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Original Article

Blunted renal dopaminergic system activity in puromycin aminonucleoside-induced nephrotic syndrome

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Abstract

Background. A primary tubular sodium handling abnormality has been implicated in the edema formation of nephrotic syndrome. Dopamine synthesized by renal proximal tubules behaves as an endogenous natriuretic hormone by activating D_1-like receptors as a paracrine/autocrine substance.

Methods. We examined the time courses of the urinary excretion of sodium, protein and dopamine in puromycin aminonucleoside (PAN)-treated and control rats. The rats were sacrificed during greatest sodium retention (day 7) as well as during negative sodium balance (day 14) for the evaluation of renal aromatic L-amino acid decarboxylase (AADC) activity, the enzyme responsible for the synthesis of renal dopamine. Also, the influence of volume expansion (VE) and the effects of the D_1-like agonist fenoldopam (10 μg/kg bw/min) on natriuresis and on proximal tubular Na^+,K^+-ATPase activity were examined on day 7.

Results. The daily urinary excretion of dopamine was decreased in PAN-treated rats, from day 5 and beyond. This was accompanied by a marked decrease in the renal AADC activity, on days 7 and 14. During VE, the fenoldopam-induced decrease in proximal tubular Na^+,K^+-ATPase activity was more pronounced in PAN-treated rats than in controls. However, the urinary sodium excretion during fenoldopam infusion was markedly increased in control rats but was not altered in PAN-treated animals.

Conclusion. PAN nephrosis is associated with a blunted renal dopaminergic system activity which may contribute to enhance the proximal tubular Na^+,K^+-ATPase activity. However, the lack of renal dopamine appears not to be related with the overall renal sodium retention in a state of proteinuria.

Keywords: aromatic L-amino acid decarboxylase (AADC); fenoldopam; Na^+,K^+-ATPase; nephrotic syndrome; renal dopamine; sodium handling

Introduction

Evidence has been gathered implicating a primary renal sodium handling abnormality in the edema formation of nephrotic syndrome. The nephrotic state was associated with enhanced sodium retention in the cortical collecting duct. It was suggested by Deschenes and Doucet [1] that the mechanism responsible for the primary distal sodium retention in nephrotic syndrome is the combination of a blunted natriuretic response to atrial natriuretic peptide (ANP) [2] and an enhanced Na^+,K^+-ATPase activity in the cortical collecting duct [3]. The ANP resistance, which occurs after ANP binding to its receptors in the collecting duct, appears to result from the activation of a phosphodiesterase responsible for the catabolism of cyclic guanosine monophosphate (cGMP), the second messenger of ANP [4]. On the other hand, a primary sodium handling abnormality in the proximal tubules has been invoked recently with the observation that sodium retention in the nephrotic syndrome may be associated with a shift of the Na^+/H^+ exchanger NHE3 from the inactive to an active pool [5]. However, the Na^+,K^+-ATPase activity in proximal convoluted tubules was shown not to differ between nephrotic and control animals [6] and, therefore, the role of the proximal tubules in the enhanced sodium retention in the nephrotic syndrome still remains to be fully elucidated.

The epithelial cells of proximal tubules are endowed with a high aromatic L-amino acid decarboxylase (AADC) activity, the enzyme responsible for the

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conversion of circulating or filtered L-3,4-dihydroxyphenylalanine (L-Dopa) to dopamine [7]. The renal dopaminergic system appears to be highly dynamic and basic mechanisms for the regulation of this system are thought to depend mainly on the availability of L-Dopa, its fast decarboxylation into dopamine and in precise and accurate cell outward amine transfer mechanisms [7,8]. Dopamine synthesized by the renal proximal tubules behaves as an endogenous natriuretic hormone by activating D_1-like receptors as a paracrine/autocrine substance [7,9]. During moderate sodium surfeit, dopamine of renal origin accounts for ~50% of sodium excretion [8,9]. Renal dopamine decreases tubular sodium reabsorption by inhibition of Na^+,K^+-ATPase activity directly or in response to the decrease in intracellular sodium following inhibition of Na^+/H^+ exchanger NHE3 [9,10]. Dopamine of renal origin can regulate sodium balance also by interaction with other natriuretic factors such as ANP [8]. In the late 1980s, several laboratories reported that the natriuretic response to ANP requires an intact renal dopaminergic system. More recently, the interaction between ANP and renal dopamine was further reinforced by the findings that ANP and its second messenger, cGMP, cause a rapid translocation of the D_1-like receptors to the plasma membrane [11].

