Deamination of newly-formed dopamine in rat renal tissues

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1 The present study has examined the formation of dopamine and 3,4-dihydroxyphenylacetic acid (DOPAC) in slices of the rat renal cortex and the renal medulla loaded with exogenous L- β -3,4-dihydroxyphenylalanine (L-DOPA). The effects of pargyline and of two selective inhibitors of monoamine oxidase (MAO) types A and B, respectively Ro 41-1049 and Ro 19-6327, on the deamination of newly-synthesized dopamine in kidney slices incubated with exogenous L-DOPA were also tested. The assay of L-DOPA, dopamine, noradrenaline and DOPAC was performed by means of h.p.l.c. with electrochemical detection.

2 Incubation of renal slices with exogenous L-DOPA resulted in a concentration-dependent accumulation of dopamine and DOPAC; the tissue levels of newly-formed dopamine and DOPAC in slices of the renal medulla were 6–8% of those in cortical slices.

3 Pargyline (0.1 mM) produced a marked decrease (84% reduction) in the formation of DOPAC in kidney slices loaded with 1.0 mM L-DOPA; this effect was accompanied by a 17% increase in the accumulation of dopamine. Similar effects were obtained at higher concentrations of pargyline (0.5 and 1.0 mM). At 5.0 and 10.0 mM pargyline, a marked decrease (46 and 76% reduction) in the accumulation of newly-formed dopamine was observed.

4 The accumulation of dopamine and DOPAC was found to be time-dependent in experiments in which tissues were incubated with 5 and 10 μ M L-DOPA for 5, 10, 20 and 30 min. Pargyline (0.1 mM) produced an increase in the accumulation of dopamine at all incubation periods and decreased the formation of DOPAC.

5 Ro 41-1049 (50, 100 and 250 nM) was found to produce a concentration-dependent increase of newly-formed dopamine (16–66% increase) and reduced DOPAC formation (44–89% reduction). Ro 19-6327 (50, 100 and 250 nM), was found not to affect the accumulation of newly-formed dopamine, but significantly reduced the formation of DOPAC.

6 It is concluded that deamination of newly-formed dopamine in kidney slices loaded with L-DOPA constitutes an important mechanism of amine inactivation. The results presented also suggest that most of the MAO, located inside the compartment where the synthesis of dopamine occurs, is of the A type.

Introduction

In kidney, a major source of dopamine derives from the decarboxylation of filtered 3,4-dihydroxyphenylalanine (DOPA) in tubular epithelial cells (Baines & Chan, 1980; Lee, 1982; Suzuki *et al.*, 1984). This is a quantitatively important process as tubular epithelial cells have a high aromatic L-amino acid decarboxylase (AAAD) activity (Adam *et al.*, 1986) and plasma levels of DOPA can attain concentrations up to 15 pmol ml⁻¹ (Cuche, 1988). More recently, evidence obtained in studies performed under *in vitro* conditions have confirmed the view that renal tissues are endowed with an enormous capacity to synthesize dopamine from exogenous L- β -3,4-dihydroxyphenylalanine (L-DOPA) and further showed that a substantial amount of newly-formed dopamine in renal tissues is deaminated to 3,4-dihydroxyphenylacetic acid (DOPAC) (Soares-da-Silva & Fernandes, 1990a). The metabolic degradation of dopamine formed in tubular epithelial cells is indeed a matter of considerable importance. Firstly, because there is evidence suggesting that in kidney, dopamine is synthesized in close proximity to the cells endowed with dopamine receptors, the activation of which results in a decrease of tubular reabsorption of sodium (Jose *et al.*, 1986; Siragy *et al.*, 1989). Secondly, there is evidence that renal tissues are endowed with one of the largest monoamine oxidase (MAO) activities in the body (Caramona & Soares-da-Silva, 1990) and deamination represents a major pathway for the inactivation of catechol-

amines within the kidney (Stöcker & Hempel, 1976; Kopin, 1985).

Most of the AAAD activity in kidney is located in the cortex (Adams *et al.*, 1986) and evidence has also been presented showing that most of the dopamine formed in renal tissues has its origin in cortical elements (Seri *et al.*, 1990; Soares-da-Silva & Fernandes, 1990b), namely in proximal convoluted tubules (Hayashi *et al.*, 1990). The aim of the present work was to compare the processes of deamination of newly-formed dopamine in slices of the rat renal cortex and the renal medulla loaded with exogenous L-DOPA and also to study the effects of non-selective and selective MAO inhibitors on the renal production of dopamine.

Methods

Male Wistar rats (Biotério do Instituto Gulbenkian de Ciência, Oeiras, Portugal) aged 45–60 days and weighing 200–280 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*. The experiments were all carried out during day time.

