Original Paper

nephron
Physiology

Nephron Physiol 2006;103:p14–p24
DOI: 10.1159/000090219

Received: December 8, 2004
Accepted: August 17, 2005
Published online: December 12, 2005

Role of Chronic Inhibition of Dopamine-Metabolizing Enzymes in the Regulation of Renal Sodium and Phosphate Excretion in the Rat Remnant Kidney

Benedita Sampaio-Maia[a] Mónica Moreira-Rodrigues[b] Manuel Pestana[b]

[a]Institute of Pharmacology and Therapeutics and [b]Unit of Research and Development of Nephrology, Faculty of Medicine, Porto, Portugal

Key Words

BIA 3-202 · Catechol-*O*-methyltransferase · DOPAC · Homovanillic acid · Monamine oxidase · Natriuresis · Phosphaturia · Remnant kidney · Renal dopamine · Ro-411049

Abstract

Background/Aims: The present study examined the effects of chronic selective or combined inhibition of type A monoamine oxidase (MAO) and catechol-*O*-methyltransferase (COMT) on daily urinary excretion of dopamine and metabolites and on natriuresis and phosphaturia in 3/4 nephrectomized (3/4nx) and Sham rats. ***Methods:*** The 3/4nx and Sham rats were placed in metabolic cages and received the MAO-A-selective inhibitor Ro-411049 (7.5 mg·kg^{-1} bid) and/or the COMT-selective inhibitor BIA 3-202 (30 mg·kg^{-1} bid) orally for 3 days during high sodium diet. ***Results:*** Selective COMT inhibition increased the urinary excretion of the deaminated metabolite (3,4-dihydroxyphenylacetic acid, DOPAC) and decreased the urinary excretion of the methylated (3-methoxytyramine, 3-MT) and deaminated plus methylated metabolite (homovanillic acid, HVA) in both groups. Selective MAO-A inhibition increased the urinary excretion of 3-MT and reduced the urinary excretion of both DOPAC and HVA in either 3/4nx or Sham rats. Combined inhibition of MAO-A and COMT did not significantly change the urinary excretion of DOPAC and markedly decreased the urinary excretion of 3-MT and HVA in both groups. Selective or combined inhibition of MAO-A and COMT did not alter the daily urinary excretion of dopamine, sodium or phosphate in either 3/4nx or Sham rats. ***Conclusions:*** Chronic selective or combined inhibition of MAO-A and COMT is not of major importance in regulating the dopamine-dependent natriuresis and phosphaturia in either 3/4nx or Sham rats.

Introduction

In kidney, dopamine is synthesized in the epithelial cells of the proximal tubules, which are endowed with a high aromatic *L*-amino acid decarboxylase (*L*-AADC) activity. Dopamine of renal origin behaves as an endogenous natriuretic and phosphaturic hormone interacting with tubular D_1-like receptors to inhibit Na^+,K^+-ATPase, Na^+-H^+ exchanger and Na^+-Pi cotransporter, as a paracrine/autocrine substance [1]. During moderate sodium surfeit, dopamine of renal origin accounts for ~50% of sodium excretion [2]. Dopamine of renal origin undergoes extensive deamination to 3,4-dihydroxyphenylacetic acid (DOPAC), *O*-methylation to 3-metoxytyramine

KARGER

Fax +41 61 306 12 34
E-Mail karger@karger.ch
www.karger.com

1660–2137/06/1031–0014$23.50/0

Accessible online at:
www.karger.com/nep

Manuel Pestana
Unit of Research and Development of Nephrology
Faculty of Medicine, University of Porto, Alameda Professor Hernani Monteiro
PT–4200-319 Porto (Portugal)
Tel. +351 919194907, Fax +351 225502023, E-Mail mvasconcelos@hsjoao.min-saude.pt

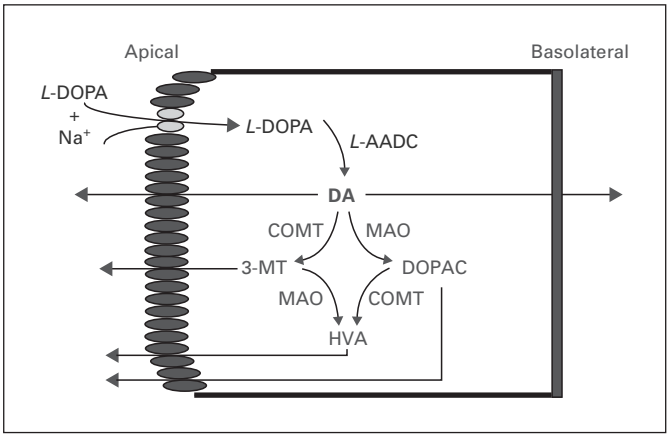

Fig. 1. Pathway for the formation and metabolism of intrarenal dopamine (DA, dopamine; DOPAC, dihydroxyphenylacetic acid; 3-MT, 3-metoxytyramine; HVA, homovanillic acid; *L*-DOPA, *L*-3,4-dihydroxyphenylalanine; *L*-AADC, aromatic *L*-amino acid decarboxylase; COMT, catechol-*O*-methyltransferase; MAO, monoamine oxidase).

(3-MT) and deamination plus *O*-methylation to homovanillic acid (HVA) [3, 4] (fig. 1) and the high levels of metabolic enzymes such as types A and B monoamine oxidases (MAO-A and MAO-B) and catechol-*O*-methyltransferase (COMT) have been considered important determinants in the overall availability of renal dopamine.

Patients suffering from chronic renal insufficiency have a reduction in the urinary excretion of dopamine and metabolites, the extent of which is related to the decrease in renal function [5, 6]. However, the residual nephrons in patients with chronic renal insufficiency maintain an intact ability to take up and decarboxylate *L*-DOPA to dopamine and deaminate the newly-formed amine to DOPAC [5, 7, 8]. The physiological role of the renal dopamine system in chronic renal insufficiency was addressed further in studies performed with animal models of reduced renal mass. In the 3/4 nephrectomized (3/4nx) rat model the absolute renal dopamine output was reduced accordingly with the decrease in renal mass [9]. However, this was accompanied in 3/4nx rats by an increased renal dopamine excretion per residual nephrons [9, 10] and dopamine-sensitive enhanced natriuresis [9] and phosphaturia [10]. This indicates that renal dopamine plays an important role in the renal handling of both sodium and phosphate during early chronic renal insufficiency. Interestingly, the remnant kidney from 3/4nx rats presented an increased renal COMT activity without changes in the renal activities of either MAO-A or MAO-B [9]. This further suggests that the overall reduced availability of renal dopamine in 3/4nx rats may result, at least in part, from enhanced renal *O*-methylation of the amine.

