British Journal of Pharmacology (1996) 117, 1187–1192

Antagonistic actions of renal dopamine and 5-hydroxytryptamine: effects of amine precursors on the cell inward transfer and decarboxylation

[1]P. Soares-da-Silva & P.C. Pinto-do-Ó

Department of Pharmacology and Therapeutics, Faculty of Medicine, 4200 Porto, Portugal

1 The present work was designed to examine the interference of L-3,4-dihydroxyphenylalanine (L-DOPA) on the cell inward transport of L-5-hydroxytryptophan (L-5-HTP) and on its decarboxylation by aromatic L-amino acid decarboxylase (AAAD) in rat isolated renal tubules.

2 The accumulation of both L-5-HTP and L-DOPA in renal tubules was found to occur through non-saturable and saturable mechanisms. The kinetics of the saturable component L-5-HTP and L-DOPA uptake in renal tubules were as follows: L-5-HTP, $V_{max}=24.9\pm4.5$ nmol mg^{-1} protein h^{-1} and $K_m=121$ (95% confidence limits: 75, 193) μM ($n=5$); L-DOPA, $V_{max}=58.0\pm4.3$ nmol mg^{-1} protein h^{-1} and $K_m=135$ (97, 188) μM ($n=5$). When the saturation curve of L-5-HTP tubular uptake was performed in the presence of L-DOPA (250 μM), the maximal rate of accumulation of L-5-HTP in renal tubules was found to be markedly ($P<0.01$) reduced ($V_{max}=10.5\pm1.7$ nmol mg^{-1} protein h^{-1}, $n=4$); this was accompanied by a significant ($P<0.05$) increase in K_m values (325 [199, 531] μM, $n=4$).

3 L-DOPA (50 to 2000 μM) was found to produce a concentration-dependent decrease (38% to 91% reduction) in the tubular uptake of 5-HTP; the K_i value (in μM) of L-DOPA for inhibition of L-5-HTP uptake was found to be 29.1 (13.8, 61.5) ($n=6$).

4 At the highest concentration tested the organic anion inhibitor, probenecid (10 μM) produced no significant ($P=0.09$) changes in L-5-HTP and L-DOPA uptake (18% and 22% reduction, respectively). The organic cation inhibitor, cyanine 863 (1-ethyl-2-[1,4-dimethyl-2-phenyl-6-pyrimidinylidene)methyl]-quinolinium) produced a potent inhibitory effect on the tubular uptake of L-5-HTP ($K_i=212$ [35, 1289] nM, $n=8$), being slightly less effective against L-DOPA uptake ($K_i=903$ [584, 1396] nM, $n=5$). The cyanine derivatives 1,1-diethyl-2,4-cyanine (decynium 24) and 1,1-diethyl-2,2-cyanine (decynium 22) potently inhibited the tubular uptake of both L-5-HTP ($K_i=100$ [49, 204] and 120 [26, 561] nM, $n=4$-6, respectively) and L-DOPA ($K_i=100$ [40, 290] and 415 [157, 1094] nM, $n=5$, respectively).

5 The V_{max} and K_m values for AAAD using L-DOPA as the substrate ($V_{max}=479.9\pm74.0$ nmol mg^{-1} protein h^{-1}; $K_m=2380$ [1630, 3476] μM; $n=4$) were both found to be significantly ($P<0.01$) higher than those observed when using L-5-HTP ($V_{max}=81.4\pm5.2$ nmol mg^{-1} protein h^{-1}, $K_m=97$ [87, 107] μM, $n=10$). The addition of 5 mM L-DOPA to the incubation medium reduced by 30% ($P<0.02$) the maximal rate of decarboxylation of L-5-HTP ($V_{max}=56.7\pm3.1$ nmol mg^{-1} protein h^{-1}, $n=10$) and resulted in a significant ($P<0.05$) increase in K_m values (249 [228, 270] μM, $n=10$).

6 The results presented suggest that L-5-HTP and L-DOPA are using the same transporter (most probably, the organic cation transporter) in order to be taken up into renal tubular cells; L-DOPA exerts a competitive type of inhibition upon the tubular uptake and decarboxylation of L-5-HTP. The decrease in the formation of 5-HT as induced by L-DOPA may also depend on a decrease in the rate of its decarboxylation by AAAD.

