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Effect of type A and B monoamine oxidase selective inhibition by Ro 41-1049 and Ro 19-6327 on dopamine outflow in rat kidney slices

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1 The influence of pargyline and of selective inhibitors of type A and B monoamine oxidase (MAO), Ro 41-1049 and Ro 19-6327 respectively, on the outflow of dopamine and 3,4-dihydroxyphenylacetic acid (DOPAC) in slices of rat renal cortex loaded with exogenous L-3,4-dihydroxyphenylalanine (L-DOPA) was examined. Dopamine and DOPAC in the tissues and in the effluent were assayed by means of h.p.l.c. with electrochemical detection.

2 The levels of newly-formed dopamine and DOPAC in the perifusate decreased progressively with time. In control conditions, DOPAC/dopamine ratios in the perifusate were 3 to 5 fold those in the tissue and were found to increase progressively with time. The addition of pargyline (100 μM), produced a marked decrease in the outflow levels of DOPAC (45 to 54% reduction) and significantly increased the levels of dopamine in the effluent (102 to 158% increase); DOPAC/dopamine ratios in the perifusate remained stable throughout the perifusion and were similar to those found in the tissues. The addition of the MAO-A inhibitor Ro 41-1049 to the perifusion fluid also significantly decreased DOPAC outflow (41% to 54% reduction) and increased dopamine outflow (19% to 80% increase). In the presence of Ro 41-1049 DOPAC/dopamine ratios in the perifusate were lower ($P<0.01$) than in controls; in contrast with the effect of pargyline, this ratio was found to increase ($P<0.01$) throughout the perifusion period. Ro 19-6327 did not reduce the outflow of DOPAC, but significantly increased (by 40–60%) that of dopamine. In the presence of Ro 19-6237, the proportion of DOPAC to dopamine in the perifusate was similar to that of controls and significantly increased throughout the perifusion; however, this increase was less than that observed in the control group.

3 When benserazide (50 μM) was added to the perifusion fluid, the levels of both dopamine and DOPAC in the effluent were similar to those observed in the absence of benserazide. However, in the presence of benserazide, DOPAC/dopamine ratios in the perifusate did not increase with time. In conditions of decarboxylase inhibition, the effects of pargyline, Ro 41-1049 and Ro 19-6327 on dopamine and DOPAC outflow were less pronounced than in experiments conducted in the absence of benserazide.

4 In conclusion, the results presented here show that the fraction of newly-formed dopamine which leaves the compartment where the synthesis has occurred is a constant source for deamination into DOPAC. The results provide evidence favouring the view that MAO-A is the main form of the enzyme involved in this process; however, the data described here suggest that dopamine would also have access to MAO-B.

Keywords: Dopamine; 3,4-dihydroxyphenylacetic acid (DOPAC); monoamine oxidase; kidney; pargyline; Ro 41-1049; Ro 19-6327; benserazide

Introduction

Evidence accumulated in recent years has favoured the view that locally formed dopamine may play a considerable role in the regulation of tubular sodium reabsorption. The inhibitory effect of dopamine on tubular sodium reabsorption has been demonstrated to be dependent on the activation of specific receptors for the amine, resulting in inhibition of both Na^+-K^+ ATPase and Na^+-H^+ antiport activities (Aperia *et al.*, 1987; Felder *et al.*, 1990). The source of dopamine responsible for the natriuretic effects of the amine is believed to reside in epithelial cells of the proximal convoluted tubules. These cells are endowed with a high aromatic L-amino acid decarboxylase (AAAD) activity and L-3,4-dihydroxyphenylalanine (L-DOPA) in the tubular filtrate represents the major source of the precursor for the amine. After being taken up into the tubular epithelial cell, L-DOPA has been shown to undergo rapid decarboxylation to dopamine. It has also been suggested that the tubular epithelial cells in which the synthesis occurs may be endowed with or be located near those endowed with dopamine receptors, leading to the possibility that dopamine in the kidney may act as a paracrine and/or autocrine substance (Siragy *et al.*, 1989).

