Uptake and intracellular fate of L-DOPA in a human intestinal epithelial cell line: Caco-2

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Vieira-Coelho, M. A., and P. Soares-da-Silva. Uptake and intracellular fate of L-DOPA in a human intestinal epithelial cell line: Caco-2. Am. J. Physiol. 275 (Cell Physiol. 44): C104–C112, 1998.—The aim of the present study was to examine the kinetic characteristics of the 3,4-dihydroxyphenylalanine (L-DOPA) transporter and the fate of newly formed dopamine in Caco-2 cells. In the presence of 50 µM benserazide (an inhibitor of aromatic L-amino acid decarboxylase), L-DOPA was rapidly accumulated in Caco-2 cells. At equilibrium (30 min of incubation) the intracellular L-DOPA concentration was 10.2 ± 0.1 µM at a medium concentration of 0.5 µM. In saturation experiments the accumulation of L-DOPA was saturable with a Michaelis-Menten constant (K_m) of 60 ± 10 µM and a maximal reaction velocity (V_max) of 6.6 ± 0.3 nmol·mg protein^{-1}·min^{-1}; at 4°C the amount of L-DOPA accumulated in the cells was nonsaturable. When cells were incubated with increasing concentrations of L-DOPA (10–100 µM) in the absence of benserazide, a substantial amount of the L-DOPA that was taken up was decarboxylated to dopamine, with an apparent K_m of 27.2 µM. In experiments performed in cells cultured in polycarbonate filters, the accumulation of L-DOPA in the presence of benserazide was greater when the substrate was applied from the basolateral cell border than when it was applied from the apical cell border. In the absence of benserazide, L-DOPA applied from the basolateral cell border resulted in a nonlinear formation of dopamine (K_m = 43 ± 7 µM, V_max = 23.7 ± 1.2 nmol·mg protein^{-1}·min^{-1}). The amount of dopamine leaving the cell through the apical cell border was lower than the amount that escaped through the basolateral cell border, and the process was saturable (K_m = 623 ± 238 µM, V_max = 0.19 ± 0.02 nmol·mg protein^{-1}·min^{-1}). In conclusion, the data presented here show that Caco-2 cells are endowed with an efficient L-DOPA uptake system, and intracellular L-DOPA was found to be rapidly converted to dopamine, some of which diffuses out of the cell. The utilization of Caco-2 cells cultured on polycarbonate filters probably provides a better way to look at processes such as the outward transfer of intracellular molecules, namely, the outward transfer of newly formed dopamine.

L-3,4-dihydroxyphenylalanine; dopamine; polycarbonate filter; basolateral cell border; apical cell border; L-amino acid decarboxylase

THE INTESTINAL TRACT has been shown to be of crucial importance in the regulation of body fluid and electrolyte homeostasis, with catecholamines assuming the role of important regulators of jejunal cell function (2). More recently, endogenous dopamine in the digestive tract has been suggested to play a role in regulating sodium absorption (1, 6). The current view of the intestinal dopaminergic system is that of a local nonneuronal system that consists of epithelial cells of intestinal mucosa rich in aromatic L-amino acid decarboxylase (AADC) activity and uses circulating or luminal L-3,4-dihydroxyphenylalanine (L-DOPA) as a source for dopamine (25). In the intestine, dopamine is particularly abundant in the mucosal cell layer (4, 5). Studies on the formation of dopamine from exogenous L-DOPA along the rat digestive tract showed that the highest AADC activity is located in the jejunum (28). Because the dopamine produced in this area is in close proximity to epithelial cells that contain receptors for the amine, it has been hypothesized that the amine may act as a paracrine or an autocrine substance (25). A high-salt diet has been found to constitute an important stimulus for the production of dopamine in rat jejunal epithelial cells, and this is accompanied, in 20-day-old animals, by a decrease in sodium intestinal absorption (6). This effect is accomplished, at the cellular level, by inhibition of Na^+–K^+–ATPase activity (26). The relative importance of this system in controlling sodium absorption assumes particular relevance in view of the findings that 40-day-old rats fed a high-salt diet have a fault in intestinal dopamine production during salt loading, in contrast to 20-day-old animals (6, 26). The lack of changes in the jejunal function in response to a high-salt diet coincides with the period in which the renal function has reached maturation (15, 16), suggesting the occurrence of complementary functions between the intestine and the kidney during development. On the other hand, this intestinal epithelial dopaminergic system presents some similarities to that described for the proximal renal tubules (25), where locally produced dopamine has long been demonstrated to play a role in the handling of sodium (9, 10, 19).

