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Na^+-Independent Transporters, LAT-2 and $b^{0,+}$, Exchange L-DOPA with Neutral and Basic Amino Acids in Two Clonal Renal Cell Lines

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Abstract. The present study examined the functional characteristics of L-DOPA transporters in two functionally different clonal subpopulations of opossum kidney (OK_{LC} and OK_{HC}) cells. The uptake of L-DOPA was largely Na^+-independent, though in OK_{HC} cells a minor component (~15%) required extracellular Na^+. At least two Na^+-independent transporters appear to be involved in L-DOPA uptake. One of these transporters has a broad specificity for small and large neutral amino acids, is stimulated by acid pH and inhibited by 2-aminobicyclo(2,2,l)-heptane-2-carboxylic acid (BCH; OK_{LC}, K_i = 291 μM; OK_{HC}, K_i = 380 μM). The other Na^+-independent transporter binds neutral and basic amino acids and also recognizes the di-amino acid cystine. [^{14}C]-L-DOPA efflux from OK_{LC} and OK_{HC} cells over 12 min corresponded to a small amount of intracellular [^{14}C]-L-DOPA. L-Leucine, nonlabelled L-DOPA, BCH and L-arginine, stimulated the efflux of [^{14}C]-L-DOPA in a Na^+-independent manner. It is suggested that L-DOPA uses at least two major transporters, systems LAT-2 and $b^{0,+}$. The transport of L-DOPA by LAT-2 corresponds to a Na^+-independent transporter with a broad specificity for small and large neutral amino acids, stimulated by acid pH and inhibited by BCH. The transport of L-DOPA by system $b^{0,+}$ is a Na^+-independent transporter for neutral and basic amino acids that also recognizes cystine. LAT-2 was found equally important at the apical and basolateral membranes, whereas system $b^{0,+}$ had a predominant distribution in apical membranes.

Key words: L-DOPA — OK cells — LAT-2 — System $b^{0,+}$ — Sodium — pH dependence

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Introduction

The renal dopaminergic system is a local non-neuronal system constituted by epithelial cells of proximal convoluted renal tubules rich in aromatic L-amino acid decarboxylase (AADC) activity and using circulating or filtered L-DOPA as a source for dopamine (Jose et al., 1992; Lee, 1993; Soares-da-Silva, 1994). Because the dopamine produced in this area is in close proximity to renal cells that contain receptors for the amine, it has been hypothesized that the amine may act as a paracrine or autocrine substance (Siragy et al., 1989). In order to overcome technical problems related to the handling of freshly isolated renal tubular epithelial cells, opossum kidney (OK) cells, which express proximal tubule cell-like properties in vitro (Koyama et al., 1978), have been used to study dopamine receptors and the renal actions of the amine. These cells have been also shown to take up (Vieira-Coelho & Soares-da-Silva, 1997) and convert intracellular L-DOPA to dopamine in a saturable manner (Soares-da-Silva, Vieira-Coelho & Serrão, 1997). Newly-formed dopamine also stimulated cAMP accumulation in OK cells (Cheng et al., 1990) and inhibited Na^+-phosphate cotransport, both of which were attenuated by carbidopa or benserazide and blocked by D_1-like receptor antagonists (Glahn et al., 1993; Perrichot et al., 1995). It appears, therefore, that in OK cells, as in epithelial cells of proximal tubules, locally formed dopamine can act as an autocrine/paracrine substance. Furthermore, the amounts of the enzymes aromatic L-amino acid decarboxylase, catechol-O-methyltransferase and monoamine oxidase found in this cell line are likely to be sufficient to reproduce, under in vitro conditions, the environment in which the renal dopaminergic system normally operates (Guimarães et al., 1997).

Although the kidney is endowed with one of the highest levels of AADC in the body and plasma levels of L-DOPA are in the nmol/ml range (Grossman et al., 1992; Soares-da-Silva et al., 1995), the rate-limiting step for the synthesis of dopamine in renal tissues is still a matter of debate. However, since K_m values for L-DOPA uptake are 10 times lower than K_m values for decarboxylation of L-DOPA, it could be possible that L-DOPA uptake rather than decarboxylation, may limit the rate of formation of dopamine. In a previous report we have concluded that OK cells take up L-DOPA through a saturable, stereoselective and temperature-dependent process when applied from the apical and basolateral cell border (Soares-da-Silva et al., 1997; Vieira-Coelho & Soares-da-Silva, 1997), this being similar to that occurring in rat renal proximal tubules (Pinto-do-Ó & Soares-da-Silva, 1996; Soares-da-Silva, Fernandes & Pinto-do-Ó, 1994). However, the transporters involved in uptake of L-DOPA by renal epithelial cells have not been identified. At present, candidate transport systems for L-DOPA may include the Na^+-dependent systems B, $B^{0,+}$ and y^+L, and the Na^+-independent systems L (LAT-1 and LAT-2) and $b^{0,+}$. Recently, both $b^{0,+}$ and LAT-1 were found to transport L-DOPA, the former in *Xenopus laevis* oocytes injected with poly A^+RNA prepared from rabbit intestinal epithelium (Ishii et al., 2000) and the latter in mouse brain capillary endothelial cells (Kageyama et al., 2000). The major involvement of Na^+-independent systems LAT-1 and $b^{0,+}$ contrast with that expected to occur at the kidney level. In fact, Na^+ is a powerful stimulus for the production of renal dopamine (Lee, 1993) and L-DOPA uptake in human and rat kidney slices is a Na^+-dependent and ouabain-sensitive process (Soares-da-Silva & Fernandes, 1992; Soares-da-Silva, Pestana & Fernandes, 1993).

The present study examined the functional characteristics and regulation of the L-DOPA transport in two functionally different clonal subpopulations of opossum kidney (OK) cells, OK_{LC} and OK_{HC} cells. These cells derive from the same original batch obtained from the American Type Culture Collection (F-12476) and are morphologically identical, but differ markedly in their ability to transport Na^+ (Gomes & Soares-da-Silva, 2000). To define the Na^+-sensitivity of the transporters involved in the uptake of L-DOPA, the effect of maneuvers that affect cellular Na^+ and H^+ gradients and the sensitivity to inhibitors of amino acid transport and Na^+ were examined. Thereafter, in order to have an insight on the molecular mechanisms governing L-DOPA uptake, the result of maneuvers that interfere with protein kinase A (PKA), protein kinase G (PKG), protein kinase C (PKC), protein tyrosine kinase (PTK) and Ca^{2+}-calmodulin mediated pathways were evaluated.

Materials and Methods

Cell Culture

OK cells (ATCC 1840-HTB) 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. OK_{LC} (passages 49 to 81) and OK_{HC} (passages 62 to 95) cells were grown in Minimum Essential Medium (Sigma, St. Louis, MO), supplemented with 100 U/ml penicillin G, 0,25 μg/ml amphotericin B, 100 μg/ml streptomycin (Sigma), 10% foetal bovine serum (Sigma) and 25 mM N-2-hydroxyethylpiperazine-N′-2-ethanosulfonic acid (HEPES; Sigma).

For subculturing, the cells were dissociated with 0.05% trypsin-EDTA, split 1:5 and subcultured in Costar flasks with 75- or 162-cm^2 growth areas (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 per well or onto collagen treated 0.2 μm polycarbonate filter supports (internal diameter 12 mm, Transwell, Costar) at a density of 13,000 cells per well. The cell medium was changed every 2 days, and the cells reached confluence after 3–5 days of incubation. For 24 hours prior to each experiment, the cell medium was free of fetal bovine serum. Experiments were generally performed 2–3 days after cells reached confluence and 6–8 days after the initial seeding and each cm^2 contained about 80–100 μg of cell protein.

Influx of L-DOPA

On the day of the experiment, the growth medium was aspirated and the cells were washed with Hanks' medium; thereafter, the cell monolayers were preincubated for 15 or 30 min in Hanks' medium (2 ml) 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 (50 μM) and tolcapone (1 μM) in order to inhibit the enzymes AADC and catechol-*O*-methyltransferase, respectively. Time-course studies were performed in experiments in which cells were incubated with 1 μM substrate for 1, 3, 6, 12, 30 and 60 min. Saturation experiments were performed in cells preincubated for 15 min and then incubated for 6 min with increasing concentrations of L-DOPA (10 to 1000 μM). In experiments performed in the presence of different concentrations of Na^+, NaCl was replaced by an equimolar concentration of choline chloride. Test substances were applied from the apical side only. Competing amino acids were present during the incubation (6 min) with L-DOPA. Modulators of PKA, PKC, PKG, PTK and Ca^{2+}-calmodulin were present during the preincubation (30 min) and incubation (6 min) periods. During preincubation and incubation, the cells were continuously shaken and maintained at 37°C. Apical uptake was initiated by the addition of 2 ml Hanks' medium with a given concentration of the substrate. 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 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.

Assay of L-DOPA

L-DOPA was quantified by means of high pressure liquid chromatography with electrochemical detection, as previously reported

(Soares-da-Silva & Serrão, 2000b). The high pressure liquid chromatography system 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^{-1}. 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.

Efflux of [^{14}C]-L-DOPA

In experiments aimed to evaluate the apical efflux of intracellular L-DOPA, cells cultured in plastic were incubated (0.5 ml) with 2.5 μM [^{14}C]-L-DOPA for 6 min, as described above. Incubation was then terminated on ice, the incubation medium aspirated and the cells were washed with 2 ml ice-cold Hanks' medium. Thereafter, 1 ml Hanks' medium was added to the cells and they were returned to the incubation bath at 37°C to monitor efflux of intracellular [^{14}C]-L-DOPA over the next 12 min, in the absence or the presence of different amino acids. At the end of the efflux collection period, the medium was rapidly removed and 250 μl of 0.1% v/v Triton X-100 (dissolved in 5 mM Tris.HCl, pH 7.4) was added to the cells. Radioactivity in efflux samples and in the cells was measured by liquid scintillation counting.

In experiments designed to study the efflux of L-DOPA through the apical and basal cell sides, cells cultured in polycarbonate filters were loaded for 6 min with 2.5 μM [^{14}C]-L-DOPA applied from both cell sides. At the end of incubation, cells were placed on ice and washed with ice-cold Hanks' medium. For the measurement of apical and basal [^{14}C]-L-DOPA efflux, the cells were incubated for 12 min with fresh Hanks' medium at 37°C in the absence and the presence of different amino acids. The medium in the apical or basal side was collected after incubation for the specified period of time, and the radioactivity was counted. The monolayers were continuously agitated during transport measurement. At the end of the efflux experiment, the medium was immediately aspirated and the cells were solubilized by 0.1% v/v Triton X-100 (dissolved in 5 mM Tris-HCl, pH 7.4). Radioactivity was measured by liquid scintillation counting.

Na^+/H^+ Exchanger Activity

Na^+/H^+ exchanger activity was assayed as the initial rate of pH_i recovery after an acid load imposed by 10 mM NH_4Cl followed by removal of Na^+ from the Krebs modified buffer solution, in the absence of CO_2/HCO_3 (Gomes, Vieira-Coelho & Soares-Da-Silva, 2001). In these experiments, NaCl was replaced by an equimolar concentration of tetramethylammonium chloride (TMA). Test compounds were added to the extracellular fluid during the acidification and Na^+-dependent pH_i recovery periods.

Measurement of Intracellular pH

In intracellular pH (pH_i) measurement experiments, OK cells were grown in 10-mm width collagen-coated glass coverslips. Intracellular pH was measured as previously described (Gomes et al., 2001). At days 6–8 after seeding, the glass coverslips were incubated at 37°C for 40 min with 5 μM of the acetoxymethyl ester of 2′,7′-bis(carboxyethyl)-5(6)-carboxyfluorescein (BCECF-AM). Coverslips were then washed twice with prewarmed dye-free modified Krebs buffer before initiation of the fluorescence recordings. The Krebs medium had the following composition (mM): NaCl 140, KCl 5.4, $CaCl_2$ 2.8, $MgSO_4$ 1.2, NaH_2PO_4 0.3, KH_2PO_4 0.3, HEPES 10, glucose 5, pH = 7.4 (adjusted with Tris base). Cells were mounted diagonally in a 1 × 1-cm acrylic fluorometric cuvette that was inserted in a Perkin-Elmer cuvette holder (model LS 50), and subsequently placed in the sample compartment of a FluoroMax-2 spectrofluorometer (Jobin Yvon-SPEX, Edison, NJ). The cuvette volume of 3.0 ml was constantly stirred and perfused at 5.0 ml min^{-1} with modified Krebs buffer prewarmed to 37°C. Under these conditions, the cuvette medium was replaced within 150 sec. After 5 min, fluorescence was measured every 5 sec alternating between 440 and 490 nm excitation (1-nm slit size) at 525 nm emission (3-nm slit size). The ratio of intracellular BCECF fluorescence at 490 and 440 nm was converted to intracellular pH values by comparison with values from an intracellular calibration curve using the nigericin (10 μM) and high-K^+ method (Thomas et al., 1979).

