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Competitive and non-competitive inhibition of L-3,4-dihydroxyphenylalanine uptake in Opossum kidney cells

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Abstract

The present study aimed to determine the kinetics of L-3,4-dihydroxyphenylalanine (L-DOPA) uptake in Opossum kidney (OK) cells and to define the type of inhibition produced by L-5-hydroxytryptophan (L-5-HTP), cyanine 863 and 3,3′-diethyloxacarbocyanine (3,3′-DOC). Non-linear analysis of the saturation curves revealed for L-DOPA a K_m (in μM) of 129 (114, 145) and a V_{max} (in nmol/mg protein per 6 min) of 30.0 ± 0.4. IC_{50} values for L-5-HTP (1454 μM) obtained in the presence of a nearly saturating (250 μM) concentration of L-DOPA were almost 4-fold those obtained when non-saturating (25 μM) concentrations of L-DOPA were used (330). IC_{50} values for cyanine 863 and 3,3′-DOC (638 and 353 μM) obtained in the presence of a nearly saturating (250 μM) concentration of L-DOPA were similar to those obtained when non-saturating (25 μM) concentrations of L-DOPA were used (654 and 339 μM). V_{max} values (in nmol/mg protein per 6 min) for L-DOPA uptake were identical in the absence (36.4 ± 0.7) and the presence of L-5-HTP (39.2 ± 1.3), but K_m values (μM) were significantly greater ($P < 0.05$) when L-DOPA uptake was studied in the presence of L-5-HTP (121 (100, 142) versus 318 (237, 399)). In contrast, the effect of cyanine 863 and 3,3′-DOC was to cause a significant reduction in V_{max} values without significant changes in K_m values. It is concluded that L-5-HTP exerts a competitive type of inhibition of L-DOPA uptake in cultured OK cells, whereas both cyanine 863, an organic cation transport inhibitor and 3,3′-DOC behave as non-competitive inhibitors.

Keywords: OK (Opossum kidney) cell; L-DOPA (L-3,4-dihydroxyphenylalanine); L-5-HTP (L-5-hydroxytryptophan); Cyanine derivative; Cellular transport

1. Introduction

Endogenous dopamine is believed to play an important role as an autocrine/paracrine hormone in regulating sodium and phosphate transport in the proximal nephron (Lee, 1993). The synthesis of dopamine in this area has been demonstrated to result from the decarboxylation of circulating or filtered L-3,4-dihydroxyphenylalanine (L-DOPA) in epithelial cells of proximal convoluted tubules (Baines and Chan, 1980; Hagege and Richet, 1985; Zimlichman et al., 1988; Goldstein et al., 1989; Hayashi et al., 1990; Soares-da-Silva and Fernandes, 1990; Soares-da-Silva, 1994). These renal tubular epithelial cells are endowed with one of the highest aromatic L-amino acid decarboxylase activities in the body. However, the conversion of circulating or filtered L-DOPA into dopamine in renal epithelial cells requires its uptake into this cellular compartment and there is evidence to suggest that the cellular uptake of L-DOPA may be the main factor determining the production of dopamine in this area (Soares-da-Silva, 1994). Early studies on the inward transport of L-DOPA conducted in rat microperfused proximal convoluted tubules demonstrated that the cellular uptake of L-DOPA occurred through an energy-dependent and stereo-selective carrier-mediated process, the rate of transport being 2 fmol/cm/s (Chan, 1976). More recently, we have shown, in isolated rat renal tubules, that the cellular uptake of L-DOPA occurs through a saturable mechanism (Soares-da-Silva et al., 1994; Soares-da-Silva and Pinto-do-O, 1996), is competitively inhibited by other neutral amino acids (Soares-da-Silva and Pinto-do-O, 1996) and is non-competitively inhibited by organic cation inhibitors (Pinto-do-O and Soares-da-Silva, 1996).