Several intestinal cell lines are often used as physiological model systems of intestinal absorptive and secretive function, because in most cases their utilization enables the evaluation of a given process in a single population of cells. Caco-2 cells are an established epithelial cell line derived from a human colon adenocarcinoma that undergoes enterocyte differentiation in culture (13). This cell line has been also suggested to possess attributes that make it a suitable in vitro model system for the investigation of transport across the small intestinal epithelium (8). To explore further the usefulness of Caco-2 cells for the study of intestinal dopaminergic physiology, we have undertaken the study of the kinetic characteristics of the L-DOPA transporter and the fate of newly formed dopamine in this cell line. We report here that Caco-2 cells take up L-DOPA through a saturable, stereoselective, and temperature-dependent process; in cells cultured in polycarbonate filters, the inward and outward transfers of L-DOPA are quantitatively more important at the basolateral than at the apical cell border. The formation of dopamine was found to be a time- and concentration-dependent process and rapidly saturated, and the newly formed amine was found to leave the intracellular compart-
ment through the apical cell border by a saturable process.

METHODS

Cell culture. The Caco-2 cell line was obtained from the American Type Culture Collection (ATCC, Rockville, MD) and maintained in a humidified atmosphere of 5% CO₂-95% air at 37°C. Caco-2 cells (ATCC 37-HTB; passages 23–30) were grown in minimal essential medium (Sigma Chemical, St. Louis, MO) supplemented with 100 U/ml penicillin G, 0.25 μg/ml amphotericin B, 100 μg/ml streptomycin (Sigma Chemical), 20% fetal bovine serum (Sigma Chemical), and 25 mM HEPES (Sigma Chemical). For subculturing, the cells were dissociated with 0.05% trypsin-EDTA, split 1:3, and subcultured in flasks with 75- or 162-cm² growth areas (Costar, Badhoevedorp, The Netherlands). For uptake studies, the cells were seeded in collagen-treated 24-well plastic culture clusters (16 mm ID, Costar) at a density of 30,000 cells/well or (2.0 × 10⁴ cells/cm²), or, depending on the experiment, onto collagen-treated 0.2-µm polycarbonate filter supports (12 mm ID, Transwell, Costar). The cell medium was changed every 2 days, and the cells reached confluence after 5–7 days of initial seeding. For 24 h before each experiment, the cell medium was free of fetal bovine serum. Experiments were generally performed 2–3 days after cells reached confluence and 7–10 days after the initial seeding, and each square centimeter contained ~100 μg of cell protein.

Transport studies. On the day of the experiment the growth medium was aspirated and the cells were washed with Hanks’ medium at 4°C; then the cell monolayers were preincubated for 15 min in Hanks’ medium at 37°C. Hanks’ medium had the following composition (in mM): 137 NaCl, 5 KCl, 0.8 MgSO₄, 0.33 Na₂HPO₄, 0.44 KH₂PO₄, 0.25 CaCl₂, 1.0 MgCl₂, 0.15 Tris·HCl, and 1.0 sodium butyrate, pH 7.4. The incubation medium also contained pargyline (100 μM) and tolcapone (1 μM) to inhibit the enzymes monoamine oxidase and catechol-O-methyltransferase, respectively; in some experiments, benserazide (50 μM) was also added to the incubation medium to inhibit AADC. During preincubation and incubation the cells were continuously shaken and maintained at 37°C.

The first series of experiments, uptake studies were performed in cells cultured in collagen-treated plastic supports, the substrates being applied from the apical cell border only. Uptake was initiated by the addition of 2 ml of Hanks’ medium with a given concentration of the substrate under study. Initial rate of uptake was determined in experiments in which cells were incubated with nonsaturating and saturating concentrations of L-DOPA (0.5, 5, and 500 μM) for 1, 3, 6, 12, 30, 60, and 120 min. Saturation experiments were performed in cells incubated for 6 min with increasing concentrations of the substrate; some experiments were conducted at 4°C. Uptake was terminated by the rapid removal of uptake solution by means of a vacuum pump connected to a pasteur pipette followed by two rapid washes with cold Hanks’ medium and the addition of 250 μl of 0.2 mM perchloric acid; the acidified samples were stored at 4°C before injection into the high-pressure liquid chromatograph for the assay of L-DOPA, D-DOPA, and dopamine.

