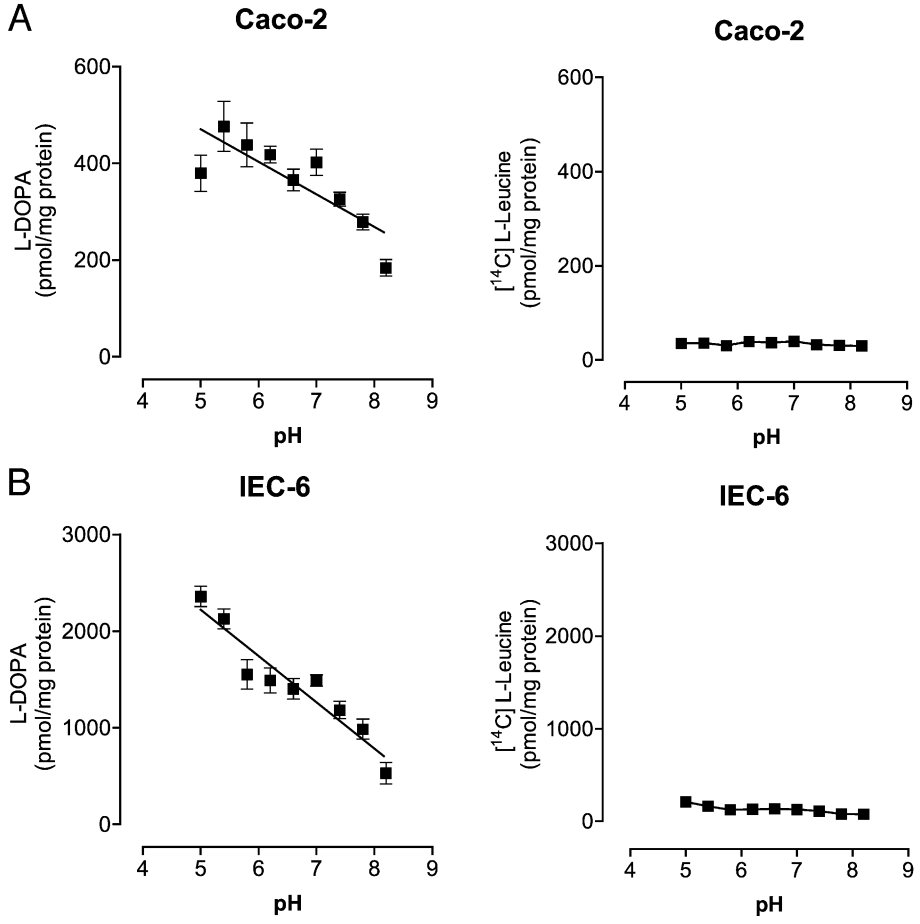

Fig. 6. Effect of pH on the uptake of L-DOPA (2.5 μM) and [^{14}C]L-leucine (0.25 μM) in (A) Caco-2 and (B) IEC-6 cells. Symbols represent the mean of eight experiments per group; vertical lines show S.E.M.

incubation medium, whereas L-DOPA transport was greater at an acidic pH. In fact, the pH-sensitive L-DOPA uptake in Caco-2 and IEC-6 cells was particularly evident being the rate of uptake 67 ± 17 and 478 ± 59 pmol/mg protein/pH unit, respectively.

Because some amino acid transporters have been shown to function as amino acid exchangers, a new series of experiments were conducted in cells loaded with [^{14}C]L-DOPA or [^{14}C]L-leucine for 6 min and then the corresponding efflux monitored over 12 min, in the absence and in presence of different amino acids and unlabeled L-leucine. As shown in Fig. 7, the efflux of [^{14}C]L-leucine from Caco-2 and IEC-6 cells over 12 min corresponded to approximately 25% of the amount of [^{14}C]L-leucine accumulated in the cells, i.e. both cell types were able to retain most of the taken up substrate. In contrast [^{14}C]L-DOPA efflux was higher particularly in IEC-6 cells with values of ~65% of the amount accumulated in the cells. Adding 1 mM L-leucine to the extracellular medium markedly increased the efflux of [^{14}C]L-DOPA and [^{14}C]L-leucine in both cell types. This is in agreement with the view that the L-DOPA as well as L-leucine transporters function as exchangers. In another series of experiments, the efflux of [^{14}C]L-DOPA and [^{14}C]L-leucine was monitored for 12 min in the absence and the presence of 1 mM BCH or 1 mM L-arginine. As shown in Fig. 7, BCH stimulated the efflux of [^{14}C]L-leucine and [^{14}C]L-DOPA in both Caco-2 and IEC-6 cells. On the other hand, L-arginine failed to stimulate the efflux in both cell lines.