In order to be active, however, the dopamine originating in tubular epithelial cells must leave the cellular compartment where the synthesis has occurred. This is a matter of considerable importance since the receptors on which the amine is supposed to act are located on the external surface of the cell membrane (Jose *et al.*, 1992). On the other hand, the amount of dopamine that is available for the activation of dopamine receptors may depend not only on the activity of AAAD and on the levels of L-DOPA in the tubular filtrate, but also on the magnitude of the metabolism to which dopamine is submitted. In fact, renal tissues are endowed with one of the highest monoamine oxidase (MAO) activities in the body (Kopin, 1985) and deamination of newly-formed dopamine into 3,4-dihydroxyphenylacetic acid (DOPAC) represents a major pathway for the inactivation of the amine (Soares-da-Silva & Fernandes, 1990).

Both the A and B forms of MAO, (MAO-A and MAO-B), have been shown to be involved in the deamination of newly-

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formed dopamine in rat kidney slices loaded with exogenous L-DOPA (Fernandes & Soares-da-Silva, 1990); however, it has been suggested that the deamination of newly-formed dopamine by these two types of MAO occurs in different compartments. Indirect evidence has suggested that MAO-A is predominantly active in the compartment where the synthesis of dopamine takes place, whereas MOA-B is thought to be mainly active outside this compartment (Fernandes & Soares-da-Silva, 1990; Fernandes *et al.*, 1991). However, the different behaviour of types A and B MAO on the deamination of newly-formed dopamine may only reflect the cellular compartmentalization of the two forms of the enzyme.

The aim of the present study was to define whether deamination of newly-formed dopamine by MAO-A or by MAO-B reflects the preferential access of the amine to one of the enzymes during the cell outflow process. For this purpose we have studied the influence of pargyline, a non-selective MAO inhibitor, and of two selective MAO-A and MAO-B inhibitors, Ro 41-1049 and Ro 19-6327 respectively (Da Prada *et al.*, 1990), on the outflow of newly-formed dopamine and of its deaminated metabolite DOPAC in slices of rat renal cortex loaded with exogenous L-DOPA. A preliminary account of some of these findings has been presented previously (Pestana & Soares-da-Silva, 1992).

Methods

Male Wistar rats (Biotério do Instituto Gulbenkian de Ciência, Oeiras, Portugal) 30–60 days old and weighing 200–250 g were used in the experiments. Animals were kept three 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.

Rats were killed by decapitation under ether anaesthesia and both kidneys removed and rinsed free from blood with saline (NaCl 0.9%). The kidneys were placed on an ice-cold glass plate and the kidney poles removed; thereafter, slices approximately 1.5 mm thick were obtained with a scalpel and four cortical fragments weighing about 60 mg were prepared and preincubated for 30 min in warm (37°C) and gassed (95% O_2 and 5% CO_2) Krebs solution. After preincubation, renal fragments were incubated for 15 min in 2 ml of warm and gassed Krebs solution with added L-DOPA (100 μM). Thereafter, the cortical slices were transferred to individual glass perifusion chambers, perifused with warm and gassed Krebs solution at a rate of 350 μl min^{-1} by means of a Gilson pump (Minipulse 2) and allowed a 30 min stabilization period. After the washing period, five consecutive 10 min perifusate samples were collected into glass tubes kept on ice and containing 500 μl of 2 M perchloric acid. The composition of the Krebs solution used in the experiments was as follows (in mM): NaCl 118; KCl 4.7; $CaCl_2$ 2.4; $MgSO_4$ 1.4; $NaHCO_3$ 25; KH_2PO_4 1.2; EDTA 0.4 and glucose 11; tropolone (50 μM) was added to the Krebs solution in order to inhibit the enzyme catechol-*O*-methyltransferase (COMT). In experiments performed with the aim of testing the influence of MAO inhibition on the outflow of dopamine and DOPAC, the MAO inhibitor pargyline (100 μM), Ro 41-1049 (250 nM) or Ro 19-6327 (250 nM) was added to the perifusion fluid. The concentration of pargyline, Ro 41-1049 and Ro 19-6327 used here was selected in the light of previous studies in renal tissues and have been shown to produce maximal inhibitory effects (Fernandes & Soares-da-Silva, 1990; Fernandes *et al.*, 1991); at high concentrations, both Ro 41-1049 and Ro 19-6327 may lose selectivity (Da Prada *et al.*, 1990; Guimarães & Soares-da-Silva, 1994). In some experiments, renal slices were perifused in the presence of 50 μM benserazide, in order to prevent the formation of dopamine during the perifusion period. At the end of the incubation period, tissues were collected and immediately placed in perifusion chambers and perifused from then onwards in the presence of benserazide. In some of these experiments, the effect of pargyline, Ro-41-1049 and Ro 19-6327 was also tested.

