Cell inward transport of L-DOPA and 3-O-methyl-L-DOPA in rat renal tubules

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1 The present study has determined the kinetics of the uptake of L-3,4-dihydroxyphenylalanine (L-DOPA) and 3-O-methyl-L-DOPA (3-OMDOPA) in rat renal tubules and examined the effect of 3-OMDOPA on the inward transport of L-DOPA and on its conversion into dopamine in kidney homogenates.

2 The accumulation of both L-DOPA and 3-OMDOPA in renal tubules was found to occur through non-saturable and saturable mechanisms. The kinetics of the saturable component of L-DOPA and 3-OMDOPA uptake in renal tubules were as follows: L-DOPA, $V_{\max} = 11.1 \text{ nmol mg}^{-1} \text{ protein h}^{-1}$ and $K_m = 216 \mu\text{M}$ ($n = 6$); 3-OMDOPA, $V_{\max} = 8.1 \text{ nmol mg}^{-1} \text{ protein h}^{-1}$ and $K_m = 231 \mu\text{M}$ ($n = 5$). The diffusion constant of the non-saturable component for the accumulation of L-DOPA and 3-OMDOPA was 0.0010 and $0.0014 \mu\text{mol}^{-1}$, respectively.

3 3-OMDOPA (100 to $2000 \mu\text{M}$) was found to produce a concentration-dependent decrease (29% to 81% reduction) of the saturable component of the tubular uptake of L-DOPA; the K_i value of 3-OMDOPA for inhibition of L-DOPA uptake was found to be $181 \mu\text{M}$ ($n = 5$). The accumulation of L-DOPA obtained in experiments conducted at 4°C was not affected by 3-OMDOPA.

4 In experiments conducted in kidney homogenates only L-DOPA (10 to $5000 \mu\text{M}$) was found to be decarboxylated. The $V_{\max}$ and K_m values for aromatic L-amino acid decarboxylase determined in the absence of 3-OMDOPA ($V_{\max} = 14.1 \text{ nmol mg}^{-1} \text{ protein h}^{-1}$; $K_m = 62 \mu\text{M}$) were not significantly different from those observed when the decarboxylation of L-DOPA was carried out in the presence of $1000 \mu\text{M}$ 3-OMDOPA ($V_{\max} = 15.7 \text{ nmol mg}^{-1} \text{ protein h}^{-1}$; $K_m = 68 \mu\text{M}$).

5 It is concluded that the tubular uptake of both L-DOPA and 3-OMDOPA occur through non-saturable and saturable mechanisms; only the saturable tubular uptake of L-DOPA was found to be inhibited by 3-OMDOPA. It is further shown that 3-OMDOPA neither undergoes decarboxylation into 3-MT nor affects the decarboxylation of L-DOPA.

Keywords: L-DOPA; 3-O-methyl-L-DOPA (3-O-methylDOPA); kidney; dopamine

Introduction

After almost three decades of clinical use, L-3,4-dihydroxyphenylalanine (L-DOPA) in combination with a peripheral aromatic L-amino acid decarboxylase (AAAD) inhibitor still remains widely used in the treatment of Parkinson's disease. Major problems with this type of therapy are the L-DOPA-induced dyskinesias which it has been suggested are related to kinetic factors that affect the absolute and relative plasma concentrations of L-DOPA and 3-O-methyl-L-DOPA (3-OMDOPA) (Furgeson *et al.*, 1976; Rivera-Calimlim *et al.*, 1977); patients with L-DOPA-induced dyskinesias have been reported to present higher plasma concentrations of 3-OMDOPA (Reilly *et al.*, 1980). Although the decarboxylation of L-DOPA is believed to represent a major pathway of its metabolism, methylation into 3-OMDOPA by catechol-O-methyltransferase (COMT) may also occur, this assuming particular relevance in conditions of AAAD inhibition (Sharpless *et al.*, 1973; Männistö & Kaakkola, 1989). The proposed mechanisms through which 3-OMDOPA may predispose to dyskinesias include inhibition of L-DOPA uptake at the blood-brain barrier and competition for decarboxylation by AAAD (Reilly *et al.*, 1980).