4. Discussion

In the present study, we show that Caco-2 and IEC-6 cells transport quite efficiently L-DOPA and L-leucine through the apical cell border and several findings demonstrate that this uptake process is a facilitated mechanism. Though most of [^{14}C]L-leucine was entering the cells in a Na^+-independent manner, a minor component of [^{14}C]L-leucine uptake (~25%) was found to require extracellular Na^+, which contrasts with the Na^+-independent L-DOPA apical transfer. Apart this, L-DOPA and [^{14}C]L-leucine uptake in Caco-2 and IEC-6 cells were both sensitive to inhibition by BCH, but not to MeAIB, and sensitive to

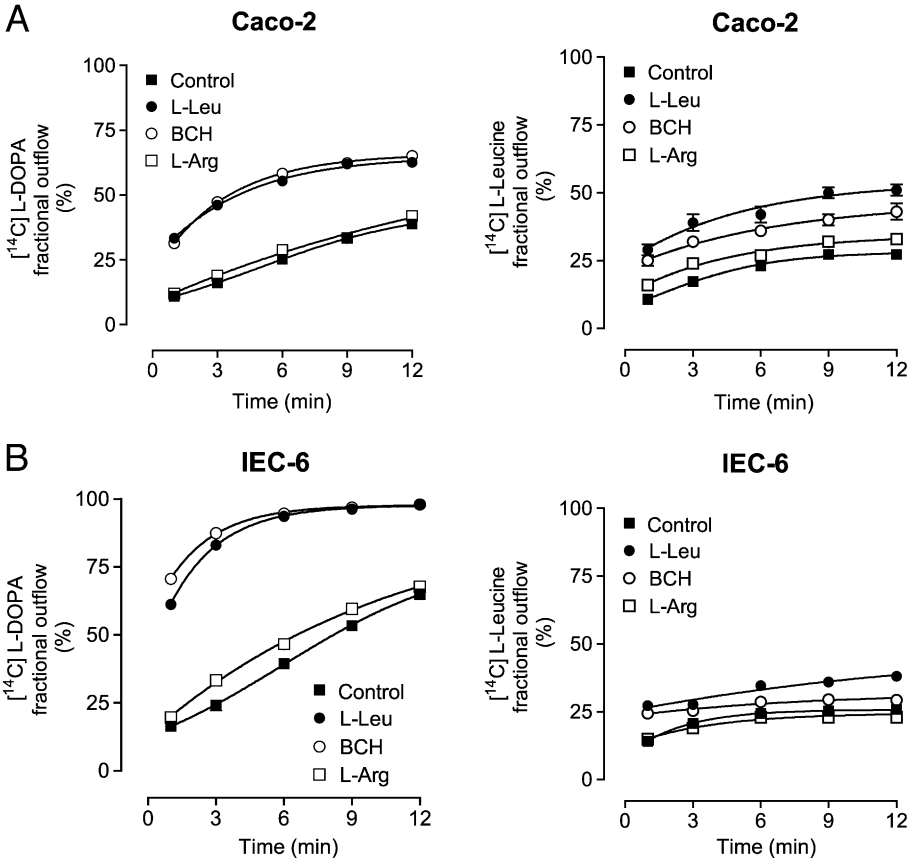

Fig. 7. Fractional outflow (%) of [^{14}C]L-DOPA and [^{14}C]L-leucine in (A) Caco-2 and (B) IEC-6 cells in the absence (control) and the presence of L-leucine (L-Leu), L-arginine (L-Arg) or 2-aminobicyclo(2,2,1)-heptane-2-carboxylic acid (BCH). Cells were incubated for 6 min in the presence of 2.5 μM [^{14}C]L-DOPA and 0.25 μM [^{14}C]L-leucine and then incubated in the absence and the presence of unlabeled L-Leu (1 mM), L-Arg (1 mM) or BCH (1 mM) for 1, 3, 6 and 12 min. Symbols represent the mean of eight experiments per group; vertical lines show S.E.M.