The assay of dopamine and DOPAC in renal slices and perifusate samples was performed by means of high pressure liquid chromatography (h.p.l.c.) with electrochemical detection, as previously described (Soares-da-Silva *et al.*, 1992). The lower limits for detection of dopamine and DOPAC were 8 and 14 pmol g^{-1}, respectively.

Dopamine hydrochloride, L-β-3,4-dihydroxyphenylalanine (L-DOPA), 3,4-dihydroxyphenylacetic acid (DOPAC), pargyline hydrochloride and tropolone hydrochloride were purchased from the Sigma Chemical Company (St Louis, MO, U.S.A.). Ro 41-1049 ((N-(2-aminoethyl)-5-(*m*-fluoro-phenyl)-4-thiazolecarboxamide hydrochloride), Ro 19-6327 ((N-(2-aminoethyl)-5-chloro-2-pyridine carboxamide hydrochloride) and benserazide were kindly donated by Prof. Mosé Da Prada, Hoffmann-La Roche (Basle, Switzerland).

Statistics

Results are means ± s.e. mean of values for the indicated number of experiments. Statistical significance was determined by the Tuckey-Kramer method (Sokal & Rohlf, 1981). A P value less than 0.05 was assumed to denote a significant difference.

Results

The levels of endogenous dopamine in the rat renal cortex (0.024 ± 0.006 nmol g^{-1}) were found to be lower than in renal cortical fragments incubated in the presence of L-DOPA (100 μM); levels of endogenous DOPAC were below the detection limit of the method (14 pmol g^{-1}). As shown in Table 1, the tissue concentration of newly-formed dopamine and DOPAC in renal cortical fragments perifused with pargyline, Ro 41-1049 and Ro 19-6327 did not differ significantly from those of corresponding controls (i.e., loaded with L-DOPA and perifused in the absence of MAO inhibitors); however, DOPAC/dopamine ratios in renal tissues perifused in the presence of Ro 41-1049 were found to be lower ($P<0.01$) than those of controls.

The outflow of dopamine and DOPAC in renal tissues loaded with L-DOPA (100 μM) was found to decrease progressively with time (Figure 1), but did not exhibit a monophasic decline, as evidenced by their upward concave shape when the levels of dopamine (from 0.84 ± 0.10 to 0.16 ± 0.03 nmol g^{-1} 10 min^{-1}) and DOPAC (0.80 ± 1.0 to 0.24 ± 0,02 nmol g^{-1} 10 min^{-1}) in the perifusate were logarithmically transformed and plotted against the time of perifusion (not shown, but see Pestana & Soares-da-Silva, 1994). Under control conditions, however, the decline of DOPAC outflow throughout the perifusion period did not follow that of dopamine; this is shown by the progressive increase of DOPAC/dopamine ratios in the perifusate (from 0.95 ± 0.07 to 1.77 ± 0.25, $n=8$, Figure 2). It is interesting to note that DOPAC/dopamine ratios in the perifusate were 3 to 5 fold those in the tissue. The addition of pargyline (100 μM), produced a marked decrease in the outflow levels of DOPAC (45 to 54% reduction) and significantly increased the levels of dopamine in the effluent (102 to 158% increase). In the presence of pargyline, in contrast to observations in controls, DOPAC/dopamine ratios in the perifusate remained stable throughout the perifusion (from 0.26 ± 0.02 to 0.33 ± 0.02) and were similar to those found to occur in the tissues (0.27 ± 0.02). As shown in Figure 1, the addition of the MAO-A inhibitor Ro 41-1049 to the perifusion fluid also significantly decreased DOPAC outflow (41% to 54% reduction) and increased dopamine outflow (19% to 80% increase). In the presence of Ro 41-1049 DOPAC/dopamine ratios in the perifusate were lower ($P<0.01$) than in controls, but, in contrast to results with pargyline, this ratio was

