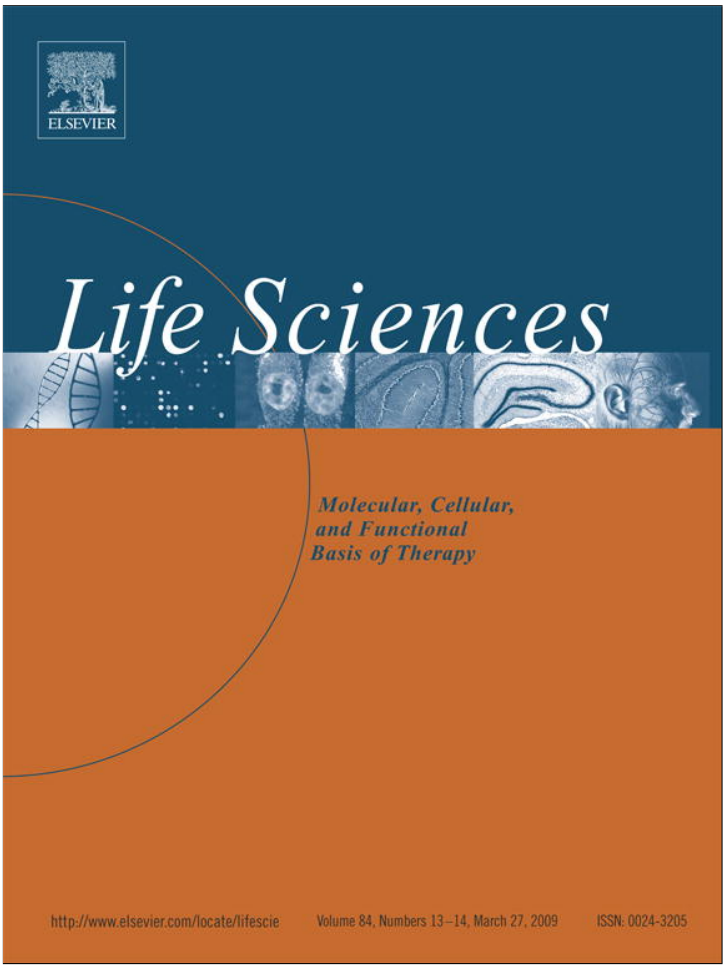

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Renal dopaminergic system activity in rat remnant kidney up to twenty-six weeks after surgery

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ABSTRACT

Aims: In 3/4 nephrectomized (3/4nx) rats the renal dopaminergic system was suggested to be involved in the adaptive increase of sodium excretion two weeks after renal mass ablation. The aim of the present study was to evaluate the renal adaptations in sodium handling and renal dopaminergic system activity in 3/4nx rats up to twenty-six weeks after surgery.
Main methods: The rats were placed in metabolic cages for the collection of 24 h urine for evaluation of sodium, dopamine, dopamine precursor and metabolites. Blood pressure, aromatic L-amino acid decarboxylase (AADC) activity in proximal tubules and the effect of dopamine D_1 receptor selective antagonist (Sch-23390) on natriuresis was evaluated.
Key findings: A time-dependent increase in both systolic and diastolic blood pressure was observed in 3/4nx rats, and this was accompanied by a decrease in urinary levels of dopamine and in renal AADC activity at twenty-six weeks after renal mass ablation. In contrast to what has been found two weeks after renal mass ablation, the natriuretic response to volume expansion was progressively reduced in 3/4nx rats at ten and twenty-six weeks after surgery and this was accompanied by insensitivity of natriuresis to Sch-23390.
Significance: In conclusion the renal dopaminergic system activity is compromised in 3/4nx rats in a time-dependent manner after renal mass ablation. It is suggested that this may contribute to compromise sodium excretion and increase blood pressure, in chronic renal insufficiency.

Introduction

The renal dopaminergic system plays an important role in the regulation of blood pressure, sodium homeostasis and kidney function (Carey et al., 1990). The epithelial cells of proximal tubules, but not of distal segments of the nephron, are endowed with a high aromatic L-amino acid decarboxylase (AADC) activity, the enzyme responsible for the conversion of circulating or filtered L-3,4-dihydroxyphenylalanine (L-DOPA) to dopamine (Hayashi et al., 1990; Soares-da-Silva and Fernandes, 1990; Soares-da-Silva et al., 1994). The renal dopaminergic system is highly dynamic and the basic mechanisms for the regulation of this system are thought to depend mainly on: 1) the availability of L-DOPA; 2) its fast decarboxylation into dopamine; 3) precise cell outward amine transfer mechanisms; 4) dopamine interaction with specific receptor and 5) accurate intracellular signal transduction (Aperia, 2000; Hussain and Lokhandwala, 1998; Soares-da-Silva et al., 1994).