Keywords: L-5-Hydroxytryptophan; L-DOPA; kidney; 5-hydroxytryptamine; dopamine

Introduction

In the kidney, dopamine has been suggested to 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-3,4-dihydroxyphenylalanine (L-DOPA) in epithelial cells of proximal convoluted tubules. This type of cell is endowed with high aromatic L-amino acid decarboxylase (AAAD) activity and the formation of dopamine is believed to occur after the uptake of L-DOPA into this cellular compartment (Soares-da-Silva, 1993). The AAAD-rich epithelial cells of proximal convoluted tubules have been also found to decarboxylate L-5-hydroxytryptophan (L-5-HTP) to 5-hydroxytryptamine (5-HT) (Stier *et al.*, 1984; Sole *et al.*, 1986). However, in contrast to dopamine, 5-HT of renal origin has been demonstrated to produce significant reductions in sodium and water excretion, accompanied by slight reductions in the clearance of *p*-aminohippurate and glomerular filtration rate (Itskowitz *et al.*, 1988; Li Kam Wa *et al.*, 1993). It is possible, therefore, that depending on the availability of L-DOPA and L-5-HTP, this type of cell will synthesize dopamine and 5-HT, the actions of which will be antagonistic in nature. When L-DOPA is infused together with L-5-HTP into the renal artery there is a lessening of these antinatriuretic and antidiuretic effects; the effects of L-DOPA occurred without changes in the urinary excretion of 5-HT, leading to the suggestion of a functional reciprocal effect of the two amines (Itskowitz *et al.*, 1988). This, however, does not exclude the possibility that L-DOPA and L-5-HTP might compete for uptake or decarboxylation by AAAD in renal tubules. In fact, L-DOPA has been demonstrated to be the preferred substrate for AAAD and to exert a competitive inhibition upon the decarboxylation of L-5-HTP in brain tissues

[1] Author for correspondence.

and liver (Hagen & Cohen, 1966). Though, to our knowledge, no evidence has been presented on the possibility that the two precursors might share the same transporter in renal tubules, the tubular uptake of both L-DOPA and L-tryptophan, the precursor of L-5-HTP, have been demonstrated to occur through energy-dependent and stereo-selective carrier-mediated processes (Williams & Huang, 1970; Chan, 1976).

The present work was designed to examine the synthesis of 5-HT in isolated proximal convoluted tubules and homogenates of renal tubules from added 5-HTP and to study the interference of L-DOPA with 5-HT formation. The decarboxylation of L-DOPA and L-5-HTP in intact renal tubules is assumed to reflect mainly the metabolism of the taken up substrates, whereas the decarboxylation of both substrates in homogenates reflects AAAD activity. A preliminary report of these results has been presented at the 8th Meeting on Adrenergic Mechanisms (Pinto-do-Ó & Soares-da-Silva, 1994).

Methods

Male Wistar rats (Biotério do Instituto Gulbenkian de Ciência, Oeiras, Portugal) 45–60 days old and weighing 180–220 g were used in the experiments. Animals were kept two per cage under controlled environmental conditions (12 h light/dark cycle and room temperature 24°C). Food and tap water were allowed *ad libitum* and the experiments were all carried out during daylight hours.

Tubule preparation and uptake studies

The preparation of renal tubules was based on the techniques described by Shah *et al.* (1979) and Bloch *et al.* (1992) with minor modifications. 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, 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 μm and then 75 μm. Unseparated cortex remained on the upper (180 μm) sieve, while the lower one (75 μm) 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*, 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, pH = 7.4. Pargyline (0.1 mM) and tolcapone (1 μM) were also added to the Hanks medium in order to inhibit the enzymes monoamine oxidase and catechol-*O*-methyltransferase, 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% w/v) exclusion method; nephron segments were pipetted to a glass slide and observed 90 s after exposure to the dye, under a Leica microscope: more than 90% of the renal tubules excluded the dye.

In experiments designed to study the uptake and decarboxylation of L-DOPA and L-5-HTP in intact renal tubules, the preparations were preincubated for 15 min in gassed (95% O_2 and 5% CO_2) and warm (37°C) Hanks medium and, thereafter, incubated for further 15 min in the presence of increasing concentrations (50 to 2000 μM) of L-DOPA or L-5-HTP, respectively. In experiments designed to study the interference of L-DOPA with the uptake of L-5-HTP, renal tubules were preincubated for 15 min in the absence of the compounds to be tested; after preincubation, renal tubules were incubated for 15 min in Hanks medium with increasing concentrations of L-5-HTP (50 to 2000 μM) alone or in the presence of 250 μM L-DOPA. In another set of experiments, renal tubules were incubated with 250 μM L-5-HTP alone or in the presence of increasing concentrations of L-DOPA, probenecid, cyanine 863 (1-ethyl-2-[1,4-dimethyl-2-phenyl-6-pyrimidinylidene)methyl]-quinolinium) and the cyanine derivatives 1,1-diethyl-2,4-cyanine (decynium 24) and 1,1-diethyl-2,2-cyanine (decynium 22). For comparison, similar experiments were performed with L-DOPA instead of L-5-HTP and the effects of probenecid, cyanine 863, decynium 24 and decynium 22 studied. 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 and maintained at 37°C. The reaction was stopped by the addition of 100 μl of 2 M perchloric acid and the preparations kept at 4°C for 60 min. The samples were then centrifuged (3000 *g*, 4 min, 4°C) and 500 μl aliquots of the supernatant filtered on Millipore microfilters (MF1) and directly injected into the high performance liquid chromatograph (h.p.l.c.) for the assay of 5-HT and dopamine.