Opossum kidney (OK) cells are an established epithelial cell line which has been used as an in vitro model for the study of renal dopaminergic physiology. These cells express proximal tubule cell-like properties in vitro, are endowed with dopamine receptors (Glahn et al., 1993; Nash et al., 1993; Perrichot et al., 1995) and have the

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synthetic and metabolic machinery needed to form and degrade dopamine (Soares-da-Silva et al., 1996; Guimarães et al., 1997). The utilisation of cultured OK cells offers the possibility to precisely study the uptake of substrates through the apical or the basolateral cell borders, which is technically very difficult in isolated renal tubules. It was, therefore, felt worthwhile to study the apical uptake of L-DOPA in OK cells in order to define its kinetics and nature. Another aspect which we would like to clarify, while using cultured OK cells, concerns the type of interaction between L-DOPA and L-5-hydroxytryptophan (L-5-HTP), another neutral amino acid which is actively taken up by renal tubular epithelial cells (Soares-da-Silva and Pinto-do-O, 1996), cyanine 863, an organic cation transport inhibitor (Rennick et al., 1956) which non-competitively inhibits L-DOPA uptake in rat renal tubules (Pinto-do-O and Soares-da-Silva, 1996) and 3,3′-diethyloxacarbocyanine (3,3′-DOC), a cyanine derivative. According to Cheng and Prusoff (1973), K_i and IC_{50} values are identical for non-competitive and uncompetitive inhibitors, but not for competitive inhibitors provided that the substrate concentration exceeds K_m values. In inhibition studies, however, the substrate concentration often does not exceed K_m values. In fact, some authors even report K_i values equal to IC_{50} values for the transporter system when non-saturating concentrations of the substrate are used (Russ et al., 1992). Thus, it was decided to determine in the present study the effects of L-5-HTP, cyanine 863 and 3,3′-DOC in OK cells incubated in the presence of non-saturating and saturating concentrations of L-DOPA.

2. Materials and Methods

2.1. Cell culture

OK cells (ATCC CRL 1840) were obtained from the American Type Culture Collection (Rockville, MD, USA) and maintained in a humidified atmosphere of 5% CO_2–95% air at 37°C. OK cells (passages 36–43) were grown in minimum essential medium without essential amino acids (Sigma, St. Louis, MO, USA) supplemented with 10% fetal bovine serum (Sigma), 100 U/ml penicillin G, 0.25 μg/ml amphotericin B, 100 μg/ml streptomycin (Sigma) and 25 mM *N*-2-hydroxyethylpiperazine-*N*′-2-ethanosulfonic acid (HEPES; Sigma). The cell medium was changed every 2 days and the cells reached confluence after 3–5 days of incubation. For subculturing, the cells were dissociated with 0.05% trypsin-EDTA (Sigma), diluted 1:5 and subcultured in petri dishes with a 21 cm^2 growth area (Costar, Badhoevedorp, The Netherlands). For uptake studies, the cells were seeded in collagen-treated 24-well plastic culture clusters (internal diameter 16 mm, Costar) at a density of 40 000 cells per well (2.0×10^4 cells/cm^2). For 24 h 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 days after initial seeding and each cm^2 contained about 100 μg of cell protein.

2.2. Transport studies

On the day of the experiment, the growth medium was aspirated and the cells were washed with Hanks medium at 4°C; thereafter, the cell monolayers were preincubated for 30 min in Hanks medium at 37°C. The Hanks' medium had the following composition (mM): NaCl, 137; KCl, 5; $MgSO_4$, 0.8; Na2HPO_4, 0.33; KH2PO_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), pargyline (100 μM) and tolcapone (1 μM) in order to inhibit the enzymes aromatic L-amino acid decarboxylase, monoamine oxidase and catechol-*O*-methyltransferase, respectively. 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 (L-DOPA). Time course studies were performed in experiments in which cells were incubated with L-DOPA (0.25 μM) for 1, 3, 6, 12, 30 and 60 min. Saturation experiments were performed in cells incubated for 6 min with increasing concentrations of L-DOPA (10 to 2500 μM). In experiments designed to study the effects of L-5-HTP/cyanine 863 and 3,3′-DOC upon the uptake of L-DOPA, OK cells were preincubated for 30 min in the presence of the compounds to be tested. After preincubation, cells were incubated for 6 min in Hanks medium with 25 or 250 μM L-DOPA. 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.