Dopamine of renal origin, by activating D_1-like receptors located at various regions in the nephron, exerts natriuretic and diuretic effects (Jose et al., 1992) accounting for up to 50% of sodium excretion, namely during moderate sodium surfeit (Carey et al., 1990; Pelayo et al., 1983). At the level of the proximal tubule, the overall increase in sodium excretion produced by dopamine results mainly from the inhibition of several sodium transporters including Na^+, K^+-ATPase and Na^+–H^+ exchanger at the basolateral and apical membranes, respectively (Felder et al., 1990). Evidence has been gathered suggesting that deficiencies in this system may be involved in sodium retention and may contribute to the increase of blood pressure with sodium sensitive characteristics (Gill et al., 1988; Jose et al., 1998).

Animal models of reduced renal mass undergo a series of adaptive mechanisms to maintain sodium homeostasis. Compensatory changes in the tubular handling of sodium include an increased excretion of sodium per nephron (Hayslett, 1979). In this way, sodium balance can be maintained, despite a diminished glomerular filtration rate. However, this does not preclude the progressive increase of blood pressure with sodium sensitive characteristics in chronic renal insufficiency (CRI). In 3/4 nephrectomized (3/4nx) rats, a model of CRI, an increased dopamine output per residual nephrons was observed two weeks after surgery (Sampaio-Maia et al., 2005), this

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being accompanied by a dopamine sensitive enhanced natriuresis, indicating that renal dopamine may play an important role in keeping 3/4nx rats within sodium balance early after renal mass ablation (Sampaio-Maia et al., 2005). However, the role of the renal dopaminergic system in sodium handling in more advanced phases after renal mass ablation still remains to be established. This is a matter of particular importance given that CRI is accompanied by increase of blood pressure with sodium sensitive characteristics, which is more pronounced in advanced stages of reduced renal function.

The aim of the present study was to evaluate renal adaptations in sodium handling, blood pressure and renal dopaminergic system activity in rats submitted to 3/4 nephrectomy up to twenty-six weeks after surgery.

Methods

In vivo studies

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

Normotensive male Wistar–Han rats (Harlan, 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/kg of sodium. Blood pressure (systolic and diastolic) and heart rate were measured throughout the study in conscious restrained animals, between 7.00 and 10.00 AM, 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.

3/4 nephrectomy

In anesthetized rats (pentobarbital sodium, 60 mg/kg; ip), a surgical ablation of the right kidney and both poles of the left kidney was performed, according to what was previously described (Sampaio-Maia et al., 2005) – 3/4 nephrectomized (3/4nx) rats. The mean percentage of remnant renal mass in 3/4nx rats was 27 ± 1% and was calculated as previously reported (Sampaio-Maia et al., 2005). 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 to 6 h), the rats were placed in animal facility, where they had free access to food and water. The survival rate in Sham rats was 100%, whereas in 3/4nx rats the survival rate was 100% at two and ten weeks after surgery and 93% at twenty-six weeks after surgery.

Metabolic study

Ten and twenty-six weeks after surgery, the rats were placed in metabolic cages (Tecniplast, Buguggiate-VA, Italy) for the collection of twenty-four hours urine for later determinations of sodium, potassium, protein, creatinine, urea and catecholamines. The vials collecting urine for quantification of catecholamines contained 1 ml hydrochloric acid (6 M), to avoid the spontaneous oxidation of the amines and its derivatives. All animals received tap water ad libitum and the daily sodium intake was similar between Sham and 3/4nx rats throughout the study. Ten and twenty-six weeks after surgery the animals were anesthetized with pentobarbital sodium (60 mg/kg; i.p.) and 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 and weighed. The outer cortex was isolated and fragments were used for later determination of AADC activity. Other fragments from renal cortex weighing around 100 mg were placed in vials containing 0.5 ml of perchloric acid 0.2 M and stored at −80 °C until quantification of catecholamines and metabolites by HPLC with electrochemical detection.

Volume expansion (VE)

In some experiments, two, ten and twenty-six weeks after the surgery, the animals were anesthetized with pentobarbital sodium (60 mg/kg followed by 20 mg/kg/h; i.p.) and placed on a thermostatically controlled heating table to maintain a rectal temperature of 37 °C. The rats were tracheostomized. The left jugular vein was catheterized by a PE50 tube (Becton Dickson, Lisboa, Portugal) for VE and infusion of Sch-23390 (30 μg/kg bolus followed by 30 μg/kg/min) 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 of Sch-23390 or vehicle started at a rate of 5 ml/kg/h 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 100 ml/kg/h during 30 min (5% of body weight); 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 5 ml/kg/h 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-VE). The animals presented the following body weights: 2 weeks – Sham, 255 ± 8 g and 3/4nx, 249 ± 5 g; 10 weeks – Sham, 420 ± 12 g and 3/4nx, 378 ± 18 g; 26 weeks – Sham, 499 ± 17 g and 3/4nx, 445 ± 20 g.