Na^+-K^+-ATPase Activity

Na^+-K^+ ATPase activity in OK cells was measured by the method of Quigley and Gotterer (1969) with minor modifications. Briefly, OK cells in suspension were permeabilized by rapid freezing in dry ice-acetone and thawing. The reaction was initiated by the addition of 4 mM ATP. For determination of ouabain-sensitive ATPase, NaCl and KCl were omitted, and Tris-HCl (150 mM) and ouabain (100 μM) were added to the assay. After incubation at 37°C for 15 min, the reaction was terminated by the addition of 50 μl of ice-cold trichloroacetic acid. Samples were centrifuged (3,000 rpm), and liberated Pi in supernatant was measured by spectrophotometry at 740 nm. Na^+-K^+ ATPase activity is expressed as nanomoles Pi per milligram protein per minute and determined as the difference between total and ouabain-insensitive ATPase. The protein content in cell suspension (approximately 2 mg/ml) was similar in all samples.

Protein Assay

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

Cell Viability

Cells cultured in collagen-treated plastic supports were preincubated for 15 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.

Data Analysis

The analysis of the time course of L-DOPA uptake in OK_{LC} and OK_{HC} cells was based on a one-compartment model (Vieira-

Coelho & Soares-da-Silva, 1997; Vieira-Coelho & Soares-da-Silva, 1998). The parameters of the equation

$$C_i/C_o = k_{in}/k_{out}(1 - e^{-k_{out}.t})$$

were fitted to the experimental data by a nonlinear regression analysis using a computed assisted method (Motulsky, 1999). C_i and C_o represent the intracellular and extracellular concentration of the substrate, respectively. k_{in} is the rate constant for inward transport, k_{out} the rate constant for outward transport and t the incubation time. k_{in} and k_{out} are given in pmol/mg protein/min. A_{max} is defined as the factor of accumulation (C_i/C_o) at equilibrium ($t \rightarrow \infty$). K_m and V_{max} values for the uptake of L-DOPA, as determined in saturation experiments, were calculated from nonlinear regression analysis using the GraphPad Prism statistics software package (Motulsky, 1999). K_i's were calculated for competitive inhibition as previously described (Cheng & Prusoff, 1973) by means of nonlinear regression analysis (Motulsky, 1999). Arithmetic means are given with SEM. Statistical analysis was performed by one-way analysis of variance (ANOVA) followed by Newman-Keuls test for multiple comparisons. A P value less than 0.05 was assumed to denote a significant difference.

Reagents

2-Aminobicyclo(2,2,l)-heptane-2-carboxylic acid (BCH), amphotericin B, benserazide, calmidazolium, chelerythrine chloride, cholera toxin, cyclic AMP sodium salt, cyclic GMP sodium salt, forskolin, genistein, genistin, isobutylmethylxanthine, L-3,4-dihydroxyphenylalanine (L-DOPA), N-(methylamino)-isobutyric acid (MeAIB), ouabain, phorbol 12,13-dibutyrate (PDBu), phorbol 12-myristate 13-acetate (PMA), 4α-phorbol 12,13-didecanoate (PDDC), sodium nitroprusside, trifluoperazine dihydrochloride, trypan blue, tyrphostin 1 and tyrphostin 25 were purchased from Sigma. LY 83583 (6-(phenylamino)-5,8-quinolinedione) and zaprinast were obtained from Research Biochemicals International (Natick, USA) and May & Backer (Dorset, England), respectively. Acetoxymethyl ester of 2′,7′-bis(carboxyethyl)-5(6)-carboxyfluorescein (BCECF-AM) and nigericin were obtained from Molecular Probes (Eugene, OR). [^{14}C]-L-DOPA, specific activity 51 mCi/mmol was purchased from Amersham Pharmacia Biotech (Little Chalfont, UK). Tolcapone was kindly donated by late Professor Mosé Da Prada (Hoffman La Roche, Basel, Switzerland).

Results

The ability of OK_{LC} and OK_{HC} cells to transport Na^+ from the apical to the basolateral cell side was assessed by measuring the activities of the apical Na^+/H^+ exchanger and the basolateral Na^+-K^+ ATPase. Na^+/H^+ exchanger activity was assayed as the initial rate of pH_i recovery measured after an acid load imposed by 10 mM NH_4Cl followed by removal of Na^+ from the Krebs modified buffer solution, in the absence of CO_2/HCO_3. The pH_i recovery rates during the linear phase of pH recovery after intracellular acidification in OK_{LC} cells were 63% lower ($P < 0.05$) than in OK_{HC} cells (Table 1). Basal Na^+-K^+ ATPase activity in OK_{LC} cells was also 48% lower ($P < 0.05$) than in OK_{HC} cells (Table 1).

Table 1. Apical Na^+/H^+ exchanger and basolateral Na^+-K^+ ATPase activities in OK_{LC} and OK_{HC} cells

Cell	Na^+/H^- Exchanger (pH units/min)	Na^+-K^+ ATPase (nmol Pi/mg protein/min)
OK_{LC}	0.094 ± 0.011	30.0 ± 0.1
OK_{HC}	0.254 ± 0.016*	57.6 ± 5.6*

Values are means ± SEM of 5 experiments per group. Significantly different from OK_{LC} cells (*$P < 0.05$).

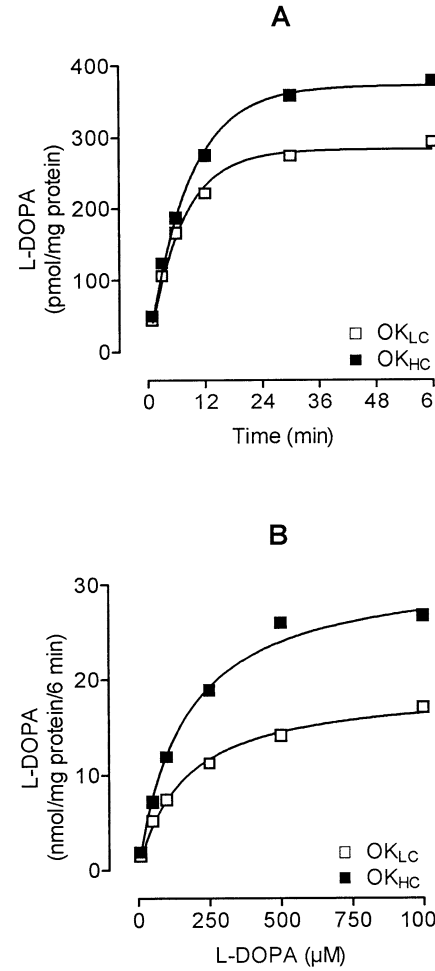

Fig. 1. (*A*) Time course and (*B*) concentration-dependent uptake of L-DOPA in OK_{LC} and OK_{HC} cells. In time-course studies, cells were incubated at 37°C with 1 μM L-DOPA applied from the apical cell border, whereas in saturation experiments increasing concentrations (10 to 1000 μM) of the substrate were added to the apical side for 6 min. Each point represents the mean of four experiments per group; SEM values were smaller than the size of the symbol.

To determine initial rates of uptake, OK_{LC} and OK_{HC} cells were incubated with a non-saturating (1 μM) concentration of L-DOPA for 1, 3, 6, 12, 30 and 60 min. In both types of cells uptake of a non-saturating concentration of L-DOPA (1 μM) was linear with time for up to 6 min of incubation (Fig. 1*A*); equilibrium was attained at 30 min of incubation.

Table 2. Apparent kinetic parameters for the uptake of L-DOPA in OK_{LC} and OK_{HC} cells

	OK_{LC}	OK_{HC}
k_{in} (pmol/mg protein/min)	20.1 ± 0.4	22.1 ± 0.6
k_{out} (pmol/mg protein/min)	5.7 ± 0.1	6.3 ± 0.2
A_{max}	40.5 ± 0.4	53.2 ± 0.1*
K_m(μM)	160 ± 20	162 ± 23
V_{max} (nmol/mg protein/6 min)	19.1 ± 0.8	31.7 ± 1.3*

Values are means ± SEM of 5–10 experiments per group.
*Significantly different from OK_{LC} cells ($P < 0.05$).

From the initial rate of uptake, k_{in}, k_{out} and an equilibrium factor of accumulation (A_{max}) were calculated. The analysis revealed similar constant rates of inward (k_{in}) and outward (k_{out}) transport for OK_{LC} and OK_{HC} cells. However, the equilibrium factor (A_{max}) in OK_{LC} cells was 24% lower ($P < 0.05$) than that in OK_{HC} cells (Table 2). At 6 min when uptake was linear and considering intracellular water as 7.0 μl/mg protein (Vieira-Coelho & Soares-da-Silva, 1997), the intracellular L-DOPA concentration was 23.6 ± 0.4 μM and 26.7 ± 0.6 μM for OK_{LC} and OK_{HC} cells, respectively, at medium concentration of 1 μM. This represented a cell concentration of L-DOPA that was 24 to 27 times higher than the corresponding medium concentration. In a subsequent set of experiments, designed to determine the apparent kinetics of the L-DOPA transporter(s), cells were incubated for 6 min with increasing concentrations (10 to 1000 μM) of the substrate (Fig. 1*B*). In both types of cells the accumulation of L-DOPA was found to be dependent on the concentration and saturable (Fig. 1*B*). However, V_{max} values in OK_{LC} cells were 40% lower ($P < 0.05$) than those in OK_{HC} cells. Apparent kinetic parameters of L-DOPA uptake (K_m and V_{max}) were determined by nonlinear analysis of the specific analysis of the saturation curves for L-DOPA and are given in Table 2.

In experiments designed to define the functional characteristics of the transporter and its molecular regulation, uptake was studied at non-saturating concentrations of L-DOPA (25 μM), which at 4°C was less than 5% of that occurring at 37°C (Table 3). Firstly, we examined the Na^+ requirements for L-DOPA uptake and then its sensitivity to inhibitors of amino acid transporters. Reducing extracellular Na^+ (from 140 mM to 0 mM) produced a slight, but significant ($P < 0.05$), reduction in the accumulation of L-DOPA in OK_{HC} cells only. Maneuvers that affect transepithelial flux of sodium, such as the addition of amphotericin B (2.5 μg/ml), amiloride (100 and 500 μM) or ouabain (100 μM), also produced slight, but significant ($P < 0.05$), reductions in the accumulation of L-DOPA (Table 3). These effects were particularly more evident in OK_{HC} than in OK_{LC} cells.

Table 3. Effect of maneuvers that affect energy-dependent process, transepithelial flux of Na^+ ions protons on the uptake of L-DOPA (25 μM)

Maneuver		OK_{LC}	OK_{HC}
Temperature	37°C	100 ± 3	100 ± 5
	4°C	3 ± 0*	2 ± 0*
Na^+	Control	100 ± 2	100 ± 1
	Na^+ 0 mM	99 ± 2	86 ± 1*
	Amphotericin B (2.5 μg/ml)	87 ± 3*	66 ± 2*
	Amiloride(100 μM)	119 ± 3*	95 ± 1*
	Amiloride (500 μM)	90 ± 1*	82 ± 2*
	Ouabain (100 μM)	105 ± 2	87 ± 1*

Values are percent of control for accumulation of L-DOPA in OK_{LC} and OK_{HC} cells (absolute levels, in pmol/mg protein, were: OK_{LC}, 2906 ± 84, $n = 22$; OK_{HC}, 3569 ± 127, $n = 31$).
Values are means ± SEM of 4 to 8 determinations per group.
*Significantly different from corresponding control values ($P < 0.05$) using the Newman-Keuls test for multiple comparisons.

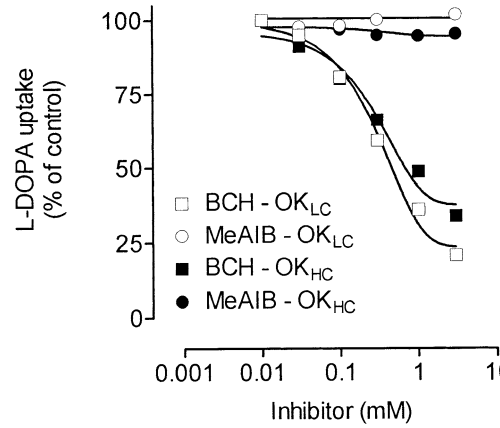

Fig. 2. Effect of increasing concentrations of BCH and MeAIB on the uptake of L-DOPA in OK_{LC} and OK_{HC} cells. Cells were incubated for 6 min at 37°C with 25 μM L-DOPA added from the apical cell border. Values are percent of control for accumulation of L-DOPA in OK_{LC} and OK_{HC} cells (absolute levels, in pmol/mg protein, were: OK_{LC}, 2567 ± 24, $n = 8$; OK_{HC} 2732 ± 51, $n = 7$). Symbols represent means of 7 to 8 experiments per group, SEM values were smaller than the size of the symbol.

