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Nature and kinetic characteristics of L-DOPA uptake in rat renal proximal tubules

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Summary. The present study was performed with the aim to determine the kinetics and the caracteristics of cellular uptake of L-3,4-dihydroxyphenylalanine (L-DOPA) in rat renal proximal tubules. Incubation of renal tubules at 4°C in the presence of increasing concentrations of L-DOPA results in a linear and concentration-dependent accumulation of the substrate. In experiments carried out at 37°C, the accumulation of L-DOPA in renal tubules was found to be greater than that occurring at 4°C and showed a trend for saturation. The saturable component of L-DOPA uptake was derived from the total amount of L-DOPA accumulated in renal tubules at 37°C subtracted with the values obtained in experiments conducted at 4°C. The V_{max} and K_m values for the saturable component of L-DOPA uptake in renal tubules were, respectively, 241 ± 32 fmol μg protein^{-1}min^{-1} and 567 ± 63 μM. Cyanine 863 (5 and 10 μM) was found to decrease the tubular uptake of L-DOPA, whereas probenecid (50 μM) did not change the rate of uptake of L-DOPA into renal tubules. The V_{max} and K_m values for the saturable component of L-DOPA uptake in renal tubules incubated in the presence of 10 μM cyanine 863 were, respectively, 97 ± 11 fmol μg protein^{-1}min^{-1} and 160 ± 22 μM. It is suggested that the anionic L-DOPA may behave as an amphoteric substance, both hydroxyl groups in the aromatic ring determining the binding of the molecule to the organic cation transporter.

Keywords: Amino acids – Renal tubules – L-DOPA – Uptake – Cyanine 863 – Probenecid

Introduction

Epithelial cells of renal proximal convoluted tubules are endowed with a high aromatic L-amino acid decarboxylase (AAAD) activity and synthetize dopamine from filtered or circulating L-3,4-dihydroxyphenylalanine (L-DOPA). Dopamine of renal origin has been demonstrated to exert marked natriuretic effects as a result of the activation of specific receptors located in

renal tubules and involving the inhibition of the Na^+-H^+ exchanger and/or inhibition of the Na^+-K^+ ATPase. Because the dopamine produced in this area is in close proximity to renal cells which contain receptors for the amine, it has been hypothetized that this dopamine may act in a cell-to-cell manner, as a paracrine or autocrine substance. The intrinsic mechanisms involved in the regulation of dopamine formation in these cells is far from being completely understood. There is, however, evidence suggesting that the synthesis of dopamine in tubular epithelial cells is in fact submitted to some sort of regulation, namely on the renal delivery of sodium (for reviews, see Jose et al., 1992; Lee, 1993). In line with this view, we have described in both human and rat kidney preparations that the production of dopamine is not only closely dependent on the extracellular sodium, but also appears to be related to the transtubular reabsorption of sodium (Soares-da-Silva and Fernandes, 1992; Soares-da-Silva et al., 1993a,b) and on the integrity of the tubular cytoskeleton and the functional integrity of the Na^+-K^+-ATPase (Soares-da-Silva, 1992).

Early studies on the inward transport of L-DOPA in tubular epithelial cells were conducted in rat microperfused proximal convoluted tubules. Using this preparation, Chan (1976) was able to demonstrate 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^{-1} s^{-1}. There is, however, lack of objective information concerning the mechanism(s) involved and the nature of the process(es) of cell membrane transport of L-DOPA in epithelial cells of renal tubules. The present work reports on the results of experiments carried out in suspensions of isolated rat renal proximal tubules with the aim to define the kinetics and caracteristics of cellular uptake of L-DOPA.