AAAD preparation and decarboxylation studies

In experiments designed to study the decarboxylation of L-5-HTP or L-DOPA and the interference of L-DOPA with the decarboxylation of L-5-HTP, homogenates of renal tubules instead of intact renal tubules were used. The tubules were homogenized in 0.5 M phosphate buffer (pH = 7.0) with Thomas teflon homogenizers and kept continuously on ice. Aliquots of 250 μl of tubular homogenate plus 250 μl incubation medium were placed in glass test tubes and preincubated for 15 min. Thereafter, L-5-HTP or L-DOPA (50 to 10,000 μM) was added to the medium for a further 15 min; the final reaction volume was 1 ml. In experiments designed to study the interference of L-DOPA with the decarboxylation of L-5-HTP, homogenates were incubated with increasing concentrations of L-5-HTP (50 to 10,000 μM) in the presence of 5 mM L-DOPA; in these experiments, the addition of L-DOPA (5 mM) to the incubation medium was performed during the incubation period only. The composition of the incubation medium was as follows (in mM): NaH_2PO_4 0.35, Na_2HPO_4 0.15, sodium borate 0.11 and pyridoxal phosphate 0.12, pH = 7.2; pargyline (100 μM) and tolcapone (1 μM) were also added to the Hanks medium in order to inhibit the enzymes catechol-*O*-methyltransferase and monoamine oxidase, respectively. The pH of the reaction medium was kept constant at an optimal pH 7.0 (Shirota & Fujisawa, 1988). During incubation, homogenates of renal tubules were continuously shaken and gassed (95% O_2 and 5% CO_2) and maintained at 37°C. The reaction was stopped by the addition of 500 μl of 2 M perchloric acid and the preparations kept at 4°C for 60 min. The samples were then centrifuged (200 *g*, 2 min, 4°C) and 500 μl aliquots of the supernatant filtered on Millipore microfilters (MF1) and injected directly into the column of a h.p.l.c. for the quantification of 5-HT and dopamine.

Assay of monoamines

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 μm 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% 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 h.p.l.c. software. The lower limits for detection of L-DOPA, L-5-HTP, dopamine and 5-HT 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.

Kinetic analysis and statistics

V_{max} and K_m values for the formation of dopamine and 5-HT in intact renal tubules and AAAD activity in tubular homogenates were calculated from non-linear regression analysis using the GraphPad Prism statistics software package (Motulsky *et al.*, 1994). For the calculation of the IC_{50}s the parameters of the equation for one site inhibition were fitted to the experimental data (Motulsky *et al.*, 1994). The K_is were calculated from the corresponding IC_{50}s as described by Cheng & Prussof (1973). Geometric means are given with 95% confidence limits and arithmetic means are given with s.e.mean. Statistical analysis was performed by one-way analysis of variance (ANOVA) followed by Student's *t* test for unpaired comparisons. A *P* value less than 0.05 was assumed to denote a significant difference.

Drugs

Cyanine 863 (1-ethyl-2-[1,4-dimethyl-2-phenyl-6-pyrimidinylidene)methyl]-quinolinium), 1,1-diethyl-2,2-cyanine, 1,1-diethyl-2,4-cyanine, L-3,4-dihydroxyphenylalanine (L-DOPA), dopamine hydrochloride, L-5-hydroxytryptophan, pargyline hydrochloride, probenecide and trypan blue were purchased from Sigma Chemical Company (St. Louis, Mo, U.S.A.); tolcapone was kindly donated by the late Professor Mosé Da Prada (Hoffmann La Roche, Basle, Switzerland).

Results

Incubation of renal tubules at 4°C in the presence of increasing concentrations of L-5-HTP and L-DOPA, resulted in a concentration-dependent accumulation of the substrates (data not shown); in these experimental conditions the accumulation of both L-5-HTP and L-DOPA was found to be linear and of similar magnitude. When the experiments were carried out at 37°C, the accumulation of 5-HTP and L-DOPA in renal tubules was found to be greater than that occurring at 4°C and showed a trend for saturation (Figure 1). The maximal rate of L-DOPA accumulation was found to be 2.5 fold that for L-5-HTP and occurred at similar concentrations of the substrate. The V_{max} and K_m values for the tubular uptake of L-5-HTP and L-DOPA in renal tubules were as follows: L-5-HTP, $V_{max}=24.9\pm4.5$ nmol mg^{-1} protein h^{-1} and $K_m=121$ (95% confidence limits: 75, 193) μM ($n=5$); L-DOPA, $V_{max}=58.0\pm4.3$ nmol mg^{-1} protein h^{-1} and $K_m=135$ (97, 188) μM ($n=5$). When the saturation curve of L-5-HTP tubular uptake was performed in the presence of L-DOPA (250 μM), the maximal rate of accumulation of L-5-HTP in renal tubules was found to be markedly ($P<0.01$) reduced ($V_{max}=10.5\pm1.7$ nmol mg^{-1} protein h^{-1}, $n=4$); this was accompanied by a significant ($P<0.05$) increase in K_m values (325 [199, 531] μM, $n=4$).