In a second series of experiments, cells were cultured in polycarbonate supports, the substrates being applied from the apical or the basal side of the monolayer. The incubation medium used in this series of experiments was similar to that described above; in some experiments the medium contained benserazide (50 μM) to inhibit AADC. The upper and lower chambers contained 100 and 600 μl, respectively. For apical uptake the uptake solution was added to the upper chamber; for basolateral uptake the uptake solution was added to the lower chamber. Cells were preincubated for 30 min and then incubated in the presence of L-DOPA, [14C]sorbitol (0.4 μM) was used to estimate paracellular fluxes and extracellular trapping of L-DOPA during L-DOPA uptake studies. At the end of incubation, cells were placed on ice, and the medium bathing the apical and basal cell borders was collected, acidified with 2 M perchloric acid, and stored at 4°C until assayed for L-DOPA and dopamine. The cells were washed with ice-cold Hanks’ medium and added with 0.2 mM perchloric acid (100 and 500 μl in the upper and lower chambers, respectively); the acidified samples were stored at 4°C before injection into the high-pressure liquid chromatograph for the assay of L-DOPA and dopamine.

AADC preparation and decarboxylation studies. Caco-2 cells were homogenized in 0.5 M phosphate buffer (pH 7.0) with a Thomas Teflon homogenizer and kept continuously on ice. Aliquots of 500 μl of cell homogenate plus 400 μl of incubation medium were placed in glass test tubes and preincubated for 15 min. Thereafter, L-DOPA (0.1–5.0 mM) was added to the medium for a further 15 min; the final reaction volume was 1 ml. The composition of the incubation medium was as follows (in mM): 0.35 NaH₂PO₄, 0.15 Na₂HPO₄, 0.11 sodium citrate and 0.12 pyridoxal phosphate, tolcapone (1 μM) and pargyline (100 μM) were also added to the medium. The pH of the reaction medium was kept constant at an optimal pH of 7.0 (18). During incubation, cell homogenates were continuously shaken and gassed (95% O₂–5% CO₂) and maintained at 37°C. The reaction was stopped by the addition of 500 μl of 2 M perchloric acid, and the preparations were kept at 4°C for 60 min. The samples were then centrifuged (200 g, 2 min, 4°C), and 500-μl aliquots of the supernatant filtered on Spin-X filter tubes (Costar) were used for the assay of dopamine.

Assay of L-DOPA, D-DOPA, and dopamine. L-DOPA, D-DOPA, and dopamine were quantified by HPLC with electrochemical detection, as previously reported (20). The high-pressure liquid chromatograph system consisted of a pump (model 302, Gilson Medical Electronics, Villiers-le-Bel, France) connected to a manometric module (model 802 C, Gilson) and a 25-cm-long stainless-steel 5-µm ODS column (Biophase, Bioanalytical Systems, West Lafayette, IN); samples were injected by means of an automatic sample injector (model 231, Gilson) connected to a dilutor (model 401, Gilson). The mobile phase was a degassed solution of citric acid (0.1 mM), sodium octylsulfate (0.5 mM), sodium acetate (0.1 M), EDTA (0.17 mM), dibutyramine (1 mM), and methanol (8% vol/vol) 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 (model 141, Gilson); the detector cell was operated at 0.75 V. The current produced was monitored using Gilson 712 HPLC software. The lower limits for detection of L-DOPA, D-DOPA, and dopamine ranged from 350 to 500 fmol.

Cell water content. Cell water content was simultaneously measured in parallel experiments with [14C]insulin as extracellular marker and tritiated water as total water marker. Intracellular water, obtained by subtracting extracellular water from total water, was expressed as microliters of cell water per milligram of protein. Subsequently, the cells were solubilized by 0.1% (vol/vol) Triton X-100 (dissolved in 5 mM Tris·HCl, pH 7.4), and radioactivity was measured by liquid scintillation counting.