As shown in Fig. 2, N-(methylamino)-isobutyric acid (MeAIB) failed to affect the uptake of L-DOPA, whereas 2-aminobicyclo(2,2,1)-heptane-2-carboxylic acid (BCH) produced a concentration-dependent inhibition of L-DOPA uptake in both cell types (geometric means [95% confidence intervals]; OK_{LC}, $K_i = 291$ [199, 425] μM; OK_{HC}, $K_i = 380$ [164, 893] μM). Taken together, these results suggest that the influx of L-DOPA in OK_{LC} and OK_{HC} cells may be largely promoted through the BCH-sensitive and Na^+-independent L-type amino-acid transporter (Christensen, 1990). Another functional characteristic

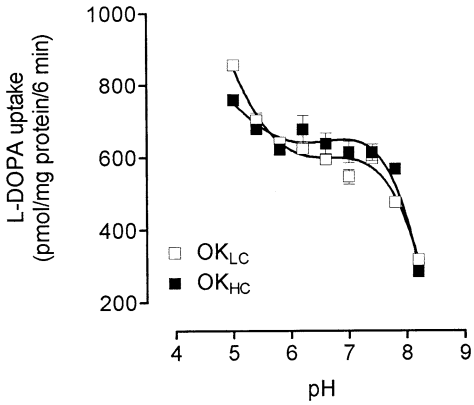

Fig. 3. Effect of extracellular pH on the accumulation of L-DOPA (25 μM) in OK_{LC} and OK_{HC} cells. Symbols represent the mean of 8 experiments per group; vertical lines show SEM.

of the mammalian system L in various cell types and tissues concerns its dependency on extracellular pH (Christensen, 1990). In fact, as shown in Fig. 3, the accumulation of L-DOPA in OK_{LC} and OK_{HC} cells was higher ($P < 0.05$) at an acidic pH. On the other hand, incubation of cells with L-DOPA (100 μM) was accompanied by a marked intracellular acidification (Fig. 4). The L-DOPA-induced rate of intracellular acidification in OK_{LC} cells was lower ($P < 0.05$) than that in OK_{HC} cells (Table 4). BCH (1 mM) attenuated the L-DOPA-induced intracellular acidification, though more markedly in OK_{HC} cells than in OK_{LC} cells (Table 4).

In order to confirm the view that the L-type system was a major transporter for L-DOPA in OK cells, the next series of experiments examined the effect of a wide range of L- and D-amino acids. As shown in Figs. 5 and 6, L-DOPA uptake was inhibited by L-isomers of small zwitterionic amino acids (alanine, serine, threonine and cysteine), L- and D-isomers of neutral amino acids (leucine, isoleucine and phenylalanine) and by L-glutamine and L-asparagine. L-Proline, the acidic amino acids (L-aspartate and L-glutamate) were not effective in reducing L-DOPA uptake. However, the basic amino acids (L-arginine and L-lysine) and L-cystine were also found to markedly inhibit L-DOPA accumulation. Basic amino acids are essentially transported by systems y^+, y^+L, b^+, $b^{0,+}$, and $B^{0,+}$ (for a review *see* Palacin et al., 1998). Systems y^+ (Christensen & Antonioli, 1969; White, Gazzola & Christensen, 1982), y^+L (Deves, Chavez & Boyd, 1992), and $b^{0,+}$ (Van Winkle, Campione & Gorman, 1988) catalyze high-affinity (K_m in the μM range) Na^+-independent transport of cationic amino acids. The transport of small and large zwitterionic amino acids by system $b^{0,+}$ is Na^+-independent (Van Winkle et al., 1988), whereas that promoted by systems y^+ and y^+L is Na^+-dependent (Angelo & Deves, 1994; Chillaron et al., 1996). Distinctive properties between systems y^+ and y^+L include insensitivity of the latter to N-ethylmaleimide (NEM) (Deves, Angelo & Chavez, 1993). System b^+ shows the narrowest specificity serving only for cationic amino acids (Van Winkle & Campione, 1990). System $B^{0,+}$ and $b^{0,+}$ show very similar broad specificity with high affinity for basic and small and large zwitterionic amino acids (Van Winkle et al., 1988; Van Winkle, Christensen & Campione, 1985). As a distinguishing feature, system $b^{0,+}$ is Na^+-independent and insensitive to BCH (Mora et al., 1996). As shown in Fig. 7, L-arginine produced a concentration-dependent inhibition of L-DOPA of similar magnitude in both OK_{LC} and OK_{HC} cells. The maximal inhibitory effect of L-arginine was attained at 1 mM and was not greater than 50% inhibition. On the other hand, inhibition of L-DOPA accumulation in both OK_{LC} and OK_{HC} cells by 1 mM basic amino acids (L-arginine and L-lysine) and L-cystine was markedly potentiated by 1 mM BCH. On the other hand, NEM (200 μM) failed to inhibit L-DOPA (25 μM) accumulation in OK cells (OK_{LC}, in pmol/mg protein: control = 3869 ± 26, $n = 4$; NEM = 3825 ± 104, $n = 4$; OK_{HC}: control = 4474 ± 46, $n = 4$; NEM = 4512 ± 53, $n = 4$). All the experiments mentioned above were performed in the presence of 140 mM Na^+.

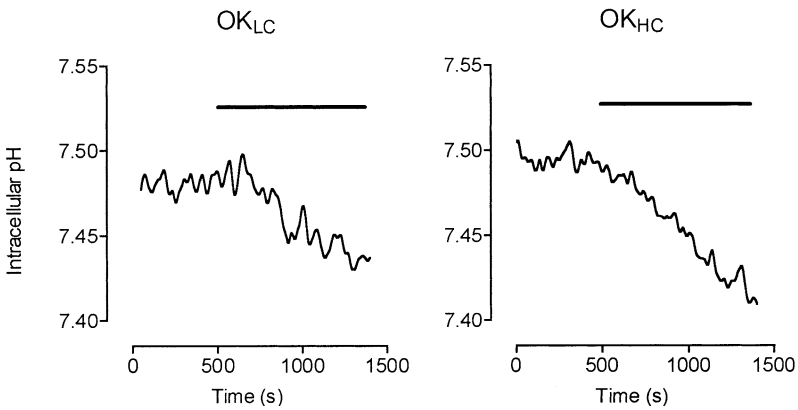

Fig. 4. Effect of of 100 μM L-DOPA (solid bar) upon steady-state intracellular pH measured in monolayers of OK_{LC} and OK_{HC} cells loaded with the pH-sensitive fluorophore BCECF. Traces are the mean of 5 to 6 independent experiments.

Table 4. Slopes of intracellular acidification [(pH units/sec) × 10^3] induced by apical Na^+ removal, L-DOPA (100 μM) and L-DOPA (100 μM) plus BCH (1 mM), in OK_{LC} and OK_{HC} cells

	L-DOPA	L-DOPA + BCH
OK_{LC}	−0.066 ± 0.011	−0.049 ± 0.005
OK_{LC}	−0.106 ± 0.011[a]	−0.058 ± 0.007[b]

Values are means ± SEM of 5 to 7 determinations per group. Significantly different ($P < 0.05$) from values in [a]OK_{LC} cells or [b]corresponding control values using the Newman-Keuls test for multiple comparisons.

Because some amino-acid transporters have been shown to function as amino-acid exchangers, a new series of experiments was conducted in cells loaded with [^{14}C]-L-DOPA and then the corresponding efflux was monitored over 12 min, in the absence and the presence of different amino acids and unlabelled L-DOPA. As shown in Fig. 8, the efflux of [^{14}C]-L-DOPA from OK_{LC} and OK_{HC} cells over 12 min corresponded to a small amount of [^{14}C]-L-DOPA accumulated in the cells, i.e., both cell types were able to retain most of the taken-up substrate. When the cells were incubated with L-leucine or unlabelled L-

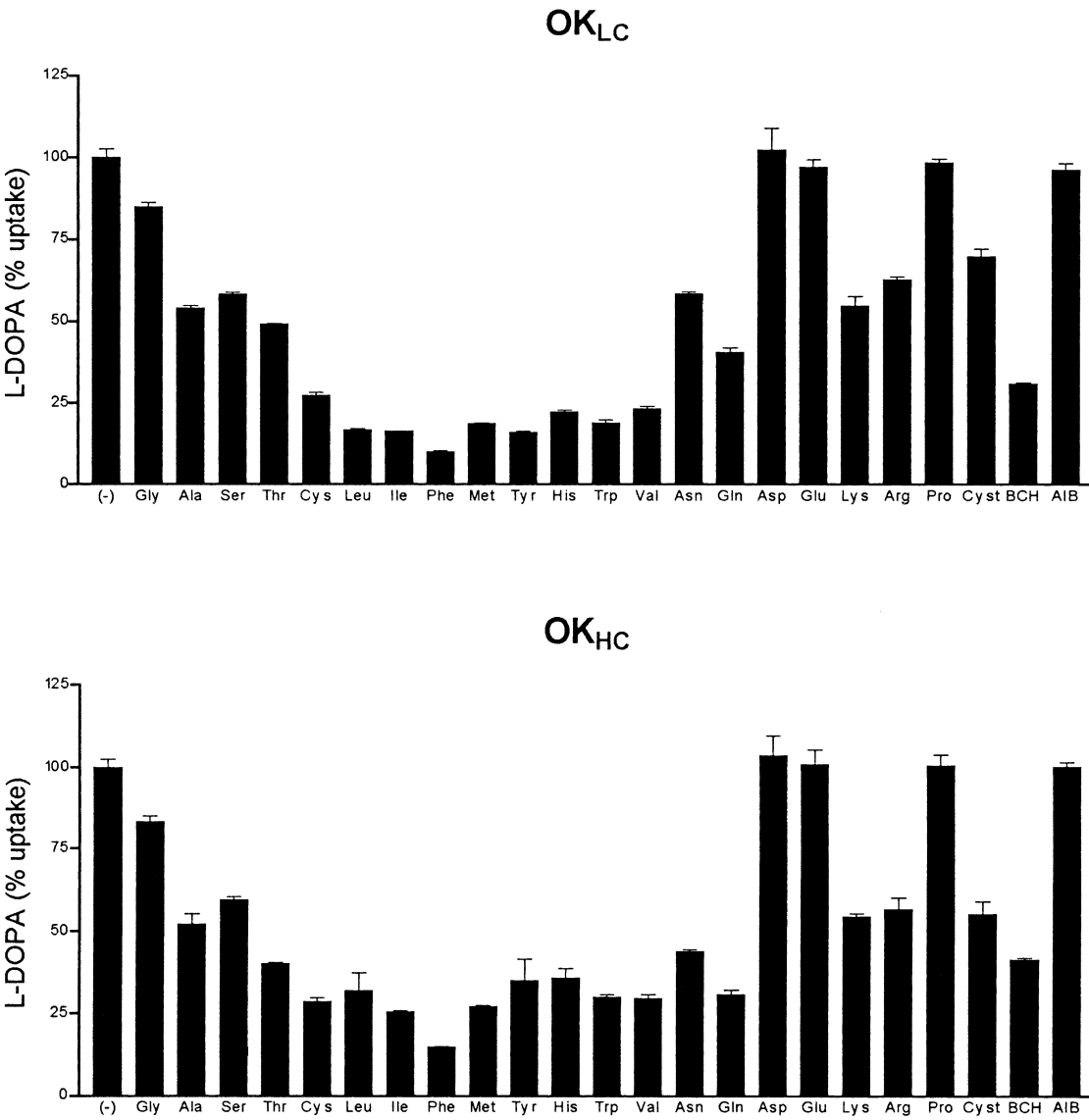

Fig. 5. Effect of L-amino acids, BCH and MeAIB (1 mM) on the accumulation of L-DOPA in OK_{LC} and OK_{HC} cells. Cells were incubated for 6 min at 37°C with 2.5 μM L-DOPA added from the apical cell border. Values are percent of control for accumulation of L-DOPA in OK_{LC} and OK_{HC} cells (absolute levels, in pmol/mg protein, were: OK_{LC}, 959 ± 30, $n = 28$; OK_{HC}, 971 ± 20, $n = 28$). Columns represent means of 4 to 8 experiments per group and vertical lines show SEM.