In kidney, it has been suggested that dopamine may be of some physiological importance in the regulation of tubular reabsorption of sodium (Lee, 1993). The synthesis of dopamine in renal tissues has been demonstrated to result from the decarboxylation of circulating or filtered L-DOPA in epithelial cells of proximal convoluted tubules. This type

of cell is endowed with high AAAD activity and the formation of dopamine is believed to occur after the uptake of L-DOPA into this cellular compartment (Soares-da-Silva & Fernandes, 1990). The tubular uptake of L-DOPA has been demonstrated to occur through an energy-dependent and stereo-selective carrier-mediated process, the rate of transport being $2 \text{ fmol cm}^{-1} \text{ s}^{-1}$ (Chan, 1976). 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. However, given the considerable high AAAD in renal tissues (Soares-da-Silva *et al.*, 1993), it is possible that the rate limiting step in the renal formation of dopamine from plasma L-DOPA resides in the cell inward transport of the substrate.

The aim of the present work was to determine the nature and the kinetic characteristics of the tubular uptake of both L-DOPA and of its O-methylated derivative 3-OMDOPA. In the studies reported here we took advantage of the fact that 3-OMDOPA is the only compound known to inhibit the cell inward transport of L-DOPA at the level of the blood-brain barrier, and decided to examine also the extent to which 3-OMDOPA is inhibiting the tubular uptake of L-DOPA. In addition, given the structural similarities between L-DOPA and 3-OMDOPA we thought it of interest to study the extent to which these compounds undergo decarboxylation by renal AAAD.

Methods

Uptake studies of L-DOPA and 3-OMDOPA into renal tubules were performed in male Wistar rats (Biotério do

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Instituto Gulbenkian de Ciência, Oeiras, Portugal), aged 45–60 days, given benzerazide (10 mg kg^{-1} , i.p.) 60 min before they were killed, in order to inhibit peripheral AAAD (DaPrada *et al.*, 1987). In some experiments, the uptake of 3-OMDOPA was performed in renal tubules obtained from rats given saline ($0.9\% \text{ NaCl}$), instead of benzerazide, 60 min before they were killed. Animals were killed by decapitation under ether anaesthesia and the kidneys removed through a midline abdominal incision, after which they were decapsulated, 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, $\text{pH} = 7.4$]. 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 $180 \mu\text{m}$ and then $75 \mu\text{m}$. Unseparated cortex remained on the upper ($180 \mu\text{m}$) sieve, while the lower one ($75 \mu\text{m}$) retained predominantly proximal nephron segments. The sieves were continuously rinsed with cold Collins solution throughout. The retained tubules were then washed off with cold Collins solution and collected into a pellet by centrifugation at 200 g , for 5 min, 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 sodium butyrate 1.0, $\text{pH} = 7.4$. Pargyline (0.1 mM) and tolcapone ($1 \mu\text{M}$) were also added to the Hanks medium in order to inhibit the enzymes, monoamine oxidase (MAO) and COMT, respectively (Fernandes *et al.*, 1991; Soares-da-Silva & Vieira-Coelho, 1993). The viability of proximal renal tubules used in this study was assessed by the trypan blue ($0.2\% \text{ w/v}$) exclusion method; nephron segments were pipetted on to a glass slide and observed 90 s after exposure to the dye, with a Leica microscope. Under these conditions, more than 90% of the renal tubules excluded the dye.