inhibition by neutral, but not acidic and basic amino acids. In addition, L-DOPA and [^{14}C]L-leucine uptake in Caco-2 and IEC-6 cells shows *trans*-stimulation by unlabelled leucine. Taken together, these findings agree with the view that L-leucine may be transported by systems B^0 (Na^+-dependent) and L (Na^+-independent), whereas L-DOPA may be transported through system L only. The sensitivity of L-DOPA and [^{14}C]L-leucine uptake to BCH, but not to MeAIB, supports the view that inward transfer in Caco-2 and IEC-6 cells is promoted neither by the A- nor the ASC-type amino acid transporter. System B^0 is a broad-specificity amino acid transport system cotransporting neutral amino acids with Na^+ into cells that also accepts BCH but not MeAIB (Palacin et al., 1998). System $B^{0,+}$ is also a Na^+-dependent transporter that has a broad-specificity for zwitterionic and basic amino acids and also accepts BCH but not MeAIB (Palacin et al., 1998). The uptake of [^{14}C]L-leucine was inhibited by neutral amino acids such as phenylalanine, leucine and tyrosine, and blocked by BCH, but not by MeAIB and the acidic and basic amino acids. For this reason it is likely that system B^0 rather than system $B^{0,+}$ might be responsible for the Na^+-dependent uptake of [^{14}C]L-leucine in both cell lines. System L transports neutral amino acids with high affinity (K_m in the μM range) with no need for Na^+ in the extracellular medium and shows very high capacity for *trans*-stimulation (Palacin et al., 1998). The finding that accumulation of L-DOPA in Caco-2 and IEC-6 cells was significantly higher at an acidic pH fits well the view that L-DOPA uptake in these cells is promoted through system L, namely the L-amino acid transporter (LAT) type 2 (LAT2). L-leucine appears to be transported by the LAT type 1 (LAT1) isoform that is characterized for being pH-insensitive (Prasad et al., 1999).

The results of [^{14}C]L-DOPA and [^{14}C]L-leucine efflux studies in Caco-2 and IEC-6 cells are also quite valuable to define the nature of transporters involved in the handling of these substrates. As most of the [^{14}C]L-DOPA and [^{14}C]L-leucine did not leak out of the cells during the 12-min incubation in amino acid-free incubation medium and measurements of efflux in the absence of extracellular amino acids did not show a consistent efflux, the results suggest that L-DOPA and L-leucine transporters function as exchangers. In fact, systems LAT1 and LAT2 function as exchangers (Chillaron et al., 1996; Rossier et al., 1999; Segawa et al., 1999) and leucine-induced outward of both substrates agree with the view that either transporter may participate in the exchange. However, amino acid specificity and affinity are different for LAT1 compared with LAT2. LAT1 induces Na^+-independent transport of large neutral amino acids with K_m values in the micromolar range. LAT2 also transports small neutral amino acids such as L-alanine, L-glycine, L-cysteine (Wagner et al., 2001), however with a lower affinity to substrate amino acids than that of LAT1 (Segawa et al., 1999). These findings are consistent with K_m values for L-DOPA and L-leucine in both cell lines. Accordingly, Caco-2 and IEC-6 cells may transport [^{14}C]L-leucine through the pH-independent LAT1 transporter whereas L-DOPA appears to be transported through the pH-dependent LAT2 transporter. Another result that supports this hypothesis is that L-leucine-stimulated outward transport of [^{14}C]L-leucine is considerably greater through LAT1 than through LAT2 (Segawa et al., 1999). LAT1-specific mRNA is expressed in most human tissues with the notable exception of the intestine (Prasad et al., 1999). This conflicts with the view that the Na^+- and pH-independent L-type amino acid transporter in Caco-2 and IEC-6 cells may not correspond to LAT1. On the other hand, the mRNA corresponding to LAT2 examined by Northern blot analysis was strongly expressed in the small intestine (Rossier et al., 1999; Segawa et al., 1999).

From a conceptual point of view, the present study adds new evidence in three important sectors. Firstly, it reveals the functional characteristics of the mechanisms governing the availability of dopamine's precursor, L-DOPA, at the intestinal level, where the amine plays the role of a local hormone regulating Na^+ absorption. Secondly, while showing that Na^+ may be not important for the uptake of L-DOPA, it suggests that the increased intestinal synthesis of dopamine following a high salt intake (Finkel et al., 1994; Lucas-Teixeira et al., 2000a) may depend on the facilitation or stimulation of mechanisms promoting the conversion of L-DOPA to dopamine, rather than stimulating the cellular uptake of L-DOPA. Finally, it indicates that competition between L-DOPA and neutral amino acids for absorption at the intestinal level, a considerable problem in parkinsonian patients on L-DOPA therapy, may result from both stimulation of L-DOPA outward transfer and competition for uptake.

It is concluded that L-DOPA and L-leucine in Caco-2 and IEC-6 cells are transported quite efficiently through the apical cell border and several findings indicate this uptake process is a facilitated mechanism involving Na^+-dependent and Na^+-independent transporters. It is likely that system B^0 may be responsible for the Na^+-dependent uptake of L-leucine. Transporter involved in Na^+-independent uptake of L-DOPA (LAT2) as well as L-leucine (LAT1) may include system L isoforms, that also function as exchangers, the activation of which results in *trans*-stimulation of L-DOPA and L-leucine outward transfer.

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