On the basis of these considerations, this study was undertaken with the aim to evaluate the role of renal dopaminergic system in the sodium retention observed in rats with puromycin aminonucleoside (PAN)-induced nephrotic syndrome. For this purpose, we examined the time courses of the urinary excretion of sodium, protein, dopamine, the precursor L-Dopa and metabolites (3,4-dihydroxyphenylacetic acid, DOPAC and homovanillic acid, HVA) in PAN-treated and control rats. The rats were sacrificed on days 7 and 14 for the evaluation of the renal AADC activity. Also, the influence of volume expansion and the effects of the D_1-like receptor agonist fenoldopam on sodium excretion and on proximal tubular Na^+,K^+-ATPase activity were examined during the phase of greatest sodium retention and ascites accumulation (day 7).

Materials and methods

In vivo studies

PAN-induced nephrosis. Normotensive male Sprague–Dawley rats (Harlan, Barcelona, Spain), weighing 200–220 g, were selected after a 7-day period of stabilization and adaptation to blood pressure measurements. The animals received a single intraperitoneal injection of 10 ml/kg bw of PAN (150 mg/kg bw) or the vehicle (NaCl 0.9%) on day 0.

Metabolic studies. The animals were kept under controlled environmental conditions (12:12 h light/dark cycle and room temperature $22 \pm 2^\circ$C); fluid intake and food consumption were monitored daily throughout the study. Two days before the PAN or vehicle injection, the rats were placed in metabolic cages (Techniplast, Buguggiate-VA, Italy). The PAN and control rats had free access to tap water. The PAN-treated rats were fed *ad libitum* throughout the study with ordinary rat chow (Panlab, Barcelona, Spain) containing 1.9 g/kg of sodium. In order to achieve the same daily sodium intake between the two groups, the control rats had only access to the mean daily rat chow intake of the PAN-treated animals. Twenty-four hour urine was collected, on even days in empty vials for later determinations of sodium, protein and creatinine and on uneven days in vials containing 1 ml hydrochloric acid 6 M (to avoid the spontaneous oxidation of the amines and its derivatives) for later determination of catecholamines. Urine volume was gravimetrically determined. Blood pressure (systolic and diastolic) and heart rate were measured daily 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).

Animals were sacrificed on day 7 and on day 14 after injection. On the days of sacrifice the animals were anaesthetized with pentobarbital sodium (50 mg/kg bw; i.p.) and the ascites volumes were measured through moistening and weighing an absorbent paper. 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. Fragments of renal cortex were used for later determination of AADC activity. Other fragments of renal cortex, weighing ~200 mg, were placed in vials containing 1 ml of 0.2 M perchloric acid, stored at -80°C until quantification of catecholamines by HPLC with electrochemical detection. Segments of jejunum, ~10 cm in length, were also removed, opened longitudinally with fine scissors and rinsed free from blood and intestinal contents with cold saline; thereafter, the jejunal mucosa was removed with a scalpel for later determination of AADC activity.

Volume expansion. In another set of experiments, 7 days after PAN or vehicle injection, the animals were anaesthetized with pentobarbital sodium (50 mg/kg bw followed by 20 mg/kg bw/h; i.p.) and were subjected to volume expansion (VE) with saline (0.9% NaCl) through a catheter in the jugular vein, as previously reported [12]. The infusion of fenoldopam (10 μg/kg bw/min) or the vehicle (0.9% NaCl) started at a rate of 5 ml/kg bw/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 increasing the infusion to a rate of 50 ml/kg bw/30 min (5% body weight, $t = 120$–150 min, VE). Thereafter, the infusion was again reduced to 5 ml/kg bw/h for 90 min; during this recovery period, urine sampling was performed every 30 min until the end of the experiment ($t = 150$–180 min, R-VE1; $t = 180$–210 min, R-VE2 and $t = 210$–240 min, R-VE3). The urine was collected in empty vials for later determinations of sodium and creatinine and in another set of experiments the urine was collected in vials containing 50 μl of hydrochloric acid 6 M for later determination of dopamine. Because the dopamine assay requires higher urine volumes, the recovery periods 2 and 3 were collected jointly. At the end of this protocol the animals were euthanized and the kidneys were removed for later determination of Na^+,K^+-ATPase activity in proximal tubular cells.