The preincubation and incubation of renal tubules ($500 \mu\text{l}$) were carried out in glass test tubes, continuously shaken and gassed ($95\% \text{ O}_2$ and $5\% \text{ CO}_2$) throughout the experiment. Some experiments were performed to define the kinetic characteristics of the tubular uptake of L-DOPA and 3-OMDOPA. The non-saturable component of L-DOPA and 3-OMDOPA uptake was determined in experiments conducted at 4°C ; the saturable component was derived from the total amount of L-DOPA and 3-OMDOPA accumulated in renal tubules at 37°C , subtracted with the values obtained for the non-saturable component. After preincubation, renal tubules were incubated for 15 min in Hanks medium with increasing concentrations (50 to $2000 \mu\text{M}$) of L-DOPA or 3-OMDOPA. The incubation was stopped by cooling and an aliquot ($300 \mu\text{l}$) of the incubation medium containing the renal tubules was immediately transferred to Spin-X (Costar) centrifuge filter ($0.22 \mu\text{m}$) tubes and centrifuged (4 min , 3000 g , 4°C); the renal tubules were washed twice with ice-cold medium ($500 \mu\text{l}$). The extraction of L-DOPA and 3-OMDOPA from renal tubules was performed by the addition of $500 \mu\text{l}$ 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 and $50 \mu\text{l}$ of the filtered aliquot containing the compounds of interest was then injected directly into the column of a high pressure liquid chromatograph for the quantification of L-DOPA and 3-OMDOPA. The filters were found not to retain L-DOPA or 3-OMDOPA and the extraction process employed was also found to remove both compounds completely from the renal tubules.

In experiments conducted with the aim of studying the interference of 3-OMDOPA with the tubular uptake of L-DOPA, renal tubules were preincubated for 15 min with increasing concentrations of 3-OMDOPA (50 , 100 , 250 , 500 , 1000 and $2000 \mu\text{M}$); the incubation was performed in the presence of $250 \mu\text{M}$ L-DOPA. 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 . At the end of the incubation, renal tubules were washed twice with ice-cold Hanks medium and the assay of L-DOPA and 3-OMDOPA was performed as described above.

In experiments designed to study the decarboxylation of L-DOPA or 3-OMDOPA and the interference of 3-OMDOPA with the decarboxylation of L-DOPA, kidney homogenates instead of renal tubules were used. Whole kidneys were homogenized in a modified Krebs solution with Duall-Kontes homogenizers and kept continuously on ice. The composition of the modified Krebs solution was as follows (in mM): NaCl 50, choline chloride 68, KCl 4.7, CaCl_2 2.4, MgSO_4 1.2, NaHCO_3 25, KH_2PO_4 1.2, EDTA 0.4, glucose 10 and sodium butyrate 1; $\text{pH} = 7.4$. L- α -Methyl-p-tyrosine ($50 \mu\text{M}$) and copper sulphate ($10 \mu\text{M}$) were also added to the Krebs solution in order to inhibit the enzyme, tyrosine hydroxylase and inhibit the endogenous inhibitors of dopamine β -hydroxylase, respectively. Aliquots of 1.0 ml of kidney homogenates plus 1.0 ml Krebs solution were placed in glass test tubes and incubated for 30 min in the presence of tolcapone ($1 \mu\text{M}$) and pargyline (0.1 mM); thereafter, L-DOPA or 3-OMDOPA (10 to $5000 \mu\text{M}$) was added to the medium for a further 15 min. In experiments in which the effect of 3-OMDOPA ($1000 \mu\text{M}$) was tested on the decarboxylation of L-DOPA, the methylated compound was present during the preincubation and incubation periods. During incubation, kidney homogenates were continuously shaken and gassed ($95\% \text{ O}_2$ and $5\% \text{ CO}_2$) and maintained at 37°C . The reaction was stopped by the addition of $250 \mu\text{l}$ of 2 M perchloric acid and the preparations kept at 4°C for 60 min; this procedure has been found to result in a complete extraction of catecholamines from the tissues (Fernandes *et al.*, 1991). The samples were then centrifuged (200 g , 2 min , 4°C) and $50 \mu\text{l}$ aliquots of the supernatant filtered on Millipore microfilters (MF1) and injected directly into the column of a high performance liquid chromatograph (h.p.l.c.) for the quantification of dopamine and 3-methoxytyramine.

The h.p.l.c. 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 $5 \mu\text{m}$ ODS column (Biophase, Bioanalytical Systems, West Lafayette, IN, U.S.A.), 25 cm in 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 ($8\% \text{ v/v}$), adjusted to $\text{pH} 3.5$ 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 h.p.l.c. software. The lower limits for detection of L-DOPA, 3-OMDOPA, dopamine and 3-methoxytyramine ranged from 350 to 500 fmol .

The protein content of the suspensions of renal tubules and kidney homogenates was determined by the method of Bradford (1976), with human serum albumin as a standard.

