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Renal Dopaminergic System Activity in the Rat Remnant Kidney

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Key Words
Renal dopamine · Remnant kidney · Natriuresis · Sch-23390 · Aromatic *L*-amino acid decarboxylase · Catechol-*O*-methyltransferase · Monoamine oxidases A and B

Abstract
Background: Renal dopamine exerts natriuretic and diuretic effects by activating D_1-like receptors. Uninephrectomy results in increased renal dopaminergic activity and dopamine-sensitive enhanced natriuresis. ***Methods:*** The present study evaluated renal adaptations in sodium handling and the role of dopamine in rats submitted to ¾ nephrectomy: right nephrectomy and excision of both poles of the left kidney (¾nx rats). ***Results:*** Two weeks after surgery the absolute urinary levels of dopamine were markedly reduced in ¾nx rats whereas the urinary dopamine excretion per % of residual nephrons was significantly increased in the remnant kidney of ¾nx rats. The V_{max} values for renal aromatic *L*-amino acid decarboxylase, the enzyme responsible for the synthesis of renal dopamine, were decreased in ¾nx rats. Renal catechol-*O*-methyltransferase activity, the enzyme responsible for the methylation of dopamine, was increased in ¾nx rats whereas the renal activities of monoamine oxidases A and B did not differ between ¾nx and Sham animals. Volume expansion (5% body weight) resulted in similar natriuretic responses in ¾nx and Sham rats. During D_1 antagonist administration (Sch-23390, 30 μg · h^{-1} · kg^{-1}) the natriuretic response to volume expansion was reduced in ¾nx rats more pronouncedly than in Sham animals. ***Conclusion:*** The decrease in absolute renal dopamine output in ¾nx rats is related with reduced renal synthesis and enhanced *O*-methylation of the amine. However, this is accompanied in ¾nx rats by increased renal dopamine excretion per residual nephrons and dopamine-sensitive enhanced natriuresis.

Introduction

Animal models of reduced renal mass undergo a series of adaptive mechanisms to maintain sodium homeostasis. Compensatory changes in the tubular sodium handling include an increased excretion of sodium per nephron and a delay in the adaptation to a low sodium diet [1]. In this way, sodium balance can be maintained despite a diminishing GFR when salt intake is unaltered in conditions of reduced renal mass. The mechanisms responsible for the increased fractional sodium excretion in the remnant kidney are still in dispute.

Tubular epithelial cells, namely those of the proximal convoluted tubules, are endowed with a high aromatic *L*-amino acid decarboxylase (AADC) activity and filtered or circulating *L*-3,4-dihydroxyphenylalanine (*L*-Dopa) can be converted to dopamine after being taken up into

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this cellular compartment [2–4]. Dopamine of renal origin exerts natriuretic and diuretic effects by activating D_1-like receptors located at various regions in the nephron [5]. At the level of the proximal tubule, the overall increase in sodium excretion produced by dopamine results from the inhibition of two main sodium transport mechanisms at the basolateral and apical membranes, Na^+,K^+-ATPase and Na^+-H^+ exchanger, respectively [6]. Dopamine of renal origin has also been found to undergo extensive deamination to 3,4-dihydroxyphenylacetic acid (DOPAC), *O*-methylation to 3-methoxytyramine (3-MT) and deamination plus *O*-methylation to homovanillic acid (HVA) [7–9] and the high levels of metabolic enzymes such as type A and B monoamine oxidases (MAO-A and MAO-B) and catechol-*O*-methyltransferase (COMT) have also been considered important determinants in the overall availability of renal dopamine.

We have shown previously in Wistar rats that uninephrectomy results in increased dopamine synthesis per nephron and dopamine-sensitive enhanced natriuresis with no changes in blood pressure values indicating that renal dopamine may play an important role in keeping uninephrectomized rats within sodium balance [10]. The aim of the present study was to evaluate the role of dopamine in rats submitted to ¾ nephrectomy, a model of chronic renal failure.

Materials and Methods

In vivo Studies

Normotensive male Wistar-Han rats (Harlan Interfauna Ibèrica, Barcelona, Spain), weighing 190–210 g, were selected after a 7-day period of stabilization and adaptation to blood pressure measurements. The animals were kept under controlled environmental conditions (12:12 h light/dark cycle and room temperature 22 ± 2°C); fluid intake and food consumption were monitored daily throughout the study. All animals were fed ad libitum throughout the study with ordinary rat chow (Panlab, Barcelona, Spain) containing 1.9 $g \cdot kg^{-1}$ of sodium. Blood pressure (systolic and diastolic) and heart rate were measured throughout the study in conscious restrained animals, between 07:00 and 10:00 h, using a photoelectric tail-cuff pulse detector (LE 5000, Letica, Barcelona, Spain). Four determinations were made each time and the means were used for further calculation.

¾ Nephrectomy. Rats were anesthetized with pentobarbital sodium (60 mg·kg bw^{-1}, i.p.), and both kidneys were exposed through an incision in abdominal wall. Thereafter, the kidneys were decapsulated and the adrenal gland and the renal pedicle avoided. The right kidney was removed and a surgical ablation of both poles of the left kidney was performed, according to what was previously described by Isaac et al. [11] – ¾ nephrectomized (¾nx) rats. The percentage of remnant renal mass was obtained assuming that both the right and the left kidneys were of the same weight [12]. The remnant renal mass was calculated by subtracting the weight of the left poles to the weight of the right kidney removed. The mean percentage of remnant renal mass in ¾nx rats was 27 ± 1%.

Uninephrectomy. In some experiments, the right kidney was removed according to what was previously described for ¾nx rats while the left kidney remained intact – uninephrectomized (Unx) rats. 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 an animal facility where they had free access to food and water. Survival rate was 100%.