found to increase ($P<0.01$) throughout the perifusion period from 0.33 ± 0.04 to 0.66 ± 0.10 (Figure 2). As shown in Figure 1, Ro 19-6327 did not reduce the outflow of DOPAC, but significantly increased by 40–60% that of dopamine. In the presence of Ro 19-6327, the proportion of DOPAC to dopamine in the perifusate was similar to that of controls, and significantly increased from 0.86 ± 0.06 to 1.19 ± 0.13 throughout the perifusion; this increase was, however, less than that observed in the control group.

Table 1 Tissue levels (in nmol g^{-1}) of dopamine and dihydroxyphenylacetic acid (DOPAC), and DOPAC/ dopamine ratios at the end of perifusions performed in the absence (**A**) and in the presence of benserazide (50 μM) (**B**)

A

	Dopamine (nmol g^{-1})	*DOPAC* nmol g^{-1})	*DOPAC/dopamine*	(*n*)
Control	3.39 ± 0.39	1.05 ± 0.15	0.31 ± 0.02	8
Pargyline	3.85 ± 0.43	1.12 ± 0.23	0.27 ± 0.03	7
Ro 41-1049	3.67 ± 0.53	0.71 ± 0.10	0.20 ± 0.02*	8
Ro 19-6327	4.07 ± 0.38	1.37 ± 0.17	0.33 ± 0.02	7

B

	Dopamine (nmol g^{-1})	*DOPAC* nmol g^{-1})	*DOPAC/dopamine*	(*n*)
Control	3.60 ± 0.37	1.13 ± 0.11	0.32 ± 0.03	4
Pargyline	4.91 ± 1.02	0.91 ± 0.32	0.17 ± 0.03*	4
Ro 41-1049	7.20 ± 2.56	1.41 ± 0.68	0.20 ± 0.04*	4
Ro 19-6327	4.42 ± 0.23	1.11 ± 0.04	0.25 ± 0.01	4

Significantly different from values of corresponding controls, *$P<0.01$.

Results are shown for observations in control conditions and in the presence of pargyline (100 μM), Ro 41-1049 (250 nM) and Ro 19-6327 (250 nM). Each value represents the mean ± s.e.mean of *n* experiments per group.

Figure 3 shows the outflow of dopamine and DOPAC in experiments carried out in the presence of benserazide (50 μM); the AAAD inhibitor was added to the perifusion fluid from the beginning of the perifusion period. In this set of experiments, the levels of both dopamine and DOPAC in the perifusate under control conditions were similar to those observed in the absence of benserazide. However, in the presence of benserazide DOPAC/dopamine ratios in the perifusate failed to show a tendency to increase with time (0.77 ± 0.12 to 1.01 ± 0.11) (Figure 4). Under conditions of AAAD inhibition, pargyline produced a marked reduction of DOPAC outflow (60% decrease) and only a slight increase (20 to 30% increase) in the outflow of dopamine (Figure 3). DOPAC/dopamine ratios in the perifusate were also markedly reduced by pargyline (Figure 4), as was observed in the absence of benserazide. The changes in the outflow levels of dopamine and DOPAC during selective MAO-A inhibition with Ro 41-1049 in the presence of benserazide, were similar to those observed with pargyline (Figures 3 and 4). DOPAC/dopamine ratios in the perifusate were reduced to an extent similar to that observed with pargyline and did not show a tendency to increase with time (Figure 4). In the presence of benserazide, Ro 19-6327 was found not to change the outflow levels of both dopamine and DOPAC, but DOPAC/dopamine ratios in the perifusate were found to be similar to those in controls, which contrasts with those observed in experiments performed in the absence of benserazide (Figure 4).