In vitro studies

AADC activity. Fragments of renal cortex and jejunal mucosa were homogenized at 4°C with a Thomas Teflon homogenizer (Poliscience Corp., IL, USA) in the incubation medium containing (in mM): 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 μM) and pargyline (100 μM) were added to the incubation medium in order to inhibit the metabolization of dopamine by catechol-*O*-methyltransferase (COMT) and monoamine-oxidase (MAO), respectively. Activity of AADC was determined as previously described by Soares-da-Silva [12] using L-Dopa (0.1–10 mM) as substrate. 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 [13].

Na^+,K^+-ATPase activity. Na^+,K^+-ATPase activity was measured by the method of Quigley and Gotterer [14] adapted in our laboratory with slight modifications. The rat renal proximal tubules were isolated, as previously described [15]. In brief, a fine paste of outer cortex was prepared and filtered sequentially through a series of Nybolt nylon sieves, first 180 μm and then 75 μm. The renal proximal tubules were retained in the 75 μm sieve and were then washed off with cold Collins solution and collected into a pellet by centrifugation at 200 g, 5 min, 4°C. Renal tubules used in incubation experiments were suspended in Hanks' medium. The Na^+,K^+-ATPase activity was determined in conditions of saturating sodium and tris salt adenosine 5′-triphosphate (ATP) concentration. The isolated renal proximal tubules were pre-incubated for 10 min at 37°C followed by rapid freezing at −80°C and subsequent thawing to allow cell permeabilization. The reaction mixture, in the final volume of 1.025 ml, contained (in mM): 37.5 imidazole buffer, 75 NaCl, 5 KCl, 1 sodium EGTA, 5 $MgCl_2$, 75 NaN_3, 75 tris(hydroxymethyl)aminomethane(tris) hydrochloride and 100 μl cell suspension. For the determination of ouabain-resistant ATPase, NaCl and KCl were omitted, and Tris-HCl (150 mM) and ouabain (1 mM) were added to the assay. The reaction was initiated by addition of 4 mM ATP. After incubation at 37°C for 20 min, the reaction was terminated by the addition of 50 μl of ice-cold trichloroacetic acid. The samples were centrifuged (4000 rpm) and liberated Pi in the supernatant was measured as the result of ATPase activity. The assay of Pi was performed by spectrophotometry. Ouabain-sensitive ATPase activity is expressed as nanomoles of Pi per miligram of protein per minute and determined as the difference between total and ouabain-resistant ATPase. The protein content in cell suspension (1.1 mg/ml) was determined by the Bradford method [13]. The relationship between the incubation time and Na^+,K^+-ATPase activity was linear between 5 and 40 min. In addition, the relationship between protein content in cell suspension and Na^+,K^+-ATPase activity was linear between 0.4 and 1.3 mg/ml.

Assay of catecholamines. The assay of catecholamines and its metabolites in urine, plasma samples, renal tissues and in samples from AADC studies were performed by HPLC with electrochemical detection, as previously described [16]. In our laboratory, the lower limit of detection of L-Dopa, dopamine, DOPAC and HVA ranged from 350 to 1000 fmol.

Plasma and urine ionogram and biochemistry. Ion-selective electrodes performed the quantifications of sodium in plasma and urine samples. Urea was measured by an enzymatic test and creatinine by the Jaffé method. Total proteins were determined by a colorimetric test, the biuret reaction. All assays were performed by Cobas Mira Plus analyser (ABX Diagnostics, Switzerland). Creatinine clearance was calculated using 24 h urinary creatinine excretion. Fractional excretion of sodium (FE_{Na+}) was calculated as previously reported [12]. Sodium balance was determined subtracting the absolute daily urinary sodium excretion (mmol/24 h) to daily sodium intake (mmol/24 h).

Drugs. The compounds ATP, DOPAC, dopamine hydrochloride, HVA, L-Dopa, ouabain, PAN, pargyline hydrochloride and fenoldopam were obtained from Sigma (St Louis, MO, USA). Tolcapone was kindly donated by the late Professor 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 Michäelis–Menten coefficient (K_m) for AADC enzymatic assay were calculated from non-linear regression analysis using GraphPad Prism statistics software package [17] and compared by one-way ANOVA followed by Student's *t*-test for unpaired comparisons. $P < 0.05$ was assumed to denote a significant difference.