The effects of MAO-A, MAO-B and COMT inhibition in the kidney have been investigated in vitro and under these conditions deamination through MAO-A appeared to be the major metabolic route [3]. However, the relative importance of MAO and COMT in the kidney in vivo and their possible roles in the regulation of the dopamine-influenced natriuresis and phosphaturia has not yet been established. The acute in vivo inhibition of COMT using nitrocatechol derivatives was reported to cause a natriuretic response whereas MAO inhibition was associated with little effect on urinary sodium excretion [4, 11]. However, the tubular Na^+,K^+-ATPase inhibition produced by the nitrocatechol derivatives was associated to the direct stimulation of the D_1-like dopamine receptors rather than to enhanced availability of renal dopamine [12]. In addition, most of the in vivo studies only examined the acute effects of the dopamine-metabolizing enzyme inhibitors [11, 13–15]. Furthermore, the role of MAO and COMT inhibition on dopamine-mediated natriuresis and phosphaturia was not evaluated in conditions of reduced renal mass.

On the basis of these considerations, this study was undertaken with the aim of evaluating the possible regulatory roles of chronic COMT and MAO-A inhibition in dopamine-influenced natriuresis and phosphaturia by the remnant kidney. For that purpose, the daily urinary excretion of dopamine and metabolites as well as the natriuresis and phosphaturia were evaluated in 3/4nx and Sham rats during selective or combined administration of the MAO-A inhibitor Ro-411049 or the novel potent, orally active and long acting COMT inhibitor BIA 3-202 [16]. Because the natriuretic effect of dopamine is more consistently demonstrated in volume-expanded conditions [17], our studies were performed in 3/4nx and Sham rats receiving a high sodium intake.

Materials and Methods

Normotensive male Wistar-Han rats (Harlan, Barcelona, Spain), weighing 215 ± 2 g were kept under controlled environmental conditions (12:12 h light/dark cycle and room temperature 22 ± 2°C) and were fed ad libitum throughout the study with ordinary rat chow (Panlab, Barcelona, Spain) containing 1.9 $g \cdot kg^{-1}$ of sodium, 5.9 $g \cdot kg^{-1}$ of phosphate and 6.7 $g \cdot kg^{-1}$ of potassium. Fluid intake and food consumption were monitored daily throughout the study.

All in vivo investigation was performed in accordance with the European Directive number 86/609, transposed to the Portuguese Law by DL 129/92 and by Portaria 1005/92.

Inhibitory Effects of BIA 3-202 and Ro-411049 on Renal MAO-A, MAO-B and COMT Activities

In one set of experiments, control rats were anesthetized with pentobarbital sodium (60 mg·kg bw^{-1}, ip) and sacrificed before (basal) or at 1, 6 and 12 h after the oral administration of the selective MAO-A inhibitor, Ro-411049 (7.5 mg·kg^{-1}, by gavage) or the selective COMT inhibitor, BIA 3-202 (30 mg·kg^{-1}, by gavage). Thereafter, fragments of renal cortex were rapidly removed for the assessment of MAO-A, MAO-B and COMT activities as described below.

3/4 Nephrectomy

The right kidney was removed and the surgical ablation of both poles of the left kidney was performed in rats anesthetized with pentobarbital sodium (60 mg·kg bw^{-1}, ip), according to what was previously described by Sampaio-Maia et al. [9] = 3/4nx rats. The mean percentage of remnant renal mass in 3/4nx rats was 28 ± 1%. Control animals were rats submitted to sham surgery under similar conditions, however their kidneys remained intact = sham-operated (Sham) rats. After total recovery from surgery (4–6 h), all groups of rats were placed in the animal facility where they had free access to food and water. Survival rate was 100%.

MAO-A and COMT Inhibition during High Sodium (HS) Intake

The 3/4nx (n = 40) and Sham (n = 38) rats were placed in metabolic cages from day 11 to day 17 after the surgery. The daily food intake did not differ between 3/4nx and Sham rats (in g·24 h^{-1}: 3/4nx, 21 ± 1; Sham, 23 ± 1). The Sham animals had 1.0% (w/v) NaCl in their drinking water. Each day, the 3/4nx rats had only access to the mean daily salt intake of the corresponding Sham rats (see table 2). The vials collecting 24-hour urine contained 1 ml hydrochloric acid (6 *M*), to avoid the spontaneous oxidation of the amines and their derivatives. Fourteen days after the surgery, the 3/4nx and Sham rats were divided in four subgroups: *Group 1*: *selective MAO-A inhibition*. In this protocol, the animals received the selective MAO-A inhibitor, Ro-411049 (7.5 mg·kg^{-1} bi-daily (bid) by gavage), for 3 days (from day 14 to day 17 after the surgery); *Group 2: selective COMT inhibition*. In this protocol, the rats received the selective COMT inhibitor, BIA 3-202 (30 mg·kg^{-1} bid by gavage), for 3 days; *Group 3: combined inhibition of MAO-A and COMT*. In this protocol, the rats received Ro-411049 (7.5 mg·kg^{-1}) + BIA 3-202 (30 mg·kg^{-1}) bi-daily by gavage, for 3 days; *Group 4*: *control*. In this protocol, the rats received the vehicle, 0.9% NaCl + 0.5% methylcellulose (bid by gavage), for 3 days. Three days after the drug or vehicle administration (17 days after the surgery) the rats from the four groups were anesthetized with pentobarbital sodium (60 mg·kg bw^{-1}, ip) and blood was collected from the heart in tubes containing lithium/heparin for later determination of plasma biochemical parameters. Thereafter, fragments of renal cortex were rapidly removed for the assessment of MAO-A and COMT activities as described below.

MAO Activity

MAO activity was determined in fragments of renal cortex, as previously described [9]. MAO activity was determined with [^{3}H]5-hydroxytryptamine (5-HT, 2–400 μ*M*) as a preferential substrate for MAO-A and [^{14}C]β-phenylethylamine (βPEA, 0.25–50 μ*M*) as a preferential substrate for MAO-B [18]. The deaminated products were extracted with ethyl acetate and measured by liquid scintillation counting.

COMT Activity

COMT activity was evaluated by the ability of fragments of renal cortex to methylate adrenaline (3–1,000 μ*M*) to metanephrine, as previously described [9]. The assay of metanephrine was performed by HPLC with electrochemical detection.

Assay of Catecholamines

The assay of catecholamines and its metabolites in urine and in samples from COMT studies were performed by HPLC with electrochemical detection, as previously described [19]. In our laboratory, the lower limit of detection of dopamine, DOPAC, HVA, metanephrine, 3-MT and noradrenaline ranged from 350 to 1,000 fmol.

Plasma and Urine Ionogram and Biochemistry

Ion-selective electrodes performed the quantifications of sodium and potassium in plasma and urine samples. Phosphate was determined by a direct photometric method. Urea was measured by an enzymatic test and creatinine by the Jaffé method. All assays were performed by Cobas Mira Plus analyser (ABX Diagnostics, Switzerland). Urine and plasma osmolality were determined by means of an osmometer (model 3 MO, Advanced Instruments, Inc.). Creatinine clearance and fractional excretion of sodium, potassium and phosphate were calculated as previously reported [9].