Metabolic Study. Twelve days after surgery, the rats were placed in metabolic cages (Tecniplast, Buguggiate-VA, Italy) for the collection of 24 h urine. The vials collecting urine contained 1 ml hydrochloric acid (6 *M*), to avoid the spontaneous oxidation of the amines and its derivatives. Animals received tap water and their daily sodium intake averaged 7.5 mmol·kg bw^{-1}. Fourteen days after surgery the animals were anesthetized with pentobarbital sodium (60 mg·kg bw^{-1}, i.p.). Blood was collected from the heart in tubes containing heparin and lithium/heparin for later determination of plasma catecholamines and biochemical parameters, respectively. The kidneys were rapidly removed, weighed and the outer cortex isolated. In ¾nx and Sham rats a fragment of liver was also removed. Fragments of renal cortex and liver were used later for determination of AADC, COMT, MAO-A and MAO-B activities. Fragments from renal cortex from ¾nx and Sham rats, weighing around 200 mg, were placed in vials containing 1 ml of 0.2 *M* perchloric acid. The samples were stored at –80°C until quantification of catecholamines and metabolites by HPLC with electrochemical detection.

Volume Expansion (VE). In another set of experiments, 14 days after the surgery, the animals were anesthetized with pentobarbital sodium (60 mg·kg bw^{-1} followed by 20 $mg \cdot kg^{-1} \cdot h^{-1}$, i.p.) and placed on a thermostatically controlled heating table to maintain a rectal temperature of 37°C. The rats were tracheotomized and the left jugular vein was catheterized by a PE50 tube (Becton-Dickson, Lisbon, Portugal) for VE and infusion of Sch-23390 (30 $\mu g \cdot kg^{-1}$ bolus followed by 30 $\mu g \cdot h^{-1} \cdot kg^{-1}$) or the vehicle (0.9% NaCl, bolus of equal volume per kg). After an abdominal incision the urinary bladder was catheterized through a suprapubic incision for urine sampling. After the completion of surgical procedures the infusion started at a rate of 0.5 $ml \cdot h^{-1} \cdot 100\ g^{-1}$ for 120 min; during this period, two consecutive 60-min urine samples were collected (t = 0–120 min, basal). After this stabilization period the VE was started by infusion of isotonic saline (0.9%) at a rate of 10 $ml \cdot h^{-1} \cdot 100\ g^{-1}$ (5% bw) during 30 min; during this phase, three consecutive urine samples were collected lasting 10 min each (t = 120–150 min, VE). Thereafter, the infusion was again reduced to 0.5 $ml \cdot h^{-1} \cdot 100\ g^{-1}$ for 90 min; during this recovery period, urine sampling was performed every 10 min until the end of the experiment (t = 150–240 min, R-VE1, R-VE2 and R-VE3).

In vitro Studies

AADC Activity. Fragments of renal cortex from ¾nx, Unx and Sham rats were homogenized at 4°C with a Thomas Teflon homogenizer (PolyScience Corp., Ill., USA) in the incubation medium containing (in m*M*): 0.35 NaH_2PO_4, 0.15 Na_2HPO_4, 0.11 $Na_2B_4O_7$ and 0.2 pyridoxal phosphate (pH 7.0). Tolcapone (1 µ*M*) and pargyline (100 µ*M*) were added to the incubation medium in order to inhibit the metabolization of dopamine by COMT and MAO, re-

spectively. Activity of AADC was determined as previously described by Soares-da-Silva et al. [13] using *L*-Dopa as substrate (100–10,000 µ*M*). In some experiments using homogenates of renal cortex from ¾nx and Sham rats, *L*-5-hydroxytryptophan (*L*-5-HTP, 10–1,000 µ*M*) was also used as substrate for AADC. The assay of dopamine or 5-hydroxytryptamine (5-HT) was performed by HPLC with electrochemical detection.

MAO Activity. Fragments of renal cortex from ¾nx and Sham rats were homogenized in 67 m*M* phosphate buffer (pH 7.2) at 4°C, with a Thomas Teflon homogenizer. MAO activity was determined with [^{3}H]5-HT (2–400 µ*M*) as a preferential substrate for MAO-A and [^{14}C]β-phenylethylamine (βPEA, 0.3–50 µ*M*) as a preferential substrate for MAO-B [14]. Aliquots of 50 µl of the homogenates were incubated for 10 min with 50 µl of each substrate. At the end of incubation period the tubes were transferred to ice and the reaction was stopped by the addition of 50 µl of perchloric acid (2 *M*). The deaminated products were extracted with ethyl acetate and measured by liquid scintillation counting.

COMT Activity. COMT activity was evaluated in the renal cortex and liver from ¾nx and Sham rats by the ability to methylate epinephrine (300 µ*M*) to metanephrine, as previously described [15]. In another set of experiments, COMT activity was evaluated in the renal cortex from ¾nx and Sham rats by the ability to methylate dopamine (1–1,000 µ*M*) to 3-MT. The composition of incubation medium was as follows (in m*M*): 5 NaH_2PO_4, 5 Na_2HPO_4, 0.1 $MgCl_2 \cdot 6H_2O$, 1 EGTA, 0.1 pargyline and a methyl donor S-adenosylmethionine 0.5 for kidney and 1 for liver. The assay of metanephrine and 3-MT was performed by HPLC with electrochemical detection.

Assay of Catecholamines. The assay of catecholamines and its metabolites in urine, plasma samples, renal tissues and in samples from AADC and COMT studies were performed by HPLC with electrochemical detection, as previously described [16, 17]. In our laboratory, the lower limit of detection of *L*-Dopa, dopamine, DOPAC, 3-MT, HVA, 5-HT, 5-HIAA, noradrenaline and metanephrine ranged from 350 to 1,000 fmol.