Results

PAN nephrosis—renal function and sodium handling

The sodium intake was similar in both PAN-treated and control animals throughout the study since the control animals had the same food intake as PAN-treated rats (Table 1). As shown in Figure 1, the PAN-treated rats developed severe proteinuria on day 4 and beyond reaching a plateau on day 8 whereas the renal protein excretion was minimal in control animals throughout the study. In parallel, the PAN-treated rats showed a marked decrease in urinary sodium excretion compared with control animals from day 2 to day 8 after drug administration (Figure 1), followed by an increase in the urinary excretion of sodium from days 12 to 14. On day 7 after injection, the PAN-treated rats exhibited a reduced FE_{Na+} accompanied with marked ascites whereas on day 14 after injection the PAN-treated rats presented an increased FE_{Na+} accompanied with ascites of much smaller magnitude (Table 1). The body weight was greater in PAN-treated rats than in control animals from day 5 to day 10 (Figure 1), showing that in PAN nephrosis the animal weight is a good index of ascites accumulation. Taken together, PAN administration resulted in the clinical equivalent of nephrotic syndrome with persistent proteinuria as well as sodium and volume retention up to day 8 after drug injection [1]. The creatinine clearance was lower in PAN-treated than in control rats either on day 7 or on day 14 (Table 1). Systolic and diastolic blood pressure did not differ significantly between nephrotic and control rats throughout the

Table 1. Body weight, metabolic balance and renal function in PAN-treated and control rats 7 and 14 days after injection

	Day 7		Day 14	
	Control	PAN	Control	PAN
Body weight, g	221 ± 5	248 ± 4*	241 ± 2	234 ± 5
Creatinine, ml/min	5.65 ± 1.38	2.24 ± 0.81*	4.51 ± 1.08	2.24 ± 0.32*
Plasma creatinine, mg/dl	0.20 ± 0.02	0.54 ± 0.06*	0.22 ± 0.02	0.32 ± 0.03*
Plasma urea, mg/dl	43 ± 2	143 ± 13*	41 ± 2	43 ± 2
Plasma proteins, g/l	46 ± 1	36 ± 1*	49 ± 2	49 ± 3
Plasma Na^+, mmol/l	137 ± 1	136 ± 1	135 ± 3	135 ± 1
Na^+ intake, mmol/24 h	1.07 ± 0.01	0.96 ± 0.06	1.31 ± 0.05	1.47 ± 0.33
Na^+ excretion, mmol/24 h	1.11 ± 0.06	0.07 ± 0.02*	1.13 ± 0.13	2.08 ± 0.29*
Na^+ balance, mmol/24 h	−0.03 ± 0.07	0.90 ± 0.07*	0.18 ± 0.13	−0.61 ± 0.38*
FE_{Na+}, %	0.14 ± 0.01	0.04 ± 0.01*	0.17 ± 0.03	0.53 ± 0.14*
Ascites, g	0.7 ± 0.1	14.5 ± 1.1*	0.6 ± 0.1	1.3 ± 0.3*
Systolic BP, mmHg	155 ± 8	147 ± 6	138 ± 4	150 ± 7
Diastolic BP, mmHg	106 ± 9	86 ± 8	86 ± 3	86 ± 7
Heart rate, beats/min	423 ± 8	390 ± 16	406 ± 9	387 ± 37

Values are means ± SE; $n = 8$ to 12 experiments per group. Creatinine = creatinine clearance, FE = fractional excretion and BP = blood pressure. *$P < 0.05$, significantly different from corresponding values in control rats.