Protein assay. The protein content of monolayers of Caco-2 cells was determined by the method of Bradford (3), with human serum albumin as a standard.
Cell viability. Caco-2 cells were preincubated for 15 min at 37°C and then incubated in the absence or the presence of L-DOPA, d-DOPA, and dopamine for a further 15 or 120 min. Subsequently, the cells were incubated at 37°C for 2 min with trypan blue (0.2% wt/vol) 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, >95% of the cells excluded the dye.

Data analysis. The analysis of the time course of L-DOPA uptake in Caco-2 cells was based on a one-compartment model. The parameters of the following equation

\[ \frac{C_i}{C_o} = \frac{k_{in}}{k_{out}} (1 - e^{-k_{out}t}) \]

were fitted to the experimental data by a nonlinear regression analysis with use of a computed assisted method (11). \( C_i \) and \( C_o \) represent the intracellular and extracellular concentration of the substrate, respectively, \( k_{in} \) and \( k_{out} \) rate constants for inward and outward transport, respectively (in pmol·mg protein\(^{-1}\)·min\(^{-1}\)), and \( t \) the incubation time. \( A_{max} \) is defined as the factor of accumulation (\( C_i/C_o \)) at equilibrium (\( t \rightarrow \infty \)). Michaelis-Menten constants (\( K_m \)) and maximal reaction velocities (\( V_{max} \)) for the uptake of substrates, as determined in saturation experiments, and decarboxylation of L-DOPA in cell homogenates were calculated from nonlinear regression analysis with use of the GraphPad Prism statistics software package (11). The rate constant of outward transfer was determined by the slope of the accumulation of substrates measured by linear regression analysis (12). Arithmetic means are given with SE. Statistical analysis was performed by one-way ANOVA followed by Newman-Keuls test for multiple comparisons. \( P < 0.05 \) was assumed to denote a significant difference.

RESULTS

To determine \( k_{in} \) and \( k_{out} \), Caco-2 cells were incubated with L-DOPA (0.5, 50, or 500 µM) for 1, 3, 6, 12, 30, 60, and 120 min in the presence of benserazide. As shown in Fig. 1, uptake of L-DOPA in Caco-2 cells was linear with time for up to 12 min of incubations in the presence of 0.5, 50, or 500 µM L-DOPA and occurred at a \( k_{in} \) of 4.2 ± 0.2, 217.4 ± 9.8, and 333.6 ± 23.9 pmol·mg protein\(^{-1}\)·min\(^{-1}\), respectively. The \( k_{out} \) was 0.61 ± 0.03, 31.1 ± 1.4, and 47.7 ± 3.4 pmol·mg protein\(^{-1}\)·min\(^{-1}\) for 0.5, 50, and 500 µM L-DOPA, respectively. The equilibrium factor (\( A_{max} \)) declined from 20.4 ± 0.3 at 0.5 µM L-DOPA to 13.5 ± 0.8 and 4.4 ± 0.1 at 50 and 500 µM L-DOPA, respectively. The intracellular water content of cell monolayers was 7.0 ± 0.6 µl/mg protein (\( n = 5 \)). At equilibrium (60 min of incubation), the intracellular L-DOPA concentration was 10.2 ± 0.1, 674.2 ± 37.3, and 2,184.7 ± 59.6 µM at medium concentrations of 0.5, 50, and 500 µM, respectively. This represented a cell concentration of L-DOPA that was 19.1 ± 0.8, 12.7 ± 0.9, and 2.8 ± 0.1 times higher than the corresponding medium concentration.

On the basis of these results, a 6-min incubation was used in all subsequent experiments designed to determine the kinetic parameters for the uptake of L-DOPA. The accumulation of L-DOPA from the apical cell border was found to be dependent on the concentration used and to be saturable at 250 µM (Fig. 2). Nonlinear analysis of the saturation curves revealed a \( K_m \) of 60 ± 10 µM and a \( V_{max} \) of 6.6 ± 0.3 nmol·mg protein\(^{-1}\)·min\(^{-1}\).
In experiments carried out at 4°C the amount of L-DOPA accumulated in the cells was markedly lower than that observed at 37°C and was found to be nonsaturable (Fig. 2). Caco-2 cells incubated at 37°C with increasing concentrations of D-DOPA, instead of L-DOPA, were found to accumulate trace amounts of the D-isomer; the cellular accumulation of D-DOPA at the highest concentration used was ~5% of the corresponding L-isomer (data not shown). The experiments shown in Figs. 1 and 2 were performed in the presence of benserazide (50 µM) to avoid the intracellular decarboxylation of incorporated L-DOPA by AADC. The effectiveness of benserazide in inhibiting L-DOPA decarboxylation was very high, since no traces of dopamine were found in these samples.