Figure 2 shows the effect of increasing concentrations (50 to 2000 μM) of L-DOPA on the tubular uptake of L-5-HTP (basal values $=5.2\pm0.7$ nmol mg^{-1} protein 15 min^{-1}, $n=5$) in renal tubules. L-DOPA (50 to 2000 μM) was found to produce a concentration-dependent decrease (38% to 91% reduction) in the tubular uptake of L-5-HTP. The lowest concentration of L-DOPA (50 μM) resulting in some inhibition of L-5-HTP uptake was found to reduce by 38% the accumulation of L-5-HTP; the greatest inhibitory effect on the tubular accumulation of L-5-HTP (91% reduction) was obtained with 2000 μM of L-DOPA. The K_i value (in μM) of L-DOPA for inhibition of L-5-HTP uptake was found to be 29.1 (13.8, 61.5) ($n=6$).

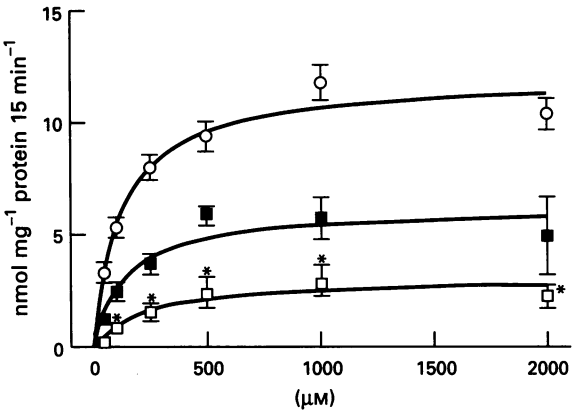

Figure 1 Formation of dopamine (○) and 5-hydroxytryptamine (■) in suspensions of rat isolated renal tubules incubated for 15 min in the presence of increasing concentrations of L-DOPA and L-5-hydroxytryptophan, respectively; (□) effect of 250 μM L-DOPA (added during the preincubation and incubation periods) on the formation of 5-hydroxytryptamine from L-5-hydroxytryptophan in renal tubules. Each point is the mean with s.e.mean of five to six experiments per group. *Significantly different ($P<0.05$) from corresponding values in the absence of L-DOPA using Student's *t* test.

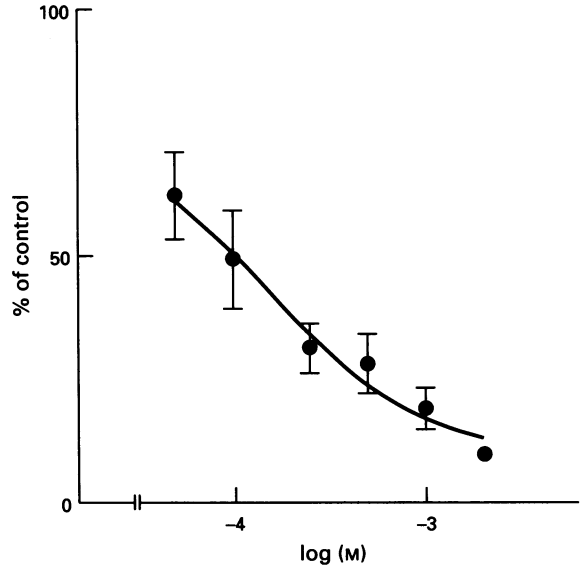

Figure 2 Concentration-dependent inhibition by L-DOPA (50 to 2000 μM) of 5-hydroxytryptamine formation in suspensions of rat isolated renal tubules incubated with 250 μM L-5-hydroxytryptophan. Each point represents the mean with s.e.mean of six experiments per group.

As shown in Figure 3a, at the highest concentration tested the organic anion inhibitor, probenecid (10 μM) produced no significant ($P=0.09$) changes in L-5-HTP and L-DOPA uptake (18% and 22% reduction, respectively). The organic cation inhibitor, cyanine 863, produced a potent inhibitory effect on the tubular uptake of L-5-HTP ($K_i=212$ [35, 1289] nM, $n=8$), being less effective against L-DOPA uptake ($K_i=903$ [584, 1396] nM, $n=5$) (Figure 3b). The cyanine derivatives 1,1-diethyl-2,4-cyanine (decynium 24) and 1,1-diethyl-2,2-cyanine (decynium 22) potently inhibited the tubular uptake of both L-5-HTP ($K_i=100$ [49, 204] and 120 [26, 561] nM, $n=4$-6, respectively) and L-DOPA ($K_i=100$ [40, 290] and 415 [157, 1094] nM, $n=5$, respectively) (Figures 3c and 3d). Basal levels of L-5-

HTP and L-DOPA in renal tubules after incubation with the corresponding substrates were 7.7 ± 0.3 and 6.9 ± 0.4 nmol mg^{-1} protein 15 min^{-1} ($n = 15-16$), respectively.