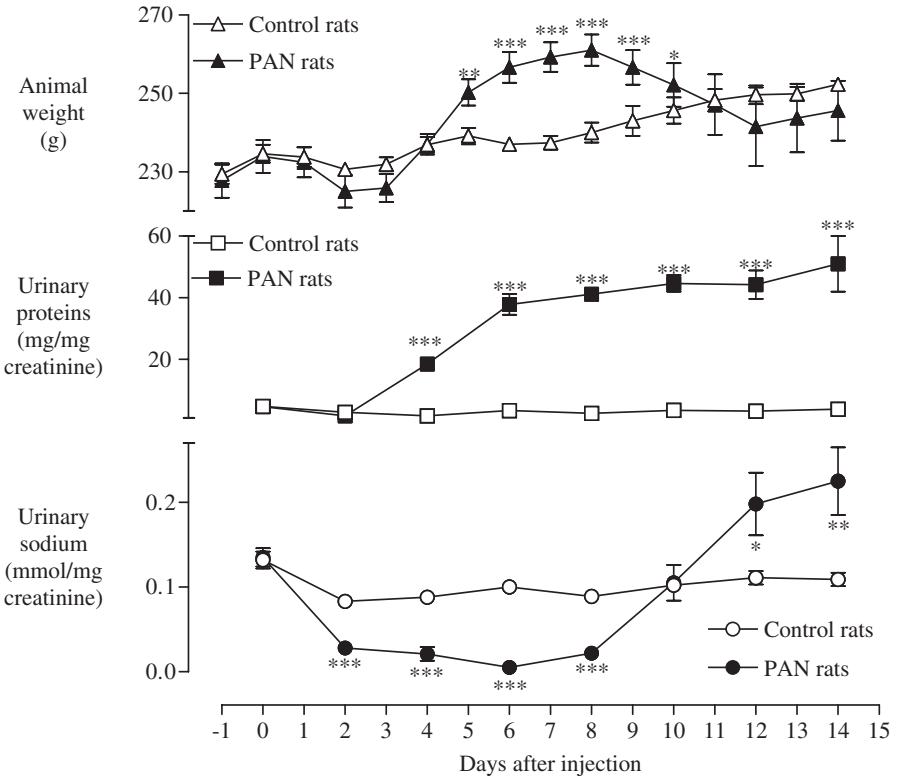

Fig. 1. Body weight, urinary proteins and urinary excretion of sodium in PAN and control rats throughout the study. Symbols represent means of 12 rats per group and error bars represent SE. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, significantly different from values in control rats.

study (Table 1). Plasma levels of sodium were similar between the two groups either on day 7 or day 14 (Table 1). Plasma levels of urea nitrogen were increased in PAN-treated rats on day 7 whereas plasma levels of creatinine were increased in nephrotic rats on both days 7 and 14 after drug injection (Table 1). Plasma protein concentration was reduced in PAN-treated rats on day 7 (Table 1).

Renal dopaminergic activity

The urinary levels of dopamine were transiently increased in PAN-treated rats during the first day after drug injection; this was followed by ~60% reduction in urinary levels of dopamine on day 5 and beyond (Figure 2). By contrast, the urinary excretion of the dopamine precursor, L-Dopa, did not differ

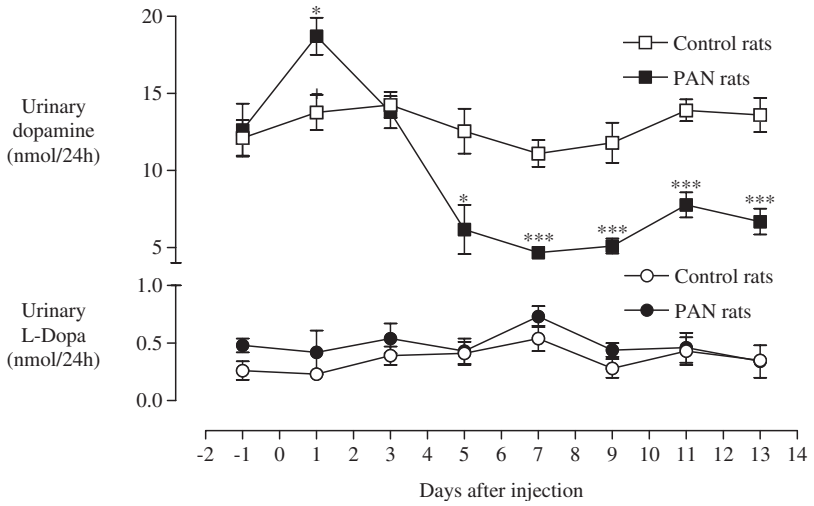

Fig. 2. Urinary levels of dopamine and L-Dopa in PAN and control rats throughout the study. Symbols represent means of 12 rats per group and error bars represent SE. $*P < 0.05$, $***P < 0.001$, significantly different from values in control rats.

between the PAN-treated rats and control animals throughout the study (Figure 2). These data suggest that the nephrotic rats might have a reduced ability to synthesize dopamine in renal proximal tubules. In agreement with this view are the results from studies evaluating the activity of AADC, the enzyme responsible for the synthesis of renal dopamine. The activity of AADC was determined in homogenates of renal cortex using L-Dopa as substrate, which resulted in a concentration-dependent formation of dopamine (Figure 3). The V_{max} values for AADC activity in renal cortex were found to be significantly lower in PAN-treated rats than in control animals on either day 7 or day 14 (Table 2); the decarboxylation reaction was a saturable process with K_m values of the same magnitude in the two groups (Table 2). In experiments performed with jejunal mucosa homogenates, no significant differences were observed in AADC activity between PAN-treated and control rats (Table 2).