V_{max} and K_{m} values for the saturable component of L-DOPA and 3-OMDOPA uptake and AAAD activity were calculated by linear regression analysis. The diffusion constant represents the slope of the accumulation of L-DOPA and 3-OMDOPA measured in experiments carried out at 4°C (Neame & Richards, 1972). For the calculation of the IC_{50} s the parameters of the Hill-equation for multisite inhibition were fitted to the experimental data (Segel, 1975). The K_i s were calculated from the corresponding IC_{50} s as described by Cheng & Prusoff (1973). Geometric means are given with 95% confidence limits and arithmetic means are given with s.e.mean.

L-3,4-dihydroxyphenylalanine (L-DOPA), dopamine hydrochloride, 3-methyl-L-DOPA, pargyline hydrochloride and trypan blue were purchased from Sigma Chemical Company (St Louis, MO, U.S.A.); 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 and 3-OMDOPA, results in a concentration-dependent accumulation of the substrates (Figure 1); under these experimental conditions the accumulation of both L-DOPA and 3-OMDOPA was found to be linear and of similar magnitude. When the experiments were carried out at 37°C, the accumulation of both L-DOPA and 3-OMDOPA in renal tubules was found to be greater than that occurring at 4°C and showed a trend for saturation. The saturable component of both L-DOPA and 3-OMDOPA uptake (Figure 1) was derived from the total amount of L-DOPA or 3-OMDOPA accumulated in renal tubules at 37°C with the values obtained in experiments conducted at 4°C subtracted. The $V_{\max}$ and K_m values for the saturable component of L-DOPA and 3-OMDOPA uptake in renal tubules were as follows: L-DOPA, $V_{\max} = 11.1 \pm 2.5 \text{ nmol mg}^{-1} \text{ protein h}^{-1}$ and $K_m = 216$ (95% confidence limits: 96,487) μM ($n = 6$); 3-OMDOPA, $V_{\max} = 8.1 \pm 2.1 \text{ nmol mg}^{-1} \text{ protein h}^{-1}$ and $K_m = 231$ (93,575) μM ($n = 5$). The diffusion constant (in μmol^{-1}) of the non-saturable component for the accumulation of L-DOPA and 3-OMDOPA

was 0.0014 (0.0011, 0.0019; $n = 6$) and 0.0010 (0.0005, 0.0019; $n = 5$), respectively.

Figure 2 shows the effect of increasing concentrations (50 to 2000 μM) of 3-OMDOPA on the saturable component of L-DOPA uptake into renal proximal tubules. 3-OMDOPA was found to produce a concentration-dependent decrease in the tubular uptake of L-DOPA. The lowest concentration of 3-OMDOPA (100 μM) resulting in some inhibition of L-DOPA uptake was found to reduce its tubular uptake by 29%; the greatest inhibitory effect on the tubular uptake of L-DOPA (81% reduction) was obtained with 1000 μM 3-OMDOPA. The K_i value (in μM) of 3-OMDOPA for inhibition of L-DOPA uptake was found to be 181 (98,333) ($n = 5$). This K_i value for 3-OMDOPA agrees with the K_m value of 3-OMDOPA uptake obtained in saturation experiments. The accumulation of L-DOPA obtained in experiments conducted at 4°C was found not to be affected by 3-OMDOPA (data not shown).

Incubation of kidney homogenates, in conditions of catechol-*O*-methyltransferase (COMT) and monoamine

Figure 1 Accumulation of (a) L-DOPA and (b) 3-OMDOPA in suspensions of rat isolated renal tubules. The non-saturable component of substrate uptake (○) was obtained in experiments conducted at 4°C and was found to be linear with the concentration of the substrate. The saturable component (■) was derived from the total (●) amount of L-DOPA or 3-OMDOPA accumulated in renal tubules at 37°C with the values obtained for the non-saturable component subtracted. Each point is the mean with s.e.mean of five to six experiments per group.

Figure 2 Concentration-dependent inhibition by 3-OMDOPA (50 to 2000 μM) of the saturable transport of L-DOPA in suspensions of rat isolated renal tubules incubated with 250 μM L-DOPA. Each point represents the mean with s.e.mean of six experiments per group.