Drugs

The compounds DOPAC, dopamine hydrochloride, HVA, metanephrine, 3-MT, noradrenaline bitartrate, and Ro-411049 were obtained from Sigma (St. Louis, Mo., USA). [^{3}H]5-HT creatinine sulfate (27.1 Ci·mmol^{-1}) and [^{14}C]βPEA hydrochloride (44.13 Ci·mmol^{-1}) were obtained from NEN Chemicals (USA). BIA 3-202 was kindly donated by BIAL (S. Mamede do Coronado, Portugal).

Statistics

Results are means ± SE of values for the indicated number of determinations. Maximal velocity (V_{max}) and Michaelis-Menten coefficient (K_m) values were calculated from non-linear regression analysis using GraphPad Prism statistics software package [20]. Statistical analysis was performed by one-way ANOVA followed by Student's t-test for unpaired comparisons ($p < 0.05$ was assumed to denote a significant difference).

Results

Ablation of renal mass had no effects on body growth, as 3/4nx rats attained the same weight at 17 days as Sham rats did (in g: 3/4nx, 263 ± 7; Sham, 269 ± 5). Kidney growth, however, was significantly altered in 3/4nx rats; 17 days after the surgery the 3/4nx rats presented a hypertrophied remnant renal mass weighing 90 ± 6% more

Table 1. Metabolic balance and renal function in sham-operated (Sham) and 3/4 nephrectomized (3/4nx) rats 3 days after vehicle administration

	Sham	3/4nx
Plasma creatinine, mg·dl^{-1}	0.22 ± 0.04	0.40 ± 0.05*
Plasma urea, mg·dl^{-1}	30.9 ± 2.5	62.7 ± 3.1*
Plasma Na^+, mmol·l^{-1}	137 ± 1	139 ± 1
Plasma K^+, mmol·l^{-1}	5.9 ± 0.5	5.5 ± 0.4
Plasma Pi, mmol·l^{-1}	2.1 ± 0.1	2.1 ± 0.1
Plasma osmolality, mosm·kg^{-1}	298 ± 2	309 ± 3*
Fluid intake, ml·24 h^{-1}	29.8 ± 1.7	53.8 ± 3.1*
Na^+ intake, mmol·24 h^{-1}	7.0 ± 0.3	6.5 ± 0.4
Urine volume, ml·24 h^{-1}	16.3 ± 1.3	38.5 ± 4.0*
Urinary Na^+, mmol·2 4 h^{-1}	6.6 ± 0.5	6.3 ± 0.7
Urinary K^+, mmol·24 h^{-1}	2.4 ± 0.2	2.7 ± 0.2
Urinary Pi, mmol·24 h^{-1}	0.25 ± 0.03	0.32 ± 0.03
Urinary osmolality, mosm·kg^{-1}	1,829 ± 83	814 ± 33*
$C_{creatinine}$, ml·min^{-1}·kg bw^{-1}	14.7 ± 3.1	7.4 ± 0.8*
FE_{Na+}, %	0.96 ± 0.18	1.82 ± 0.37*
FE_{K+}, %	7.96 ± 1.31	20.20 ± 4.30*
FE_{Pi}, %	2.14 ± 0.51	8.38 ± 1.23*

Values are means ± SE; n = 6–9 experiments per group. $C_{creatinine}$ = Creatinine clearance; FE = fractional excretion; bw = body weight.

* Significantly different from corresponding values in Sham rats ($p < 0.05$).

than on the day of surgery. Plasma levels of sodium, potassium and phosphate were similar in 3/4nx and Sham rats (table 1). Fluid intake and urine volume were greater in 3/4nx rats than in Sham rats, however, the 3/4nx rats had only access to the mean daily salt intake of the corresponding Sham rats and no significant differences were observed between the two groups in the daily urinary excretion of sodium (table 1). Also, the daily urinary excretion of potassium and phosphate did not differ between 3/4nx and Sham rats. The 3/4nx rats presented with significant increases in both plasma creatinine and urea levels, this being associated with a ~50% decrease in creatinine clearance values in comparison with Sham rats (table 1). The fractional excretion of sodium, potassium and phosphate were increased in 3/4nx animals by 90, 154 and 292%, respectively. The urine osmolality was reduced in 3/4nx rats whereas the plasma osmolality was slightly increased in 3/4nx animals (table 1).

The daily urinary excretion of dopamine and metabolites in 3/4nx and Sham rats during HS intake is depicted in table 2. As shown, on day 0 (before drug administration) the absolute urinary dopamine, DOPAC and HVA levels were lower in 3/4nx rats than in Sham animals. However, no significant differences were observed in the urinary excretion of 3-MT between the two groups. The

Table 2. Daily urinary levels of dopamine, DOPAC, HVA, 3-MT and noradrenaline in sham-operated (Sham) and 3/4 nephrectomized (3/4nx) rats on day 0 (before drug administration) and on day 3 of vehicle, Ro-411049 (7.5 mg·kg^{-1}, bid), BIA 3-202 (30 mg·kg^{-1}, bid) and Ro-411049+BIA 3-202 (Ro+BIA) administration

		Day 0	Day 3			
			vehicle	Ro-411049	BIA 3-202	Ro + BIA
Dopamine	Sham	19.8 ± 1.0	17.6 ± 1.9	20.7 ± 1.6	21.1 ± 3.7	19.0 ± 1.7
	3/4nx	8.8 ± 0.6[c]	8.1 ± 0.9[c]	9.5 ± 1.2[c]	10.5 ± 3.5[c]	11.9 ± 1.9[c]
DOPAC	Sham	39.2 ± 2.1	37.9 ± 6.0	23.2 ± 2.6[a, b]	101.9 ± 15.2[a, b]	29.7 ± 2.3[a]
	3/4nx	30.6 ± 1.3[c]	29.2 ± 2.4	19.5 ± 2.7[a, b]	85.3 ± 10.0[a, b]	32.3 ± 2.5
HVA	Sham	337 ± 26	364 ± 56	180 ± 51[a, b]	140 ± 44[a, b]	98 ± 14[a, b]
	3/4nx	244 ± 21[c]	265 ± 28	51 ± 10[a, b]	143 ± 46[b]	28 ± 5[a–c]
3-MT	Sham	11.4 ± 2.4	10.3 ± 2.3	21.8 ± 1.6[a, b]	ND	ND
	3/4nx	13.1 ± 1.4	13.7 ± 1.2	27.4 ± 4.5[a, b]	ND	ND
Noradrenaline	Sham	8.7 ± 0.8	7.1 ± 0.5	6.3 ± 1.0	7.6 ± 1.2	10.1 ± 1.1
	3/4nx	3.2 ± 0.4[c]	2.8 ± 0.3[c]	2.2 ± 0.3[c]	2.1 ± 0.5[c]	3.4 ± 0.3[c]

Values are means ± SE; n = 6–9 experiments per group. Values are expressed in nanomoles per 24 h. DOPAC = 3,4-Dihydroxyphenylacetic acid; HVA = homovanillic acid; 3-MT = 3-metoxytyramine; ND = levels not detectable.

[a] Significantly different from corresponding values on day 0 ($p < 0.05$).

[b] Significantly different from corresponding values in vehicle-treated rats ($p < 0.05$).