Plasma and Urine Ionogram and Biochemistry. The quantifications of sodium, potassium and chloride in plasma and urine samples were performed by ion-selective electrodes. 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). Creatinine clearance was calculated 14 days after surgery using 24-hour urine creatinine excretion. Fractional excretion (FE) of sodium, potassium and phosphate was calculated 14 days after surgery using the formula: $FE_X = [(U_X/P_X)/(U_{creat}/P_{creat})] \cdot 100$, where U_X is the urinary concentrations of sodium, potassium or phosphate, U_{creat} is the urinary creatinine concentration, P_X is the plasma concentrations of sodium, potassium or phosphate, and P_{creat} is the plasma creatinine concentration.

Drugs. The compounds DOPAC, dopamine hydrochloride, HVA, 5-HT, 5-HIAA, *L*-5-HTP, *L*-Dopa, *L*-adrenaline bitartrate, noradrenaline bitartrate, pargyline and Sch-23390 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). Tolcapone was kindly donated by the late Prof. Mosé Da Prada (Hoffmann-La Roche, Basel, Switzerland).

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 nonlinear regression analysis using GraphPad Prism statistics software package [18]. 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 ¾nx and Unx rats attained the same weight at 2 weeks as did Sham rats. Kidney growth, however, was significantly altered in both Unx and ¾nx rats; 14 days after surgery the Unx and ¾nx rats presented a hypertrophied remnant renal mass, weighing 56 ± 3 and 97 ± 9% more, respectively, than on the day of surgery (table 1). Plasma levels of electrolytes (sodium, potassium and chloride) and phosphate were similar in the three groups. In addition, no significant differences were observed between the three groups in either daily intake and urinary excretion of sodium. Fluid intake and urine volume did not differ between Sham and Unx rats; however, both fluid intake and urine volume were greater in ¾nx rats than in the other two groups (table 1). Uninephrectomy led to small but statistically significant increases in both plasma creatinine and urea nitrogen, this being accompanied by a 32% reduction in creatinine clearance values; the ¾nx rats presented greater increases in both plasma creatinine and urea nitrogen, this being accompanied by a 61% reduction in creatinine clearance values in comparison with Sham rats (table 1). The fractional excretion of sodium and potassium was higher in Unx rats than in Sham rats and was further increased in ¾nx animals, which might explain why the urinary excretion of these electrolytes was similar among the three groups. Systolic and diastolic blood pressure and heart rate did not differ between Unx and Sham rats. By contrast, both systolic and diastolic blood pressures were higher in ¾nx rats than in the other two groups (table 1).

Absolute urinary dopamine excretions, calculated based on the urinary dopamine concentrations and urinary volumes, were significantly reduced in ¾nx rats (fig. 1A). Because hypertrophy that occurs in the remnant kidney from ¾nx rats is due to increases in nephron size rather than in number [19, 20], the percentage of residual nephrons for ¾nx rats was calculated based on nephron counts reported by Kaufman et al. [12]. These authors studied an identical ablative remnant kidney model with 28% residual renal mass and they found that the % of remnant kidney residual nephrons was only 14.1%. In the

Table 1. Body and kidney weight, metabolic balance, renal function and blood pressure in sham-operated, uninephrectomized and ¾ nephrectomized rats 14 days after surgery

	Sham-operated	Uninephrectomized	¾ nephrectomized
Day of surgery			
Body weight, g	197 ± 3	202 ± 3	206 ± 3
Remnant renal mass, %	100 ± 0	50 ± 0	27 ± 1
14 days after surgery			
Body weight, g	255 ± 5	259 ± 3	245 ± 5
Increase of renal mass, %		56 ± 3	97 ± 9
Plasma urea, $mg \cdot dl^{-1}$	32.4 ± 1.5	41.5 ± 1.6*	65.6 ± 3.8*, †
Plasma creatinine, $mg \cdot dl^{-1}$	0.35 ± 0.02	0.46 ± 0.02*	0.73 ± 0.05*, †
Plasma Na^+, $mmol \cdot l^{-1}$	139.3 ± 0.6	138.5 ± 0.5	139.5 ± 0.4
Plasma K^+, $mmol \cdot l^{-1}$	5.1 ± 0.1	4.8 ± 0.1	4.9 ± 0.1
Plasma Cl^-, $mmol \cdot l^{-1}$	105.3 ± 0.8	103.6 ± 0.5	104.1 ± 0.5
Plasma Pi, $mmol \cdot l^{-1}$	2.4 ± 0.1	2.6 ± 0.1	2.4 ± 0.1
Fluid intake, $ml \cdot kg\ bw^{-1} \cdot day^{-1}$	98.3 ± 6.3	111.9 ± 7.4	144.1 ± 7.8*, †
Na^+ intake, $mmol \cdot kg\ bw^{-1} \cdot day^{-1}$	7.40 ± 0.24	7.37 ± 0.21	7.53 ± 0.26
Urine volume, $ml \cdot kg\ bw^{-1} \cdot day^{-1}$	44.8 ± 5.9	48.3 ± 4.7	70.9 ± 4.7*, †
Urinary Na^+, $mmol \cdot kg\ bw^{-1} \cdot day^{-1}$	6.6 ± 0.2	6.5 ± 0.2	6.5 ± 0.3
Urinary K^+, $mmol \cdot kg\ bw^{-1} \cdot day^{-1}$	7.5 ± 0.7	8.5 ± 0.6	7.8 ± 0.8
Urinary Pi, $mmol \cdot kg\ bw^{-1} \cdot day^{-1}$	5.1 ± 1.6	6.0 ± 1.7	5.7 ± 1.0
C-creatinine, $ml \cdot min^{-1} \cdot kg\ bw^{-1}$	9.08 ± 0.91	6.17 ± 0.36*	3.57 ± 0.29*, †
FE_{Na+}, %	0.39 ± 0.04	0.54 ± 0.04*	0.95 ± 0.06*, †
FE_{K+}, %	12.9 ± 1.6	20.6 ± 1.1*	32.7 ± 4.0*, †
FE_{Pi}, %	1.5 ± 0.5	3.2 ± 1.1	4.9 ± 0.9*
Systolic BP, mm Hg	123 ± 3	122 ± 2	139 ± 4*, †
Diastolic BP, mm Hg	80 ± 3	81 ± 3	106 ± 4*, †
Heart rate, $beats \cdot min^{-1}$	415 ± 12	404 ± 8	473 ± 32