The rats were killed by decapitation under ether anaesthesia and both kidneys removed and rinsed free from blood with saline (0.9% w/v NaCl solution). The kidneys were placed on an ice cold glass plate, the kidney poles removed and slices of renal cortex and renal medulla approximately 1.5 mm thick and weighing 70–80 mg wet weight were prepared with a scalpel. Thereafter, slices of the renal cortex and the renal

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medulla were preincubated individually for 60 min in warm (37°C) and gassed (95% O₂ and 5% CO₂) 10 ml Krebs solution; the pH of the incubation medium was 7.4 for both cortical and medullary slices. After preincubation, renal slices were incubated for 15 min in gassed and warm Krebs solution with added L-DOPA (10–2500 µM); up to a concentration of 5 mM L-DOPA, the pH of the incubation medium remained stable at 7.4. In studies of MAO inhibition, kidney slices containing both the cortex and the medulla were preincubated for 60 min in the presence of the inhibitor, pargyline (0.1–10.0 mM). After preincubation, renal slices were incubated for 15 min in Krebs solution with added L-DOPA (1.0 mM) and containing pargyline, as in the preincubation period. In some experiments, kidney slices (containing both the cortex and the medulla) were incubated for 5, 10, 20 or 30 min with L-DOPA (5 and 10 µM) in the presence of pargyline (0.1 mM). Selective inhibition of type A and B MAO was performed with compounds Ro 41-1049 and Ro 19-6327, respectively (Da Prada *et al.*, 1990). Slices containing both the cortex and the medulla were preincubated for 60 min in the presence of increasing concentrations of the inhibitors (50, 100 and 250 nM); after preincubation, renal tissues were incubated for 15 min with added L-DOPA (10 µM) in the presence of the selective MAO inhibitors. The Krebs solution had the following composition (mM): NaCl 118, KCl 4.7, CaCl₂ 2.4, MgSO₄ 1.2, NaHCO₃ 25, KH₂PO₄ 1.2, EDTA 0.4, ascorbic acid 0.57 and glucose 11; 1- α -methyl-*p*-tyrosine (50 µM), copper sulphate (10 µM) and tropolone (30 µM) were added to the Krebs solution in order to prevent the synthesis of endogenous catecholamines from tyrosine and to inhibit the endogenous inhibitors of dopamine β -hydroxylase and the enzyme catechol-*O*-methyltransferase (COMT), respectively. The preincubation and incubation were carried out in a shaking water bath at 37°C, in an atmosphere of 95% O₂ and 5% CO₂; renal slices were incubated individually in glass vials containing 10 ml Krebs solution. After the incubation, renal slices were collected, washed for 30 min in ice cold Krebs solution, blotted with filter paper, weighed, minced with fine scissors and placed in 2 ml of 0.2 M perchloric acid at 4°C for the next 24 h, till quantification of tissue catecholamines.

The assay of L-DOPA, dopamine, noradrenaline (NA) and DOPAC in renal tissues was performed by means of high performance liquid chromatography with electrochemical detection (h.p.l.c.-e.c.d.). Aliquots of 1.5 ml perchloric acid in which tissues have been kept were placed in 5 ml conical-based glass vials with 50 mg alumina and the pH of the sample immediately adjusted to pH 8.6 by the addition of Tris buffer. Mechanical shaking for 10 min was followed by centrifugation and the supernatant discarded. The adsorbed catecholamines were then eluted from the alumina with 200 µl of 0.2 M perchloric acid on Millipore microfilters (MF1); 50 µl of the eluate was injected into the h.p.l.c.-e.c.d. system. The h.p.l.c.-e.c.d. 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, Indiana, U.S.A.) 25 cm in length. Samples were injected into the column by means of an automatic sample injector (Gilson model 231) connected to a Gilson dilutor (model 401). The detection was carried out electrochemically by means of a thin-layer cell with a glassy carbon working electrode, an Ag/AgCl reference electrode, and an amperometric detector (model 304 LC-4A; Bioanalytical Systems). The detector cell was operated at 0.75 V. The current produced was monitored with a Spectra-Physics integrator (model SP4270; Spectra-Physics, Darmstadt-Kranichstein, F.R.G.). 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 (10% v/v), pH 3.5 and pumped at a rate of 1.0 ml min⁻¹. Standard solutions of L-DOPA, NA, dopamine, DOPAC and dihydroxybenzylamine (internal standard) were injected at different concentrations and peak height increased linearly. The

interassay coefficient of variation was less than 5%. The lower limits for detection of L-DOPA, NA, dopamine and DOPAC were 1.0, 0.9, 1.4 and 2.5 pmol g⁻¹, respectively.