Figure 3 Decarboxylation of 3-OMDOPA (■) and L-DOPA alone (○) or in combination with 3-OMDOPA (1000 μM ; ●) in rat kidney homogenates incubated with increasing concentrations (10 to 5000 μM) of the substrates. The results show levels (in $\text{mol mg}^{-1} \text{ protein 15 min}^{-1}$) of dopamine and 3-methoxytyramine formed, respectively, from added L-DOPA and 3-OMDOPA during a 15 min incubation period. Since no formation of 3-methoxytyramine from added 3-OMDOPA was found to occur, the levels indicated in the figure are the detection limit of the method for 3-methoxytyramine. Each point represents the mean with s.e.mean of four experiments per group.

Figure 4 Accumulation of 3-OMDOPA in suspension of rat isolated renal tubules obtained from control animals (●) and benserazide-treated animals (○). The results are levels (in nmol mg^{-1} protein 15 min^{-1}) of 3-OMDOPA accumulated in renal tubules through the saturable transporter. The saturable component was derived from the total amount of 3-OMDOPA accumulated in renal tubules at 37°C with the values obtained for the non-saturable component subtracted. Each point is the mean with s.e.mean of five experiments per group.

oxidase (MAO) inhibition, with L-DOPA (10 to $5000 \mu\text{M}$) resulted in a concentration-dependent formation of dopamine, as shown in Figure 3. However, the incubation of kidney homogenates with 3-OMDOPA (10 to $5000 \mu\text{M}$) did not result in the formation of 3-methoxytyramine, the compound expected to result from the decarboxylation of 3-OMDOPA. The addition of $1000 \mu\text{M}$ 3-OMDOPA to the incubation medium did not affect the formation of dopamine in kidney homogenates incubated with increasing concentrations of L-DOPA (10 to $5000 \mu\text{M}$). The V_{max} and K_m values for AAAD determined in the absence of 3-OMDOPA ($V_{\text{max}} = 14.1 \pm 0.6 \text{ nmol mg}^{-1} \text{ protein h}^{-1}$; $K_m = 62$ [43, 87] μM) were not significantly different from those observed when the decarboxylation of L-DOPA was carried out in the presence of $1000 \mu\text{M}$ 3-OMDOPA ($V_{\text{max}} = 15.7 \pm 1.0 \text{ nmol mg}^{-1} \text{ protein h}^{-1}$; $K_m = 68$ [54, 87] μM).

In Figure 4 are shown the results on the saturable component of 3-OMDOPA uptake in renal tubules obtained from non-treated rats and animals given benserazide 60 min before they were killed. As indicated in the figure, no significant difference was observed on the tubular uptake of 3-OMDOPA in control and benserazide-treated rats. The accumulation of 3-OMDOPA through the non-saturable uptake component was similar in benserazide treated rats to that observed in the corresponding control animals (data not shown).

Discussion

The data presented here show that both L-DOPA and 3-OMDOPA are taken up into renal tubules through saturable and non-saturable mechanisms. The maximum rate of uptake of these two substrates through the saturable transporter and their affinity for the transporter unit, as shown by the V_{max} and K_m values, were found to be similar. The finding that 3-OMDOPA exerts a concentration-dependent inhibition upon the saturable component of tubular transport of L-DOPA, suggests that the two compounds are taken up by the same transporter. This suggestion is further supported by the result that 3-OMDOPA does not affect the non-saturable accumulation of L-DOPA, which is also in agreement with the view that the accumulation of both L-DOPA and 3-OMDOPA in renal tubules may not be related to the binding of these compounds to tissue components. At concentrations

of the substrates approaching half saturation, diffusional uptake accounts for 49% and 36% of the total accumulation of 3-OMDOPA and L-DOPA, respectively. On the other hand, the similarity between K_i values of 3-OMDOPA for inhibition of L-DOPA uptake and K_m values of 3-OMDOPA tubular uptake agree with the view that 3-OMDOPA exerts a competitive type of inhibition upon the tubular uptake of L-DOPA. The results reported here for renal tissues are in accordance with those obtained in brain tissues, where it has been demonstrated that 3-OMDOPA competes with L-DOPA for its transport at the level of the blood-brain barrier (Wade & Katzman, 1975). Like that found to occur in renal tubules, the transport of both 3-OMDOPA and L-DOPA was found to occur through saturable and non-saturable mechanisms. In brain, however, the affinity of 3-OMDOPA ($K_i = 0.13 \text{ mM}$) for the saturable transporter was found to be higher than that for L-DOPA ($K_i = 0.34 \text{ mM}$).