Incubation of homogenates of renal tubules with L-5-HTP or L-DOPA (50 to 10,000 μM) resulted in a concentration-dependent formation of 5-HT and dopamine, respectively (Figure 4). The V_{max} and K_m values for AAAD using L-DOPA as the substrate ($V_{max} = 479.9 \pm 74.0$ nmol mg^{-1} protein h^{-1}; $K_m = 2380$ [1630, 3476] μM; $n = 4$) were both found to be significantly ($P < 0.01$) higher than those observed when using L-5-HTP ($V_{max} = 81.4 \pm 5.2$ nmol mg^{-1} protein h^{-1}; $K_m = 97$ [87, 107] μM, $n = 10$). The addition of 5 mM L-DOPA to the incubation medium reduced by 30% ($P < 0.02$) the maximal rate of decarboxylation of L-5-HTP ($V_{max} = 56.7 \pm 3.1$ nmol mg^{-1} protein h^{-1}, $n = 10$) and resulted in a significant ($P < 0.05$) increase of K_m values (249 [228, 270] μM, $n = 10$). The addition of

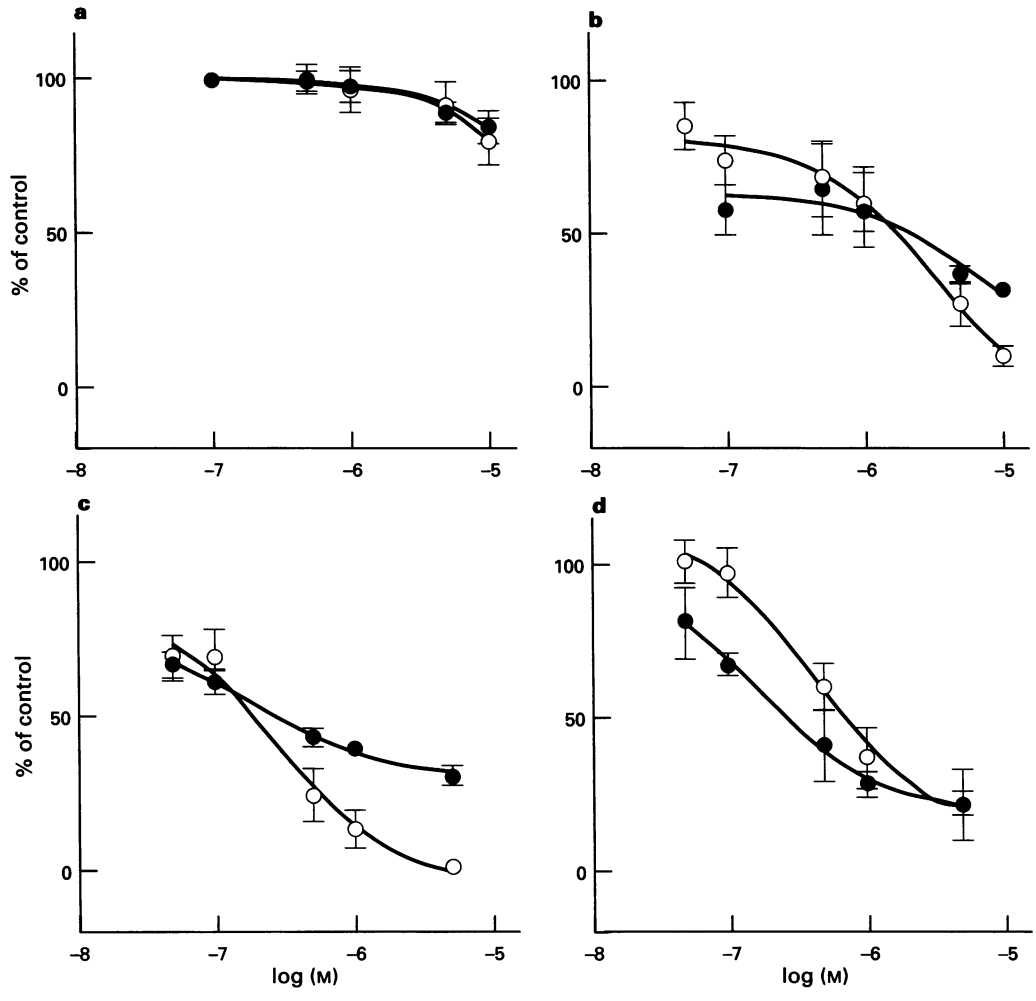

Figure 3 Effect of increasing concentrations of (a) probenecid, (b) cyanine 863, (c) decynium 24 and (d) decynium 22 on the saturable uptake of 250 μM L-5-hydroxytryptophan (closed symbols) and 250 μM L-DOPA (open symbols) in rat isolated renal tubules. Each point represents the mean with s.e.mean of six experiments per group.