Mean values $\pm$ s.e.mean of *n* experiments are given. Significance of differences between two means was estimated by Student's *t* test for unpaired data. Significance of difference between one control and several experimental groups was evaluated by Tuckey-Kramer method (Sokal & Rohlf, 1981). A *P* value < 0.05 was taken to denote a significant difference.

1- α -Methyl-*p*-tyrosine, dopamine hydrochloride, (–)-noradrenaline bitartrate, L- β -3,4-dihydroxyphenylalanine (L-DOPA), 3,4-dihydroxyphenylacetic acid (DOPAC), pargyline hydrochloride and tropolone hydrochloride were purchased at Sigma Chemical Company (St Louis, MO, U.S.A.) and copper sulphate at May and Baker (Dagenham, England). Ro 41-1049 (N-(2-aminoethyl)-5-(*m*-fluoro-phenyl)-4-thiazole carboxamide hydrochloride) and Ro 19-627 (N-(2-aminoethyl)-5-chloro-2-pyridine carboxamide hydrochloride) were kindly donated by Prof. Mosé Da Prada from Hoffman-La Roche, Basle, Switzerland.

Results

As shown in Figure 1a, the accumulation of L-DOPA in kidney slices was found to be dependent on the concentration of the amino acid added to the medium; this was much more evident in cortical slices than in slices containing the renal medulla, as evidenced by the tendency for saturation in the

Figure 1 Tissue levels (in nmol g⁻¹) of (a) L- β -3,4-dihydroxyphenylamine (L-DOPA), (b) dopamine and (c) 3,4-dihydroxytryphenylacetic acid (DOPAC) in slices of the (■) rat renal cortex and the (□) renal medulla incubated with increasing concentrations of exogenous L-DOPA (10–2500 µM) for 15 min. Each point represents the mean of four experiments per group; vertical lines show s.e.mean.

accumulation of L-DOPA in this renal area. Incubation of cortical slices with increasing concentrations of L-DOPA for 15 min also resulted in a concentration-dependent accumulation of newly-formed dopamine; the amount of the amine accumulated in the tissue reached values as high as 480 nmol g^{-1} when 2.5 mM L-DOPA was added to the medium (Figure 1b). The production of dopamine from added L-DOPA in slices containing the renal medulla was about 6–8% of that occurring in the renal cortex at 2.5 mM L-DOPA (Figure 1b). The accumulation of DOPAC, the deaminated metabolite of dopamine, in cortical slices was also found to be dependent on the concentration of L-DOPA used and reached values as high as 62 nmol g^{-1} when 2.5 mM L-DOPA was used (Figure 1c). As has been found for dopamine, the formation of DOPAC in the renal medulla was approximately 8% of that occurring in cortex. However, although in the renal cortex there was a trend for saturation in the formation of DOPAC, in the renal medulla such a tendency was not observed. In kidney slices incubated in the absence of exogenous L-DOPA the tissue levels of NA were 1.3 ± 0.14 and $0.40 \pm 0.06 \text{ nmol g}^{-1}$ ($n = 5$), respectively in the cortex and the medulla; DOPAC was not detectable in these experimental conditions. The tissue levels of NA in kidney slices did not change significantly in the course of these experiments even when 2.5 mM L-DOPA was used (data not shown).

Incubation of slices containing both the renal cortex and the medulla with 1.0 mM L-DOPA resulted in the accumulation of newly-formed dopamine and DOPAC. The addition of 0.1 mM pargyline, the non-selective MAO inhibitor, to the incubation medium resulted in a marked decrease (84% reduction) in the formation of DOPAC; this effect was accompanied by a non-significant rise (17% increase) in the accumulation of newly-formed dopamine in kidney slices (Figure 2). The addition of increasing concentrations (0.5, 1.0, 5.0 and

10.0 mM) of pargyline similarly reduced the formation of DOPAC (85–95% reduction). This effect was accompanied by a decrease in the accumulation of newly-formed dopamine in the presence of 5.0 and 10.0 mM pargyline when tissue levels of dopamine in kidney slices were respectively 54% and 22% of those in control conditions (Figure 2).