Values are means ± SE; n = 6–12 experiments per group. C-creatinine = Creatinine clearance; FE = fractional excretion; BP = blood pressure.

* Significantly different from corresponding values in sham-operated rats ($p < 0.05$).

† Significantly different from corresponding values in uninephrectomized rats ($p < 0.05$).

Table 2. Urinary levels of *L*-Dopa, DOPAC, 3-MT, HVA, 5-HT, 5-HIAA and noradrenaline in sham-operated and ¾ nephrectomized rats 14 days after surgery

	Sham-operated	¾ nephrectomized
L-Dopa	0.27 ± 0.06	0.23 ± 0.06
DOPAC	35.0 ± 2.5	26.4 ± 1.9*
3-MT	3.4 ± 0.9	5.2 ± 1.1
HVA	159.4 ± 3.6	158.0 ± 7.6
5-HT	10.9 ± 2.6	12.3 ± 3.1
5-HIAA	212.0 ± 11.9	190.6 ± 10.3
Noradrenaline	7.3 ± 0.8	2.2 ± 0.2*

Values are means ± SE; n = 6–11 experiments per group. Values are expressed in nanomoles per day. *L*-Dopa = 3,4-Dihydroxyphenylalanine; DOPAC = 3,4-dihydroxyphenylacetic acid; 3-MT = 3-methoxytyramine; HVA = homovanillic acid; 5-HT = 5-hydroxytryptamine; 5-HIAA = 5-hydroxyindoleacetic acid.

* Significantly different from corresponding values in sham-operated rats ($p < 0.05$).

present study, the remnant renal mass in ¾nx rats was 27% and the calculated percentage of residual nephrons in ¾nx rats was 13.6%. Based on these findings, the urinary dopamine excretion expressed per percentage of residual nephrons in the remnant kidney from ¾nx rats was significantly higher than urinary dopamine excreted by 13.6% of the nephrons in sham control rats (fig. 1B). The urinary excretion of the dopamine precursor, *L*-Dopa, did not differ between ¾nx and Sham rats (table 2).

The activity of AADC was determined in homogenates of renal cortex with *L*-Dopa (100–10,000 µ*M*), which resulted in a concentration-dependent formation of dopamine (fig. 2). The V_{max} values for AADC activity in renal cortex were found to be significantly lower in ¾nx rats than in Sham rats whereas the V_{max} values for AADC activity in renal cortex of Unx animals were higher than in Sham rats (table 3). The decarboxylation reaction was a saturable process, with K_m values of the same magni-

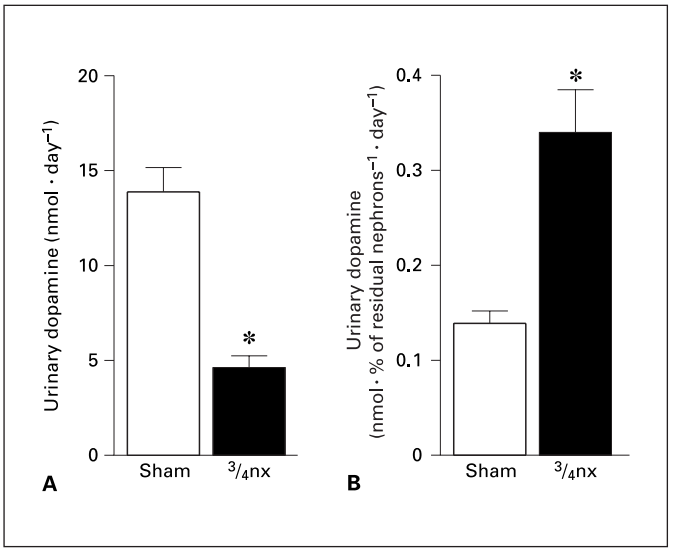

Fig. 1. Absolute urinary dopamine excretion (**A**) and calculated urinary dopamine excretion per % of residual nephrons (**B**) in sham-operated rats with intact kidneys (Sham, open bars) and in ¾ nephrectomized rats (¾nx, closed bars). The percentage of residual nephrons for ¾nx rats was calculated based on nephron counts reported by Kaufman et al. Bars represent means of 6–11 experiments per group, and error bars represent SE. * Significantly different from values in sham-operated rats ($p < 0.05$).