2.3. Assay of L-DOPA

L-DOPA was quantified by means of high pressure liquid chromatography with electrochemical detection, as previously reported (Pinto-do-O and Soares-da-Silva, 1996; Soares-da-Silva and Pinto-do-O, 1996). The high-pressure liquid chromatograph 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, USA) 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), dibuty-

lamine (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. Detection was carried out electrochemically with a glassy carbon electrode, an Ag/AgCl reference electrode and an amperometric detector (Gilson model 141); the detector cell was operated at 0.75 V. The current produced was monitored using the Gilson 712 HPLC software. The lower limits for detection of L-DOPA ranged from 350 to 500 fmol.

2.4. Cell water content

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

2.5. 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.

2.6. Cell viability

OK cells were preincubated for 30 min (in the presence of L-5-HTP or 3,3′-DOC) at 37°C and then incubated in the absence or the presence of L-DOPA for a further 6 min. Subsequently the cells were incubated at 37°C for 2 min with Trypan blue (0.2%, w/v) in phosphate buffer. Incubation was stopped by rinsing the cells twice with Hanks medium and the cells were examined using a Leica microscope. Under these conditions, more than 95% of the cells excluded the dye.

2.7. Data analysis

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

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

were fitted to the experimental data by a non-linear regression analysis using a computed assisted method (Motulsky et al., 1994). 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 per min. A_{max} is defined as the factor of accumulation (C_i/C_o) at equilibrium (t). K_m and V_{max} values for the uptake of L-DOPA, as determined in saturation experiments, were calculated by non-linear regression analysis, using the GraphPad Prism statistics software package (Motulsky et al., 1994). For the calculation of the IC_{50} values the parameters of the equation for one site inhibition were fitted to the experimental data (Motulsky et al., 1994). K_i values were calculated as defined by Cheng and Prusoff (1973) for competitive or non-competitive inhibition. Arithmetic means are given with S.E.M. or geometric means with 95% confidence values. Statistical analysis was done with a 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.

2.8. Drugs

Drugs used were: Cyanine 863 (Sigma), 3,3′-diethyloxacarbocyanine iodide (Aldrich, Milwaukee, WI, USA), L-DOPA (Sigma), L-5-hydroxytryptophan (Sigma), pargyline hydrochloride (Sigma), tolcapone (kindly donated by the late Professor Mosé Da Prada, Hoffman La Roche, Basle, Switzerland).

3. Results

To determine the rate constant of total inward (k_{in}) and outward (k_{out}) transport, OK cells were incubated with L-DOPA (0.25 μM) for 1, 3, 6, 12, 30 and 60 min (Fig. 1). L-DOPA was rapidly accumulated in OK cells and the accumulated substrate was found not to be decarboxylated to its corresponding amine; equilibrium was attained at 30 min of incubation. From the initial rate of uptake, k_{in}, k_{out} and an equilibrium factor of accumulation (A_{max}) were calculated: $k_{in} = 8.9 \pm 0.5$ pmol/mg protein per min, $k_{out} = 2.5 \pm 0.1$ pmol/mg protein per min and $A_{max} = 50.3 \pm 4.6$. The intracellular water content of OK cell monolayers

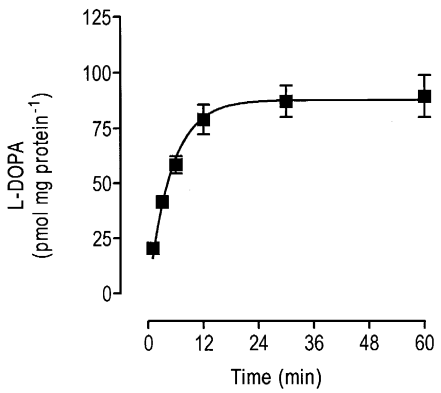

Fig. 1. Time-course of L-DOPA accumulation in OK cells. Cells were incubated at 37°C with 0.25 μM L-DOPA. The results reflect levels (in pmol/mg protein) of L-DOPA accumulated. Each point represents the mean of five experiments per group; vertical lines show S.E.M.