Assuming that the compartment where renal synthesis of dopamine takes place might be of limited storage capacity, it was decided to perform additional experiments with a lower concentration of L-DOPA ($10 \mu\text{M}$) and different periods of incubation (5, 10, 20 and 30 min) were also tested. The accumulation of newly-formed dopamine and DOPAC was shown to be time-dependent (Figure 3). In the presence of 0.1 mM pargyline, the increase in the accumulation of dopamine (20–32% increase; Figure 3a) in kidney slices loaded with $10 \mu\text{M}$ L-DOPA was less pronounced than the reduction of DOPAC tissue levels (54–55% reduction; Figure 3b). However, when tissues have been incubated for 30 min, the decreasing effect of pargyline upon DOPAC levels (62% decrease) was similar to its increasing effect upon dopamine tissue levels (56% increase) (Figure 3). When kidney slices were incubated with $5 \mu\text{M}$ L-DOPA (data not shown), the magnitude of the effect of pargyline (0.1 mM) in increasing dopamine tissue levels (20–32% increase) was similar to the magnitude of its effect in decreasing DOPAC tissue levels (33–60% reduction).

The addition of increasing concentrations (50, 100 and 250 nM) of the selective MAO-A inhibitor, Ro 41-1049, resulted in a concentration-dependent increase of newly-formed dopamine tissue levels (16–66% increase) in kidney slices incubated with $10 \mu\text{M}$ L-DOPA (Figure 4a). This effect was accompanied by a concentration-dependent decrease

Figure 2 Effect of increasing concentrations of pargyline (0.1, 0.5, 1.0, 5.0 and 10.0 mM) on the tissue levels (in nmol g^{-1}) of (a) dopamine and (b) 3,4-dihydroxyphenylacetic acid (DOPAC) in slices containing both the renal cortex and renal medulla incubated with exogenous L- β -3,4-dihydroxyphenylalanine (L-DOPA) (1.0 mM) for 15 min. Each column represents the mean of three experiments per group; vertical lines show s.e.mean. Significantly different from corresponding values of control by the Tuckey-Kramer method (* $P < 0.01$). Control, open columns; pargyline, hatched columns.

Figure 3 Effect of pargyline (0.1 mM) on the time-dependent accumulation of (a) dopamine and (b) 3,4-dihydroxyphenylacetic acid (DOPAC) (in nmol g^{-1}) in slices containing both the renal cortex and renal medulla incubated with exogenous L- β -3,4-dihydroxyphenylalanine (L-DOPA, $10 \mu\text{M}$) for 5, 10, 20 and 30 min. Each column represents the mean of four experiments per group; vertical lines show s.e.mean. Significantly different from corresponding values of control by Student's t test (* $P < 0.01$). Control, open columns; pargyline, hatched columns.

Figure 4 Tissue levels (in nmol g^{-1}) of (a) dopamine and (b) 3,4-dihydroxyphenylacetic acid (DOPAC) in slices containing both the renal cortex and renal medulla incubated with exogenous L- β -3,4-dihydroxyphenylalanine (L-DOPA, $10 \mu\text{M}$) for 10 min in control conditions and in the presence of Ro 41-1049 (50, 100 and 250 nM) and Ro 19-6327 (50, 100 and 250 nM). Each column represents the mean of five to six experiments per group; vertical lines show s.e.mean. Significantly different from corresponding values of control by the Tuckey-Kramer method (* $P < 0.01$). Control, open columns; Ro 41-1049, cross hatched columns, Ro 19-6327, solid columns.

(44–89% reduction) of DOPAC tissue content (Figure 4b). Incubation of kidney slices in the presence of the MAO-B selective inhibitor, Ro 19-6327 (50, 100 and 250 nM), was found not to affect the accumulation of newly-formed dopamine, but reduced DOPAC formation (Figure 4). In fact, at 50 nM, Ro 19-6327 did not affect DOPAC formation, but at higher concentrations (100 and 250 nM) it decreased (40–49% reduction) DOPAC accumulation. The tissue contents of both NA and L-DOPA in kidney slices at the end of incubation were unaffected by the presence of either Ro 41-1049 or Ro 19-6327 (data not shown).

Discussion

The results described here are in agreement with previous observations showing that most AAAD activity in renal tissues is located in cortical elements. The finding that a considerable amount of newly-formed dopamine in kidney slices is deaminated to DOPAC gives further support to the view that MAO plays a major role in the disposition of the amine in renal tissues (Stöcker & Hempel, 1976; Kopin, 1985). The present results suggest that both types of MAO may be involved in the deamination of newly-formed dopamine but that most of the MAO located inside the compartment where the synthesis of dopamine takes place appears to be of the A type.