[c] Significantly different from corresponding values in Sham rats ($p < 0.05$).

daily urinary excretion of noradrenaline was significantly lower in 3/4nx rats than in Sham animals (table 2).

Effects of BIA 3-202 and Ro-411049 Administration on Renal MAO-A, MAO-B and COMT Activities

The time courses of the effects of BIA 3-202 and Ro-411049 on the renal activities of COMT, MAO-A and MAO-B (V_{max} values) in control animals are depicted in table 3. As it can be observed, the effect of BIA 3-202 (30 mg·kg^{-1}) on the renal COMT activity was a ~95% decrease up to 6 h after administration and a reduction of ~50% at 12 h after drug administration. Ro-411049 (7.5 mg·kg^{-1}) produced a ~30% decrease in renal MAO-A activity, which was maintained constant up to 12 h after administration. As shown, Ro-411049 and BIA 3-202 administration did not change the renal activities of COMT and MAO-A, respectively (table 3). Also, the selective MAO-A or COMT inhibitors did not change the renal MAO-B activity. Moreover, neither BIA 3-202 nor Ro-411049 administration changed the K_m values for MAO-A, MAO-B or COMT (data not shown).

The effects of chronic BIA 3-202 and Ro-411049 administration on the renal activities of COMT and MAO-A (V_{max} values) in Sham and 3/4nx animals are depicted in figure 2. As it can be observed, renal COMT activity in 3/4nx rats was higher than in Sham animals. Three days after BIA 3-202 and Ro-411049 administration the renal COMT activity was markedly reduced by ~85% in both Sham and 3/4nx rats. In addition, chronic BIA 3-202 and Ro-411049 administration reduced by ~45% the renal MAO-A activity in both Sham and 3/4nx rats.

Effect of COMT and MAO-A-Selective Inhibition during HS Intake in 3/4nx Rats

Chronic administration of the selective MAO-A inhibitor (Ro-411049) significantly reduced by ~40% the urinary levels of DOPAC in both Sham and 3/4nx rats; however, the urinary levels of dopamine were not altered during selective MAO-A inhibition in both groups (table 2). This resulted in marked decreases in the urinary DOPAC/dopamine ratios in both 3/4nx and Sham rats (fig. 3a). In addition, the urinary levels of HVA were markedly reduced during Ro-411049 administration in both groups (Sham rats, ~50% reduction; 3/4nx rats, ~80% reduction) (table 2). This was accompanied in both 3/4nx and Sham rats by a 2-fold increase in both the urinary excretion of 3-MT and in the urinary 3-MT/dopamine ratios (fig. 3b).

Chronic administration of the selective COMT inhibitor (BIA 3-202) completely abolished the urinary levels of the methylated metabolite 3-MT and markedly reduced the urinary levels of the methylated plus deaminated metabolite HVA (Sham rats, ~60% reduction; 3/4nx rats, ~40% reduction) (table 2). However, BIA 3-202 administration did not change the urinary levels of dopamine in both Sham and 3/4nx rats. The selective COMT inhibitor increased by 2.5-fold the urinary excretion of DOPAC in either 3/4nx or Sham. This resulted in a ~80% decrease in the urinary HVA/DOPAC ratios (fig. 4a) as well as in a 2.5-fold increase in the urinary DOPAC/dopamine ratios in both groups (fig. 4b).

Combined administration of Ro-411049 and BIA 3-202 completely abolished the urinary levels of 3-MT in both groups (table 2) and markedly decreased in the urinary excretion of HVA (Sham, ~70% reduction; 3/4nx, ~80% reduction). In addition, combined administration of Ro-411049 and BIA 3-202 slightly reduced by ~25% the urinary excretion of DOPAC in Sham rats and did not alter the urinary excretion of DOPAC in 3/4nx rats. However, the urinary levels of dopamine were not changed by the combined inhibition of COMT and MAO-A, in either Sham or 3/4nx rats (table 2).

Table 3. Effects of BIA 3-202 (30 mg·kg^{-1}) and Ro-411049 (7.5 mg·kg^{-1}) on renal COMT, MAO-A and MAO-B activities (V_{max} values in nmol·mg protein^{-1}·h^{-1}) in homogenates of renal cortex from control animals in basal conditions and at 1, 6 and 12 h after the drug administration

	Basal	BIA 3-202			Ro-411049		
		1 h	6 h	12 h	1 h	6 h	12 h
COMT	24.3 ± 3.1	0.76 ± 0.02*	1.16 ± 0.03*	11.2 ± 1.6*	25.6 ± 3.4	25.4 ± 3.4	24.9 ± 3.4
MAO-A	10.6 ± 0.5	10.3 ± 0.6	11.5 ± 2.1	11.4 ± 0.2	8.2 ± 1.0*	7.0 ± 0.5*	7.7 ± 1.0*
MAO-B	3.0 ± 0.1	3.5 ± 0.1	3.4 ± 0.3	2.7 ± 0.2	3.2 ± 0.5	3.4 ± 0.2	2.8 ± 0.1

Values are means ± SE; n = 4 experiments per group. * Significantly different from basal values ($p < 0.05$).