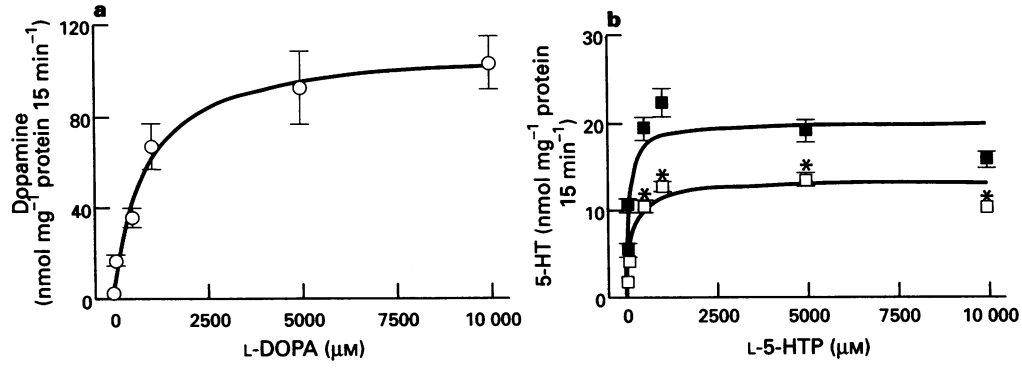

Figure 4 Decarboxylation of (a) L-DOPA (○) and (b) L-5-hydroxytryptophan alone (■) or in combination with 10 mM L-dopa (□) in homogenates of renal tubules incubated with increasing concentrations (50 to 10,000 μM) of the substrates. The results are levels (in nmol mg^{-1} protein 15 min^{-1}) of dopamine and 5-hydroxytryptamine formed, respectively, from added L-DOPA and L-5-hydroxytryptophan during a 15 min incubation period. Each point represents the mean with s.e.mean of four to ten experiments per group. *Significantly different ($P < 0.05$) from corresponding values in the absence of L-DOPA using Student's t test.

decynium 24 (5 μM) to the incubation medium was found to change neither the maximal rate of L-5-HTP decarboxylation by homogenates of isolated renal tubules nor K_m values for AAAD (V_{max} = 104.7 ± 6.3 nmol mg^{-1} protein h^{-1}, K_m values (84 [80, 89] μM, n = 4).

Discussion

The data presented here show that both L-5-HTP and L-DOPA are taken up into renal tubules and subsequently decarboxylated to 5-HT and dopamine, respectively. It is suggested that L-5-HTP and L-DOPA share the same tubular transporter, though L-DOPA appears to be a better substrate than 5-HTP for tubular uptake and decarboxylation by AAAD. However, considering the high rate of decarboxylation in tubular epithelial cells, the decrease in the formation of 5-HT as induced by L-DOPA most probably reflects a reduction in the intracellular availability of L-5-HTP.

Assuming that the kinetic parameters for the formation of both 5-HT and dopamine in intact renal tubules reflect the kinetics of the tubular transport system for the corresponding substrates, it is interesting to underline the similarity of K_m values between L-5-HTP (121 [75, 193] μM) and L-DOPA (135 [97, 188] μM). This suggests that both substrates share the same transporter for uptake into renal tubules. This is not unique since at the level of the blood-brain barrier a similar situation was recognized by Wurtman *et al.* (1981). The result that the concentration-dependent uptake of L-5-HTP in intact renal tubules is markedly reduced by 250 μM L-DOPA, a concentration which is twice the K_m value for its uptake, suggests the latter is exerting a competitive inhibition upon the tubular uptake of L-5-HTP. This is also shown by the increase in K_m values for uptake of L-5-HTP in the presence of L-DOPA. The finding that K_i values of L-DOPA for inhibition of L-5-HTP uptake were lower than the K_m values of L-5-HTP uptake in renal tubules also agrees with the view that L-DOPA (1) exerts a competitive type of inhibition upon the tubular uptake of L-5-HTP and (2) may have a higher affinity for the transporter than L-5-HTP. The observation that cyanine 863, but not probenecid, reduces the tubular uptake of L-DOPA and L-5-HTP suggests the involvement of an organic cation transport system in the tubular uptake of both substrates. The result that the two cyanine derivatives, decynium 22 and decynium 24, also produce potent inhibitory effects on the tubular uptake of L-DOPA and L-5-HTP can also be used as an argument suggesting that both amino acids are sharing the same transporter. This view is reinforced by the fact that K_i values for cyanine 863, decynium 22 and decynium 24 are similar when L-DOPA or L-5-HTP are used as substrates.

The finding that the maximal rate of formation of dopamine in intact renal tubules was 2.5 fold that for 5-HT cannot be used as a direct indication that L-DOPA is transported into the cell at a higher rate than L-5-HTP. These differences may to a certain extent reflect the rate at which the transported L-DOPA or L-5-HTP are decarboxylated by AAAD. In agreement with this view are the results obtained in homogenates of renal tubules; the maximal rate of decarboxylation of L-DOPA was found to be 6.1 fold that for L-5-HTP. This suggests that L-DOPA is a better substrate for AAAD than 5-HTP, though the affinity of 5-HTP for the enzyme, as indicated by the K_m values, would favour the decarboxylation of the latter. This discrepancy has been described in several tissues, both in the central nervous system and the periphery, by several groups of researchers over the past 30 years (Lovenberg *et al.*, 1962; Hagen & Cohen, 1966; Bender & Coulson, 1972; Sims *et al.*, 1973; Shirota & Fujisawa, 1988; Sumi *et al.*, 1990). The different kinetic parameters for decarboxylation of L-DOPA and L-5-HTP have even been considered as evidence for the presence of similar but different enzymes (Hagen & Cohen, 1966; Sims *et al.*, 1973; Sourkes, 1977). Recently, however, it has been demonstrated that the purified rat renal AAAD preferentially decarboxylates L-DOPA in comparison to L-5-HTP (Shirota & Fujisawa, 1988). Similar results have been obtained by Sumi *et al.* (1990) using a recombinant human AAAD expressed in COS cells. The expressed enzyme decarboxylates both L-DOPA and L-5-HTP; however, differences concerning the kinetic properties for decarboxylation of L-DOPA and L-5-HTP were found to be similar to those described in crude homogenates or preparations with different degrees of enzyme purification (Lovenberg *et al.*, 1962; Hagen & Cohen, 1966; Bender & Coulson, 1972; Sims *et al.*, 1973). The mutual competitive inhibition between L-DOPA and L-5-HTP described in several studies and the present one in the kidney is consistent with the finding of a single catalytic unit. According to Bender & Coulson (1972), it is possible for L-DOPA and L-5-HTP to be arranged in such a manner that the α-amino and the α-carboxyl groups and the 3-hydroxyl group of L-DOPA and the 5-hydroxyl group of L-5-HTP occupy corresponding sites. With such an arrangement the aromatic rings of both substances would no longer occupy corresponding planes or sites. It is possible that this different arrangement may explain differences in kinetic properties for the two substrates.