Methods

Uptake studies of L-DOPA into renal tubules were performed in male Wistar rats (Biotério do Instituto Gulbenkian de Ciência, Oeiras, Portugal), aged 45–60 days, given benserazide (10 mg kg^{-1}, i.p.) 30 min before the sacrifice, in order to inhibit AAAD. Animals were sacrificed by decapitation under ether anaesthesia and the kidneys removed through a midline abdominal incision, after which they were decapsulated and cut in half and placed in ice-cold Collins solution [containing (in mM): KH_2PO_4 15, K_2HPO_4 50, KCl 15, $NaHCO_3$ 15, $MgSO_4$ 60 and glucose 140]. The outer cortex was cut out with fine scissors and minced with a scalpel into a fine paste. The cortical paste was filtered sequentially through a series of Nybolt nylon sieves, first 180 μm and then 75 μm. Unseparated cortex remained on the upper (180 μm) sieve, while the lower (75 μm) one retained predominantly proximal nephron segments. The sieves were continuously rinsed with cold Collins solution through the procedure. The retained tubules were then washed off with cold Collins solution and collected into a pellet by centrifugation at 200 g, 4°C; renal tubules used in incubation experiments were suspended in Hanks' medium. 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 $C_4H_7NO_2$ 1.0; tolcapone (1 μM) was also added to the Hanks' medium in order to inhibit the enzyme catechol-O-methyltransferase. The viability of proximal renal tubules used in this study was assessed by the tripan blue (0.2% wt/vol) exclusion method; nephron segments were pipetted onto

a glass slide and observed 90 s after exposure to the dye, using a Leica inverted microscope. Under these conditions, greater than 90% of the renal tubules excluded the dye.

The preincubation and incubation of renal tubules (500 µl) were carried out in glass test tubes, continuously shaken and gassed (95% O_2 and 5% CO_2) throughout the experiment. Some experiments were performed with the aim to define the kinetic characteristics of the tubular uptake of L-DOPA. After preincubation, renal tubules were incubated for 15 min in Hanks' medium with increasing concentrations (10 to 2000 µM) of L-DOPA. The non-saturable component of L-DOPA uptake was determined in experiments conducted at 4°C; the saturable component was derived from the total amount of L-DOPA accumulated in renal tubules at 37°C, subtracted with the values obtained for the non-saturable component. In experiments performed to test the influence of cyanine 863 (5 and 10 µM) and probenecid (50 µM) on the tubular uptake of L-DOPA, the compounds were present during preincubation and incubation. As mentioned above, the saturable component of L-DOPA uptake was derived from the total amount of L-DOPA accumulated in renal tubules at 37°C subtracted with the values obtained in paired experiments conducted at 4°C. The incubation was stopped by cooling and an aliquot (300 µl) of the incubation medium containing the renal tubules was immediately transfered to Spin-X (Costar) centrifuge filter (0.22 µm) tubes and centrifuged (4 min, 3000°C); the renal tubules retained in the filter were washed twice with ice-cold Hanks' medium (500 µl). The extraction of L-DOPA from renal tubules was performed by the addition of 500 µl of 0.2 M perchloric acid to the Spin-X centrifuge filter tubes at the end of the second washing period; the centrifuge filter tubes were then centrifuged (4 min, 3000 g, 4°C); the filtered aliquot was then injected directly into the column of a high pressure liquid chromatograph for the quantification of L-DOPA. The filters were found not to retain L-DOPA and the extraction process employed was also found to completely remove it from the renal tubules. No formation of dopamine was also found to occur, even when 2000 µM L-DOPA was used.

The assay of L-DOPA was performed by means of high pressure liquid cromatography with electrochemical detection, as previously described (Soares-da-Silva et al., 1993a). The lower limit for detection of L-DOPA was 500 fmol. The protein content of the suspensions of renal tubules was determined by the method of Bradford (1976), with human serum albumin as a standard.

Statistical analysis of the data was performed using the Tuckey-Kramer method and expressed as mean ± s.e. mean. AP value less than 0.05 was assumed to denote a significant difference. V_{max} and K_m values for the saturable component of L-DOPA uptake was calculated by linear analysis regression.

L-3,4-dihydroxyphenylalanine (L-DOPA), cyanine 863 and probenecid were purchased from Sigma Chemical Company (St. Louis, Mo, USA); tolcapone was kindly donated by the producer (Hoffmann La Roche, Basle, Switzerland).