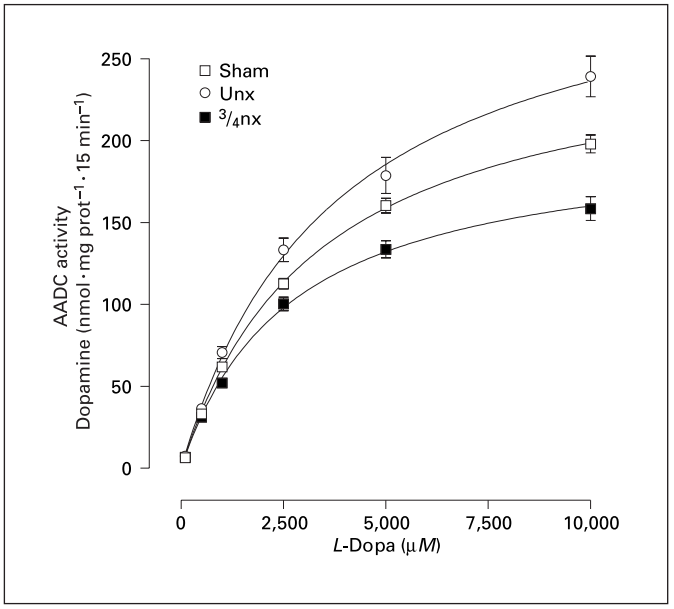

Fig. 2. Aromatic *L*-amino acid decarboxylase (AADC) activity in homogenates of renal cortex obtained from ¾ nephrectomized rats (¾nx, ■), uninephrectomized rats (Unx, ○) and sham-operated rats (Sham, □). AADC activity is expressed as the rate of formation of dopamine vs. concentration of *L*-Dopa. Symbols represent means of 6 experiments per group, and error bars represent SE.

tude in three groups (table 3). Similar to that observed using *L*-Dopa as substrate, the V_{max} values for renal AADC when using *L*-5-HTP were significantly lower in ¾nx rats than in Sham controls without changes in K_m values between the two groups (table 3).

The urinary excretion of deaminated metabolite DOPAC was lower in ¾nx rats than in Sham rats whereas the urinary excretion of the methylated and deaminated plus methylated metabolites, 3-MT and HVA respectively, did not differ between the two groups (table 2). Thus, we felt it was worthwhile to examine the activities of the enzymes responsible for these metabolic transformations (MAO-A, MAO-B, and COMT). The activities of both type A and B monoamine oxidases (MAO-A and MAO-B) in renal cortex did not differ between Sham and ¾nx rats (table 4). By contrast, the V_{max} values for renal COMT were significantly higher in ¾nx rats than in Sham animals (table 4). The activity of COMT was determined in homogenates of renal cortex with dopamine (1–1,000 μ*M*) as substrate, which resulted in a concentration-dependent formation of 3-MT (fig. 3A). The methylation reaction was a saturable process, with K_m values of the same magnitude in the two groups. In ex-

Table 3. Kinetic parameters (V_{max} and K_m) of AADC activities in homogenates of renal cortex from sham-operated, uninephrectomized and ¾ nephrectomized rats 14 days after surgery

	Sham-operated	Uninephrectomized	¾ nephrectomized
L-*Dopa as substrate*			
V_{max}, nmol · mg prot^{-1} · 15 min^{-1}	267 ± 3	329 ± 12*	204 ± 3*, †
K_m, m*M*	2.1 ± 0.1	2.3 ± 0.3	1.9 ± 0.2
L-*5-HTP as substrate*			
V_{max}, nmol · mg prot^{-1} · 15 min^{-1}	154 ± 3	–	99 ± 3*
K_m, μ*M*	165 ± 12	–	180 ± 19

Values are means ± SE; n = 6 experiments per group. V_{max} = Maximal velocity; K_m = Michaelis-Menten constant; AADC = aromatic *L*-amino acid decarboxylase.

* Significantly different from corresponding values in sham-operated rats ($p < 0.05$).

† Significantly different from corresponding values in uninephrectomized rats ($p < 0.05$).