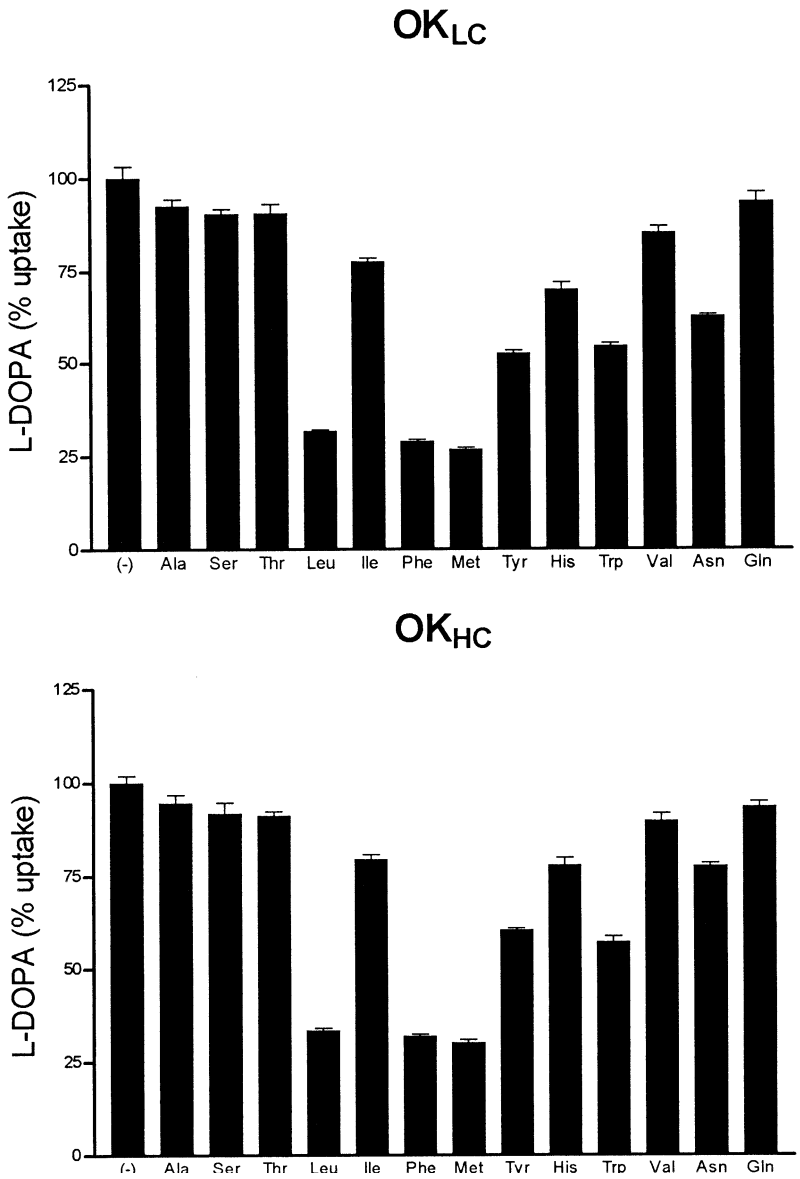

Fig. 6. Effect of D-amino acids (1 mM) on the accumulation of L-DOPA in OK_{LC} and OK_{HC} cells. Cells were incubated for 6 min at 37°C with 2.5 μM L-DOPA added from the apical cell border. Values are percent of control for accumulation of L-DOPA in OK_{LC} and OK_{HC} cells (absolute levels, in pmol/mg protein, were: OK_{LC}, 959 ± 30, n = 28; OK_{HC}, 971 ± 20, n = 28). Columns represent means of 4 to 8

DOPA, the efflux of [^{14}C]-L-DOPA was markedly increased, evidenced by increases of [^{14}C]-L-DOPA in the medium and decreases of [^{14}C]-L-DOPA in the cell. This is in agreement with the view that the L-DOPA transporter functions as an exchanger. In another series of experiments, the [^{14}C]-L-DOPA efflux was monitored for 12 min in the absence and the presence of L-arginine, BCH or L-arginine plus BCH. As shown in Figure 9, BCH stimulated the efflux of [^{14}C]-L-DOPA in both OK_{LC} and OK_{HC} cells, with potency similar to that of L-leucine and L-DOPA. The effect of L-arginine on the efflux of [^{14}C]-L-DOPA in both OK_{LC} and OK_{HC} cells was less marked than that observed with L-DOPA, L-leucine and BCH. However, particularly evident in OK_{LC} cells, BCH potentiated the efflux induced by L-arginine. Another interesting feature of the outward transport of L-DOPA concerned its Na^+ dependence. In contrast with that observed for the inward transport of L-DOPA, the spontaneous and L-leucine- and L-arginine-stimulated outward transfer of [^{14}C]-L-DOPA was largely Na^+-independent in both OK_{LC} and OK_{HC} cells (Fig. 10). In this particular set of experiments, cells were loaded with [^{14}C]-L-DOPA for 6 min and then the corresponding efflux was monitored for 12 min, in the absence and the presence of 140 mM Na^+. Several of these amino-acid exchangers have been shown to be associated with the single transmembrane domain 4F2hc or the 4F2hc-related rBAT protein through an intermolecular disulfide bond (Palacin et al., 1998; Verrey et al., 1999, 2000). Hg^{2+} and other organic mercury compounds have been demonstrated to inactivate the transporter by covalently modifying cysteine residues, this being re-

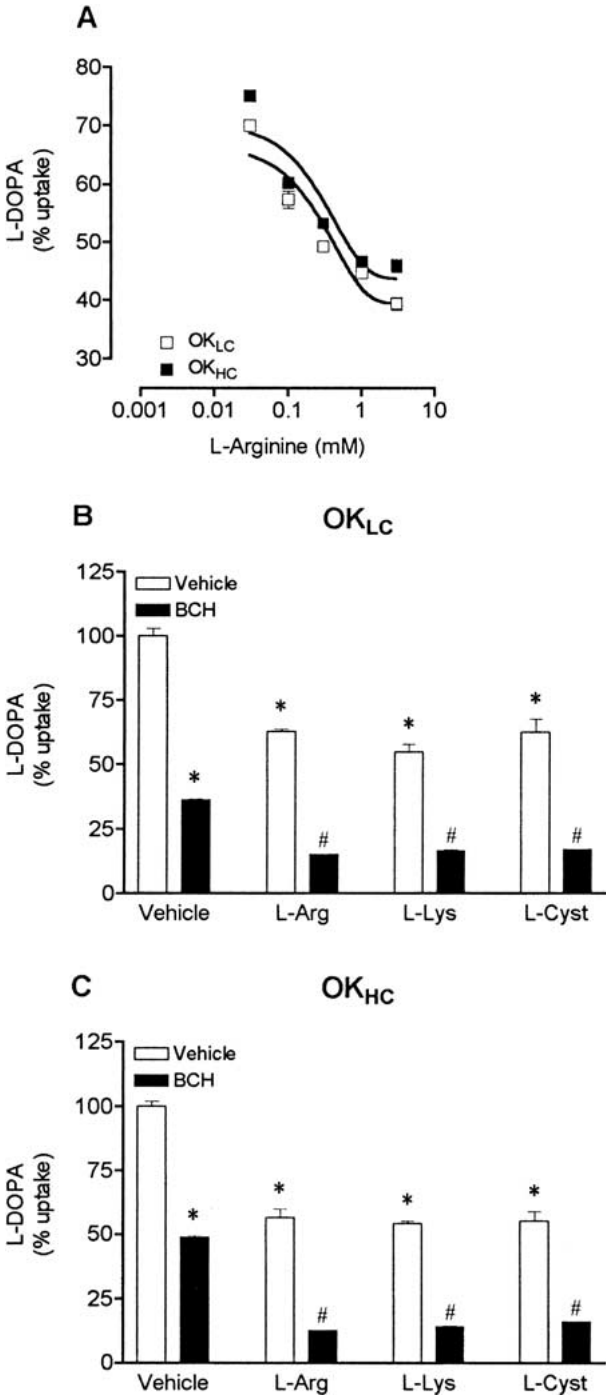

Fig. 7. (*A*) Concentration-dependent inhibition of L-DOPA by increasing concentrations of L-arginine in OK_{LC} and OK_{HC} cells. (*B*) and (*C*): Effects of basic L-amino acids (L-arginine, L-Arg; L-lysine, L-Lys; L-cystine, L-Cyst) and BCH (1 mM) on the accumulation of L-DOPA in (*B*) OK_{LC} and (*C*) OK_{HC} cells. Cells were incubated for 6 min at 37°C with 2.5 μM L-DOPA added from the apical cell border. Values are percent of control for accumulation of L-DOPA in OK_{LC} and OK_{HC} cells (absolute levels, in pmol/mg protein, were: OK_{LC}, 959 ± 30, $n = 28$; OK_{HC}, 971 ± 20, $n = 28$). Columns represent means of 4 to 6 experiments per group and vertical lines show SEM. Significantly different ($P < 0.05$) from *Control values or #values for L-arginine, L-lysine and L-cystine alone.

verted by the reducing agent β-mercaptoethanol (Estevez et al., 1998). As shown in Fig. 11, Hg^{2+} was found to completely prevent the L-leucine- and L-arginine-stimulated outward transfer of [^{14}C]-L-DOPA in both OK_{LC} and OK_{HC} cells, this being reverted by the reducing agent β-mercaptoethanol. Taken together, the results presented here suggest that efflux of L-DOPA is promoted through Na^+-independent transporters that recognize neutral amino acids, BCH and basic amino acids. In addition, cysteine residues appear to be essential for the outward transfer of L-DOPA, which may be due to covalent modification of the cysteine residue of the light chain involved in the intermolecular disulfide bridge or covalent modification of other cysteine residues of the light chain.

In experiments designed to study the efflux of L-DOPA through the apical and basal cell sides, cells cultured in polycarbonate filters were loaded for 6 min with 2.5 μM [^{14}C]-L-DOPA applied from both cell sides. For the measurement of apical and basal [^{14}C]-L-DOPA efflux, the cells cultured in polycarbonate filters were incubated for 12 min with Hanks' medium in the absence or the presence of L-leucine (1 mM) or L-arginine (1 mM). As shown in Fig. 12, the spontaneous apical fractional outflow of [^{14}C]-L-DOPA in OK_{LC} cells was half that through the basal cell border, whereas in OK_{HC} cells, the spontaneous apical fractional outflow of [^{14}C]-L-DOPA was identical to that through the basal cell side. The addition of L-leucine increased 3-fold the apical and basal efflux of [^{14}C]-L-DOPA in both cell lines. By contrast, the L-arginine-stimulated increase in the fractional outflow of [^{14}C]-L-DOPA through the apical cell side was twice that observed through the basal cell side.