In vitro studies

AADC activity

The AADC activity was determined at two, ten and twenty-six weeks after surgery in fragments of renal cortex as previously described (Soares-da-Silva et al., 1998) using L-Dopa as substrate (100 to 10,000 μM). The assay of dopamine was performed by HPLC with electrochemical detection. The protein content in cell suspension (1.5 mg/ml) was determined by the Bradford method (Bradford, 1976).

Assay of catecholamines

The assay of catecholamines and derivatives in urine, plasma samples, renal tissues and in samples from AADC studies was performed by HPLC with electrochemical detection, as previously described (Pestana et al., 1995; Vieira-Coelho et al., 1999). In our laboratory, the lower limit of detection of dopamine, dopamine metabolites (DOPAC, 3,4-dihydroxyphenylacetic acid; 3-MT, 3-metoxytyramine and HVA, homovanillic acid) and noradrenaline ranged from 350 to 1000 fmol.

Plasma and urine ionogram and biochemistry

The quantification of sodium, potassium, urea, creatinine and total proteins in plasma and urine samples, were performed by Cobas Mira Plus analyzer (ABX Diagnostics, Geneva, Switzerland) as previously described (Sampaio-Maia et al., 2005). Creatinine clearance and fractional excretion of sodium and potassium were calculated as previously reported (Sampaio-Maia et al., 2005).

Drugs

The compounds DOPAC; dopamine hydrochloride; HVA; L-Dopa; 3-MT; noradrenaline bitartrate and Sch-23390 were obtained from Sigma (St. Louis, MO, USA).

Statistics

Results are means ± SE of values for the indicated number of determinations. Maximal velocity (Vmax) and Michäelis–Menten

Table 1
Body weight, increase in renal mass, metabolic balance and renal function in sham-operated (Sham) and 3/4 nephrectomized (3/4nx) rats at ten and twenty-six weeks after surgery

	Ten weeks		Twenty-six weeks	
	Sham	3/4nx	Sham	3/4nx
Body weight, g	420 ± 8	409 ± 15	472 ± 22	462 ± 7
Plasma urea, mg/dl	39.0 ± 1.2	73.5 ± 3.8*	37.6 ± 1.6	92.6 ± 14.4*
Plasma creatinine, mg/dl	0.51 ± 0.08	0.95 ± 0.07*	0.58 ± 0.03	1.44 ± 0.23*
Plasma protein, g/l	51.3 ± 1.6	48.9 ± 0.9	55.7 ± 0.5	53.2 ± 1.1
Plasma Na^+, mmol/l	138.6 ± 0.6	137.3 ± 1.0	137.0 ± 1.5	138.8 ± 1.3
Plasma K^+, mmol/l	5.2 ± 0.2	5.7 ± 0.3	6.2 ± 0.5	6.1 ± 0.4
Fluid intake, ml/day	20.2 ± 1.1	32.1 ± 1.1*	15.9 ± 1.0	30.8 ± 1.7*
Na^+ intake, mmol/day	1.8 ± 0.1	1.7 ± 0.1	1.1 ± 0.1	1.0 ± 0.1
Urine volume, ml/day	10.3 ± 0.6	22.0 ± 1.2*	9.4 ± 1.5	23.3 ± 1.5*
Urine protein, mg/day	61.5 ± 3.6	117.8 ± 12.0*	47.9 ± 5.2	192.1 ± 16.7*
Urine Na^+, mmol/day	1.8 ± 0.1	1.6 ± 0.1	1.2 ± 0.1	1.0 ± 0.1
Urine K^+, mmol/day	1.5 ± 0.1	2.1 ± 0.1*	1.3 ± 0.1	1.6 ± 0.1*
Ccreat, ml/min	2.2 ± 0.2	1.0 ± 0.1*	2.2 ± 0.1	0.9 ± 0.1*
FE_{Na+}, %	0.25 ± 0.01	0.92 ± 0.05*	0.29 ± 0.03	1.03 ± 0.28*
FE_{K+}, %	5.9 ± 0.5	20.6 ± 4.2*	6.8 ± 0.9	23.5 ± 4.4*

Values are means ± SE; $n = 9$ to 12 experiments per group. Ccreat, creatinine clearance; FE, fractional excretion. *Significantly different from values in sham-operated rats ($p < 0.05$).

coefficient (Km) were calculated from nonlinear regression analysis using GraphPad Prism statistics software package (Motulsky et al., 1994). Statistical analysis was performed by one-way ANOVA followed by Student's *t*-test for unpaired comparisons. A $p < 0.05$ was assumed to denote a significant difference.

Results

Ablation of renal mass in 3/4nx rats had no significant effects on body growth at ten and twenty-six weeks after surgery. Kidney growth, however, was significantly altered in 3/4nx rats. Ten and twenty-six weeks after surgery the 3/4nx rats presented a hypertrophied remnant renal mass with a 191 ± 17% and 257 ± 15% weight increase, respectively.