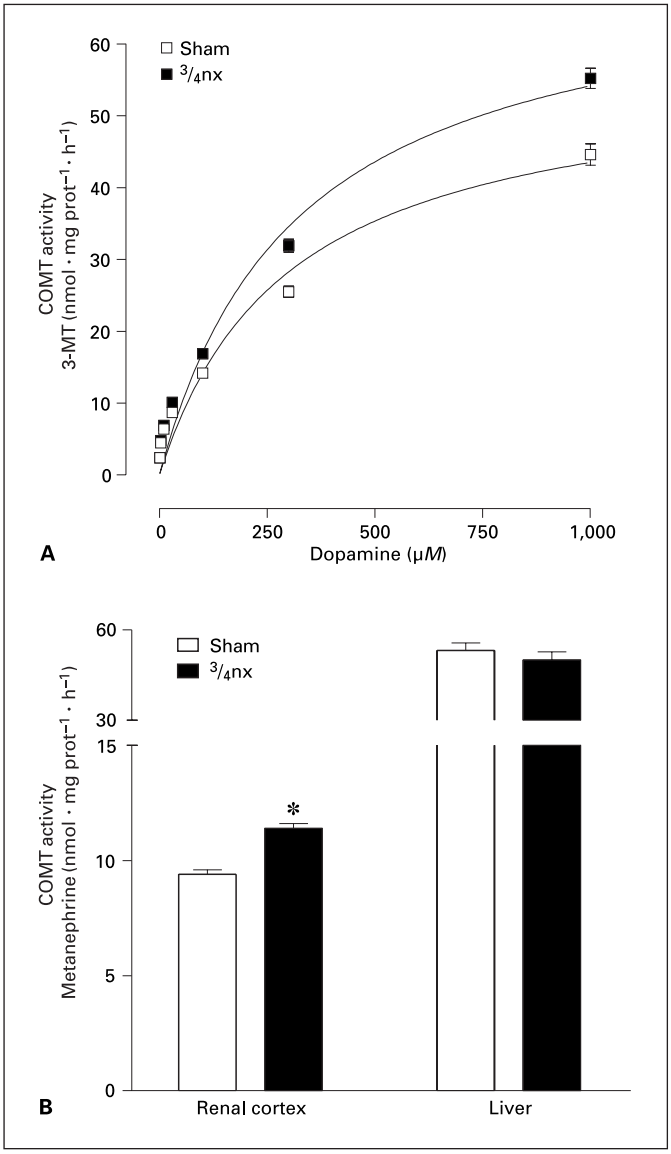

Fig. 3. Catechol-*O*-methyltransferase (COMT) activity. **A** COMT activity in homogenates of renal cortex obtained from ¾ nephrectomized rats (¾nx, ■) and sham-operated rats (Sham, □). COMT activity is expressed as the rate of formation of 3- methoxytyramine (3-MT) vs. concentration of dopamine. Symbols represent means of 6 experiments per group, and error bars represent SE. **B** COMT activity in homogenates of renal cortex and liver obtained from ¾nx (closed bars) and Sham rats (open bars). COMT activity is expressed as the rate of formation of metanephrine from substrate epinephrine (300 m*M*). Bars represent means of 4–6 experiments per group, and error bars represent SE. * Significantly different from values in sham-operated rats ($p < 0.001$).

Table 4. Kinetic parameters (V_{max} and K_m) of COMT and MAO activities in homogenates of renal cortex from sham-operated and ¾ nephrectomized rats 14 days after surgery

	Sham-operated	¾ nephrectomized
COMT		
V_{max}, nmol·mg prot^{-1}·h^{-1}	57 ± 8	72 ± 3*
K_m, μ*M*	300 ± 15	324 ± 30
MAO-A		
V_{max}, pmol·mg prot^{-1}·h^{-1}	5,113 ± 308	4,972 ± 143
K_m, μ*M*	82 ± 15	79 ± 7
MAO-B		
V_{max}, pmol·mg prot^{-1}·h^{-1}	1.98 ± 0.30	1.42 ± 0.19
K_m, μ*M*	0.68 ± 0.52	0.39 ± 0.28

Values are means ± SE; n = 6 experiments per group. COMT = Catechol-*O*-methyltransferase; MAO = monoamine oxidase. * Significantly different from corresponding values in sham-operated rats ($p < 0.05$).

periments performed with liver homogenates, no significant differences were observed in COMT activity between ¾nx and Sham rats suggesting that the increase in renal COMT activity observed in ¾nx rats was the result of local adaptations (fig. 3B).

Tissue levels of *L*-Dopa (in pmol·g^{-1}, 100.6 ± 41.6 vs. 71.1 ± 5.9) and dopamine (43.3 ± 4.7 vs. 58.5 ± 6.1) in fragments of renal cortex did not differ between the ¾nx and Sham rats. No significant differences were observed in plasma levels of *L*-Dopa (in pmol·ml^{-1}, 2.84 ± 0.29 vs. 3.07 ± 0.38) and dopamine (1.15 ± 0.12 vs. 0.70 ± 0.25) between the two groups.

The urinary excretion of noradrenaline was found to be markedly lower in ¾nx than in Sham rats (table 2) and this was accompanied with a similar decrease in tissue levels of noradrenaline in the renal cortex of ¾nx rats (in pmol·g^{-1}, 731 ± 101 vs. 2,070 ± 92). By contrast, no significant differences were observed in noradrenaline plasma levels between ¾nx and Sham rats (in pmol·ml^{-1}, 29.1 ± 4.9 vs. 22.8 ± 4.9).

The urinary sodium excretion before (t = 0–120 min), during (t = 120–150 min) and after (t = 150–240 min) isotonic saline VE (5% bw) in Sham, Unx and ¾nx rats is depicted in figure 4. The natriuretic response to VE was similar among the three groups. The accumulated urinary sodium excretion in Sch-23390-treated rats from the three groups is depicted in figure 5. As can be observed, the effect of Sch-23390 was a marked decrease in the ac-

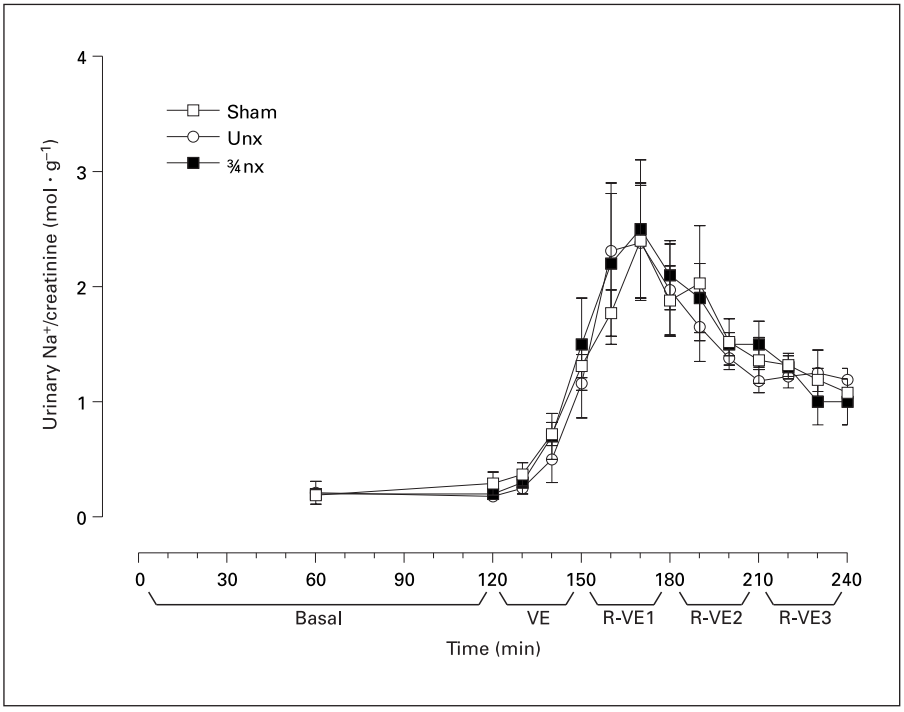

Fig. 4. Urinary sodium excretion (mol · g creat^{-1}) in sham-operated (Sham, □), uninephrectomized (Unx, ○) and ¾ nephrectomized (¾nx, ■) rats before (t = 0–120 min, Basal), during (t = 120–150 min, VE) and after (t = 150–180 min, R-VE1; t = 180–210 min, R-VE2; t = 210–240, R-VE3) 5% volume expansion with isotonic saline. Symbols represent means of 4 experiments per group, and error bars represent SE.