The next series of experiments explored the role of intracellular regulatory pathways in the cellular uptake of L-DOPA. Involvement of a PKA-mediated pathway in the regulation of L-DOPA uptake was tested by examining the effect of pretreating cells for 30 min with compounds that are known to increase intracellular cAMP levels. Dibutyryl cyclic AMP (1 mM), the adenylyl cyclase stimulant forskolin (30 μM), the phosphodiesterase inhibitor isobutylmethylxanthine (IBMX, 1 mM) and cholera toxin (3 μg/ml) failed to affect the accumulation of a non-saturating (2.5 μM) concentration of L-DOPA in OK_{LC} and OK_{HC} cells (Table 5). In another series of experiments, we tested the involvement of a PKG-mediated pathway in the regulation of L-DOPA uptake. Cyclic GMP (1 mM), the selective cGMP phosphodiesterase inhibitor zaprinast (30 μM), the guanylyl cyclase inhibitor LY 83583 (6-(phenylamino)-5,8-quinolinedione; 30 μM) and sodium nitroprusside (100 μM) failed to affect the accumulation of a non-saturating concentration of L-DOPA (Table 5). The possible role of PKC in the regulation of L-DOPA uptake in OK_{LC} and OK_{HC} cells was tested by examining the effect of

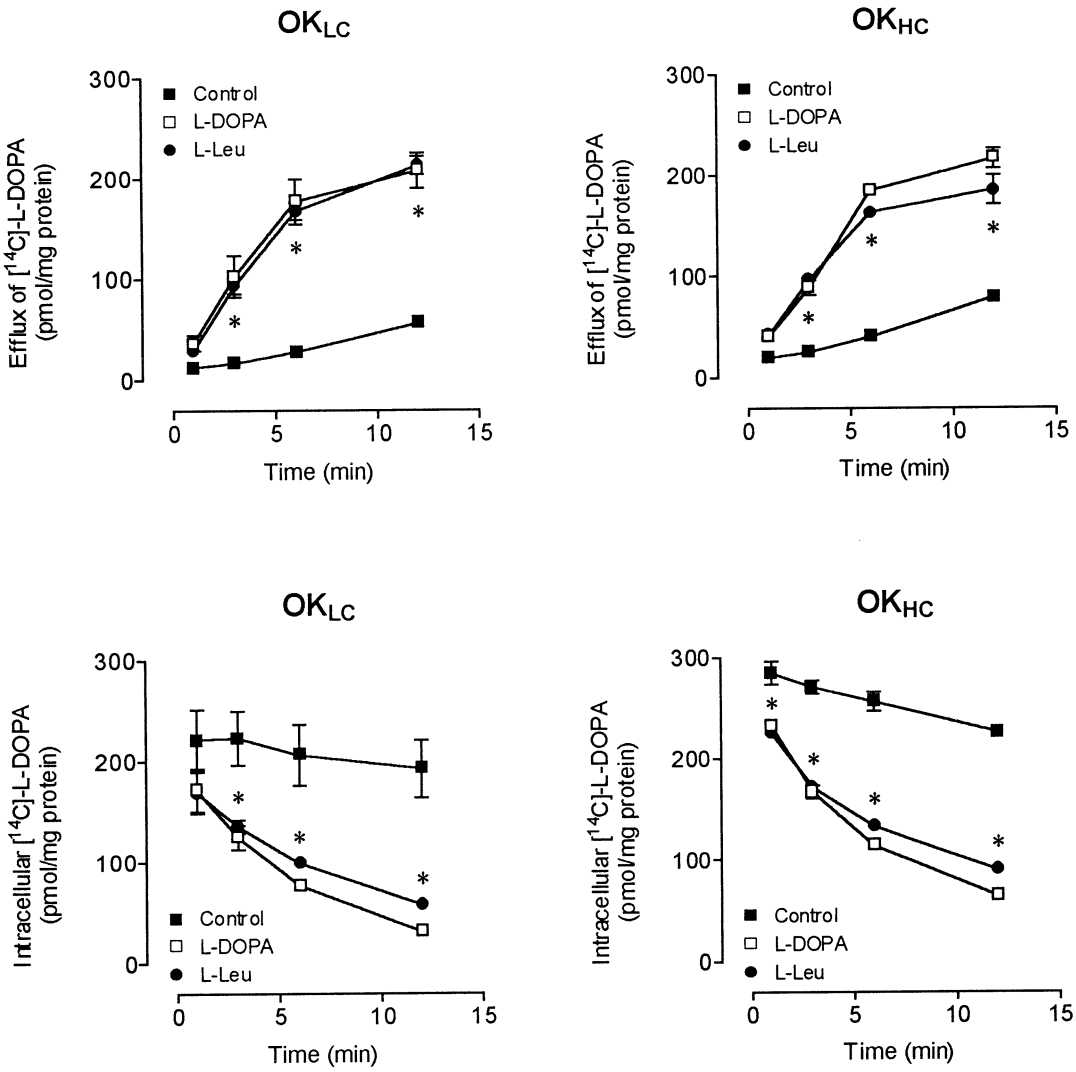

Fig. 8. Efflux and intracellular levels of [^{14}C]-L-DOPA in OK_{LC} and OK_{HC} cells in the absence (control) and the presence of 1 mM L-DOPA and 1 mM L-leucine (L-Leu). Cells were incubated for 6 min in the presence 2.5 μM [^{14}C]-L-DOPA and then incubated in the absence or the presence of unlabeled L-DOPA (1 mM) or L-Leu (1 mM) for 1, 3, 6 and 12 min. Symbols represent the mean of 8 experiments per group; vertical lines show S.E.M.. *Significantly different from control values ($P < 0.05$).

pretreating cells with either PKC activators or inhibitors. The PKC activators phorbol 12,13-dibutyrate (PDBu, 1 μM) and phorbol 12-myristate 13-acetate (PMA, 5 μM), the inactive phorbol ester 4α-phorbol 12,13-didecanoate (PDDC, 1 μM) and the PKC inhibitors staurosporine (3 μM) and chelerythrine (10 μM) failed to affect the accumulation of L-DOPA in OK_{LC} cells (Table 5). In contrast, in OK_{HC} cells PDBu, PMA, staurosporine and chelerythrine were found to produce significant decreases in L-DOPA accumulation (Table 5). In another study, we tested the involvement of PTK in the regulation of L-DOPA uptake by OK_{LC} and OK_{HC} cells. Basically, the effects of PTK inhibitors genistein and tyrphostin 25 were found not to differ from those exerted by their negative controls genistin and tyrphostin 1, suggesting the lack of involvement of PTK in the regulation of L-DOPA accumulation (Table 5). In the final series of experiments, the role of Ca^{2+}-calmodulin-mediated pathways in the regulation of L-DOPA uptake by OK_{LC} and OK_{HC} cells was tested by examining the effect of pretreating the cells with the calmodulin inhibitors calmidazolium and trifluoperazine. Both compounds produced concentration-dependent inhibition of L-DOPA (2.5 μM) uptake, with IC_{50}'s (in μM; given as geometric means with 95% confidence intervals in brackets; n = 4–6) in the same range of magnitude (calmidazolium; OK_{LC} 32 [10,105], OK_{LC}-65 [18, 236]: trifluoperazine; OK_{LC}-70 [22, 225], OK_{LC}-53 [21, 131]). However, the calcium ionophore A23187 (calcimicin) and thapsigargin, an inhibitor of the endoplasmic reticulum Ca^{2+} pump

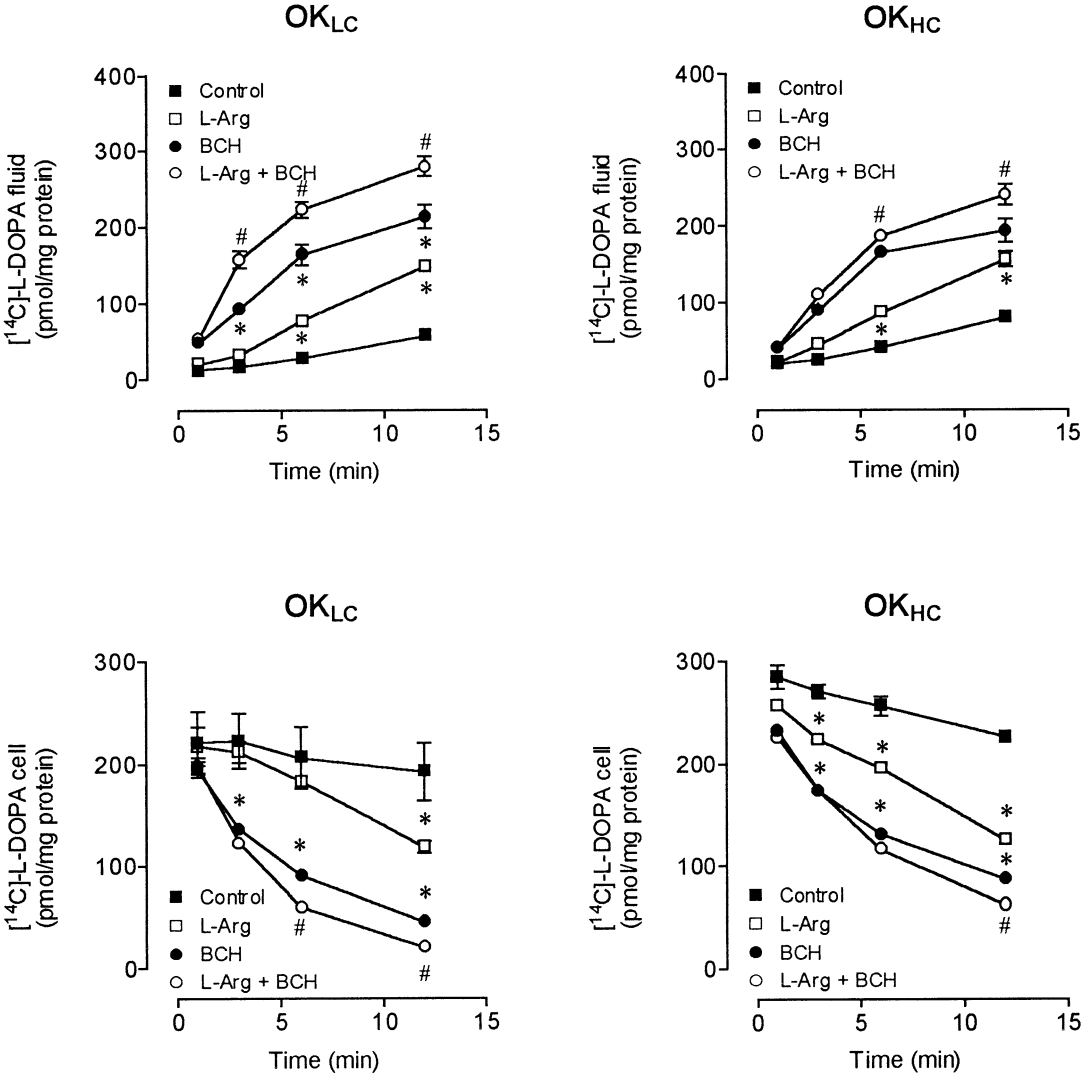

Fig. 9. Efflux and intracellular levels of [^{14}C]-L-DOPA in OK$_{LC}$ and OK$_{HC}$ cells in the absence (control) and the presence of L-arginine (L-Arg), BCH or L-Arg plus BCH. Cells were incubated for 6 min in the presence 2.5 μM [^{14}C]-L-DOPA and then incubated in the absence or the presence of L-Arg (1 mM), BCH (1 mM) or L-Arg plus BCH for 1, 3, 6 and 12 min. Symbols represent the mean of 8 experiments per group; vertical lines show SEM. Significantly different ($P < 0.05$) from control values or *values for BCH alone.

(Lytton, Westlin & Hanley, 1991; Thastrup et al., 1990), failed to alter L-DOPA accumulation in OK$_{LC}$ and OK$_{HC}$ cells (Table 5).

Discussion

The results presented here show that L-DOPA crosses the apical and the basolateral membrane of OK$_{LC}$ and OK$_{HC}$ cells using at least two major transporters. One of these transporters corresponds to a Na^+-independent transporter with a broad specificity for small and large neutral amino acids, stimulated by acid pH and inhibited by BCH, and the other is a Na^+-independent transporter for neutral and basic amino acids that also recognizes the di-amino acid cystine. Transporters with such functional characteristics correspond to systems LAT-2 and $b^{0,+}$ respectively. A third transporter, responsible for Na^+-dependent uptake of L-DOPA plays a minor or no role at all in OK$_{HC}$ and OK$_{LC}$ cells, respectively. At present, this Na^+-dependent component can not be ascribed to any particular transport system, though it correlates well with the enhanced ability of OK$_{HC}$ cells to transport Na^+.