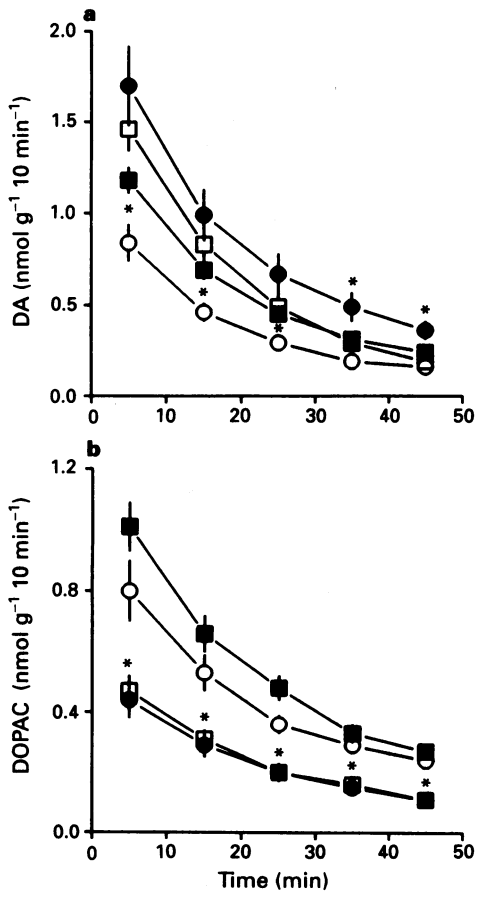

Figure 1 Dopamine (a) and DOPAC (b) outflow levels (in nmol g^{-1} 10 min^{-1}) in 10 min perifusate samples of renal cortical fragments in control conditions (○) and in the presence of pargyline (100 μM; ●), Ro 41-1049 (250 nM; □) or Ro 19-6327 (250 M; ■). Each point represents the mean ± s.e.mean of 7 to 8 experiments per group. Significantly different from corresponding control values, *$P<0.05$.

Discussion

The results presented here are in agreement with previous observations showing that renal tissues are endowed with a high capacity to synthesize dopamine from L-DOPA and that MAO plays a major role in the inactivation of the amine (Stöker & Hempel, 1976; Kopin, 1985; Fernandes *et al.*, 1991). The analysis of the outflow of dopamine and DOPAC suggests that the amine released from tubular epithelial cells is a constant source for deamination into DOPAC and that MAO-A is the main form of enzyme involved in the

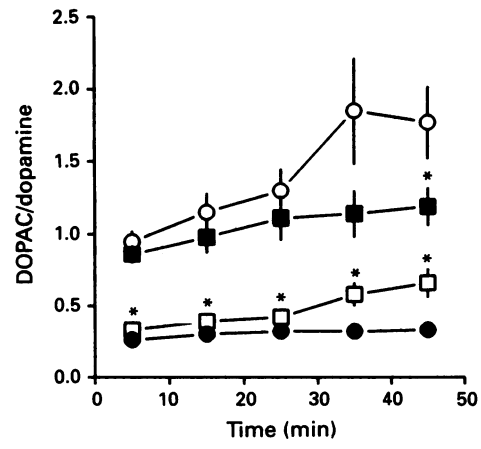

Figure 2 DOPAC/dopamine ratios in 10 min perifusate samples collected from renal cortical fragments in control conditions (○) and in the presence of pargyline (100 μM; ●), Ro 41-1049 (250 nM; □) or Ro 19-6327 (250 nM; ■). Each point represents the mean ± s.e.mean of 7 to 8 experiments per group. Significantly different from corresponding control values, *$P<0.05$.