cumulated urinary sodium excretion in ¾nx rats (35–55% reduction) (fig. 5C) whereas in Sham rats the decrease in the accumulated urinary sodium excretion (20–35% reduction) did not attain a significant difference throughout the experiment (fig. 5A). During Sch-23390 administration, the natriuretic response to VE was also attenuated in Unx rats (fig. 5B). However, the decrease in the accumulated urinary sodium excretion in Unx Sch-23390-treated rats was only significant during the first collection of the recovery period following the end of VE (t = 150–180 min).

Discussion

The findings of the present study show that ¾nx rats presented the known consequences of partial renal ablation, namely (1) compensatory renal growth, (2) significant azotemia, (3) significant increase in fractional excretion of sodium, and (4) consistent increase in systemic blood pressure. In association with these findings we report that the ¾nx rats presented a decrease in the absolute renal dopamine output, which appears to result from both a reduced renal synthesis of dopamine and an enhanced renal *O*-methylation of the amine. However, infusion of the D_1 receptor antagonist (Sch-23390) markedly decreased sodium excretion in ¾nx rats before, during and after VE, suggesting that dopamine of renal origin contributes importantly to the enhanced fractional excretion of sodium of the remnant kidney. The enhanced sensitivity of natriuresis to D_1 receptor blockade in ¾nx rats when viewed collectiv ely with the increased fractional excretion of sodium by the ¾nx rat model may be related to an enhanced dopaminergic tonus per residual nephron.

The overall reduced renal dopamine synthesis in ¾nx rats was evidenced by low AADC activity and low urinary levels of dopamine and DOPAC. Increasing evidence suggests that renal AADC corresponds to a single enzyme entity responsible not only for the renal synthesis of dopamine but also for the decarboxylation of *L*-5-HTP to 5-HT [21]. Similar to what has been found when using *L*-Dopa, the renal AADC activity was markedly lower in ¾nx than in Sham rats when *L*-5-HTP was the substrate used. Taken together, these results reinforce the existence of a reduced renal AADC activity in ¾nx rats. The reduced AADC activity in the remnant kidney from ¾nx rats contrasts with the observation, in the present and previous studies [10], showing that uninephrectomy is accompanied with increased renal AADC activity. It is noteworthy that the compensatory growth that occurs in the remnant kidney is well established to be due mainly