No significant differences were observed between 3/4nx and Sham rats in either daily intake or urinary excretion of sodium throughout the study (Table 1). In addition, plasma levels of sodium and potassium were similar in Sham and 3/4nx rats at ten and twenty-six weeks after surgery (Table 1). However, fluid intake and urine volume were

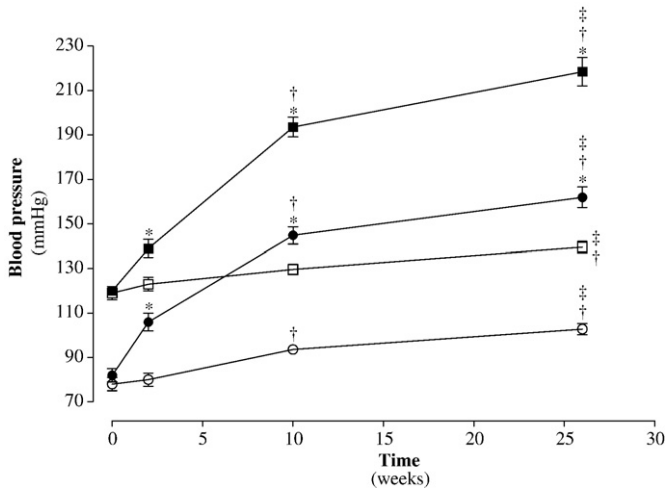

Fig. 1. Systolic (■,□) and diastolic (●,○) blood pressure in 3/4 nephrectomized (3/4nx, ■,●) and sham-operated (Sham, □,○) rats at two, ten and twenty-six weeks after surgery. Symbols represent means of 9 to 12 experiments per group, and error bars represent SE. *Significantly different from values in sham-operated rats ($p < 0.05$). †Significantly different from values before and at two weeks after surgery ($p < 0.05$). ‡Significantly different from values at ten weeks after surgery ($p < 0.05$).

Table 2
Urinary levels of dopamine, L-Dopa, DOPAC, 3-MT, HVA and noradrenaline in sham-operated (Sham) and 3/4 nephrectomized (3/4nx) rats at ten and twenty-six weeks after surgery

	Ten weeks		Twenty-six weeks	
	Sham	3/4nx	Sham	3/4nx
Dopamine, nmol/day	10.7 ± 1.1	7.3 ± 1.0*	10.2 ± 1.7	3.2 ± 1.2*
L-Dopa, nmol/day	0.17 ± 0.02	0.22 ± 0.02	0.20 ± 0.03	0.23 ± 0.05
DOPAC, nmol/day	21.0 ± 3.7	25.4 ± 2.4	20.0 ± 2.4	12.1 ± 3.6
3-MT, nmol/day	18.5 ± 2.4	12.3 ± 2.5	21.3 ± 2.9	22.8 ± 2.5
HVA, nmol/day	205.1 ± 12.5	221.1 ± 10.3	207.4 ± 9.6	179.4 ± 6.1
Noradrenaline, nmol/day	7.1 ± 0.2	3.5 ± 0.5*	7.4 ± 1.0	2.0 ± 0.6*

Values are means ± SE; $n = 9$ to 12 experiments per group. L-Dopa, L-3,4-dihydroxyphenylalanine; DOPAC, 3,4-dihydroxyphenylacetic acid; 3-MT, 3-methoxytyramine; HVA, homovanilic acid. *Significantly different from values in sham-operated rats ($p < 0.05$).

greater in 3/4nx rats than in Sham rats throughout the study (Table 1). The 3/4nx rats presented increased plasma creatinine and urea values and decreased creatinine clearance both at ten and twenty-six weeks after surgery (Table 1). The fractional excretion of both sodium and potassium were greater in 3/4nx animals, throughout the study (Table 1). In addition, the 3/4nx rats presented a time-dependent increase in urinary protein excretion (Table 1).

A time-dependent increase in both systolic and diastolic blood pressure was observed in both groups throughout the study (Fig. 1). However, blood pressure, both systolic and diastolic, was higher in 3/4nx rats than in Sham rats at two, ten and twenty-six weeks after surgery.

Absolute daily urinary dopamine excretion was significantly lower in 3/4nx rats than in Sham rats at ten and twenty-six weeks after surgery (Table 2). In addition, the reduction of urinary dopamine in 3/4nx rats was more marked at twenty-six than at ten weeks after surgery (68 ± 9% vs 31 ± 12%). Daily urinary excretion of the dopamine precursor (L-Dopa) and metabolites (DOPAC, HVA and 3-MT) were similar between 3/4nx and Sham rats at ten and twenty-six weeks after surgery (Table 2).