Similar to that observed with urinary dopamine, the urinary excretion of the deaminated metabolite DOPAC was transiently increased in PAN-treated rats on day 1, followed by ~50% reduction on day 5 and beyond (Figure 4). By contrast, the urinary excretion of the deaminated plus methylated metabolite, HVA, did not differ between the two groups of rats throughout the study (Figure 4).

Since the purpose of our study was to evaluate the possible role of the renal dopaminergic system in the sodium retention observed in PAN nephrosis, we examined the relationship between urinary dopamine and sodium excretion and found no linear relation between the two parameters ($r^2 = 0.06$) throughout the study. Also, no linear relationship was observed between urinary DOPAC and urinary sodium excretion ($r^2 = 0.01$) throughout the study.

Notwithstanding the differences observed in urinary levels of dopamine as well as in the renal AADC activity between the two experimental groups, the plasma and renal tissue levels of dopamine and the

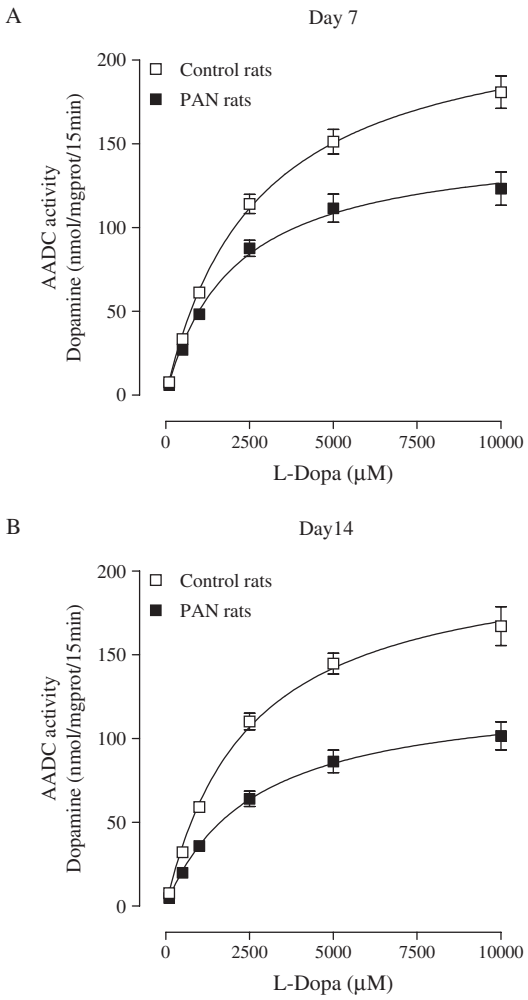

Fig. 3. Aromatic L-amino acid decarboxylase (AADC) activity in homogenates of renal cortex obtained from PAN and control rats (**A**) 7 days and (**B**) 14 days after injection. AADC activity is expressed as the rate of formation of dopamine *vs* concentration of L-Dopa. Symbols represent means of 7–12 experiments per group and error bars represent SE.

Table 2. Kinetic parameters (V_{max} and K_m) of AADC activities in homogenates of renal cortex and jejunal mucosa from PAN-treated and control rats 7 and 14 days after injection

	Day 7		Day 14	
	Control	PAN	Control	PAN
Renal cortex				
V_{max}, nmol/mg prot/15 min	234 ± 13	159 ± 14*	218 ± 15	134 ± 10*
K_m, mM	2.8 ± 0.2	2.1 ± 0.1	2.5 ± 0.2	2.8 ± 0.2
Jejunal mucosa				
V_{max}, nmol/mg prot/15 min	105 ± 10	105 ± 24	103 ± 5	83 ± 11
K_m, mM	2.5 ± 0.1	2.2 ± 0.2	2.9 ± 0.2	2.5 ± 0.3

Values are means ± SE; $n = 7$–12 experiments per group. *$P < 0.01$, significantly different from corresponding values in control rats.