Incubation of homogenates of Caco-2 cells with L-DOPA (0.1–5.0 mM) resulted in a concentration-dependent formation of dopamine (Fig. 3). The decarboxylation process was nonsaturable up to 1 mM L-DOPA and showed a trend for saturation at 2 mM L-DOPA. Nonlinear analysis of the saturation curves revealed a \( K_m \) of 1.0 ± 0.4 mM and a \( V_{\text{max}} \) of 22.5 ± 0.6 nmol·mg protein\(^{-1}\)·h\(^{-1}\).

As shown in Fig. 4, decarboxylation of L-DOPA (0.5 µM) in monolayers of Caco-2 cells was a time-dependent process, and most of the incorporated L-DOPA was decarboxylated to dopamine; only small amounts of L-DOPA remained in the intracellular compartment without undergoing decarboxylation to dopamine. Most of the newly formed amine escaped into the medium bathing the apical cell border. The amount of newly formed dopamine that remained in the cell attained equilibrium 30 min after the addition of the substrate. In saturation experiments (Fig. 5) a 6-min incubation period was chosen. In this set of experiments the formation of dopamine from increasing concentrations of L-DOPA (10–100 µM) followed nonlinear kinetics.

Again, most of the incorporated L-DOPA was decarboxylated to dopamine; only small amounts of L-DOPA escaped decarboxylation (Fig. 5A). The process of L-DOPA decarboxylation in Caco-2 cells was, however, rapidly saturated at low concentrations of L-DOPA with an apparent \( K_m \) of 27.2 ± 3.8 µM and a \( V_{\text{max}} \) of 6.4 ± 0.3 nmol·mg protein\(^{-1}\)·6 min\(^{-1}\). Some of newly formed dopamine escaped into the incubation medium, and this process was nonsaturable, the rate constant of outward transfer being 4.4 ± 0.6 mmol\(^{-1}\).
The next series of experiments was performed in cells cultured in polycarbonate filters. L-DOPA was applied from the apical or the basal border; intracellular L-DOPA and L-DOPA that had escaped into the basal or the apical bathing fluid were also measured. Paracellular leakage measured by the fluxes of $[^{14}C]$sorbitol from either side was minimal and represented 0.1% of the amount applied at the cell surface. The first series of experiments was carried out in the presence of benserazide (50 µM), and L-DOPA (0.5 µM) was applied from the apical or the basal cell border for increasing periods of time (1–60 min). As shown in Fig. 6, L-DOPA applied from the apical or the basal cell border was rapidly accumulated in Caco-2 cells, equilibrium being attained at 12 min of incubation, and no flux of L-DOPA across the cell monolayer was detected. The $k_{in}$, $k_{out}$, and $A_{max}$ for the apical application of L-DOPA (38.7 ± 18.0 pmol·mg protein$^{-1}$·min$^{-1}$, 11.1 ± 5.2 pmol·mg protein$^{-1}$·min$^{-1}$, and 42.7 ± 5.6, respectively) were lower ($P < 0.05$) than those observed for the basal application (183.5 ± 50.8 pmol·mg protein$^{-1}$·min$^{-1}$, 52.4 ± 16.5 pmol·mg protein$^{-1}$·min$^{-1}$, and 64.7 ± 5.4, respectively) of the substrate. When the substrate was applied from the apical side, at equilibrium (30 min of incubation), the intracellular concentration of L-DOPA was also lower than that observed for the basal application of the substrate (15.9 ± 3.0 vs. 31.7 ± 1.5 µM) at medium concentration of 0.5 µM. In benserazide-treated cells the accumulation of L-DOPA applied from the basal side was also greater than that from the apical side; $V_{max}$ values were substantially greater for the basal application than for the apical application (78.1 ± 5.4 and 14.6 ± 2.1 nmol·mg protein$^{-1}$·6 min$^{-1}$), as revealed by nonlinear analysis (Fig. 7A). In both cases the uptake was a saturable process with $K_m$ of 601 ± 67 and 482 ± 118 µM for the basal and apical application, respectively. A considerable amount of intracellular L-DOPA, applied from the basal or the apical side, left the cell. In both experimental conditions (apical and basal application of L-DOPA) the outward transfer of intracellular L-DOPA was nonsaturable (Fig. 7B). However, the rate constant of outward transfer of intracellular L-DOPA was greater at the basolateral cell border than at the apical cell border (11.9 ± 1.9 and 3.7 ± 0.2 mmol·6 min$^{-1}$). The rate constant of outward transfer was determined by the slope of the levels of L-DOPA in the incubation medium measured by linear regression analysis (12).