The AADC activity was determined in homogenates of renal cortex with L-Dopa as substrate, which resulted in a concentration-dependent formation of dopamine. The decarboxylation reaction was a saturable process, with Km values of the same magnitude in all groups (Table 3). At ten weeks after surgery, the Vmax values for renal AADC activity were significantly higher in 3/4nx rats than in Sham rats, whereas at twenty-six weeks after surgery a marked decrease in the renal AADC activity was observed in 3/4nx rats (Table 3).

Tissue levels of L-Dopa in fragments of renal cortex did not differ between the 3/4nx and Sham rats at ten and twenty-six weeks after surgery (Table 4). By contrast, the tissue levels of dopamine in fragments of renal cortex were significantly reduced in 3/4nx rats throughout the study (Table 4). This resulted in decreased renal dopamine/L-DOPA ratios in the renal cortex of 3/4nx rats at twenty-six weeks after surgery (Table 4).

The daily urinary excretion and the renal tissue levels of noradrenaline were significantly reduced in 3/4nx rats throughout the study (Tables 2 and 4). By contrast, no significant differences were

Table 3
Kinetic parameters (Vmax and km) of aromatic L-amino acid decarboxylase (AADC) activity in homogenates of renal cortex from sham-operated (Sham) and 3/4 nephrectomized (3/4nx) rats ten and twenty-six weeks after surgery

	Ten weeks		Twenty-six weeks	
	Sham	3/4nx	Sham	3/4nx
Vmax, pmol mg prot/15 min	214 ± 7	284 ± 23*	248 ± 22	117 ± 21*
Km, mM	2.6 ± 0.4	2.9 ± 0.4	2.6 ± 0.3	2.2 ± 0.3

Values are means ± SE; $n = 9$ to 12 experiments per group. Vmax, Maximal velocity; Km, Michaelis–Menten constant. *Significantly different from values in sham-operated rats ($p < 0.05$).

Table 4
Levels of dopamine, L-Dopa, noradrenaline and dopamine/L-Dopa ratios in renal cortex from sham-operated (Sham) and 3/4 nephrectomized (3/4nx) rats at ten and twenty-six weeks after surgery

	Ten weeks		Twenty-six weeks	
	Sham	3/4nx	Sham	3/4nx
Dopamine, pmol/g	88.3 ± 8.9	59.8 ± 5.1*	73.0 ± 3.3	33.6 ± 5.8*
L-Dopa, pmol/g	159.1 ± 16.7	130.4 ± 8.9	146.2 ± 15.5	142.2 ± 26.0
Dopamine/L-Dopa	0.76 ± 0.11	0.55 ± 0.07	0.53 ± 0.05	0.24 ± 0.06*
Noradrenaline, pmol/g	1661 ± 59	802 ± 60*	1558 ± 229	600 ± 81*

Values are means ± SE; $n = 5$ to 12 experiments per group. L-Dopa, L-3,4-dihydroxyphenylalanine. *$p < 0.05$, Significantly different from corresponding values in sham-operated rats.

observed in plasma levels of noradrenaline between 3/4nx and Sham rats at ten (2.8 ± 0.9 vs 2.6 ± 0.5; pmol/ml) and twenty-six (1.9 ± 0.9 vs 1.7 ± 0.3; pmol/ml) weeks after surgery.

The urinary sodium excretion before ($t = 0$–120 min, basal), during ($t = 120$–150 min, VE) and after ($t = 150$–240 min, R-VE) VE in 3/4nx and Sham rats at two, ten and twenty-six weeks after surgery is depicted in Fig. 2. Two weeks after surgery (Fig. 2A), the natriuretic response to VE was slightly decreased in 3/4nx when compared with Sham rats, whereas at ten and twenty-six weeks after surgery (Fig. 2B and C) the natriuretic response to VE was markedly decreased in 3/4nx rats. Moreover, the compromised natriuretic response to VE in 3/4nx rats was more pronounced at twenty-six weeks than at ten weeks after surgery (Fig. 2B and C).

The effect of Sch-23390 on the accumulated urinary sodium excretion before, during and after VE in Sham and 3/4nx rats at two, ten and twenty-six weeks after surgery is depicted in Fig. 3. As can be observed, in 3/4nx rats two weeks after renal mass ablation, Sch-23390 significantly decreased the accumulated urinary sodium excretion before, during and after VE (Fig. 3A), whereas at ten and twenty-six weeks after surgery the infusion of Sch-23390 did not significantly change the urinary sodium excretion (Fig. 3B and C).