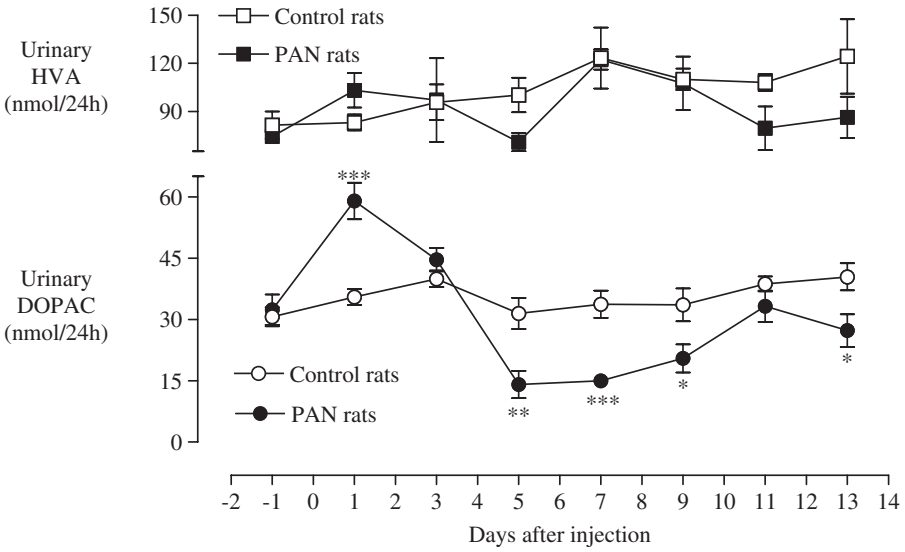

Fig. 4. Urinary levels of HVA and DOPAC in PAN and control rats throughout the study. Symbols represent means of 12 rats per group and error bars represent SE. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, significantly different from values in control rats.

precursor L-Dopa did not differ between the PAN-treated and control animals, either on day 7 or day 14 (Table 3).

Volume expansion and assessment of D_1 receptor-mediated natriuresis

The urinary dopamine excretion was markedly increased by 130% both during and after VE in control rats, whereas in PAN-treated animals the urinary dopamine output did not increase significantly either during or after VE (Figure 5). This resulted in the urinary dopamine excretion being markedly lower in PAN-treated rats than in control animals, before (basal), during and after VE (Figure 5). The urinary sodium excretion was markedly lower in PAN-treated rats than in control animals before (basal, $P < 0.05$), during (VE, $P < 0.01$) and after (R-VE1, $P < 0.0001$; R-VE2, $P < 0.0001$; R-VE3, $P < 0.0001$) VE in vehicle-treated rats (Figure 6). Fenoldopam induced a 20–80% increase in the accumulated urinary sodium excretion in control rats whereas the D_1 receptor agonist did not significantly change the urinary sodium excretion in PAN-treated animals throughout the study (Figure 6). Fenoldopam did not alter the urinary dopamine excretion in either control or PAN-treated rats throughout the study (data not shown).

Renal proximal tubular Na^+,K^+-ATPase activity

The Na^+,K^+-ATPase activity in renal proximal tubules was determined in PAN-treated and control rats after VE with the infusion of fenoldopam or the vehicle. As shown in Figure 7, the Na^+,K^+-ATPase activity after vehicle infusion did not differ between PAN-treated and control rats. The effect of the dopamine D_1 receptor agonist infusion was a decrease in Na^+,K^+-ATPase activity in both groups, which was more pronounced in PAN-treated rats than in control animals (49 ± 6% *vs* 31 ± 4%, $P < 0.05$).

Discussion

The present study was undertaken with the aim of clarifying the possible role of the renal dopaminergic

Table 3. Levels of dopamine and L-Dopa in plasma and in renal cortex of PAN-treated and control rats 7 and 14 days after injection

	Day 7		Day 14	
	Control	PAN	Control	PAN
Plasma levels				
Dopamine	0.89 ± 0.10	0.87 ± 0.15	0.87 ± 0.19	0.61 ± 0.12
L-Dopa	6.08 ± 0.47	5.30 ± 0.32	6.05 ± 0.37	6.16 ± 1.16
Renal cortex levels				
Dopamine	44.7 ± 3.7	58.4 ± 17.0	37.6 ± 6.7	29.4 ± 9.1
L-Dopa	73.2 ± 15.5	77.9 ± 13.7	102.3 ± 18.3	103.8 ± 19.0

Values are means ± SE; $n = 5$–12 experiments per group. Values are expressed for plasma in picomoles per millilitre and for renal cortex in picomoles per gram.