An additional mechanism by which 3-OMDOPA has been suggested to interfere with the action of L-DOPA is that concerning the competition between the two compounds for AAAD (Reilly *et al.*, 1980). The experiments described here on kidney homogenates show, in contrast, that 3-OMDOPA was neither decarboxylated by AAAD nor interfered with the decarboxylation of L-DOPA. These results agree with those of Ferrini & Glasser (1964) while showing that 3-OMDOPA is in fact a poor substrate for AAAD, as determined in the guinea-pig kidney and mouse brain. After confirming that 3-OMDOPA does not undergo decarboxylation by renal AAAD it was decided to test the possibility of the interference of benserazide on the tubular accumulation of this compound. In experiments carried out in renal tubules prepared from renal tissues obtained from benserazide-treated and the corresponding controls (animals given the vehicle) it was found that the rate of maximum uptake of 3-OMDOPA through the saturable transporter was similar in both experimental conditions and the diffusional uptake of 3-OMDOPA was also not affected by pretreatment with the AAAD inhibitor. The dose of benserazide used has been demonstrated by other authors (DaPrada *et al.*, 1987) to produce an almost complete inhibition of the enzyme in peripheral tissues; the finding that no formation of dopamine was observed in renal tubules incubated with L-DOPA agrees with the view that this dose of benserazide is effective in inhibiting renal AAAD. Thus, in spite of the structural similarities between benserazide and L-DOPA or 3-OMDOPA, it might be suggested that the AAAD inhibitor does not interfere with the cell inward transport of these compounds.

The functional relevance of 3-OMDOPA is most probably shown in parkinsonian patients treated with L-DOPA in combination with a peripheral AAAD inhibitor, a condition which facilitates the *O*-methylation of L-DOPA (Männistö & Kaakkola, 1989). Even so, the plasma levels of 3-OMDOPA after the administration of 500 mg of L-DOPA to non-parkinsonian subjects has been found to exceed by far those of L-DOPA, dopamine or the amine metabolites, 3-methoxytyramine, 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) (Shigetomi *et al.*, 1991). In contrast to that described for normotensive subjects, salt-sensitive essential hypertensive patients fail to excrete larger amounts of dopamine in the urine, in conditions of increased renal delivery of sodium (for a review, see Lee, 1993) and appear to present an enhanced ability to deaminate and *O*-methylate renal dopamine (Shigetomi *et al.*, 1991). Apparently no differences were observed between the pattern of 3-OMDOPA formation after the administration of L-DOPA between control subjects and hypertensive patients (Shigetomi *et al.*, 1991). Plasma 3-OMDOPA does not, however, reflect exclusively the ability of renal tissues to *O*-methylate L-DOPA and it remains to be determined whether the increased renal COMT activity suggested to occur in hypertensive patients is accompanied by an increased *O*-methylation of endogenous circulating L-DOPA; if this

should occur, then the increased availability of 3-OMDOPA at the renal tubules would tend to decrease the tubular uptake of L-DOPA and the formation of renal dopamine. Circulating L-DOPA has been demonstrated to be converted to dopamine in the human kidney (Zimlichman *et al.*, 1988; Wolfovitz *et al.*, 1993), where the amine is expected to exert a natriuretic effect (Lee, 1993), and thereafter is excreted into the urine.

In conclusion, the results presented suggest that L-DOPA and 3-OMDOPA are using the same transporter in order to

be taken up into renal tubular cells, 3-OMDOPA exerts a competitive type of inhibition upon the tubular uptake of L-DOPA. It is further shown that 3-OMDOPA neither undergoes decarboxylation into 3-MT nor affects the decarboxylation of L-DOPA.

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