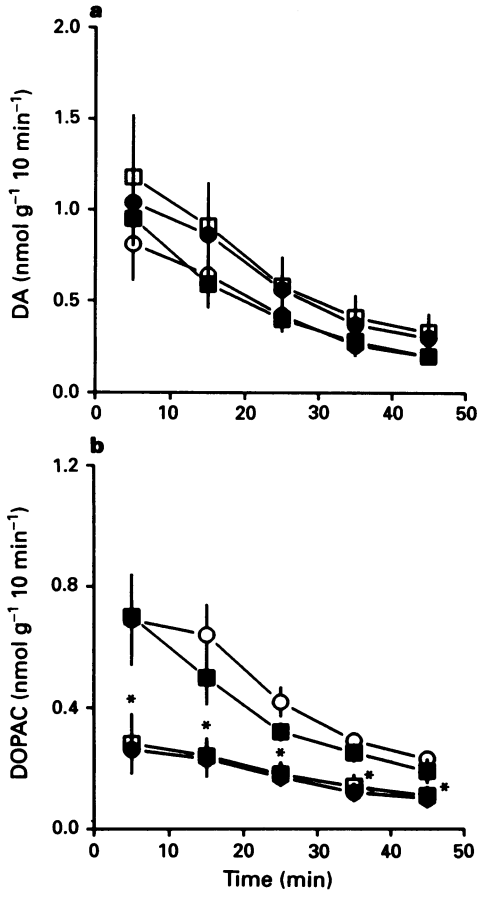

Figure 3 Dopamine (a) and DOPAC (b) outflow levels (in nmol g^{-1} 10 min^{-1}) in 1 min perifusate samples of renal cortical fragments in the presence of benserazide (50 μM). Results are shown for observations in control conditions (○), and in the presence of pargyline (100 μM; ●), Ro 41-1049 (250 nM; □) or Ro 19-6327 (250 nM; ■). Each point represents the mean ± s.e.mean of 4 experiments per group. Significantly different from corresponding control values, $*P<0.05$.

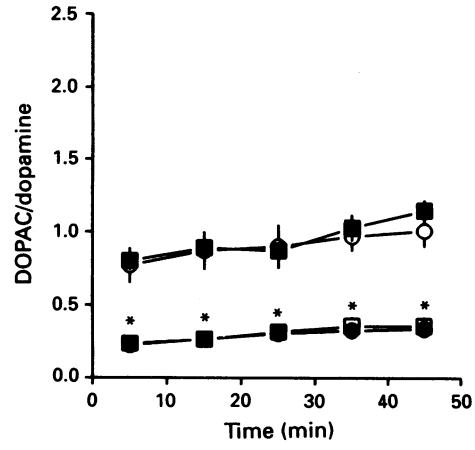

Figure 4 DOPAC/dopamine ratios in 10 min perifusate samples collected from renal cortical fragments in the presence of benserazide. Results are shown for observations in control conditions (○) and in the presence of pargyline (100 μM; ●), Ro 41-1049 (250 nM; □) or Ro19-6327 (250 nM; ■). Each point represents the mean ± s.e.mean of 4 experiments per group. Significantly different from corresponding control values, $*P<0.05$.

deamination of newly-formed dopamine, although the amine might also have a limited access to MAO-B.

Most probably, the formation of the dopamine and DOPAC present in kidney slices loaded with exogenous L-DOPA occurred during the incubation period and not during the subsequent period of perifusion. The main argument favouring this suggestion is that dopamine and DOPAC outflow and the levels of both compounds in renal tissues perifused in the presence of benserazide were similar to those obtained in experiments performed in the absene of AAAD inhibition. The effect of benserazide (added to the perifusion fluid) was mainly a reduction in DOPAC/dopamine ratios in the last two collection periods. This, as discussed below, suggests that a minor amount of the dopamine appearing in the effluent was formed during the perifusion period, after the incubation with L-DOPA and this dopamine has an easy access to MAO. Another argument favouring the view that a considerable amount of the DOPAC in the effluent (up to 50%) was formed during the incubation period is that neither pargyline nor Ro 41-1049 decreased the tissue levels of the metabolite, but significantly decreased DOPAC outflow. In agreement with this suggestion are the results of previous work from our laboratory where it has been consistently shown that a significant amount (up to 30%) of the toal dopamine formed in kidney slices loaded with L-DOPA undergoes rapid deamination to DOPAC (Fernandes & Soares-da-Silva, 1990; Fernandes *et al.*, 1991; Fernandes & Soares-da-Silva, 1993). Of course it could be argued also that the lack of effect of MAO inhibitors in reducing the tissue accumulation of DOPAC is a concentration-related problem. In our hands, however, when added to the incubation medium before loading with L-DOPA, all the three MAO inhibitors, at these very same concentrations, were found to decrease DOPAC formation (100 μM pargyline, 84% reduction; 250 nM Ro 41-1049, 86% reduction; 250 nM Ro 19-6327, 79% reduction) (Fernandes & Soares-da-Silva, 1990; Fernandes *et al.*, 1991).