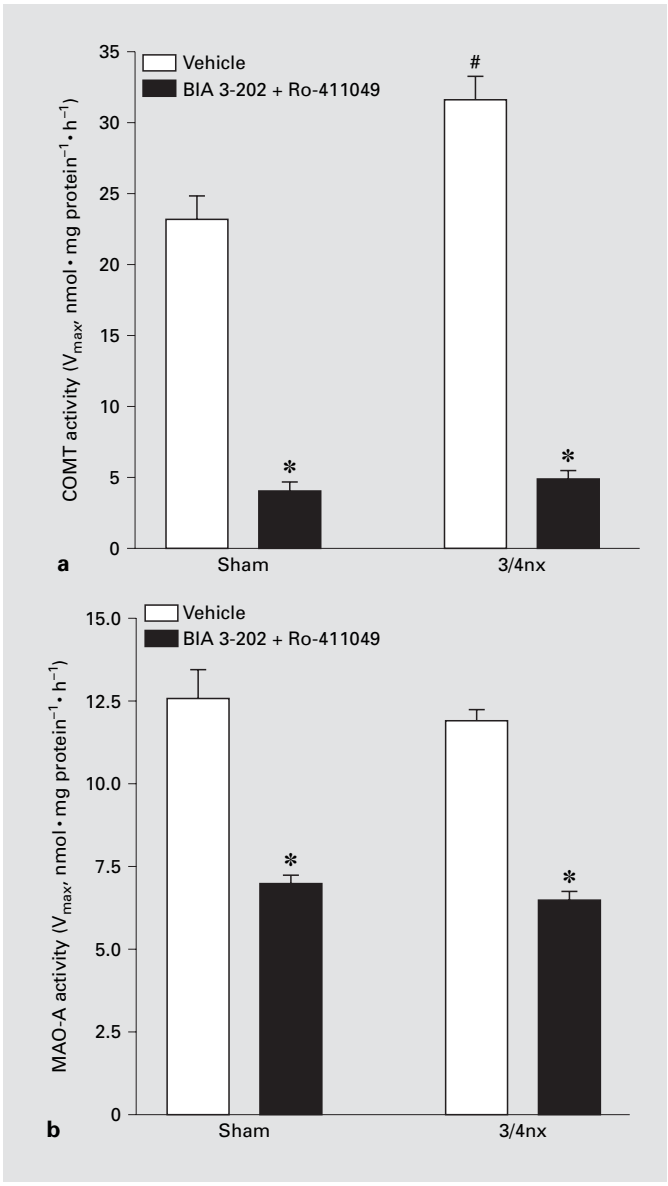

Fig. 2. Effect of 3 days of chronic administration of BIA 3-202 (30 mg·kg^{-1}) and Ro-411049 (7.5 mg·kg^{-1}) on renal COMT (**a**) and MAO-A (**b**) activities (V_{max} values in nmol·mg protein^{-1}·h^{-1}) in homogenates of renal cortex from Sham and 3/4nx rats. Bars represent means of 8–10 experiments per group, and error bars represent SE. * Significantly different from corresponding values in vehicle-treated rats ($p < 0.0001$). # Significantly different from values in Sham rats ($p < 0.05$).

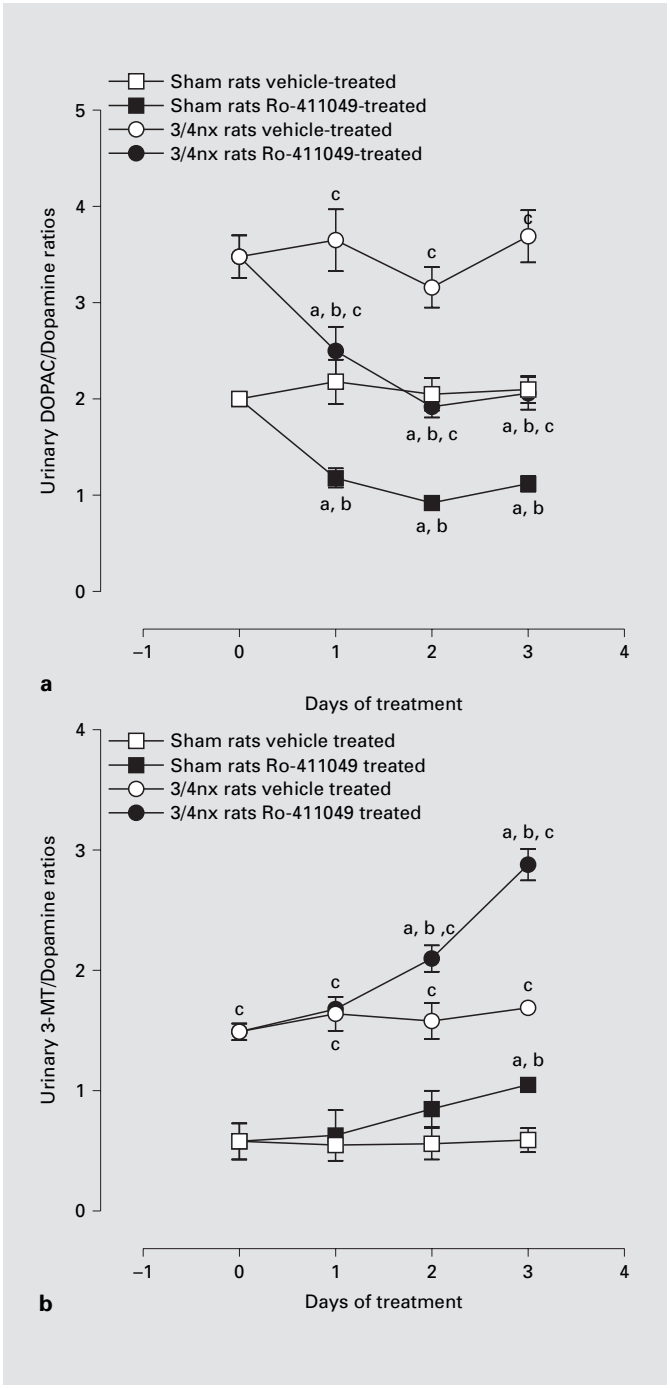

Fig. 3. Effect of Ro-411049 (7.5 mg·kg^{-1}, bid) administration in daily urinary DOPAC/dopamine ratios (**a**) and in daily urinary 3-MT/dopamine ratios (**b**) in Sham and 3/4nx rats. Symbols represent means of 6–9 experiments per group and error bars represent SE. [a] Significantly different from corresponding values on day 0. [b] Significantly different from corresponding values in vehicle-treated rats. [c] Significantly different from corresponding values in Sham rats ($p < 0.05$).