In the absence of benserazide, Caco-2 cells cultured in polycarbonate filters and incubated with L-DOPA (applied from either cell side for 6 min) converted the substrate to dopamine, some of which remained in the intracellular compartment, and a small amount left the cell. Figure 8 shows intracellular levels of newly formed dopamine in Caco-2 incubated with increasing concentrations of L-DOPA (5–250 µM). The intracellular accumulation of dopamine differed markedly according to

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Fig. 5. A: intracellular levels of L-DOPA taken up (○) and newly formed dopamine (●) and extracellular levels of dopamine (□) in Caco-2 cells. B: extracellular levels of newly formed dopamine plotted against intracellular dopamine. Cells were preincubated in absence of benserazide for 30 min and incubated for 6 min with increasing concentrations of L-DOPA; substrate was applied from apical cell border. Symbols represent means of 4 experiments/group; vertical lines show SE.

Fig. 6. Time course of L-DOPA accumulation in Caco-2 cells cultured in polycarbonate filters. Cells were incubated at 37°C with 0.5 µM L-DOPA. Exponential saturation curve was fitted to experimental data. L-DOPA applied from apical (●) or basal cell border (□) was rapidly accumulated in Caco-2 cells, equilibrium being attained at 12 min of incubation; no flux of L-DOPA across cell monolayer could be detected. $k_{in}$, $k_{out}$, and $A_{max}$ values for apical application of L-DOPA were lower than those observed for basal application of substrate. Symbols represent means of 4 experiments/group; vertical lines show SE.
the side of the cell used for L-DOPA application (Fig. 8). Levels of newly formed dopamine were higher and showed a trend for saturation (apparent $K_m$ 5.43 ± 0.67 µM) when L-DOPA was applied from the basal side. In contrast, the levels of newly formed dopamine were lower when L-DOPA was applied from the apical side, and the process of amine formation was nonsaturable up to 250 µM L-DOPA. Table 1 shows the percentage of intracellular L-DOPA decarboxylated to dopamine when the substrate was applied from either side. The amount of intracellular L-DOPA that undergoes decarboxylation to dopamine was approximately the same over a wide range of substrate concentrations, although at the highest concentrations of extracellular L-DOPA the decarboxylation was less pronounced. On the other hand, the decarboxylation of L-DOPA was greater when L-DOPA was applied from the basal side ($P < 0.05$) than from the apical side (Table 1).

As mentioned above, a small amount of newly formed dopamine escaped into the incubation medium. As shown in Fig. 9, the amount of dopamine that escaped through the apical cell border was lower than the amount leaving the cell through the basolateral cell border and was a saturable process; nonlinear analysis of the saturation curve revealed a $K_m$ of 623 ± 238 µM and a $V_{max}$ of 0.19 ± 0.02 nmol·mg protein$^{-1}$·min$^{-1}$. The dopamine leaving the cell through the basolateral cell border was not directly related to the intracellular levels of the amine. In these two examples, dopamine levels in the medium were measured in experiments in which cells were incubated with L-DOPA from the opposite cell border.

### DISCUSSION

The data presented here show that Caco-2 cells efficiently take up L-DOPA, and several findings demonstrate that this uptake process was a carrier-mediated mechanism. First, steady-state uptake of nonsaturating concentrations of L-DOPA showed a curvilinear dependence on incubation time. Second, at an initial rate of uptake (6 min of incubation) the cellular transport of L-DOPA showed a curvilinear dependence on L-DOPA concentration.