Discussion

This study has focused upon the impairment of sodium excretion and increase of blood pressure in rats submitted to 3/4 nephrectomy and the possible role of abnormal renal dopaminergic system activity. Two weeks after renal mass ablation, both systolic and diastolic blood pressure was slightly increased in 3/4nx rats. This was accompanied by reasonable well preserved natriuretic response to volume expansion going along with dopamine sensitive enhanced natriuresis. By contrast, ten and twenty-six weeks after renal mass ablation, a time-dependent increase in both systolic and diastolic blood pressure was accompanied by a blunted natriuretic response to volume expansion going along with insensitivity of natriuresis to D1 dopamine blockade. Taken together, our results suggest that the time-dependent decrease of renal dopaminergic system activity in 3/4nx rats may underlie the impaired control of sodium homeostasis and increase of blood pressure after renal mass ablation.

Renal parenchymal diseases are the most common cause of secondary hypertension, accounting for up to 5% of all cases (Smith and Dunn, 1995). However, despite much interest and investigation, the precise pathophysiological mechanisms that produce hypertension in patients with kidney disease remains to be completely elucidated. Although renal parenchymal hypertension most probably represents the combined interactions of multiple independent mechanisms, several lines of evidence suggest that impaired sodium handling leading to positive sodium balance contribute importantly to this condition (Preton and Epstein, 1995). The finding that pressure natriuresis is abnormal in renal parenchymal hypertension emphasizes the importance of long term study of natriuretic systems in renal parenchymal diseases (Anderson et al., 1995).

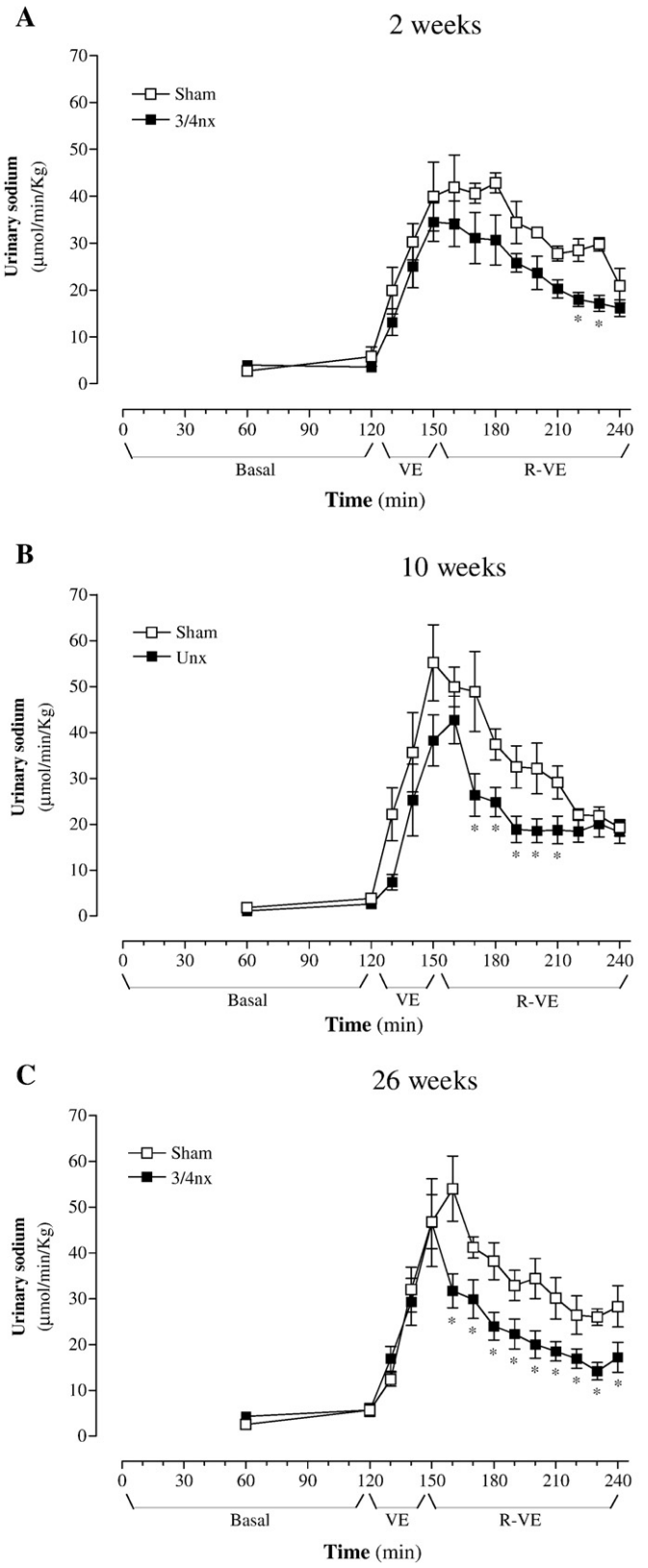

Fig. 2. Urinary sodium excretion (μmol/min/kg) in sham-operated (Sham, □) and 3/4 nephrectomized (3/4nx, ■) rats before ($t = 0$–120 min, Basal), during ($t = 120$–150 min, VE) and after ($t = 150$–240 min, R-VE) 5% volume expansion with isotonic saline at two (A), ten (B) and twenty-six (C) weeks after surgery. Symbols represent means of 5 to 8 experiments per group, and error bars represent SE. *Significantly different from values in sham-operated rats ($p < 0.05$).