The major component of L-DOPA uptake in OK cells is Na^+-independent, sensitive to BCH, but not to MeAIB, sensitive to neutral and basic, but not acidic, amino acids and shows *trans*-stimulation by both neutral and basic amino acids in a Na^+-independent manner. The influx of L-DOPA can be fractionated with neutral and basic amino acids into two components. The fraction that interacts with

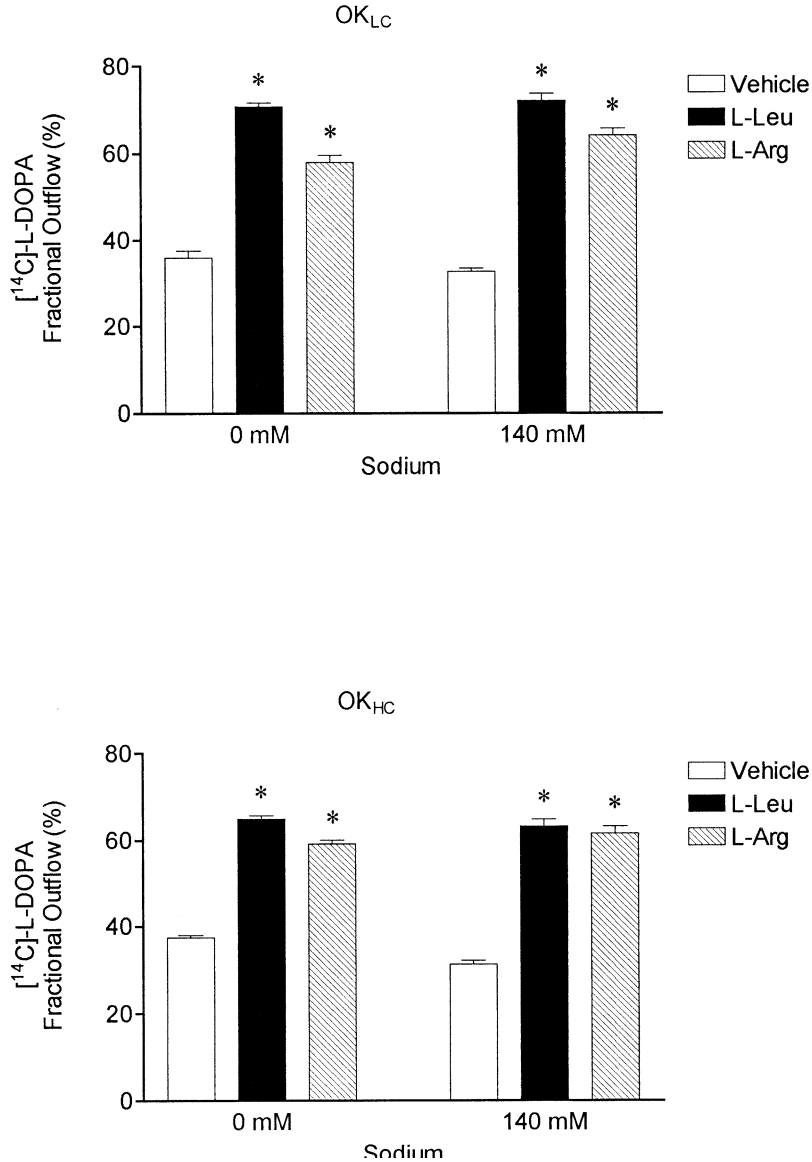

Fig. 10. Spontaneous and L-leucine (L-Leu)-or L-arginine (L-Arg)-stimulated fractional outflow of [^{14}C]-L-DOPA in OK_{LC} and OK_{HC} cells in the absence and the presence of Na^+ (140 mM). Cells were incubated for 6 min in the presence 2.5 μM [^{14}C]-L-DOPA and then incubated in the absence or the presence of L-Leu (1 mM) or L-Arg (1 mM) for 12 min. Columns represent the mean of 4 to 8 experiments per group; vertical lines show SEM. *Significantly different from control values ($P < 0.05$).

basic amino acids and cystine exhibits the properties of system $b^{0,+}$. This is substantiated by the following arguments: 1) the activity is inhibited by neutral and basic amino acids (Van Winkle et al., 1988); 2) it recognizes cystine, a typical substrate of system $b^{0,+}$ (Bertran et al., 1993); it functions as a tightly coupled exchanger (Mora et al., 1996); 3) it binds BCH with low affinity (Mora et al, 1996); and 4) it is Na^+-independent for the binding of neutral amino acids (Van Winkle et al., 1988; Van Winkle et al., 1985). System y^+L also transports basic amino acids with high affinity (K_m in the μM range) and transports small and large zwitterionic amino acids (Angelo & Deves, 1994; Chillaron et al., 1996). However, system y^+L can be excluded as a major component of L-DOPA transport in OK cells because 1) the transport of L-DOPA through this system would be Na^+-dependent (Angelo & Deves, 1994) and 2) leucine stimulated L-DOPA efflux in the absence of Na^+. This is in agreement with the report of Ishii et al. (2000) who suggested that system y^+L is not involved in the transport of L-DOPA. The fraction that does not recognize basic amino acids, but is competitively inhibited by BCH may correspond to LAT-2. This is substantiated by the following observations: 1) it is selective for neutral amino acids (Pineda et al., 1999); 2) it is relatively nonspecific, binding both small and large amino acids (Pineda et al., 1999), unlike LAT-1 (Kanai et al., 1998; Pineda et al., 1999); 3) it is stimulated by acid pH (Christensen, 1990; Segawa et al., 1999), unlike LAT-l (Prasad et al., 1999); and 4) it functions as a tightly coupled exchanger (Pineda et al., 1999). The uptake of L-DOPA by mouse brain capillary endothelial cells was found to occur through LAT-l (Kageyama et al., 2000). One pos-

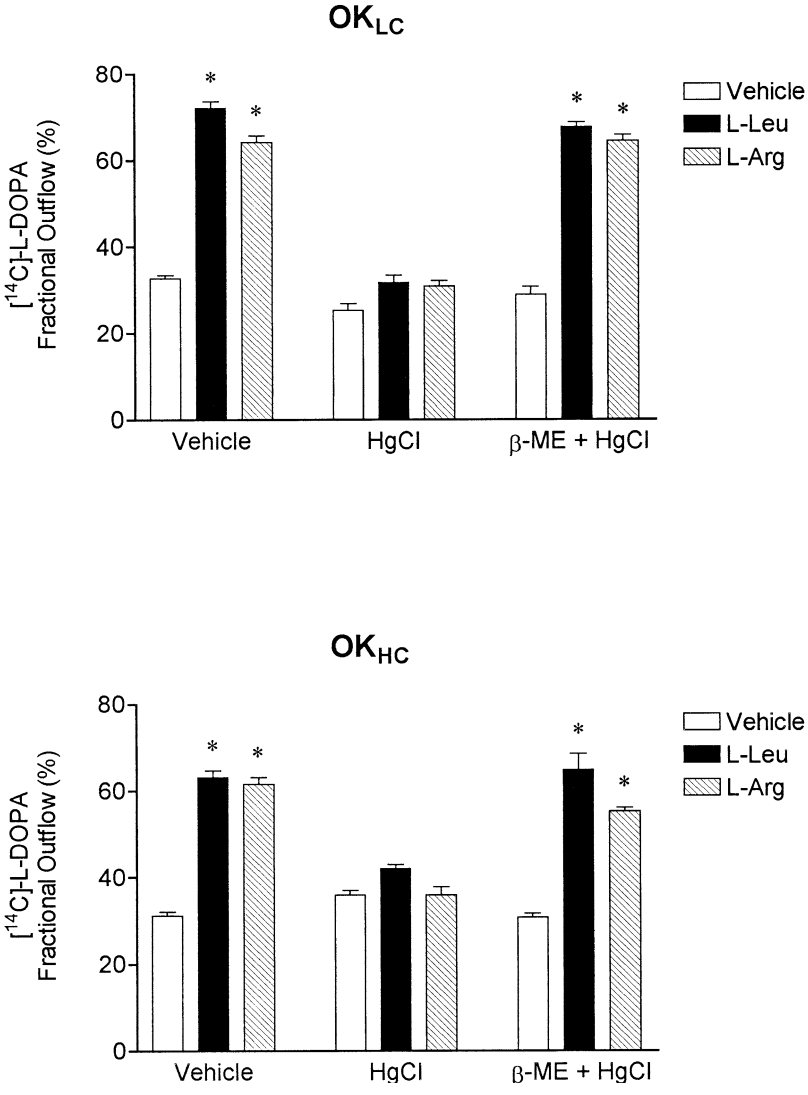

Fig. 11. Spontaneous and L-leucine (L-Leu)-or L-arginine (L-Arg)-stimulated fractional outflow of [^{14}C]-L-DOPA in OK_{LC} and OK_{HC} cells in the absence and the presence of HgCl (200 μM) or HgCl (200 μM) plus β-mercaptoethanol (5 mM). Cells were incubated for 6 min in the presence 2.5 μM [^{14}C]-L-DOPA and then incubated in the absence or the presence of L-Leu (1 mM) or L-Arg (1 mM) for 12 min. Columns represent the mean of 4 to 12 experiments per group; vertical lines show SEM. *Significantly different from control values ($P < 0.05$).

sible explanation for the major involvement of LAT-1 in the transport of L-DOPA by brain capillary endothelial cells may concern the fact that LAT-1 is the functionally predominant isoform expressed at the blood-brain barrier, being cerebrovascular LAT-2 insignificant or absent (Killian & Chikhale, 2001).

The results of [^{14}C]-L-DOPA efflux studies in OK_{LC} and OK_{HC} cells are also quite valuable to define the nature of transporters involved in the handling of L-DOPA. Both systems $b^{0,+}$ and LAT-2 function as exchangers (Chillaron et al., 1996; Rossier et al., 1999; Segawa et al., 1999) and L-arginine- and L-leucine-induced efflux of [^{14}C]-L-DOPA agree with the view that both transporters may participate in the exchange of [^{14}C]-L-DOPA. The finding that BCH potentiated the L-arginine-induced efflux of [^{14}C]-L-DOPA reinforces this suggestion. As the labelled L-DOPA virtually does not leak out of the cells during the 12-min incubation in amino acid-free buffer, and measurements of efflux in the absence of extracellular amino acids did not show a consistent efflux, the results suggest that L-DOPA transporters function as exchangers. System $b^{0,+}$ and LAT-2 have been shown to associate with different amino-acid transporter-associated glycoproteins, namely rBAT and 4F2hc (Verrey et al., 1999, 2000). rBAT glycoprotein is expressed in the brush border plasma membrane of both the renal proximal tubules and the small intestine. OK cells have been shown to express the rBAT gene and hybrid depletion of OK mRNA with an antisense oligonucleotide complementary to rBAT cDNA specifically blocked expression of $b^{0,+}$ transport (Mora et al., 1996). Furthermore, an antisense oligonucleotide of rBAT was recently found to almost completely suppress the L-DOPA uptake activities in *Xenopus* oocytes injected with a poly A^+RNA from rabbit intestinal epithelium (Ishii et al., 2000). The mRNA corresponding to LAT-2 examined by Northern blot analysis was strongly expressed in kidney and small intestine (Pineda et al.,

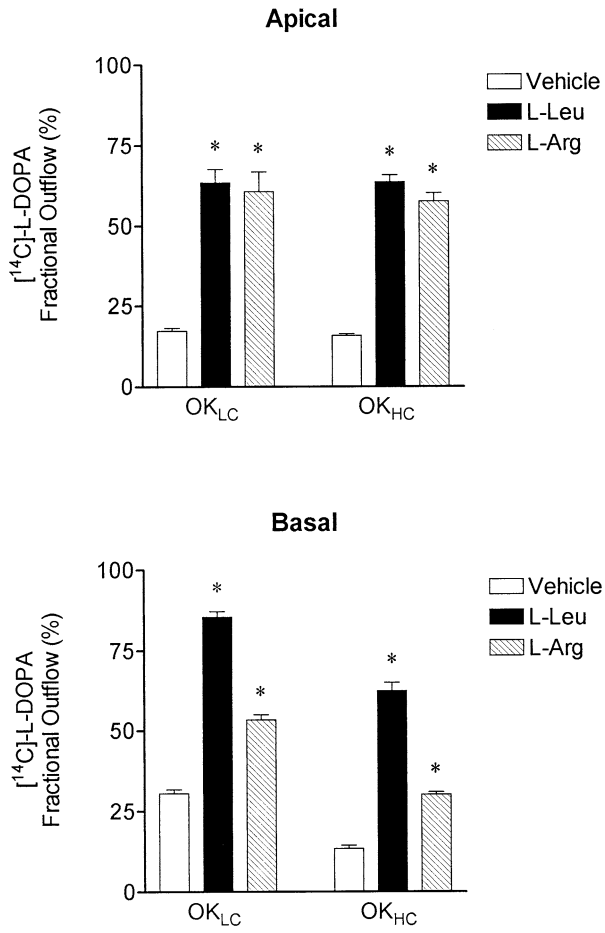

Fig. 12. Spontaneous and L-leucine (L-Leu)-or L-arginine (L-Arg)-stimulated fractional outflow of [^{14}C]-L-DOPA in OK_{LC} and OK_{HC} cells through the apical or basal cell sides. Cells were incubated for 6 min in the presence 2.5 μM [^{14}C]-L-DOPA and then incubated in the absence or the presence of L-Leu (1 mM) or L-Arg (1 mM) for 12 min. Columns represent the mean of 4 experiments per group; vertical lines show SEM. *Significantly different from control values ($P < 0.05$).