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Values are means ± SE of 4 experiments/group expressed as percentages. Cells were preincubated in the absence of benserazide for 30 min and incubated for 6 min with increasing concentrations of L-DOPA; substrate was applied from apical or basal cell border. *Significantly different from corresponding values for apical application of L-DOPA ($P < 0.05$, by Newman-Keuls test). †Significantly different from 5 µM L-DOPA ($P < 0.05$, by Newman-Keuls test).
L-DOPA medium concentration, suggesting that the uptake was saturable. Third, the cellular transport of L-DOPA was nearly abolished at 4°C, demonstrating that the uptake was energy dependent. The efficiency of this L-DOPA transport in Caco-2 cells can also be evidenced by the ratio of L-DOPA concentration in cellular water to medium concentration. The intracellular L-DOPA concentration at equilibrium was higher than that which could be expected by passive equilibration of L-DOPA. At steady state of L-DOPA uptake the mean intracellular concentration of L-DOPA was 50 times higher than the L-DOPA concentration in the incubation medium. Finally, the finding that d-DOPA transport was nonsaturable indicates that the uptake of L-DOPA is stereoselective, in agreement with previous evidence on the uptake of L-DOPA obtained in other epithelia (14, 20–22, 27).

When nonsaturating concentrations (0.5 µM) of L-DOPA are used in time course experiments, the result is a rapid accumulation of the substrate until equilibrium is reached, at ~30 min of incubation. Under these experimental conditions and with 1 mg of cell protein, Caco-2 cells cleared through the L-DOPA transporter ~8.5 ± 0.4 µl·mg protein⁻¹·min⁻¹ of incubation medium containing 0.5 µmol/l L-DOPA (~4.2 ± 0.2 pmol·mg protein⁻¹·min⁻¹). The clearance values were markedly reduced to 4.4 ± 0.2 and 0.7 ± 0.1 µl·mg protein⁻¹·min⁻¹ when half-saturating and nearly saturating concentrations of L-DOPA, 50 and 500 µM, respectively, were used. Under similar experimental conditions we were able to demonstrate that OK and LLC-PK₁ cells take up 36 and 7 µl·mg protein⁻¹·min⁻¹, respectively, of a solution containing 0.5 µmol/l L-DOPA (23, 27). Other authors (17, 24) used the same approach to study the uptake of different substrates and found that the L-arginine and glucose transport systems in OK cells clear ~130 and 5.9 µl/min incubation medium of L-arginine and α-methylglucosidase, respectively. On the other hand, Amax values for L-DOPA in Caco-2 cells (20.4 ± 0.3) were similar to those described for LLC-PK₁ cells (15.9 ± 0.9) but lower than those for OK cells (75.0 ± 5.0) (23, 27). Therefore, it can be suggested that the L-DOPA transporter in Caco-2 cells is quite efficient and the clearance values are within the range observed for other substrates and the same substrate (L-DOPA) in other cell types. This, however, does indicate that L-DOPA is neither free in cellular water nor distributed to a single compartment. This type of data gives only an indication concerning the kinetic characteristics of the transporter but is unable to provide information related to the intracellular distribution of the substrate or even its compartmentalization. Intracellular concentration of L-DOPA of 10 µM at a medium substrate concentration of 0.5 µM assumes that L-DOPA is free in intracellular water and is, therefore, a mean value of substrate concentration inside the cell. If we hypothesize that intracellular L-DOPA is unequally distributed, then this value can vary (even higher or substantially lower) according to the degree of compartmentalization of the substrate. The possibility that intracellular L-DOPA may be subjected to some sort of compartmentalization is discussed below.