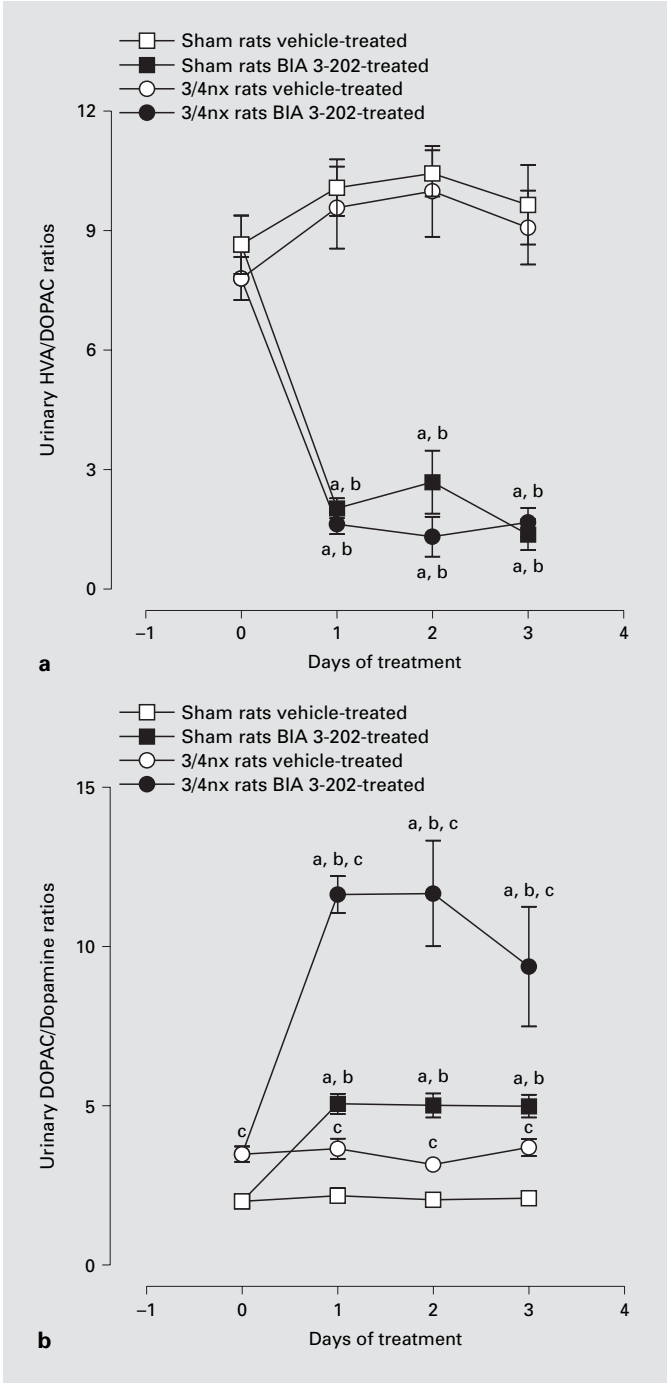

Fig. 4. Effect of BIA 3-202 (30 mg·kg^{-1}, bid) administration in daily urinary HVA/DOPAC ratios (**a**) and in daily urinary DOPAC/dopamine ratios (**b**) in Sham and 3/4nx rats. Symbols represent means of 6–9 experiments per group and error bars represent SE. [a] Significantly different from corresponding values on day 0. [b] Significantly different from corresponding values in vehicle-treated rats. [c] Significantly different from corresponding values in Sham rats ($p < 0.05$).

The urinary excretion of noradrenaline was not altered during the selective or combined administration of Ro-411049 and BIA 3-202, in either Sham or 3/4nx rats (table 2).

The creatinine clearance (in ml·min^{-1}·kg bw^{-1}) was not changed by the selective or combined administration of Ro-411049 and BIA 3-202 (3/4nx rats: vehicle, 7.4 ± 0.8; Ro-411049, 6.9 ± 1.3; BIA 3-202, 5.2 ± 1.8; Ro-411049+BIA 3-202, 5.7 ± 2.1; Sham rats: vehicle, 14.7 ± 3.1; Ro-411049, 14.7 ± 2.6; BIA 3-202, 13.6 ± 1.5; Ro-411049+BIA 3-202, 15.5 ± 5.0). In addition, the selective or combined administration of Ro-411049 and BIA 3-202 did not alter the urinary excretion of either sodium or phosphate in both Sham and 3/4nx animals (table 4). Also, the fractional excretion of both sodium and phosphate were not changed by the selective or combined inhibition of MAO-A and COMT in either 3/4nx or Sham rats (data not shown).

The effects of BIA 3-202 and Ro-411049 during the first 12-hour catecholamine excretion, natriuresis and phosphaturia were also examined in 3/4nx and Sham rats (tables 5, 6). As it can be observed, the acute effects of BIA 3-202 and Ro-411049 on catecholamine excretion did not differ from those observed after chronic MAO-A and COMT inhibition, in either 3/4nx or Sham rats (table 5). In addition, similarly to that observed after 3 days of BIA 3-202 and Ro-411049 administration, neither natriuresis nor phosphaturia were significantly altered during the first 12 h of drug administration in either 3/4nx or Sham rats (table 6).

Discussion

This study was undertaken with the aim of clarifying the possible regulatory roles of COMT and MAO-A in dopamine-influenced natriuresis and phosphaturia by the remnant kidney. Because the natriuretic effect of dopamine is more consistently demonstrated in volume-expanded conditions [17], our studies were performed in rats receiving a HS intake. The results showed that chronic treatment of 3/4nx and Sham rats with BIA 3-202 and/or Ro-411049 at doses producing an effective inhibition of renal COMT and MAO-A, respectively, was not accompanied by increases in the urinary excretion of dopamine, notwithstanding the marked changes observed in the urinary excretion of the corresponding metabolites, 3-MT, DOPAC and HVA. In addition, no significant changes were observed in the urinary excretion of either sodium or phosphate during the selective or combined

Table 4. Daily urinary excretion of sodium and phosphate in sham-operated (Sham) and 3/4 nephrectomized (3/4nx) rats on day 0 (before drug administration) and on day 3 of vehicle, Ro-411049 (7.5 mg·kg^{-1}, bid), BIA 3-202 (30 mg·kg^{-1}, bid) and Ro-411049+BIA 3-202 (Ro+BIA) administration

		Day 0	Day 3			
			vehicle	Ro-411049	BIA 3-202	Ro + BIA
Sodium	Sham	7.7 ± 0.4	6.6 ± 0.5	7.4 ± 0.6	6.8 ± 0.8	7.8 ± 1.4
	3/4nx	7.3 ± 0.4	6.3 ± 0.7	8.4 ± 0.8	5.9 ± 0.1	7.2 ± 1.0
Phosphate	Sham	0.27 ± 0.02	0.25 ± 0.03	0.31 ± 0.03	0.22 ± 0.02	0.36 ± 0.05
	3/4nx	0.32 ± 0.02	0.32 ± 0.03	0.38 ± 0.06	0.29 ± 0.01	0.32 ± 0.03

Values are means ± SE; n = 6–9 experiments per group. Values are expressed in millimoles per 24 h.

Table 5. Twelve-hour urinary excretion of dopamine, DOPAC, HVA, 3-MT and noradrenaline in sham-operated (Sham) and 3/4 nephrectomized (3/4nx) rats after vehicle and Ro-411049 (7.5 mg·kg^{-1}) + BIA 3-202 (30 mg·kg^{-1}) (Ro+BIA) administration

		12 h	
		vehicle	Ro + BIA
Dopamine	Sham	4.49 ± 0.45	4.7 ± 0.7
	3/4nx	2.6 ± 0.15[b]	2.18 ± 0.25[b]
DOPAC	Sham	8.36 ± 0.4	6.56 ± 0.01[a]
	3/4nx	9.8 ± 0.8	9.4 ± 1.2[b]
HVA	Sham	146.6 ± 15.1	39.2 ± 6.8[a]
	3/4nx	107.1 ± 10.1	16.1 ± 6.0[a, b]
3-MT	Sham	7.1 ± 1.3	3.3 ± 0.3[a]
	3/4nx	6.8 ± 1.9	ND
Noradrenaline	Sham	2.36 ± 0.12	2.91 ± 0.4
	3/4nx	1.25 ± 0.4[b]	1.07 ± 0.06[b]

Values are means ± SE; n = 4–5 experiments per group. Values are expressed in nanomoles per 12 h. DOPAC = 3,4-Dihydroxyphenyl-acetic acid; HVA = homovanillic acid; 3-MT = 3-metoxytyramine; ND = levels not detectable.

[a] Significantly different from corresponding values in vehicle-treated rats ($p < 0.05$). [b] Significantly different from corresponding values in Sham rats ($p < 0.05$).

Table 6. Twelve-hour urinary excretion of sodium and phosphate in sham-operated (Sham) and 3/4 nephrectomized (3/4nx) rats after vehicle and Ro-411049 (7.5 mg·kg^{-1}) + BIA 3-202 (30 mg·kg^{-1}) (Ro+BIA) administration

		12 h	
		vehicle	Ro + BIA
Sodium	Sham	1.36 ± 0.22	1.25 ± 0.05
	3/4nx	1.43 ± 0.16	1.23 ± 0.18
Phosphate	Sham	0.064 ± 0.011	0.051 ± 0.07
	3/4nx	0.098 ± 0.018	0.094 ± 0.27

Values are means ± SE; n = 4–5 experiments per group. Values are expressed in millimoles per 24 h.

administration of BIA 3-202 and Ro-411049. Taken together, our findings strongly suggest that chronic inhibition of renal COMT and/or MAO-A activities are not of major importance in regulating the dopamine-dependent natriuresis and phosphaturia in either 3/4nx or Sham rats.