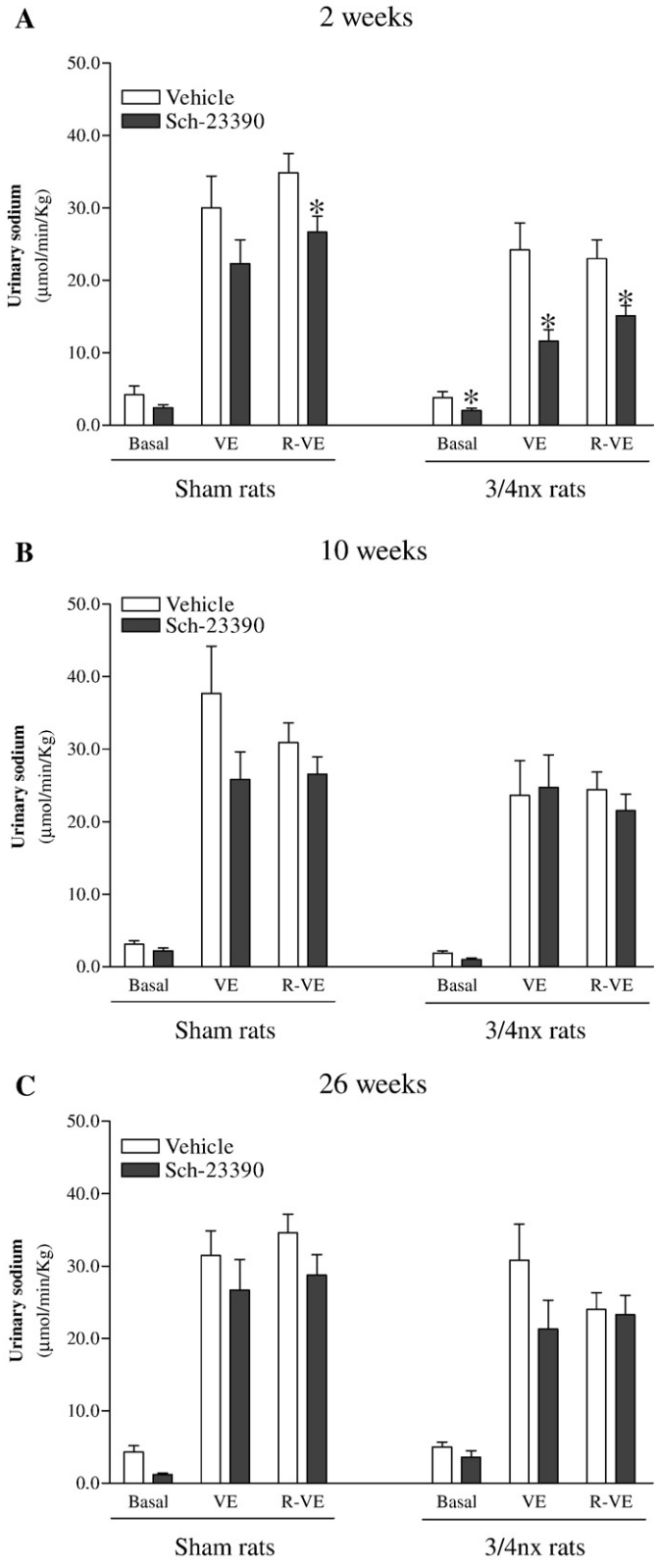

Fig. 3. Accumulated urinary sodium excretion (μmol/min/kg) in vehicle-treated (open bars) and Sch-23390 treated (closed bars) sham-operated (Sham) and 3/4 nephrectomized (3/4nx) rats before ($t=0$–120 min, Basal), during ($t=120$–150 min, VE) and after ($t=150$–240 min, R-VE) 5% volume expansion with isotonic saline at two (A), ten (B) and twenty-six (C) weeks after surgery. Bars represent means of 5 to 8 experiments per group and error bars represent SE. *Significantly different from values in vehicle-treated rats ($p<0.05$).

Renal dopamine behaves as an endogenous natriuretic hormone namely during sodium loading, and several lines of evidence suggest that renal dopamine has a key role in the pathophysiology of salt sensitive hypertension (Holtback and Aperia, 2008). Two fundamental defects in the renal dopamine system have been suggested to result in sodium retention and hypertension: 1) deficient renal dopamine production due to reduced renal uptake and/or decarboxylation of L-Dopa and 2) defective D1-like receptor G protein coupling such that renal dopamine is ineffective in transmitting a signal to increase sodium excretion (Jose et al., 1998).