The accumulation of L-DOPA in experiments performed in cells cultured in polycarbonate filters differed markedly from that observed in cells cultured in collagen-treated plastic. This was particularly evident for the apical application of the substrate, the main difference being that Kₘ values for L-DOPA were approximately eight times higher in cells cultured in polycarbonate filters than in cells cultured in plastic cells grown in collagen-treated plastic also accumulated more L-DOPA. The most likely explanation for this discrepancy concerns the possibility that large quantities of L-DOPA can be easily extruded from the cell at the basolateral cell side; a fully functional basolateral side provides an easy way to extrude intracellular L-DOPA (much more important than extrusion at the apical cell border), thereby reducing the saturability of the system. In fact, the outward transfer of intracellular L-DOPA through the basolateral cell border was markedly greater than that observed through the apical side, although at both cell sides the outward transfer was a nonsaturable process. By contrast, the apical and basal uptakes of L-DOPA were found to be saturable pro-
cesses with similar $K_m$ values ($482 \pm 118$ and $601 \pm 67$ µM), but $V_{\text{max}}$ values were substantially greater for the basal than for the apical application ($78.1 \pm 5.4$ and $14.6 \pm 2.1$ nM·mg protein$^{-1}$·min$^{-1}$). Taken together these data suggest that inward and outward transfers of L-DOPA are quantitatively more important at the basolateral than at the apical cell border. This is in agreement with the data of Hidalgo and Brochardt (7), who used phenylalanine, a large neutral amino acid, as the substrate. Obviously, this would favor the accumulation of L-DOPA at the basal pole of the cell, even with the assumption that there are no intracellular stores for the substrate. Time course experiments in cells cultured in polycarbonate filters also show that intracellular L-DOPA is greater when a nonsaturating concentration of the substrate (0.5 µM) is applied from the basolateral side than from the apical side.

Another crucial step in the whole process of dopamine formation is the decarboxylation of intracellular L-DOPA. Experiments conducted in cell homogenates showed that Caco-2 cells are endowed with a high AADC activity and the efficiency of decarboxylation process, as indicated by $K_m$ values, is quite similar to that observed for the rat renal and jejunal epithelial cells (25). When cell monolayers were loaded with L-DOPA, in the absence of benserazide, Caco-2 cells also synthesized dopamine. The formation of dopamine was a time- and concentration-dependent process and rapidly saturated, with an apparent $K_m$ of 27 µM; most of the L-DOPA taken up was, in fact, decarboxylated to dopamine. Again it is interesting to observe (Table 1) that L-DOPA applied from the basolateral side is considerably more decarboxylated than that applied from the apical side. This would fit the hypothesis that L-DOPA uptake at the basolateral pole is quantitatively more important than that at the apical cell border, as discussed above. Because AADC is a cytosolic enzyme, these cells are believed to synthesize dopamine as a result of the availability of L-DOPA in the cytosol.

The intracellular fate of newly formed dopamine is another interesting point to discuss. In the first set of experiments in cells cultured in collagen-treated plastic, the outward transfer of newly formed dopamine through the apical cell border was found to be a diffusional process. On the other hand, in polycarbonate-cultured cells loaded with L-DOPA from the basolateral cell border, the apical outward transfer of newly formed dopamine was found to be a saturable process. The magnitude of the outward transfer of newly formed dopamine differed markedly depending on the technique used, being considerably greater in cells cultured on plastic. In polycarbonate-cultured cells the outward transfer of newly formed dopamine through the basolateral cell border (evaluated in cells loaded with L-DOPA from the apical side) was 5–10 times greater than that through the apical cell border (evaluated in cells loaded with L-DOPA from the basal side) and did not depend on the intracellular concentration of dopamine. This, again, shows that the basolateral cell border in Caco-2 cells is quite permeable and may constitute an important route for intracellular molecules to leave this compartment. It is possible that the presence of a fully functional basolateral cell border may explain, as well, the discrepant outward transfer of dopamine through the apical cell border observed in Caco-2 cells cultured on plastic and polycarbonate filters. Although the present study was intended to define the nature of the apical outward dopamine transfer, the finding that this is a process with a $K_m$ of 623 ± 238 µM suggests that this does not correspond to the dopamine transporter found in neuronal and nonneuronal cells.

In conclusion, the data presented here show that Caco-2 cells are endowed with an efficient L-DOPA uptake system and considerable AADC activity. Intracellular L-DOPA was found to be rapidly converted to dopamine, some of which diffuses out of the cell. The utilization of Caco-2 cells cultured on polycarbonate filters probably provides a better way to look at processes such as the outward transfer of intracellular molecules, namely, the outward transfer of newly formed dopamine. Our observations also support the use of Caco-2 cells as in vitro models for the study of the intestinal dopaminergic physiology, although this may not reflect what happens in the intact tissue.

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REFERENCES