Incubation of rat kidney slices with subtype-selective MAO inhibitors indicated that in the rat, MAO-A, in contrast to MAO-B, is preferentially present in the cellular compartment where dopamine is formed and MAO-A might thus be more important than MAO-B in the regulation of dopamine levels [21, 22]. In addition, MAO-A is more than 3-fold as abundant in the rat renal cortex and greatly predominates over MAO-B in the rest of the kidney [21]. Furthermore, no significant changes were observed between 3/4nx and Sham animals in the renal activities of both types A and B MAO [9]. Hence, for the purpose of the present investigation, it seemed more important to obtain a satisfactory MAO-A inhibition. Our results showed that the urinary dopamine output did not increase during treatment with the selective MAO-A inhibitor in both Sham and 3/4nx rats. In addition, neither sodium nor phosphate excretion changed during administration of Ro-411049, in both Sham and 3/4nx rats. We found that in the rat renal cortex the dose of 7.5 mg·kg bw^{-1} of Ro-411049 inhibited the MAO-A activity by ~30% at 6 and 12 h after administration and by ~45%

after 3 days of chronic drug administration. It might be argued that this degree of inhibition is not sufficient to form a basis for conclusions. However, the urinary DOPAC/dopamine ratios as well as the urinary levels of both DOPAC and HVA were markedly decreased in both 3/4nx and Sham rats, suggesting that MAO activity was significantly inhibited throughout the study. Few in vivo studies have addressed the renal effects of chronic selective and non-selective MAO inhibition in rats with well-preserved renal function [13, 23, 24] and none has shown increases in the urinary sodium excretion. Thus, our present findings strengthen the suggestion that chronic MAO-A inhibition is not of major importance in regulating the dopamine-dependent natriuresis and further extend this observation to the enhanced natriuresis and phosphaturia in the remnant kidney from 3/4nx rats.

The role of acute COMT inhibition in regulating the dopamine-dependent natriuresis has been investigated in several in vivo studies using different inhibitors. In some studies, there was a natriuretic response to COMT inhibition probably due to attenuation of Na^+/K^+-ATPase [11, 14, 15]. These effects were prevented by selective blockade of dopamine D_1-like receptors but were not accompanied by parallel increases in the urinary excretion of dopamine [11, 14, 15]. In addition, pyridine derivatives also endowed with marked inhibitory effects on COMT failed to affect the urinary excretion of either sodium or dopamine [14]. More recently, evidence has been gathered that the natriuresis produced by nitrocatechol derivatives may be not dependent on the enhanced availability of renal dopamine but rather to direct stimulation of D_1-like dopamine receptors [12]. In the present study, the dose of 30 $mg \cdot kg\ bw^{-1}$ of BIA 3-202 inhibited COMT activity in the rat renal cortex by ~95% at 6 h and by ~50% at 12 h. In addition, 3 days after chronic BIA 3-202 administration the renal COMT activity was inhibited by ~85%, resulting in markedly reduced urinary levels of 3-MT and HVA in both 3/4nx and Sham rats. At this dose, when most of enzyme activity is inhibited, the urinary excretion of both sodium and phosphate were not altered in either 3/4nx or Sham rats. In addition, the urinary excretion of dopamine did not change during BIA 3-202 administration, in either 3/4nx or Sham rats. Since the dose of BIA 3-202 employed in this study is expected to originate high levels of the compound in the tubular filtrate, the absence of associated natriuretic or phosphaturic responses suggests that, in these conditions, BIA 3-202 did not act as D_1 dopamine receptor agonist apart from inhibition of COMT. Taken together, our present findings suggest that chronic COMT inhibition is not of major importance in regulating the dopamine-dependent natriuresis and further extend this observation to the enhanced natriuresis and phosphaturia in the remnant kidney from 3/4nx rats.

One possible explanation for the absence of changes in the urinary excretion of dopamine during chronic Ro-411049 or BIA 3-202 administration could be related with the trend towards the renal *O*-methylation or deamination of dopamine, respectively. This suggestion is supported by the finding that Ro-411049 administration increased both the urinary excretion of the methylated metabolite 3-MT and the urinary 3-MT/dopamine ratios whereas BIA 3-202 administration was accompanied with a marked increase in both the urinary excretion of the deaminated metabolite DOPAC and in the urinary DOPAC/dopamine ratios. Thus, we found that it was worthwhile to study the effect of the combined inhibition of MAO-A and COMT on renal dopamine system activity as well as on the natriuresis and phosphaturia. In these studies, the daily urinary excretion of dopamine did not increase despite the accompanied marked decreases in the urinary levels of both 3-MT and HVA. Moreover, the daily urinary excretion of either sodium or phosphate was not significantly altered during combined administration of Ro-411049 and BIA 3-202.

Several challenges arise from the findings of the present study. MAO-B was not inhibited and therefore, one cannot exclude that MAO-B may have been involved in the deamination of the amount of dopamine that escaped inactivation by both MAO-A and COMT. This may be, however, of minor metabolic importance considering that the urinary excretion of deaminated plus methylated metabolite HVA was markedly decreased during combined inhibition of MAO-A and COMT and this was not accompanied with an increase in the urinary excretion of deaminated metabolite DOPAC. In addition, in rat epithelial cells, newly-formed dopamine was found to be mainly deaminated by MAO-A [3, 21, 22] and inhibition of MAO-B did not affect the urinary excretion of sodium in both man [25] and rat [24]. Urine dopamine output was not significantly altered during inhibition of MAO-A and/or COMT notwithstanding the marked changes observed in the urinary levels of the corresponding metabolites, 3-MT, DOPAC and HVA. Because the amount of newly-formed dopamine extruded by the apical cell border is not taken up by the renal tubular cells after being released [26], our results suggest that the metabolization of renal dopamine by both MAO-A and COMT is mainly related with the portion of the dopamine that is extruded by the basolateral cell border. Thus, it can be hy-

pothesized that MAO-A and COMT may behave as a metabolic barrier crossed by the amine before reaching the renal interstitial fluid. This hypothesis would be in agreement with the previous results showing that the fraction of newly-formed dopamine leaving the cell through the basolateral border is a constant source for deamination to DOPAC [22]. What could be the fate of the amount of dopamine extruded by the basolateral cell border that escaped inactivation during selective or combined MAO-A and COMT inhibition? Dopamine-3-*O*-sulfate was demonstrated to account for most of conjugated dopamine in renal tissues and administration of *L*-DOPA to rats was found to result in a marked increase in plasma levels of free and conjugated (sulfate) dopamine, the proportion of free to conjugated being 1:10 [27]. Therefore it is expected that the amount of dopamine escaping deamination and/or methylation could be markedly conjugated to dopamine-3-*O*-sulfate. The sulfo-conjugation of the amount of dopamine leaving the renal tubular cells through the basolateral border would contribute to the absence of changes in the urinary excretion of dopamine as well as in the natriuresis and phosphaturia, during selective and combined MAO-A and COMT inhibition. One cannot exclude, however, that increased delivery of sodium or phosphate from the proximal tubule during dopamine-metabolizing enzymes inhibition may be offset by increased reabsorption by more distal nephron segments.