Results

Incubation of renal tubules at 4°C in the presence of increasing concentrations of L-DOPA results in a linear and concentration-dependent accumulation of the substrate (Figure 1). When the experiments were carried out at 37°C, the accumulation of L-DOPA in renal tubules was found to be greater than that occurring at 4°C and showed a trend for saturation (Fig. 1). The saturable component of L-DOPA uptake (Fig. 2) was derived from the total amount of L-DOPA accumulated in renal tubules at 37°C subtracted with the values obtained in experiments conducted at 4°C. The V_{max} and K_m values for the saturable component of L-DOPA uptake in renal tubules were, respectively, 241 ± 32 fmol µg protein^{-1}min^{-1} and 567 ± 63 µM.

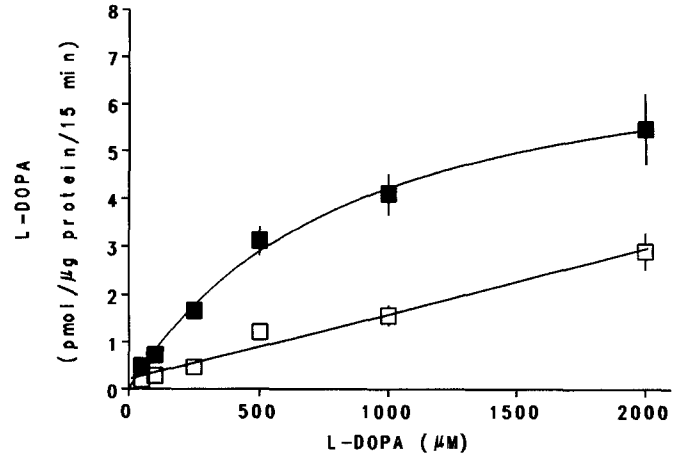

Fig 1. Accumulation of L-DOPA in suspensions of isolated rat proximal convoluted tubules obtained in experiments conducted at 4°C (open squares) and at 37°C (closed squares). Each point is the mean of five experiments per group; vertical lines show s.e. mean

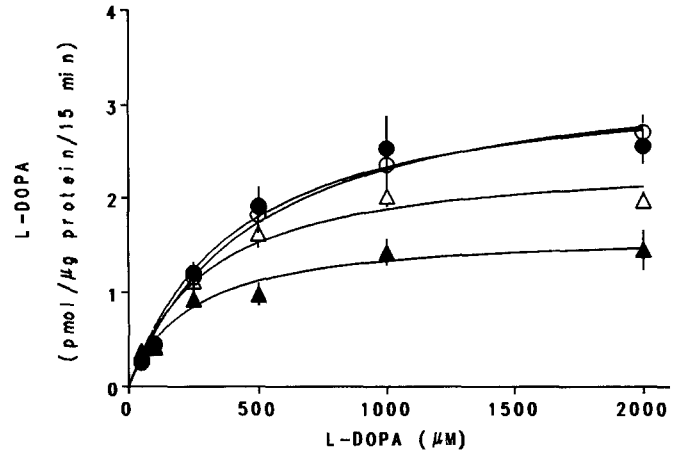

Fig. 2. Uptake of L-DOPA through a saturable transport system in suspensions of isolated rat proximal convoluted tubules in control conditions (closed circles) and in the presence of probenecid (50 µM, open circles) or cyanine 863 (5 µM, open triangles; 10 µM, closed triangles). The saturable component was derived from the total amount of L-DOPA accumulated in renal tubules at 37°C subtracted with the values obtained for the non-saturable component. The non-saturable component of L-DOPA accumulation was obtained in paired experiments conducted at 4°C and was found to be linear with the concentration of the substrate. The V_{max} and K_m values for the saturable component of L-DOPA uptake in renal tubules incubated in control conditions were, respectively, 241 ± 32 fmol µg protein^{-1}min^{-1} and 567 ± 63 µM and in the presence of 10 µM cyanine 863, respectively, 97 ± 11 fmol µg protein^{-1}min^{-1} and 160 ± 22 µM. Each point is the mean of five to seven experiments per group; vertical lines show S.E.M.