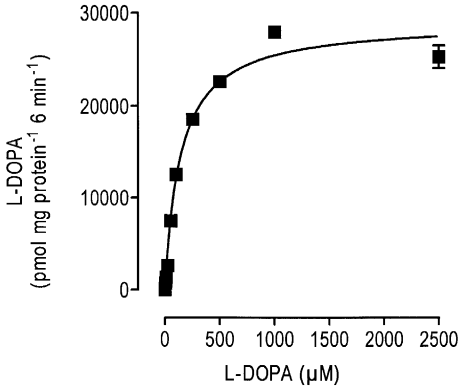

Fig. 2. Accumulation of L-DOPA in OK cells. Cells were incubated for 6 min at 37°C and increasing concentrations (10–2500 μM) of the substrate were applied from the apical border. The results reflect levels (in pmol/mg protein per 6 min) of accumulated L-DOPA. Each point represents the mean of five experiments per group; vertical lines show S.E.M.

was 6.8 ± 0.7 μl/mg protein ($n = 12$). At equilibrium (30 min incubation), the intracellular L-DOPA concentration in OK cells was 12.6 ± 1.2 μM, at a medium concentration of 0.25 μM. This represented a cell concentration of L-DOPA in OK cells that was 50 times higher than the medium concentration.

In time-course experiments, the accumulation of L-DOPA increased linearly with time for several minutes (see Fig. 1). Thus, in all subsequent experiments designed to determine the kinetic parameters of L-DOPA uptake, the cells were incubated for 6 min with increasing concentrations (10 to 2500 μM) of the substrate. The accumulation of L-DOPA was found to be dependent on the concentration used and to be saturable at nearly 500 μM (Fig. 2). Again, accumulated L-DOPA was found not to be decarboxylated to its corresponding amine, dopamine. Non-linear analysis of the saturation curve revealed for L-DOPA a K_m of 129 μM (114, 145; 95% confidence limits, $n = 5$) and a V_{max} of 30.0 ± 0.4 nmol/mg protein per 6 min.

Fig. 3A shows inhibition curves for L-5-HTP obtained in the presence of a non-saturating (25 μM) and a nearly saturating (250 μM) concentration of L-DOPA. The inhibition curve obtained with a saturating concentration of

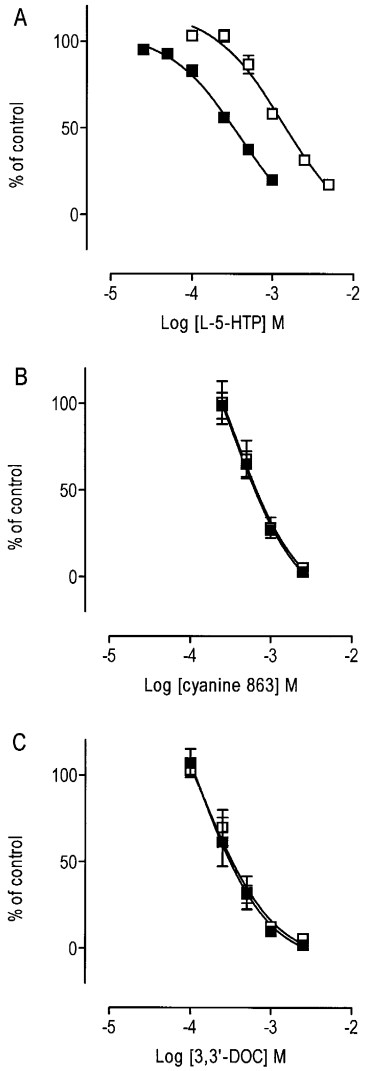

Fig. 3. Inhibition curve of L-DOPA uptake (closed squares, 25 μM; open squares, 250 μM) by (A) L-5-HTP, (B) cyanine 863 and (C) 3,3′-diethyloxacarbocyanine (3,3′-DOC) in OK cells; the substrates were applied from the apical cell border only. Symbols represent means of five experiments per group; vertical lines show S.E.M.