The deamination of newly-formed dopamine in kidney slices loaded with exogenous L-DOPA is rather unlikely to occur in a neuronal compartment. In fact, previous studies have shown that renal denervation does not affect the deamination of dopamine to DOPAC in rat kidney slices loaded with L-DOPA (Soares-da-Silva *et al.*, 1992). this also agrees with the evidence that renal denervation does not affect both MAO-A and MAO-B activities, suggesting that in the kidney, most MAO is located extraneuronally (Caramona & Soares-da-Silva, 1990, Soares-da-Silva *et al.*, 1992). Although this does not completely rule out the possibility that some of the renal dopamine might have access to neuronal MAO, there is evidence to suggest that the neuronal uptake of newly-formed dopamine in kidney slices is most probably an irrelevant process. When levels of dopamine in kidney slices increase up to 480 nmol g^{-1} (i.e., 6000 fold the levels of endogenous dopamine), as has found to occur in the presence of 2.5 mM L-DOPA, the levels of endogenous noradrenaline (1.3 nmol g^{-1}) in kidney slices do not change (Fernandes *et al.*, 1991).

The suggestion that dopamine is intensely deaminated to DOPAC before reaching the perifusion medium is supported by the findings that: (1) DOPAC/dopamine ratios in the perifusate were significantly higher than those in the tissues (0.95 vs 0.30) and (2) this ratio progressively increased with time (from 0.95 to 1.77). Non selective inhibition of MAO with pargyline was found to increase the levels of dopamine in the effluent and to reduce the outflow of DOPAC. However, the tissue levels of both dopamine and DOPAC in the presence of pargyline did not significantly differ from those of controls. This would suggest that pargyline inhibits mainly the deamination of that dopamine leaving the compartment where the synthesis has occurred or the deamination of that dopamine which crosses a compartment rich in MAO activity before reaching the perifusion medium. This conclusion is further reinforced by the finding that the proportion of DOPAC to dopamine in the effluent, in the presence of

pargyline, was drastically reduced and did not differ from that found in the tissues. It can be hypothesized that the cellular compartment crossed by the amine before reaching the perifusate is the very same in which dopamine is synthesized; however, this would imply that the amine would be taken up into the original cellular structure, after being released. Considering that the luminal cell border has been shown not to take up dopamine (Chan, 1976) and this cell border has been found to be collapsed when renal slices are used (Wedeen & Weiner, 1973), it might then be suggested that the released dopamine would be taken up into tubular epithelial cells through the basolateral membrane. This hypothesis might explain why the proportion of DOPAC to dopamine is significantly higher in the perifusate than that found in the tissues and would also support the results presented here on the influence of selective and non selective MAO inhibitors in the outflow levels of dopamine and DOPAC.

The changes in the outflow of both dopamine and DOPAC produced by the selective MAO-A inhibitor, Ro 41-1049, were similar to those observed with pargyline. In addition, the significant reduction in DOPAC/dopamine ratios in the perifusate produced by Ro 41-1049 was similar to that observed with pargyline. These results suggest that MAO-A is the form of the enzyme located in the compartment where the synthesis of dopamine has occurred and in the cellular compartment rich in MAO crossed by the amine before it has reached the perifusion medium. However, the finding that the increase with time of DOPAC/dopamine ratios in the perifusate was found to be abolished by pargyline, but not by Ro 41-1049, suggests that MAO-B would be the form of the enzyme responsible for the increased deamination of dopamine taking place in the last two collection periods. In agreement with this suggestion are the results that Ro 19-6327 does, in fact, reduce the increase with time of DOPAC/dopamine ratios in the perifusate.