The results presented here showed that two weeks after renal mass ablation the urinary sodium excretion in 3/4nx rats was sensitive to D1 receptor blockade. At two weeks after surgery the effect of Sch-23390 in 3/4nx rats was a ~34 ± 6% reduction in urinary excretion of sodium, whereas in Sham rats the effect of Sch-23390 was a ~23 ± 6% reduction. In this respect, it is interesting to note that uninephrectomized dogs, but not Sham dogs, responded to Sch-23390 with antinatriuresis (Siragy et al., 1989); this corresponded to the first observation on the tonic role of endogenous renal dopamine as a local natriuretic hormone. Our present findings in 3/4nx rats at two weeks after surgery agree well with the previous reports indicating that early after renal mass ablation, the remnant kidney exhibits increased renal dopamine output per nephron and augmented dopamine-sensitive phosphaturic and natriuretic responses to parathyroid hormone and volume expansion, respectively (Isaac et al., 1993; Sampaio-Maia et al., 2005; Vieira-Coelho et al., 2000).

Unlike the findings observed two weeks after renal mass ablation, ten and twenty-six weeks after surgery a blunted increase in urinary excretion of sodium during isotonic saline volume expansion was observed and this was accompanied by a progressive increase in both systolic and diastolic blood pressure in 3/4nx rats. Interestingly, ten and twenty-six weeks after renal mass ablation a time-dependent decrease of renal dopamine activity was observed in 3/4nx rats as evidenced by both decreased dopaminuric response and insensitivity of natriuresis to D1 receptor blockade. Thus, it is likely that the contribution of the renal dopaminergic system to the adaptations of renal function after renal mass ablation may be diminished over time. Because high blood pressure and sodium retention may be linked to abnormalities in the function of the renal dopaminergic system, one can hypothesize that the blunted natriuretic response and the progressive increase of blood pressure at ten and twenty-six weeks after renal mass ablation may be associated with inability of the remnant kidney to increase dopamine synthesis and/or with deficient coupling of dopamine receptors to effector mechanisms (Jose et al., 2002; Jose et al., 1998; Pestana et al., 2001).

The renal adaptations in sodium handling and renal dopaminergic system activity was previously examined in uninephrectomized rats at two, ten and twenty-six weeks after surgery (Moreira-Rodrigues et al., 2007). It is interesting to note that unlike the findings of compensatory increase of renal dopaminergic system at two weeks after uninephrectomy, at ten and twenty-six weeks after uninephrectomy a blunted renal dopaminergic system was observed (Moreira-Rodrigues et al., 2007). It seems, therefore, that the contribution of renal dopamine to the adaptations of renal function after renal mass ablation may change over time, assuming particular importance in the early phases (two weeks), but not in later phases (ten and twenty-six weeks).

Patients suffering from chronic renal parenchymal diseases showed a close relationship between the decline of renal function and the decrease in the renal production of natriuretic dopamine (Pestana et al., 1998). Interestingly, the dopaminuric response to sodium loading was related to salt sensitivity of blood pressure in patients with chronic kidney disease presenting slightly compromised renal function (Pestana et al., 2001), whereas in more advanced phases of renal insufficiency salt loading was associated with failure to mobilize dopamine in kidney (Casson et al., 1983). Those findings in

patients with chronic kidney disease, when viewed collectively with the present observations in 3/4nx rats up to twenty-six weeks after surgery suggests that distinctly to what occurs in the early phases, in later phases of chronic renal insufficiency an attenuation of renal dopaminergic activity cannot be responsible for the increased natriuresis per nephron and may instead favor sodium retention and increase blood pressure.

Another interesting observation was that the urinary excretion of noradrenaline was marked lower in 3/4nx rats than in Sham animals. This is in agreement with a previous report of our group, in 3/4nx rats, two weeks after surgery (Sampaio-Maia et al., 2005). One possible explanation could be the ablation of renal nerves due to the marked reduction in renal mass. Indeed, tissue noradrenaline concentration was also significantly lower in the remnant kidney from 3/4nx rats than in Sham rats. The decreased noradrenaline concentrations in the remnant kidney of 3/4nx rats is in agreement with the findings of others (Isaac et al., 1993) and strongly suggests that by increasing sodium excretion the reduced sympathetic tone may contribute to maintain sodium balance by the remnant kidney. The finding that plasma levels of noradrenaline were similar between 3/4nx and Sham rats 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 (Esler, 2000).

Conclusion

It is concluded that, in 3/4nx rats, the role of the renal dopaminergic system in the control of blood pressure and sodium balance may differ over time. Unlike the compensatory response at two weeks after renal mass ablation, at ten and twenty-six weeks after surgery a blunted dopaminuric response is observed. We suggest that this may contribute to the impairment of sodium homeostasis and increase of blood pressure in 3/4nx rats at ten and twenty-six weeks after renal mass ablation.

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