We conclude that chronic treatment of 3/4nx and Sham rats with BIA 3-202 and/or Ro-411049 at doses producing an effective inhibition of renal COMT and MAO-A, respectively, did not increase the daily urinary excretion of dopamine, sodium or phosphate. Taken together, these findings suggest that inhibition of renal COMT and/or MAO-A activities are not of major importance in regulating the dopamine-dependent natriuresis and phosphaturia in either 3/4nx or Sham rats.

Acknowledgments

We thank the technical assistance of Manuela Moura, Gracieth Oliveira and Isaura Oliveira. Benedita Sampaio-Maia was supported by SFRH/BD/1479/2000 and this study was supported by grant POCTI/FCB/45660/2002 from 'Fundação para a Ciência e a Tecnologia/FEDER'.

References

1 Lee MR: Dopamine and the kidney: ten years on. Clin Sci (Lond) 1993;84:357–375.
2 Doggrell SA: The therapeutic potential of dopamine modulators on the cardiovascular and renal systems. Expert Opin Investig Drugs 2002;11:631–644.
3 Fernandes M, Soares-da-Silva P: Sequential Involvement of Monoamine Oxidase and Catechol-*O*-Methyltransferase in the Metabolism of Newly-Formed Dopamine in Rat Renal Tissues. Cardiovascular and Renal Actions of Dopamine. London, Pergamon Press, 1993, pp 21–30.
4 Aperia AC: Intrarenal dopamine: a key signal in the interactive regulation of sodium metabolism. Annu Rev Physiol 2000;62:621–647.
5 Pestana M, Jardim H, Serrao P, Soares-da-Silva P, Guerra L: Reduced urinary excretion of dopamine and metabolites in chronic renal parenchymal disease. Kidney Blood Press Res 1998;21:59–65.
6 Itskovitz HD, Wynn NC: Renal functional status and patterns of catecholamines excretion. J Clin Hypertens 1985;1:223–227.
7 Pestana M, Jardim H, Correia F, Vieira-Coelho MA, Soares-da-Silva P: Renal dopaminergic mechanisms in renal parenchymal diseases and hypertension. Nephrol Dial Transplant 2001;16:53–59.
8 Pestana M, Santos J, Santos A, Coroas A, Correia F, Serrao P, Valbuena C, Soares-da-Silva P: Renal dopamine and salt sensitivity of blood pressure in IgA nephropathy. Kidney Blood Press Res 2004;27:78–87.
9 Sampaio-Maia B, Serrao P, Guimaraes JT, Vieira-Coelho MA, Pestana M: Renal dopaminergic system activity in the rat remnant kidney. Nephron Exp Nephrol 2005;99:e46–e55.
10 Isaac J, Berndt TJ, Thothathri V, Tyce GM, Knox FG: Catecholamines and phosphate excretion by the remnant kidney. Kidney Int 1993;43:1021–1026.
11 Eklof AC, Holtback U, Sundelof M, Chen S, Aperia A: Inhibition of COMT induces dopamine-dependent natriuresis and inhibition of proximal tubular Na+,K+-ATPase. Kidney Int 1997;52:742–747.
12 Vieira-Coelho MA, Gomes P, Serrao MP, Soares-da-Silva P: D_1-like dopamine receptor activation and natriuresis by nitrocatechol COMT inhibitors. Kidney Int 2001;59:1683–1694.
13 Odlind C, Reenila I, Mannisto PT, Ekblom J, Hansell P: The role of dopamine-metabolizing enzymes in the regulation of renal sodium excretion in the rat. Pflügers Arch 2001;442:505–510.
14 Hansell P, Odlind C, Mannisto PT: Different renal effects of two inhibitors of catechol-*O*-methylation in the rat: entacapone and CGP 28014. Acta Physiol Scand 1998;162:489–494.
15 Odlind C, Goransson V, Reenila I, Hansell P: Regulation of dopamine-induced natriuresis by the dopamine-metabolizing enzyme catechol-*O*-methyltransferase. Exp Nephrol 1999;7:314–322.
16 Learmonth DA, Vieira-Coelho MA, Benes J, Alves PC, Borges N, Freitas, AP, Soares-da-Silva P: Synthesis of 1-(3,4-dihydroxy-5-nitrophenyl)-2-phenyl-ethanone and derivatives as potent and long-acting peripheral inhibitors of catechol-*O*-methyltransferase. J Med Chem 2002;45:685–695.
17 Hansell P, Fasching A: The effect of dopamine receptor blockade on natriuresis is dependent on the degree of hypervolemia. Kidney Int 1991;39:253–258.
18 Fernandes MH, Soares-da-Silva P: Type A and B monoamine oxidase activities in the human and rat kidney. Acta Physiol Scand 1992;145:363–367.
19 Pestana M, Vieira-Coelho MA, Pinto-do OP, Fernandes MH, Soares-da-Silva P: Assessment of renal dopaminergic system activity during cyclosporine A administration in the rat. Br J Pharmacol 1995;115:1349–1358.

20 Motulsky H, Spannard P, Neubig R: GraphPad Prism. San Diego, GraphPad Prism Software, 1994.
21 Fernandes MH, Pestana M, Soares-da-Silva P: Deamination of newly-formed dopamine in rat renal tissues. Br J Pharmacol 1991;102:778–782.
22 Pestana M, Soares-da-Silva P: 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. Br J Pharmacol 1994;113:1269–1274.
23 Soares-da-Silva P, Pinto-do OP: Antagonistic actions of renal dopamine and 5-hydroxytryptamine: effects of amine precursors on the cell inward transfer and decarboxylation. Br J Pharmacol 1996;117:1187–1192.
24 Vieira-Coelho MA, Fernandes MH, Pestana M: Effect of type A and B monoamine oxidase inhibitors on the urinary excretion of dopamine, 5-hydroxytryptamine and amine metabolites. Br J Pharmacol 1993;36:153P.
25 Freestone S, Li Kam TC, Lee MR: Further Studies on Gludopa in Man: The Effect of the Monoamine Oxidase Inhibitor Selegiline. Oxford, Pergamon Press, 1993.
26 Chan YL: Cellular mechanisms of renal tubular transport of *L*-dopa and its derivatives in the rat: microperfusion studies. J Pharmacol Exp Ther 1976;199:17–24.
27 Buu NT, Duhaime J, Kuchel O: Effects of *L*-DOPA on the concentrations of free and sulfoconjugated catecholamines in plasma, cerebrospinal fluid, urine, and central and peripheral nervous system tissues of the rat. J Neurochem 1985;44:787–792.