The functional relevance of the competitive inhibitory effect of L-DOPA upon the decarboxylation of L-5-HTP by AAAD is most probably meaningless; the concentrations of L-DOPA (at least 5 mM) needed to obtain a 30% reduction in the decarboxylation of L-5-HTP are well above the levels of endogenous L-DOPA (~10 μM). In order to occur, a competitive inhibitory effect of L-DOPA upon L-5-HTP would have to be at its tubular uptake, since the concentrations of the inhibitor (L-DOPA) would be not far from those occurring under physiological conditions (Wolfovitz *et al.*, 1993). On the other hand, it should always be kept in mind that the cellular transport of one of these two large neutral amino acids is dependent on the relative concentration of the other (Wurtman, 1981). Endothelial cells of cerebral blood vessels and of the choroid plexus are known to take up L-DOPA and L-5-HTP through the same carrier (Maruki *et al.*, 1984; DeFeudis, 1986); furthermore, the rate of transport of L-5-HTP by endothelial cells of the choroid plexus in hyperphenylalaninaemic rats is considerably impaired (Piel *et al.*, 1982).

In conclusion, the results presented here suggest that L-5-HTP and L-DOPA are using the same transporter in order to be taken up into renal tubular cells; L-DOPA exerts a competitive type of inhibition upon the tubular uptake of L-5-HTP. It is further shown that this inhibitory effect is only observed when the concentration of the inhibitor is approaching half saturation concentrations for uptake. The functional consequences of this interaction between L-DOPA and L-5-HTP at the level of tubular uptake may be of some relevance for the overall understanding of the renal effects of dopamine and 5-HT, as described in the accompanying papers (Soares-da-Silva *et al.*, 1996a,b).

The present study was supported by grant number PBIC/C/CEN 1139/92 from the Junta Nacional de Investigação Cientifica e Tecnológica (JNICT). P.C.P-d.-Ó is the recipient of a fellowship sponsored by the 'American Portuguese Biomedical Research Fund'.

References

BENDER, D.A. & COULSON, W.F. (1972). Variations in aromatic amino acid decarboxylase activity towards dopa and 5-hydroxytryptophan caused by pH changes and denaturation. *J. Neurochem.*, **19**, 2801–2810.

BLOCH, R.D., ZIKOS, D., FISHER, K.A., SCHLEICHER, L., OYAMA, M., CHENG, J.-C., SKOPICKI, H.A., SUKOWSKI, E.J., CRAGOE Jr., E.J. & PETERSON, D.R. (1992). Activation of proximal tubular Na^+-H^+ exchange by angiotensin II. *Am. J. Physiol.*, **263**, F135–F143.

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.

CHAN, Y.L. (1976). Cellular mechanisms of renal tubular transport of L-DOPA and its derivatives in the rat: microperfusions studies. *J. Pharmacol. Exp. Ther.*, **199**, 17–24.

CHENG, Y.-C. & PRUSSOFF, W.H. (1973). Relationship between the inhibition constant (K_i) and the concentration of the inhibitor which causes 50 per cent inhibition (IC_{50}) of an enzymatic reaction. *Biochem. Pharmacol.*, **22**, 3099–3108.

DEFEUDIS, F.V. (1986). New studies on the cerebrovascular endothelium - possible relevance to the interpretation of 'precursor-loading' experiments. *Trends Pharmacol. Sci.*, **7**, 51–52.

FERNANDES, M.H., PESTANA, M. & SOARES-DA-SILVA, P. (1991). Deamination of newly-formed dopamine in rat renal tissues. *Br. J. Pharmacol.*, **102**, 778–782.

HAGEN, P.B. & COHEN, L.H. (1966). Biosynthesis of indolalkylamines. Physiological release and transport of 5-hydroxytryptamine. In *5-Hydroxytryptamine and related Indole Alkylamines*. ed. Eichler, O. & Farah, A. *Handbook of Experimental Pharmacology*, Vol 19. pp. 182–211. Berlin: Springer-Verlag.

ITSKOWITZ, H.D., CHEN, Y.-H. & STIER, Jr., C.T. (1988). Reciprocal effects of dopamine and 5-hydroxytryptamine formed within the kidney. *Clin. Sci.*, **75**, 503–507.