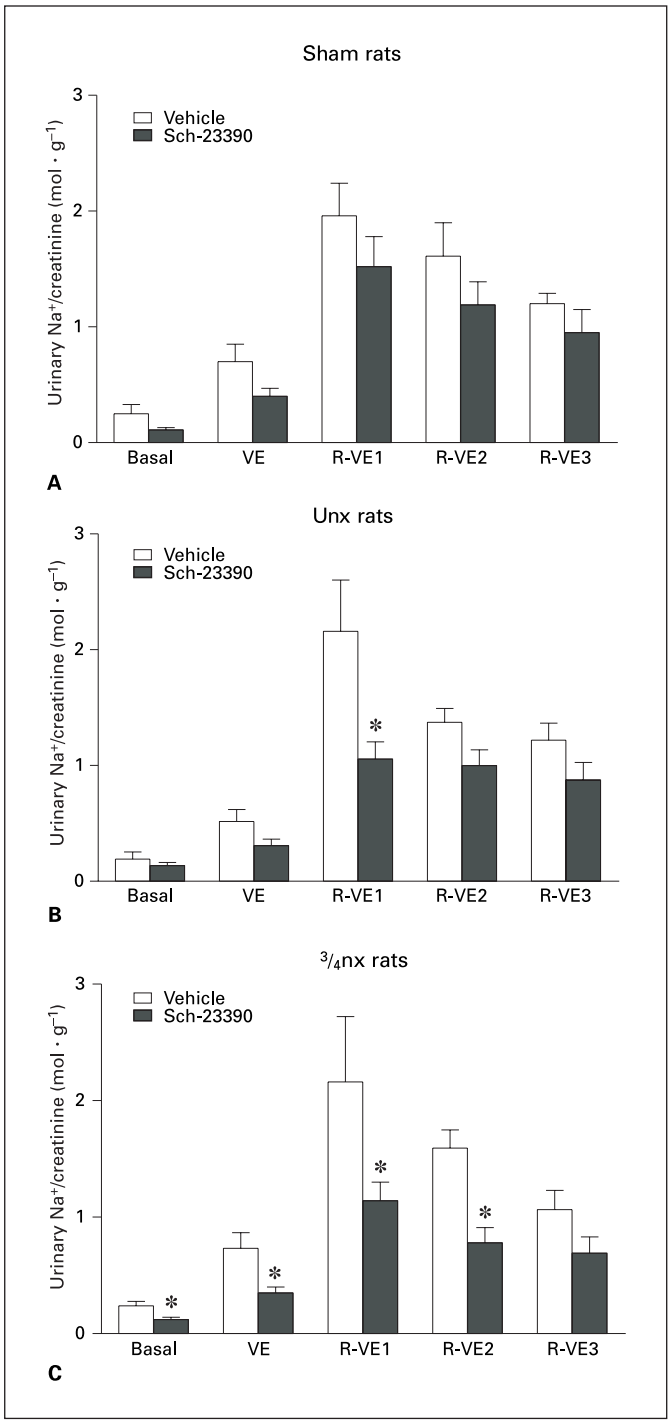

Fig. 5. Accumulated urinary sodium excretion (mol·g creat^{-1}) in untreated (open bars and Sch-23390-treated (30 µg·h^{-1}·kg^{-1}, closed bars) sham-operated (Sham, **A**), uninephrectomized (Unx, **B**) and ¾ nephrectomized (¾nx, **C**) rats before (t = 0–120 min, Basal), during (t = 120–150 min, VE) and after (t = 150–180 min, R-VE1; t = 180–210 min, R-VE2; t = 210–240, R-VE3) 5% volume expansion with isotonic saline. Bars represent means of 4–7 experiments per group and error bars represent SE. * Significantly different from values in vehicle-treated rats ($p < 0.05$).

to increases in nephron size rather than in number [19, 20]. In rats submitted to uninephrectomy, the decrease in nephron number is proportional to the reduction in renal mass. By contrast, Kaufman et al. [12] found in rats submitted to renal ablation of the same magnitude as in ¾nx rats in the present study that the reduction in the number of residual nephrons far outweighed the decrease in renal mass of the remnant kidney (e.g. in rats with 28% of residual renal mass the number of residual nephrons was found to be only 14.1%). Therefore, the decreased renal AADC activity in ¾nx rats may simply denote the fact that AADC is confined to a disproportionately reduced number of proximal tubular cells. Since in ¾nx rats urinary dopamine is synthesized by only 13.6% of the total nephron population, the daily urinary dopamine excretion was corrected per percentage of residual nephrons. Urinary dopamine excretion expressed per residual nephron in the remnant kidney from ¾nx rats was significantly higher than urinary dopamine excreted per nephron in Sham control animals.

The urinary excretion of dopamine metabolites was expressed in nmol·day^{-1} because there is an important contribution from extrarenal sources to the urine content. Similar to the decrease in the urinary excretion of dopamine, when expressed in nmol·day^{-1}, the urinary excretion of DOPAC was significantly reduced in ¾nx rats. The accompanying finding that no significant changes were observed between ¾nx and Sham animals in the renal activities of both type A and B monoamine oxidases suggests that deamination of dopamine was not compromised in ¾nx animals. By contrast, the urinary excretion of both 3-MT and HVA did not follow the decrease in the daily urinary excretion of dopamine in ¾nx rats and this was accompanied by an increase in COMT activity in the remnant kidney from ¾nx rats. It is interesting to note that COMT activity in liver, which is endowed with markedly greater COMT activity than in the renal cortex [22], failed to change in ¾nx rats suggesting that the increase in enzyme activity observed in renal tissues is the result of local adaptations.

The reduced renal dopamine output in ¾nx rats was not accompanied by changes in the tissue levels of dopamine. The most likely explanation for this apparent discrepancy might have to do with the nature of this non-neuronal dopaminergic system [23]. The amine storage structures normally present in monoaminergic neuronal systems and the classical mechanisms for the regulation of amine formation and release do not appear to be present or in operation; the basic mechanisms for the regulation of this system appear to depend on the availability

of *L*-Dopa, its fast decarboxylation into dopamine and in precise and accurate cell outward amine transfer mechanisms [23].

In additional experiments performed in ¾nx rats, infusion of the D_1 receptor antagonist, Sch-23390, markedly attenuated the VE-induced natriuresis in basal conditions as well as during and following VE when compared to saline-infused control rats. These findings strongly suggest that an increased dopamine tonus per nephron is associated with the enhanced fractional excretion of sodium exhibited by the remnant kidney. In Unx and Sham rats, the D_1 antagonist also attenuated sodium excretion. However, the antinatriuretic effect of the D_1 antagonist did not reach significant difference in Sham rats and only attained significant difference in Unx rats during the first period after VE (t = 150–180 min). These results fit well with the previous findings from our group when Unx and Sham rats received the D_1 receptor antagonist (Sch-23390) during an oral sodium load; the D_1 antagonist reduced the urinary excretion of sodium by 31% in Unx rats whereas in Sham rats the urinary excretion of sodium nonsignificantly decreased by 19%. Taken together, our findings provide evidence indicating an important involvement of renal dopamine in the increased natriuresis per nephron observed in ¾nx rats.

Another interesting observation was that the urinary excretion of noradrenaline was markedly lower in ¾nx rats than in Sham animals. One possible explanation could be the renal denervation in ¾nx rats due to the markedly reduction in renal mass especially because the tissue noradrenaline concentration was also significantly lower in the remnant kidney from ¾nx rats than in the Sham control intact kidney. The decreased tissue noradrenaline concentrations in ¾nx rats are in agreement with the findings of others [11] and strongly suggest that by increasing sodium excretion the decreased renal tubular sympathetic tone may contribute to maintain sodium balance by the remnant kidney. The finding that plasma levels of noradrenaline in ¾nx and Sham rats were similar can be explained on the basis that circulating levels of noradrenaline mainly reflect systemic release of the amine during a limited period of time, whereas urinary levels give a better indication of renal sympathetic tone over a longer period of time.

We conclude that the decrease in absolute renal dopamine output in ¾nx rats is related with reduced renal synthesis and enhanced renal *O*-methylation of the amine. However, this is accompanied in the remnant kidney from ¾nx rats by increased renal dopamine excretion per residual nephrons and dopamine-sensitive enhanced natriuresis indicating that renal dopamine plays an important role in renal sodium handling during early chronic renal insufficiency.

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