1999; Rossier et al., 1999). In situ hybridization studies specifically localized the renal expression of LAT-2 mRNA to the epithelial cells of proximal tubules (Pineda et al., 1999). A similar pattern of expression has been shown in immunolocalization of 4F2hc protein in kidney cortex (Quackenbush et al., 1986; Rossier et al., 1999), suggesting an association between LAT-2 and 4F2hc. In fact, as described for other transporters (Mastroberardino et al., 1998), 4F2hc was found to bring LAT-2 to the oocyte plasma membrane (Pineda et al., 1999). Subsequently, it was demonstrated that LAT-2 forms a disulfide-bond heterodimeric complex with 4F2hc (Rossier et al., 1999), as has been found to occur with other amino-acid transporters and their associated glycoproteins (Verrey et al., 1999, 2000). One potential point of conflict derives from the fact that 4F2hc has a basolateral location (Quackenbush et al., 1986; Rossier et al., 1999), which contrasts with the suggested role of LAT-2 as an apical transporter for L-DOPA in OK cells. Though the majority of studies reported here were performed in cells plated on plastic supports, some experiments were carried out in cells cultured in polycarbonate filters with access to both the apical and basal cell sides. The finding that increases in the fractional outflow of [^{14}C]-L-DOPA by L-leucine were identical at both cell sides suggests that LAT-2 in OK cells may be present in both the apical and basolateral membranes. However, this contrasts with the finding that L-arginine-stimulated increase in the fractional outflow of [^{14}C]-L-DOPA through the apical cell side was twice that through the basal cell side. This indicates that system $b^{0,+}$ may have a polarized distribution in OK cells.

Although most of L-DOPA was entering the cells in a Na^+-independent manner, a minor component of L-DOPA uptake (~15%) was found to require extracellular Na^+. Maneuvers that alter Na^+ gradients, such as those resulting from the application of amiloride (an inhibitor of the Na^+/H^+ exchanger), ouabain (an inhibitor of the Na^+-K^+ ATPase) and amphotericin B (a Na^+ ionophore), were also found to significantly affect L-DOPA uptake. This was particularly evident in OK_{HC} cells, whereas in OK_{LC} cells Na^+ removal, amiloride, ouabain and amphotericin B were found not to affect L-DOPA accumulation. One possible explanation for differences in the magnitude of the Na^+-sensitive uptake of L-DOPA between and OK_{LC} and OK_{HC} cells could be related to differences in their ability to translocate Na^+ from the apical to the basal cell side. In fact, Na^+/H^+ exchange and Na^+-k^+-ATPase activity in OK_{HC} cells was nearly twice that in OK_{LC} cells. Alternatively, OK_{LC} cells may not express the transporter responsible for Na^+-dependent uptake of L-DOPA. The finding that L-DOPA uptake in OK cells is largely promoted through the Na^+-insensitive L-type amino acid transporter contrasts with the role of Na^+ in the formation of renal dopamine (Lee, 1993). Studies from our group using human and rat kidney slices also showed that L-DOPA uptake is a Na^+-dependent and ouabain-sensitive process (Soares-da-Silva & Fernandes, 1992; Soares-da-Silva et al., 1993), suggesting that the Na^+-dependent increase in urinary dopamine depends on enhanced uptake of L-DOPA into tubular epithelial cells. However, L-DOPA uptake in both renal (LLC-PK_1 cells) and non-renal (RBE4, DITNC1 and Neuro 2A) cultured cells has been recently shown to be largely Na^+-independent, BCH-sensitive and greater at acid pH (Sampaio-Maia, Serrão & Soares-da-Silva, 2001; Soares-da-Silva & Serrão, 2000a). Altogether, this suggests that OK cells may lack or have lost the Na^+-dependent transporter, but behave similarly to other types of cells in culture.

Table 5. Effect of modulators of PKA, PKG, PKC and PTK on the uptake of L-DOPA (2.5 μM)

	Modulator	OK_{LC}	OK_{HC}
PKA	Control	100 ± 3	100 ± 3
	Cyclic AMP (1 mM)	125 ± 7	93 ± 3
	Forskolin (30 μM)	94 ± 4	85 ± 1
	IBMX (1 mM)	108 ± 2	105 ± 2
	Cholera toxin (3 μg/ml)	96 ± 5	92 ± 9
PKG	Control	100 ± 2	100 ± 2
	Cyclic GMP(1 mM)	97 ± 3	98 ± 4
	Zaprinast (30 μM)	92 ± 4	99 ± 10
	LY 83583 (30 μM)	100 ± 3	97 ± 1
	Na^+ Nitroprusside (100 μM)	92 ± 2	102 ± 1
PKC	Control	100 ± 4	100 ± 4
	PDBu (1 μM)	112 ± 1	82 ± 2*
	PMA (5 μM)	110 ± 4	78 ± 3*
	PDDc (1 μM)	90 ± 2	92 ± 4
	Staurosporine (3 μM)	99 ± 2	83 ± 1*
	Chelerythrine (10 μM)	95 ± 8	81 ± 4*
PTK	Control	100 ± 2	100 ± 4
	Genistein (30 μM)	97 ± 3	85 ± 2*
	Genistin (30 μM)	103 ± 4	84 ± 2*
	Tyrphostin 25 (100 μM)	88 ± 4	72 ± 2*
	Tyrphostin 1 (100 μM)	102 ± 9	91 ± 2
Calcium	Control	100 ± 1	100 ± 3
	A23187 (10 μM)	98 ± 1	91 ± 3
	Thapsigargin (10 μM)	97 ± 3	99 ± 3

Values are percent of control for accumulation of L-DOPA in OK_{LC} and OK_{HC} cells (absolute levels, in pmol/mg protein, were: OK_{LC}, 536 ± 16, $n = 62$; OK_{HC}, 498 ± 16, $n = 59$).
Values are mean ± SEM of 4 to 8 determinations per group. * Significantly different from corresponding control values ($P < 0.05$) using the Newman-Keuls test for multiple comparisons.

The efficiency of the L-DOPA transport in OK_{LC} and OK_{HC} cells can be evidenced by the ratio of L-DOPA concentration in cellular water to medium concentration. The intracellular L-DOPA concentration at 6 min, when uptake was linear, was ~25 times greater than that which could be expected by passive equilibration of L-DOPA. Since influx of L-DOPA is promoted by at least two transporters that act as tightly coupled exchangers, it is likely that the high efficiency to take up L-DOPA may result from exchange with intracellular amino acids. On the other hand, the relationship between the initial influx of L-DOPA and the substrate concentration was hyperbolic, suggesting that the uptake was saturable. However, the kinetic parameters derived from these experiments reflect the activities of at least two or three transport agencies, as mentioned above. The enhanced ability of OK_{HC} cells to take up L-DOPA is indicated by higher A_{max} and V_{max} values, compared with those in OK_{LC} cells. Considering that apparent kinetics (k_{in}, k_{out} and K_m values) for individual transporters were identical in OK_{HC} and OK_{LC} cells, it is suggested that OK_{HC} cells have more transport units than OK_{LC} cells. The finding that uptake of L-DOPA in OK cells was accompanied by intracellular acidification, this being prevented by BCH, strongly suggests that these cells are provided with an L-DOPA-H^+ cotransport system. This would agree with the observation that the enhanced accumulation of L-DOPA at acidic pH may result from *trans*-stimulation of L-DOPA inward transfer by an imposed H^+ gradient. Similar findings were reported before for L-leucine in Chang liver cells (Mitsumoto et al., 1986). However, the observation that the L-DOPA-induced intracellular acidification was prevented by BCH does not necessarily indicate that BCH is not transported by LAT-2 in these cells. In fact, we do provide evidence suggesting that BCH is transported into the cells as indicated by BCH-induced increases in [^{14}C]-L-DOPA efflux. At present, no clear explanation is available for these apparently discrepant findings.

After defining the mechanism of uptake, we then examined the regulation of L-DOPA uptake in OK_{LC} and OK_{HC} cells. Using relatively specific modulators we found that PKA-, PKG-, PKC- and PTK-mediated pathways appear to have no role in regulating L-DOPA uptake in OK cells. This is in line with recent evidence obtained in other types of cells (Sampaio-Maia et al., 2001; Soares-da-Silva & Serrão, 2000a). However, it has been reported that amino-acid transport system L is modulated by PKC-dependent

phosphorylation (Christensen, 1990; Ramamoorthy et al., 1992; Shotwell, Kilberg & Oxender, 1983). PKC-dependent phosphorylation sites are predicted on the LAT-2 amino-acid sequence, namely in the predicted intracellular loops (Segawa et al., 1999). Although the regulation by tyrosine phosphorylation has not been demonstrated for system L, there is an interesting observation that the monoclonal antibody against 4F2hc causes the tyrosine kinase-dependent phosphorylation of an ~125-kDa protein (Warren et al., 1996). On the other hand, compounds that interfere with Ca^{2+}/calmodulin-mediated pathways, such as calmidazolium and trifluoperazine, caused a concentration-dependent reduction in L-DOPA uptake in both types of cells. Similar findings were observed in human renal and intestinal epithelial cells where calmidazolium, trifluoperazine and KN-62 were found to markedly reduce riboflavin accumulation (Kumar et al., 1998; Said & Ma, 1994; Said et al., 2000). Previous work from our group has also shown that the Na^+-independent and BCH-sensitive uptake of L-DOPA in renal and non-renal cells may be under the control of Ca^{2+}/calmodulin mediated pathways (Sampaio-Maia et al., 2001; Soares-da-Silva & Serrão, 2000a). However, with the exception of astrocytes all other types of cells failed to respond to increases in intracellular Ca^{2+} (Sampaio-Maia et al., 2001). This may suggest that the effects of calmidazolium and trifluoperazine upon the L-DOPA transporter may be due to a direct interaction with the transporter rather than inhibition of Ca^{2+}/calmodulin.

We concluded that L-DOPA in OK_{LC} and OK_{HC} cells is transported quite efficiently through the apical cell border and several findings indicate that L-DOPA uses at least two major transporters, systems LAT-2 and $b^{0,+}$. The transport of L-DOPA by LAT-2 corresponds to a Na^+-independent transporter with a broad specificity for small and large neutral amino acids, stimulated by acid pH and inhibited by BCH. The transport of L-DOPA by system $b^{0,+}$ is a Na^+-independent transporter for neutral and basic amino acids that also recognizes the di-amino acid cystine. Transporters involved in Na^+-independent uptake of L-DOPA (systems LAT-2 and $b^{0,+}$) also function as a tightly coupled exchangers.

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References

Angelo, S., Deves, R. 1994. Amino acid transport system y+L of human erythrocytes: specificity and cation dependence of the translocation step. *J. Membrane Biol.* **141:**183–192

Bertran, J., Werner, A., Chillaron, J., Nunes, V., Biber, J., Testar, X., Zorzano, A., Estivill, X., Murer, H., Palacin, M. 1993. Expression cloning of a human renal cDNA that induces high affinity transport of L-cystine shared with dibasic amino acids in *Xenopus* oocytes. *J. Biol. Chem.* **268:**14842–14849

Bradford, M.M. 1976. A rapid method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.* 72:248–254

Cheng, L., Precht, P., Frank, D., Liang, C.T. 1990. Dopamine stimulation of cAMP production in cultured opossum kidney cells. *Am. J. Physiol.* **258:**F877–F882

Cheng, Y.-C., Prusoff, W.H. 1973. Relationship between the inhibition constant (Ki) and the concentration of the inhibitor which causes 50 per cent inhibition (IC50) of an enzymatic reaction. *Biochem. Pharmacol.* 22:3099–3108

Chillaron, J., Estevez, R., Mora, C., Wagner, C.A., Suessbrich, H., Lang, F., Gelpi, J.L., Testar, X., Busch, A.E., Zorzano, A., Palacin, M. 1996. Obligatory amino acid exchange via systems $b^{0,+}$-like and y^+ L-like. A tertiary active transport mechanism for renal reabsorption of cystine and dibasic amino acids. *J. Biol. Chem.* **271:**17761–17770

Christensen, H.N. 1990. Role of amino acid transport and countertransport in nutrition and metabolism. *Physiol. Rev.* **70:**43–77

Christensen, H.N., Antonioli, J.A. 1969. Cationic amino acid transport in the rabbit reticulocyte. Na^+-dependent inhibition of Na^+-independent transport. *J. Biol. Chem.* **244:**1497–1504

Deves, R., Angelo, S., Chavez, P. 1993. N-ethylmaleimide discriminates between two lysine transport systems in human erythrocytes. *J. Physiol.* **468:**753–766

Deves, R., Chavez, P., Boyd, C.A. 1992. Identification of a new transport system (y+L) in human erythrocytes that recognizes lysine and leucine with high affinity. *J. Physiol.* **454:**491–501

Estevez, R., Camps, M., Rojas, A.M., Testar, X., Deves, R., Hediger, M.A., Zorzano, A., Palacin, M. 1998. The amino acid transport system y+L/4F2hc is a heteromultimeric complex. *FASEB J.* **12:**1319–1329

Glahn, R.P., Onsgard, M.J., Tyce, G.M., Chinnow, S.L., Knox, F.G., Dousa, T.P. 1993. Autocrine/paracrine regulation of renal Na(+)-phosphate cotransport by dopamine. *Am. J. Physiol.* **264**:F618–F622