This raises the question of the relative importance of the two forms of MAO, types A and B, in the deamination of renal dopamine. Previous studies in kidney slices loaded with L-DOPA have shown that both MAO-A and MAO-B are involved in the deamination of newly-formed dopamine and that MAO-B would be particularly involved in the deamination of the amine outside the compartment where the synthesis takes place (Fernandes & Soares-da-Silva, 1990; Fernandes *et al.*, 1991). In these experimental conditions, the cellular structures potentially involved in the handling of renal dopamine may theoretically include all those present in the renal cortical slices of the rat kidney: glomeruli, tubules, vascular tissues and support connective tissue. The renal handling of dopamine is expected, however, to involve mainly tubular epithelial cells, namely those of proximal convoluted tubules. These are the cells involved in the synthesis of renal dopamine as a result of the decarboxylation of circulating L-DOPA (Baines & Chan, 1980; Lee, 1982; Suzuki *et al.*, 1984) and a major area in cortical slices is occupied by the renal tubules. The presence of type A and type B MAO in different cellular compartments has been suggested in radioautographic studies showing that MAO-A is homogenously distributed in both the cortex and the medulla, while MAO-B is heterogenously distributed throughout the renal cortex (Saura *et al.*, 1992). Although it could be hypothesized that some of the released dopamine might have been deaminated by MAO-B in non tubular epithelial cellular structures, rat proximal convoluted renal tubules were shown recently to be endowed with both MAO-A and MAO-B activities and 250 nM Ro 19-6327 was found to reduce by 70% the deamination of [^{14}C]-β-phenylethylamine, a specific substrate of MAO-B (Guimarães & Soares-da-Silva, 1994). Therefore, our hypothesis, considering all data available on this matter, is that in rat epithelial cells of cortical renal tubules, newly-formed dopamine is mainly deaminated by MAO-A.

The released dopamine appears to be taken up into a MAO-A rich compartment, whereas the deamination of newly-formed dopamine by MAO-B is dictated by a slow diffusion of the amine towards the enzyme. There is evidence indicating that MAO-B is located in renal tubular cells endowed with the ability to synthesize dopamine (Guimarães & Soares-da-Silva, 1994), but the data presented here cannot exclude the presence of a multi-compartment system where the MAO-B involved in this process might be located also in non-tubular cells. In fact, the analysis of the outflow of dopamine and DOPAC does not reveal the presence of a monocompartmental system with MAO activity, as indicated by the upward concave shape of the efflux curves, and, therefore, is not in a steady state of efflux (Pestana & Soares-da-Silva, 1994). This may be, however, of minor metabolic importance as indicated by the results that in both man (Freestone *et al.*, 1993) and rat (Vieira-Coelho *et al.*, 1993), inhibition of MAO-B was shown not to affect the urinary excretion of sodium, the main renal effect of tubular dopamine (Lee, 1993).

In some experiments renal slices were perifused in the presence of benserazide, a well-known inhibitor of the enzyme AAAD. After incubation with L-DOPA, renal tissues were immediately placed in the perifusion chambers and perifused onwards in the presence of 50 μM benserazide. These experiments were performed with the aim of preventing the synthesis *de novo* of dopamine during the perifusion period; under these experimental conditions the amount of dopamine available for release is, therefore, only that which has been synthesized during the incubation period. The synthesis *de novo* of dopamine during the perifusion appears, however, not to be of great importance, since benserazide failed to decrease the outflow of both dopamine and DOPAC. The most relevant results obtained in the presence of benserazide were the following: (1) inhibition of the increase of DOPAC/dopamine ratios in the perifusate and (2) inhibition of the increase of the outflow levels of dopamine, as induced by pargyline, Ro 41-1049 and Ro 19-6327. This suggests that the dopamine formed during the perifusion period has easy access to both forms of MAO, especially that of type B.

In conclusion, the results presented here show that the fraction of newly-formed dopamine which leaves the compartment where the synthesis has occurred is a constant source for deamination into DOPAC and also provide evidence favouring the view that MAO-A is the main form of the enzyme involved in this process; however, the data reported here suggest that dopamine also has acces to MAO-B, although this may be a minor metabolic pathway.

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