L-DOPA was shifted to the right from that obtained with 25 μM L-DOPA and IC_{50} values were almost 4-fold those obtained when non-saturating concentrations of the sub-

Table 1
IC_{50} and/or K_i values for inhibition of L-DOPA uptake by L-5-HTP, cyanine 863 and 3,3′-diethyloxacarbocyanine, determined in the presence of non-saturating (25 μM) or nearly saturating (250 μM) concentrations of the substrate in cultured OK cells. Results are geometric means with 95% confidence values

Substrate	L-DOPA (μM)	IC_{50} (μM)	K_i (μM)
L-5-HTP	25	330 (293, 371)	264 (234, 297)
	250	1454 (1214, 1742)	415 (347, 498)
Cyanine 863	25	654 (502, 852)	
	250	638 (430, 947)	
3,3́-diethyloxacarbocyanine	25	339 (184, 627)	
	250	353 (234, 531)	

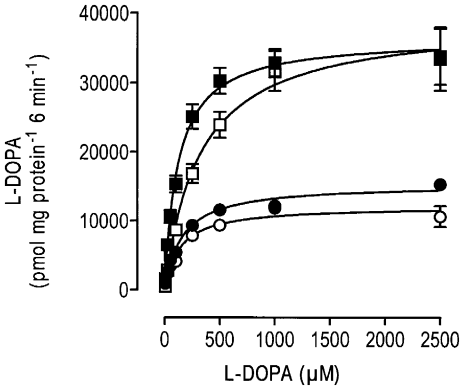

Fig. 4. Accumulation of L-DOPA in the absence (closed squares) and the presence of 250 μM L-5-HTP (open squares), 600 μM cyanine 863 (closed circles) and 300 μM 3,3′-diethyloxacarbocyanine (open circles) in OK cells. Cells were incubated for 6 min at 37°C and increasing concentrations (10 to 2500 μM) of the substrate were applied from the apical border. Symbols represent means of five experiments per group; vertical lines show S.E.M.

strate were used (see Table 1). According to Cheng and Prusoff (1973), this is the profile for a competitive type of inhibition. For the calculation of K_i values we then used the expression $K_i = IC_{50}/(1 + S/K_m)$, which applies for competitive inhibitors. Table 1 shows IC_{50} and K_i values for L-5-HTP and it is interesting to note that for non-saturating concentrations of the substrate IC_{50} values equalled K_i values, but for the nearly saturating concentration of the inhibitor IC_{50} values were greater than K_i values. Fig. 3B and C show inhibition curves for cyanine 863 and 3,3′-DOC obtained in the presence of 25 and 250 μM L-DOPA. For both compounds, the inhibition curves recorded in the presence of 25 and 250 μM L-DOPA were coincident and IC_{50} values quite similar (see Table 1). Cyanine 863 was, however, less potent than 3,3′-DOC. According to Cheng and Prusoff (1973), this is the profile for a non-competitive type of inhibition and thus $K_i = IC_{50}$.

Fig. 4 shows saturation curves for the uptake of L-DOPA in the absence and the presence of a concentration of L-5-HTP, cyanine 863 or 3,3′-DOC equal to the K_i value, as determined in inhibition studies. As can be observed in this figure, V_{max} values (in nmol/mg protein per 6 min) for L-DOPA uptake were identical in the absence (36.4 ± 0.7) and the presence of L-5-HTP (39.2 ± 1.3), but K_m values (μM) were significantly greater ($P < 0.05$) when L-DOPA uptake was studied in the presence of L-5-HTP (121 (100, 142) versus 318 (237, 399)). In contrast, the effect of cyanine 863 and 3,3′-DOC was to cause a significant reduction in V_{max} values without causing significant changes in K_m values (Table 2).