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

LI KAM WA, T.C., FREESTONE, S., SAMSON, R.R., JOHNSON, N.R. & LEE, M.R. (1993). A comparison of the renal and neuroendocrine effects of two 5-hydroxytryptamine prodrugs in normal man. *Clin. Sci.*, **85**, 607–614.

LOVENBERG, W., WEISSBACH, H. & UDENFRIEND, S. (1962). Aromatic L-amino acid decarboxylase. *J. Biol. Chem.*, **237**, 89–93.

MARUKI, C., SPATZ, M., UEKI, Y., NAGATSU, I. & BEMBRY, J. (1984). Cerebrovascular endothelial cell culture: Metabolism and synthesis of 5-hydroxytryptamine. *J. Neurochem.*, **43**, 316–319.

MOTULSKY, H.J., SPANNARD, P. & NEUBIG, R. (1994). *GraphPad Prims* (Version 1.0). San Diego, U.S.A.: GraphPad Prism Software Inc.

PIEL, N., LANE, J.D., HUTHER, G. & NEUHOFF, V. (1982). Impaired permeability of the blood-cerebrospinal fluid barrier in hyperphenylalaninemia. *Neuropediatrics*, **13**, 88–92.

PINTO-DO-Ó, P.C. & SOARES-DA-SILVA, P. (1994). L-DOPA interferes with the renal handling of 5-hydroxy-L-tryptophan. *J. Auton. Pharmacol.*, **14**, 25.

SHAH, S.V., NORTHRUP, T.E., HUI, Y.S.F. & DOUSA, T.P. (1979). Action of serotonin (5-hydroxytryptamine) on cyclic nucleotides in glomeruli of rat renal cortex. *Kidney Int.*, **15**, 463–472.

SHIROTA, K. & FUJISAWA, H. (1988). Purification and characterization of aromatic L-amino acid decarboxylase from rat kidney and monoclonal antibody to the enzyme. *J. Neurochem.*, **51**, 426–434.

SIMS, K.L., DAVIS, G.A. & BLOOM, F. (1973). Activities of 3,4-dihydroxy-L-phenylalanine and 5-hydroxy-L-tryptophan decarboxylases in rat brain: Assay characteristics and distribution. *J. Neurochem.*, **20**, 449–464.

SOARES-DA-SILVA, P. (1993). Enhanced protein kinase C mediated inhibition of renal dopamine synthesis during high sodium intake. *Biochem. Pharmacol.*, **45**, 1791–1800.

SOARES-DA-SILVA, P., PINTO-DO-Ó, P.C. & BERFORALLO, A.M. (1996a) Antagonistic action of renal dopamine and 5-hydroxytryptamine: increase in Na^+, K^+ ATPase activity in renal proximal tubules via activation of 5-HT_{1A} receptors. *Br. Pharmacol.*, **117**, 1199–1203.

SOARES-DA-SILVA, P. & VIEIRA-COELHO, M.A. (1993). Increased sensitivity of renal catechol-O-methyltransferase to inhibition by tolcapone. *J. Am. Soc. Nephrol.*, **4**, 896 (abstract).

SOARES-DA-SILVA, P., VIEIRA-COELHO, V.A. & PESTANA, M. (1996b). Antagonistic actions of renal dopamine and 5-hydroxytryptamine: role of endogenous 5-HT and 5-HT_{1A} receptors in antinatriaresis during high sodium intake. *Br. J. Pharmacol.*, **117**, 1193–1198.

SOLE, M.J., MADAPALLIMATTAM, A. & BAINES, A.D. (1986). An active pathway for serotonin synthesis by renal proximal tubules. *Kidney Int.*, **29**, 689–694.

SOURKES, T.L. (1977). Enzymology of aromatic amino acid decarboxylases. In *Structure and Function of Monoamine Enzymes*, ed. Usdin, E., Weiner, N. & Youdim, M.B.H. pp. 477–496. New York: Marcell Decker.

STIER, Jr., C.T., MCKENDALL, G. & ITSKOVITZ, H. (1984). Serotonin formation in non-blood perfused rat kidneys. *J. Pharmacol. Exp. Ther.*, **228**, 53–56.

SUMI, C., ICHINOSE, H. & NAGATSU, T. (1990). Characterization of recombinant human aromatic L-amino acid decarboxylase expressed in COS cells. *J. Neurochem.*, **55**, 1075–1078.

WILLIAMS, W.M. & HUANG, K.G. (1970). In vitro and in vivo renal tubular transport of tryptophan derivatives. *Am. J. Physiol.*, **219**, 1468–1474.

WOLFOVITZ, E., GROSSMAN, E., FOLIO, C.J., KEISER, H.R., KOPIN, I.J. & GOLDSTEIN, D.S. (1993). Derivation of urinary dopamine from plasma dihydroxyphenylalanine in humans. *Clin. Sci.*, **84**, 549–557.

WURTMAN, R.J., HEFTI, F. & MELAMED, F. (1981). Precursor control of neurotransmitter synthesis. *Pharmacol. Rev.*, **32**, 315–335.

(Received July 13, 1995
Revised November 10, 1995
Accepted November 13, 1995)