The synthesis of dopamine, in renal tissues, has been suggested to occur either at neuronal and non-neuronal elements: in noradrenergic neurones, dopamine is the precursor for NA; in independent dopaminergic neurones, dopamine may serve as the transmitter substance (Bell, 1987) and there is evidence

to suggest that in tubular epithelial cells, dopamine may act as a paracrine substance (Lee, 1982; Siragy *et al.*, 1989). Thus, it might be suggested that decarboxylation of L-DOPA in renal tissues loaded with the amino acid can take place in each of these three cellular elements. However, Baines & Chan (1980) showed that kidney denervation does not affect the formation of dopamine from exogenous L-DOPA; this strongly suggests that most of the decarboxylation of exogenous L-DOPA does occur in non-neuronal structures, namely in tubular epithelial cells (Hayashi *et al.*, 1990). The finding that tissue levels of NA in kidney slices did not change even when 2.5 mM L-DOPA was added to the incubation medium, strongly supports the view that L-DOPA is not decarboxylated inside noradrenergic neurones. Moreover, this further suggests that newly-formed dopamine is not taken up into sympathetic nerve terminals (see Soares-da-Silva, 1988). The possibility that a major proportion of exogenous L-DOPA is decarboxylated inside dopaminergic neurones is also rather unlikely, given the limited size of this hypothetical compartment and the large amounts of L-DOPA which are decarboxylated into dopamine.

Deamination represents a major pathway in the inactivation of catecholamines within the kidney (Stöcker & Hempel, 1976; Kopin, 1985) and renal tissues are endowed with one of the largest MAO activities in the body (Caramona & Soares-da-Silva, 1990). The results presented here show that a substantial amount of newly-formed dopamine is deaminated to DOPAC; this occurs largely in the renal cortex where saturation of deamination of dopamine could be observed, but also to some extent in the medulla and this difference may be related to the limited production of dopamine in the rat renal medulla. Alternatively, it could be hypothesized that in the rat renal medulla the specific activity of MAO may exceed that in the renal cortex, as has been found to occur in the kidney of the cat (Caramona & Soares-da-Silva, 1990). Furthermore, the results indicate a limited ability of MAO to deaminate newly-formed dopamine in renal tissues. In fact, the accumulation of dopamine obtained in the presence of 1.0 or 2.5 mM L-DOPA was far greater than that of DOPAC, whereas with concentrations of 5 and $10 \mu\text{M}$ L-DOPA the amount of DOPAC accumulated in the tissue was about 80% of that of dopamine.

When kidney slices were loaded with $10 \mu\text{M}$ L-DOPA the effect (20–32% increase) of 0.1 mM pargyline on the accumulation of dopamine was considerable lower than its decreasing effects on the formation of DOPAC (54–55% reduction). Since pargyline is a non-selective MAO inhibitor, the different effects on the accumulation of dopamine and DOPAC could be the result of the interference with the two different types of MAO, A and B, located in different cellular compartments. For this reason, it was decided to test the effects of selective inhibitors of type A and B MAO, Ro 41-1049 and Ro 19-6327 respectively, on the deamination of newly-formed dopamine. Ro 41-1049 was found to produce a concentration-dependent increase in the accumulation of newly-formed dopamine; this effect was accompanied by a parallel reduction of DOPAC tissue levels. By contrast, incubation of kidney slices in the presence of the MAO-B selective inhibitor, Ro 19-6327, did not affect the accumulation of dopamine in kidney slices, but decreased the formation of DOPAC, at concentrations of 100 and 250 nM. These results appear to suggest that type A and B MAO do not occur in the same cellular compartments. It is possible that most of type A MAO is located in the compartment where the synthesis of dopamine takes place, whereas type B MAO is mainly located outside this compartment and would be responsible for the deamination of dopamine leaking from the storage compartment since MAO-B inhibition does not increase dopamine accumulation but does reduce DOPAC formation.

In recent years, several lines of evidence have suggested that dopamine may exert considerable effects within the kidney and contribute actively in the maintenance of blood pressure at levels considered as acceptable (Lee, 1982; Yoshimura & Takahashi, 1988; Bertorello *et al.*, 1988). The activation of

type D₁ dopamine receptors in the renal vasculature produced vasodilatation resulting in increased renal blood flow and glomerular filtration rate (Felder *et al.*, 1988). Tubular epithelial cells are also endowed with type D₁ dopamine receptors (Hedge *et al.*, 1989) and it has been suggested that dopamine of tubular origin would activate these receptors resulting in an increased sodium excretion (Siragy *et al.*, 1989). Therefore, it might be suggested that inhibition of an important mechanism responsible for the inactivation of tubular dopamine would

result in a potentiation of diuretic and natriuretic effects of the amine. Pargyline may not be the most effective MAO inhibitor for this purpose, because of its non-selective effects, but the use of a selective MAO-A inhibitor would be preferred if MAO-A predominates in the compartment where the synthesis of dopamine takes place.

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