Table 2
K_m and V_{max} values for the saturable component of L-DOPA uptake in cultured OK cells under control conditions and in the presence of L-5-HTP, cyanine 863 and 3,3′-diethyloxacarbocyanine (3,3′-DOC). Values are arithmetic means ± S.E.M. (V_{max}) or geometric means with 95% confidence limits (K_m) of 6 to 12 experiments per group

	K_m (μM)	V_{max} (nmol/mg protein per 6 min)
Control	121 (100, 142)	36.5 ± 0.7
L-5-HTP	318 (237, 399) [a]	39.2 ± 1.3
Cyanine 863	160 (98, 222)	15.3 ± 0.7 [a]
3,3′-DOC	139 (67, 211)	12.1 ± 0.7 [a]

[a] Significantly different ($P < 0.05$) from corresponding control values using the Newman–Keuls test.

4. Discussion

The results presented here show that OK cells transport quite efficiently L-DOPA through the apical cell border and several findings demonstrate that this uptake process is a facilitated mechanism. Firstly, steady-state uptake of non-saturating concentrations of L-DOPA showed a curvilinear dependence on incubation time. Secondly, at an initial rate of uptake (6 min incubation) the cellular transport of L-DOPA showed a curvilinear dependence on L-DOPA medium concentration, suggesting that the uptake was saturable. Thirdly, the uptake of L-DOPA was markedly inhibited by L-5-HTP, cyanine 863 and 3,3′-DOC. The efficiency of this L-DOPA transport in OK cells can be also 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 a 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.

Several findings suggest that the type of inhibition by L-5-HTP on L-DOPA uptake is of the competitive type. Firstly, IC_{50} values for L-5-HTP were significantly greater when inhibition curves were recorded in the presence of a nearly saturating concentration of the substrate. This is in full agreement with the finding that only K_m values, but not V_{max} values, for L-DOPA uptake were changed when saturation experiments were performed in the presence of a concentration of the inhibitor equal to K_i values. By contrast, cyanine 863 and 3,3′-DOC are suggested to produce a non-competitive inhibition of L-DOPA uptake. IC_{50} values for cyanine 863 and 3,3′-DOC were similar when the corresponding inhibition curves were recorded in the presence of non-saturating or nearly saturating concentrations of L-DOPA. This agrees with the findings that only V_{max} values, but not K_m values, for L-DOPA uptake were changed when saturation experiments are performed in the presence of a concentration of the inhibitor equal to the K_i value. The most likely explanation for the competitive inhibition produced by L-5-HTP upon L-DOPA uptake is that both compounds share the same transporter(s) for uptake, which is probably due to their structural similarities (Fig. 5). In fact, rat renal tubular epithelial cells have been reported to take up L-5-HTP and L-DOPA with similar kinetics and L-DOPA has been shown to inhibit the

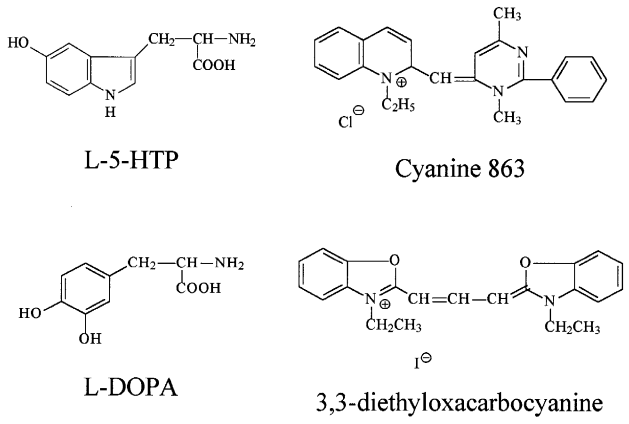

Fig. 5. Structural formulae of L-DOPA, L-5-HTP, cyanine 863 and 3,3′-diethyloxacarbocyanine.