Figure 2 shows the effect of cyanine 863 (5 and 10 µM) and probenecid (50 µM) on the saturable component of L-DOPA uptake into renal proximal tubules. Cyanine 863 was found to decrease in a concentration dependent manner the tubular uptake of L-DOPA, whereas probenecid did not change

the uptake of L-DOPA into renal tubules. The V_{max} and K_m values for the saturable component of L-DOPA uptake in renal tubules incubated in the presence of 10 μM cyanine 863 were, respectively, 97 ± 11 fmol μg protein^{-1}min^{-1} and 160 ± 22 μM; both values were found to be significantly different from those obtained in control conditions ($P < 0.01$). The accumulation of L-DOPA obtained in experiments conducted at 4°C was found not to be affected by cyanine 863 (data not shown).

Discussion

The results presented here show that the tubular uptake of L-DOPA occurs through non-saturable and saturable mechanisms. Only the saturable tubular uptake of L-DOPA was found to be inhibited by cyanine 863, probenecid being ineffective in reducing the accumulation of L-DOPA occurring through the saturable transporter.

To our knowledge this is the first study reporting on the kinetics and characteristics of L-DOPA uptake in isolated renal proximal tubules and provides evidence in favour of the view that the tubular uptake of L-DOPA, rather than its decarboxylation into dopamine, may function as the rate limiting step in the formation of renal dopamine. In fact, the rate of decarboxylation of L-DOPA (V_{max} = 5.5 ± 0.8 pmol μg protein^{-1}min^{-1}; unpublished observations) is 22-fold the rate of its uptake through the saturable component (V_{max} = 241 ± 32 fmol μg protein^{-1}min^{-1}). In previous studies, we have used kidney slices instead of isolated renal tubules and information on the tubular transport of L-DOPA was indirectly obtained from the formation of dopamine, since no inhibition of AAAD was performed. The results obtained in these experiments have suggested that the tubular uptake of L-DOPA was a sodium-dependent and ouabain-sensitive mechanisms (Soares-da-Silva and Fernandes, 1992; Soares-da-Silva et al., 1993a,b). Both the sodium-dependent and the ouabain-sensitive mechanisms of dopamine formation were found to be completely abolished by anoxia (Soares-da-Silva and Fernandes, 1992). However, almost up to 50% of dopamine formation in kidney slices loaded with L-DOPA was affected neither by ouabain nor by anoxia; this was considered to reflect the amount of L-DOPA entering the tubular epthelial cells through diffusion. The results presented here in isolated proximal renal tubules show that the magnitude of saturable tubular uptake of L-DOPA is of considerable proportions, at the highest concentrations of L-DOPA used (1 and 2 mM), being similar to that occurring through the non-staurable component. It should be considered, however, that some of the L-DOPA accumulated in renal tubules in experiments conducted at 4°C could be related to the binding of the substrate to cell structures.

The finding that cyanine 863, but not probenecid, was found to reduce the tubular uptake of L-DOPA through the saturable transporter, without effect upon the accumulation of the susbtrate in experiments carried out at 4°C, suggests the involvement of an organic cation transport system in the tubular uptake of L-DOPA. The type of inhibition appears to be a non-competitive

one as evidenced by the simultaneous reduction of V_{max} and K_m values. This is an interesting finding if one particularly considers the fact that L-DOPA is classically assumed as an organic anion, which is related to the presence of a terminal carboxyl group in the lateral chain of the molecule. It could be argued, however, that L-DOPA for its transport in renal tubules behaves as an amphoteric substance, both hydroxyl groups in the aromatic ring determining the binding of the molecule to the organic cation transporter. Another aspect which we have programmed to evaluate in future work concerns the possibility that the inhibitory effect of cyanine 863 on the uptake of L-DOPA is related to the inhibition of the extraneuronal uptake system for catecholamines. In fact, this compound has been recently demonstrated to inhibit this uptake system with an inhibitory potency greater than that of corticosterone (Russ et al., 1992); however, L-DOPA lacks an amine group in its molecule which is believed to be crucial for the binding to the transporter.

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