Gomes, P., Soares-da-Silva, P. 2000. Transepithelial flux of sodium and handling of L-DOPA in renal epithelial cells. *Br. J. Pharmacol.* **129:**170P

Gomes, P., Vieira-Coelho, M.A., Soares-Da-Silva, P. 2001. Ouabain-insensitive acidification by dopamine in renal OK cells: primary control of the Na^+/H^+ exchanger. *Am. J. Physiol.* **281:**R10–R18

Grossman, E., Goldstein, D.S., Hoffman, A., Wacks, I.R., Epstein, M. 1992. Effects of water immersion on sympathoadrenal and dopa-dopamine systems in humans. *Am. J. Physiol.* **262:**R993–R999

Guimarães, J.T., Vieira-Coelho, M.A., Serrão, M.P., Soares-da-Silva, P. 1997. Opossum kidney (OK) cells in culture synthesize and degrade the natriuretic hormone dopamine: a comparison with rat renal tubular cells. *Int. J. Biochem. Cell Biol.* **29**:681–688

Ishii, H., Sasaki, Y., Goshima, Y., Kanai, Y., Endou, H., Ayusawa, D., Ono, H., Miyamae, T., Misu, Y. 2000. Involvement of rBAT in Na(+)-dependent and -independent transport of the neurotransmitter candidate L-DOPA in *Xenopus laevis* oocytes injected with rabbit small intestinal epithelium poly A(+) RNA. *Biochim. Biophys. Acta* **1466:**61–70

Jose, P.A., Raymond, J.R., Bates, M.D, Aperia, A., Felder, R.A., Carey, R.M. 1992. The renal dopamine receptors. *J. Am. Soc. Nephrol.* **2:**1265–1278

Kageyama, T., Nakamura, M., Matsuo, A., Yamasaki, Y., Takakura, Y., Hashida, M., Kanai, Y., Naito, M., Tsuruo, T.,

Minato, N., Shimohama, S. 2000. The 4F2hc/LAT1 complex transports L-DOPA across the blood-brain barrier. *Brain Res.* **879:**115–121

Kanai, Y., Segawa, H., Miyamoto, K., Uchino, H., Takeda, E., Endou, H. 1998. Expression cloning and characterization of a transporter for large neutral amino acids activated by the heavy chain of 4F2 antigen (CD98). *J. Biol. Chem.* **273:**23629–23632

Killian, D.M., Chikhale, P.J. 2001. Predominant functional activity of the large, neutral amino acid transporter (LAT1) isoform at the cerebrovasculature. *Neurosci. Lett.* **306:**1–4

Koyama, H., Goodpasture, C., Miller, M.M., Teplitz, R.L., Riggs, A.D. 1978. Establishment and characterization of a cell line from the American opossum (*Didelphys virginiana*). *In Vitro* **14:**239–246

Kumar, C.K., Yanagawa, N., Ortiz, A., Said, H.M. 1998. Mechanism and regulation of riboflavin uptake by human renal proximal tubule epithelial cell line HK-2. *Am. J. Physiol.* **274:**F104–F110

Lee, M.R. 1993. Dopamine and the kidney: ten years on. *Clin. Sci.* **84:**357–375

Lytton, J., Westlin, M., Hanley, M.R. 1991. Thapsigargin inhibits the sarcoplasmic or endoplasmic reticulum Ca-ATPase family of calcium pumps. *J. Biol. Chem.* **266:**17067–17071

Mastroberardino, L., Spindler, B., Pfeiffer, R., Skelly, P.J., Loffing, J., Shoemaker, C.B., Verrey, F. 1998. Amino-acid transport by heterodimers of 4F2hc/CD98 and members of a permease family. *Nature* **395:**288–291

Mitsumoto, Y., Sato, K., Ohyashiki, T., Mohri, T. 1986. Leucine-proton cotransport system in Chang liver cell. *J. Biol. Chem.* **261:**4549–4554

Mora, C., Chillaron, J., Calonge, M.J., Forgo, J., Testar, X., Nunes, V., Murer, H., Zorzano, A., Palacin, M. 1996. The rBAT gene is responsible for L-cystine uptake via the $b^{0,+}$-like amino acid transport system in "renal proximal tubular" cell line (OK cells). *J. Biol. Chem.* **271:**10569–10576

Motulsky, H.J. 1999. Analyzing data with GraphPad Prism. GraphPad Prism Software, Inc., San Diego, CA

Palacin, M., Estevez, R., Bertran, J., Zorzano, A. 1998. Molecular biology of mammalian plasma membrane amino acid transporters. *Physiol. Rev.* **78:**969–1054

Perrichot, R., Garciaocana, A., Couette, S., Comoy, E., Amiel, C., Friedlander, G. 1995. Locally Formed Dopamine Modulates Renal Na-P-I Co-Transport Through Da(1) and Da(2) Receptors. *Biochem. J.* **312:**433–437

Pineda, M., Fernandez, E., Torrents, D., Estevez, R., Lopez, C., Camps, M., Lloberas, J., Zorzano, A., Palacin, M. 1999. Identification of a membrane protein, LAT-2, that co-expresses with 4F2 heavy chain, an L-type amino acid transport activity with broad specificity for small and large zwitterionic amino acids. *J. Biol. Chem.* **274:**19738–19744

Pinto-do-Ó, P.C., Soares-da-Silva, P. 1996. Studies on the pharmacology of the a inward transport of L-DOPA in rat renal tubules. *Br. J. Pharmacol.* **118:**741–747

Prasad, P.D., Wang, H., Huang, W., Kekuda, R., Rajan, D.P., Leibach, F.H., Ganapathy, V. 1999. Human LAT1, a subunit of system L amino acid transporter: molecular cloning and transport function. *Biochem. Biophys. Res. Commun.* **255:**283–288

Quackenbush, E.J., Gougos, A., Baumal, R., Letarte, M. 1986. Differential localization within human kidney of five membrane proteins expressed on acute lymphoblastic leukemia cells. *J. Immunol.* **136:**118–124

Quigley, J.P., Gotterer, G.S. 1969. Distribution of Na,K-stimulated ATPase activity in rat intestinal mucosa. *Biochim. Biophys. Acta* **173:**456–468

Ramamoorthy, S., Leibach, F.H., Mahesh, V.B., Ganapathy, V. 1992. Modulation of the activity of amino acid transport system L by phorbol esters and calmodulin antagonists in a human placental choriocarcinoma cell line. *Biochim. Biophys. Acta* 1**136:**181–188

Rossier, G., Meier, C., Bauch, C., Summa, V., Sordat, B., Verrey, F., Kuhn, L.C. 1999. LAT2, a new basolateral 4F2hc/CD98-associated amino acid transporter of kidney and intestine. *J. Biol. Chem.* **274:**34948–34954

Said, H.M., Ma, T.Y. 1994. Mechanism of riboflavin in uptake by Caco-2 human intestinal epithelial cells. *Am. J. Physiol.* **266:**G15–G21

Said, H.M., Ortiz, A., Moyer, M.P., Yanagawa, N. 2000. Riboflavin uptake by human-derived colonic epithelial NCM460 cells. *Am. J. Physiol.* **278:**C270–C276

Sampaio-Maia, B., Serrão, M.P., Soares-da-Silva, P. 2001. Regulatory pathways and uptake of L-DOPA by capillary cerebral endothelial cells, astrocytes, and neuronal cells. *Am. J. Physiol.* **280:**C333–C342

Segawa, H., Fukasawa, Y., Miyamoto, K., Takeda, E., Endou, H., Kanai, Y. 1999. Identification and functional characterization of a Na^+-independent neutral amino acid transporter with broad substrate selectivity. *J. Biol. Chem.* **274:**19745–19751

Shotwell, M.A., Kilberg, M.S., Oxender, D.L. 1983. The regulation of neutral amino acid transport in mammalian cells. *Biochim. Biophys. Acta* **737:**267–284

Siragy, H.M., Felder, R.A., Howell, N.L., Chevalier, R.L., Peach, M.J., Carey, R.M. 1989. Evidence that intrarenal dopamine acts as a paracrine substance at the renal tubule. *Am. J. Physiol.* **257:**F469–F477

Soares-da-Silva, P. 1994. Source and handling of renal dopamine: Its physiological importance. *News in Physiological Sciences* **9:**128–134

Soares-da-Silva, P., Fernandes, M.H. 1992. Sodium-dependence and ouabain-sensitivity of the synthesis of dopamine in renal tissues of the rat. *Br. J. Pharmacol.* **105:**811–816

Soares-da-Silva, P., Fernandes, M.H., Pinto-do-Ó, P.C. 1994. Cell inward transport of L-DOPA and 3-O-methyl-L-DOPA in rat renal tubules. *Br. J. Pharmacol.* **112:**611–615

Soares-da-Silva, P., Pestana, M., Fernandes, M.H. 1993. Involvement of tubular sodium in the formation of dopamine in the human renal cortex. *J. Am. Soc. Nephrol.* **3:**1591–1599

Soares-da-Silva, P., Pestana, M., Vieira-Coelho, M.A., Fernandes, M.H., Albino-Teixeira, A. 1995. Assessment of renal dopaminergic system activity in the nitric oxide-deprived hypertensive rat model. *Br. J. Pharmacol.* **114:**1403–1413

Soares-da-Silva, P., Serrão, M.P. 2000a. Molecular modulation of inward and outward apical transporters of L-DOPA in LLC-PK(1) cells. *Am. J. Physiol.* **279:**F736–F746

Soares-da-Silva, P., Serrão, M.P. 2000b. Outward Transfer of Dopamine Precursor L-3,4-Dihydroxyphenylalanine (L- dopa) by Native and Human P-glycoprotein in LLC-PK_1 and LLC-GA5 Col300 Renal Cells. *J. Pharmacol. Exp. Ther.* **293:**697–704

Soares-da-Silva, P., Vieira-Coelho, M.A., Serrão, M.P. 1997. Uptake of L-3,4-dihydroxyphenylalanine and dopamine formation in cultured renal epithelial cells. *Biochem. Pharmacol* **54:**1037–1046

Thastrup, O., Cullen, P.J., Drobak, B.K., Hanley, M.R., Dawson, A.P. 1990. Thapsigargin, a tumor promoter, discharges intracellular Ca^{2+} stores by specific inhibition of the endoplasmic reticulum Ca^{2+}-ATPase. *Proc. Natl. Acad. Sci. USA* **87:**2466–2470

Thomas, J.A., Buchsbaum, R.N., Zimniak, A., Racker, E. 1979. Intracellular pH measurements in Ehrlich ascites tumor cells

utilizing spectroscopic probes generated in situ. *Biochemistry* **18:**2210–2218

Van Winkle, L.J., Campione, A.L. 1990. Functional changes in cation-preferring amino acid transport during development of preimplantation mouse conceptuses. *Biochim. Biophys. Acta* 1028:165–173

Van Winkle, L.J., Campione, A.L., Gorman, J.M. 1988. Na^+-independent transport of basic and zwitterionic amino acids in mouse blastocysts by a shared system and by processes which distinguish between these substrates. *J. Biol. Chem.* **263:**3150–3163

Van Winkle, L.J., Christensen, H.N., Campione, A.L. 1985. Na^+-dependent transport of basic, zwitterionic, and bicyclic amino acids by a broad-scope system in mouse blastocysts. *J. Biol. Chem.* **260:**12118–12123

Verrey, F., Jack, D.L., Paulsen, I.T., Saier, M.H., Jr., Pfeiffer, R. 1999. New glycoprotein-associated amino acid transporters. *J. Membrane Biol.* **172:**181–192

Verrey, F., Meier, C., Rossier, G., Kuhn, L.C. 2000. Glycoprotein-associated amino acid exchangers: broadening the range of transport specificity. *Pfluegers Arch.* **440:**503–512

Vieira-Coelho, M.A., Soares-da-Silva, P. 1997. Apical and basal uptake of L-dopa and L-5-HTP and their corresponding amines, dopamine and 5-HT, in OK cells. *Am. J. Physiol.* **272:**F632–F639

Vieira-Coelho, M.A., Soares-da-Silva, P. 1998. Uptake and intracellular fate of L-DOPA in a human intestinal epithelial cell line: Caco-2. *Am. J. Physiol.* **275:**C104–C112

Warren, A.P., Patel, K., McConkey, D.J., Palacios, R. 1996. CD98: A type II transmembrane glycoprotein expressed from the beginning of primitive and definitive hematopoiesis may play a critical role in the development of hematopoietic cells. *Blood* **87:**3676–3687

White, M.F., Gazzola, G.C., Christensen, H.N. 1982. Cationic amino acid transport into cultured animal cells. I. Influx into cultured human fibroblasts. *J. Biol. Chem.* **257:**4443–4449