uptake of L-5-HTP (Soares-da-Silva and Pinto-do-O, 1996). The inhibitory profile of cyanine 863 and 3,3′-DOC is, in contrast to that for L-5-HTP, that of a non-competitive inhibition. Cyanine 863 is a well known organic cation transport inhibitor (Rennick et al., 1956) and evidence recently available shows that this compound exerts an even more potent inhibitory effect upon the extraneuronal uptake of catecholamines (Martel et al., 1994; Russ et al., 1993). It is not known whether this inhibitory effect of cyanine 863 on catecholamine uptake is competitive or non-competitive. However, cyanine 863 was demonstrated to potently inhibit L-DOPA uptake by rat renal tubular epithelial cells, the effect being a non-competitive one (Pinto-do-O and Soares-da-Silva, 1996). In rat renal tubular epithelial cells, cyanine 863 was 300-fold more potent than in OK cells; the type of mechanism involved in inhibition of L-DOPA uptake is poorly understood, but it is pH dependent (Pinto-do-O and Soares-da-Silva, 1996). The present work shows that 3,3′-DOC, a cyanine 863-related compound (see Fig. 5), was 2-fold more potent than cyanine 863 in inhibiting L-DOPA uptake in OK cells and that this inhibitory effect had the characteristics of non-competitive inhibition. It is suggested that both compounds may constitute interesting research tools in the study of L-DOPA cellular transport mechanisms. This is obviously of relative importance considering the view that patients with salt-sensitive hypertension are believed to have a defect in renal tubular uptake/decarboxylation of filtered L-DOPA (Shikuma et al., 1986; Shigetomi et al., 1991; Kuchel and Shigetomi, 1992; Lee, 1993; Sakamoto et al., 1994).

The data presented here also give the opportunity to address the question concerning the relationship between IC_{50} values and K_i values and their utility in the study of a given inhibitor. IC_{50} values depend on the concentration of the substrate used and the use of K_i values offers a way to compare data from different laboratories. However, the meaning of IC_{50} values and K_i values may be questionable if no information is available on the type of inhibition produced by a given compound. It is classically accepted that a competitive inhibitor does not change V_{max} values and increases K_m values, as a result of a decrease in the affinity of the transporter or enzyme for the substrate. Similarly, for a competitive inhibitor IC_{50} values will be greater than K_i values when the concentration of the substrate is reaching saturation. IC_{50} values will equal K_i values when non-saturating concentrations of the substrate are used. This is valid for a competitive inhibitor, but not for non-competitive inhibitors. For non-competitive inhibition, the affinity of the substrate for the transporter or the enzyme is not altered (K_m values do not change), but the V_{max} values are reduced. In this particular case, K_i values will equal IC_{50} values, despite the concentration of the substrate. One might conclude, therefore, for the sake of simplification of the analysis of the inhibitory behaviour of a given compound that by measuring inhibition curves at non-saturating and saturating concentrations of the substrate one will obtain the very same type of information which can be obtained by measuring saturation curves in the absence and the presence of the inhibitor. The main disadvantage of the classical method (the saturation curve in the presence of the inhibitor) is that one has first to determine which is the best concentration of the inhibitor to be used and this may cause some concern in certain circumstances. The example reported here also provides some information on this and shows that a concentration of the inhibitor equal to the corresponding K_i value is enough to produce the expected changes in V_{max} or K_m, if the compound is behaving as a non-competitive or competitive inhibitor, respectively. This line of reasoning does not apply to other type of inhibitors, such as tight-binding and slow-binding inhibitors (Cha, 1975)

Another point worth mentioning concerns the use of OK cells as model of renal proximal tubular cells for the study of L-DOPA uptake. It is interesting to note that in isolated rat renal tubules K_m values (in μM) for the uptake of L-DOPA (135 (97, 188)) reported by our group (Soares-da-Silva and Pinto-do-O, 1996) are not significantly different from those reported here for OK cells. This agrees with the accumulated evidence suggesting that OK cells might constitute an interesting in vitro model for the study of renal dopaminergic mechanisms (Glahn et al., 1993; Nash et al., 1993; Perrichot et al., 1995; Soares-da-Silva et al., 1996; Guimarães et al., 1997).

In conclusion, OK cells transport L-DOPA quite efficiently through the apical cell border and L-5-HTP exerts a competitive type of inhibition of L-DOPA uptake, whereas both cyanine 863 and 3,3′-DOC behave as non-competitive inhibitors.

Acknowledgements

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