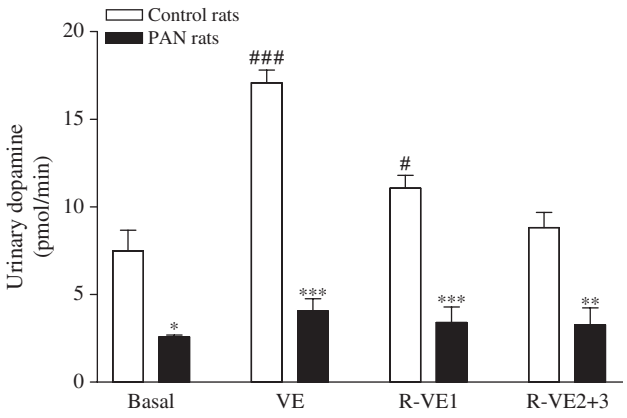

Fig. 5. Accumulated urinary dopamine excretion in PAN and control rats before ($t = 0$–120 min, Basal), during ($t = 120$–150 min, VE) and after ($t = 150$–180 min, R-VE1; $t = 180$–240 min, R-VE2+3) volume expansion, 7 days after injection. Bars represent means of 5–6 experiments per group and error bars represent SE. $*P < 0.05$, $**P < 0.01$, $***P < 0.001$, significantly different from values in control rats; $\#P < 0.05$, $\#\#\#P < 0.001$, significantly different from basal levels in corresponding group of rats.

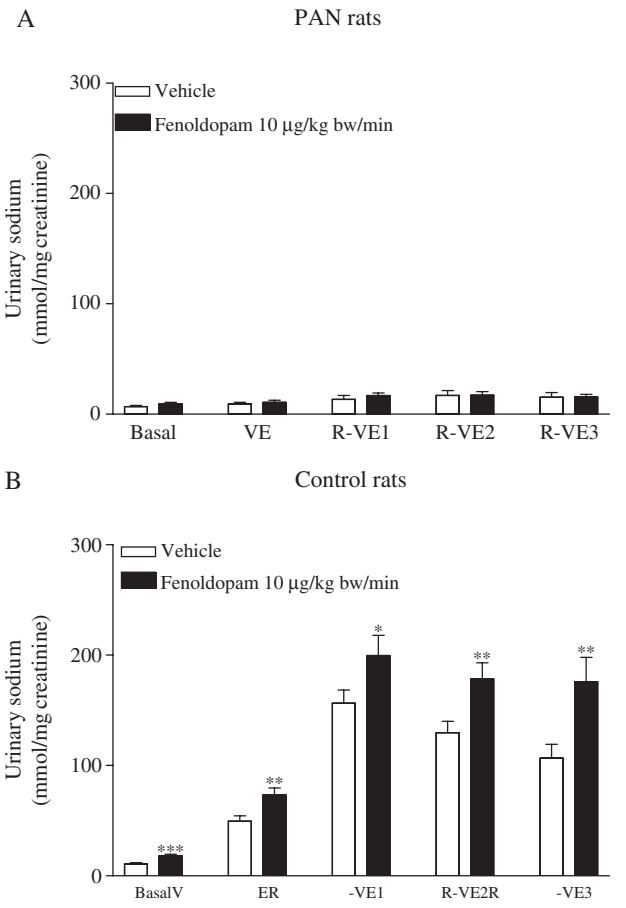

Fig. 6. Accumulated urinary sodium excretion in vehicle-treated and fenoldopam-treated (10 µg/kg bw/min) PAN (**A**) and control (**B**) rats before ($t = 0$–120 min, Basal), during ($t = 120$–150 min, VE) and after ($t = 150$–180 min, R-VE1; $t = 180$–210 min, R-VE2; $t = 210$–240, R-VE3) volume expansion, 7 days after injection. Bars represent means of 6–12 experiments per group and error bars represent SE. $*P < 0.05$, $**P < 0.01$, $***P < 0.001$, significantly different from values in vehicle-treated rats.

system in the renal sodium retention of the nephrotic syndrome. Our results provided evidence for a markedly reduced renal dopamine synthesis in PAN-treated rats. The enhanced proximal Na^+,K^+-ATPase sensitivity to inhibition by fenoldopam observed in PAN-treated rats suggests that the lack of renal dopamine may contribute to increase the proximal tubular sodium absorption in PAN nephrosis. However, the reduced renal dopaminergic activity appears not to be an important mechanism related to the overall renal sodium retention in PAN nephrosis because: (1) the decrease in sodium excretion in PAN-treated rats preceded the decrease in urine dopamine output; (2) no correlation was observed between urine dopamine output and sodium excretion (or sodium balance) throughout the study; and (3) a blunted natriuretic response to the infusion of fenoldopam was observed in PAN-treated rats during greatest sodium retention and ascites accumulation.

As previously reported [1], the urinary protein excretion increased 4 days after injection of PAN and remained elevated throughout the duration of the study. In addition, the time courses of variations of sodium retention and ascites accumulation in PAN nephrosis were divided into two phases. The first phase, from day 2 to day 8, was marked by the decrease of

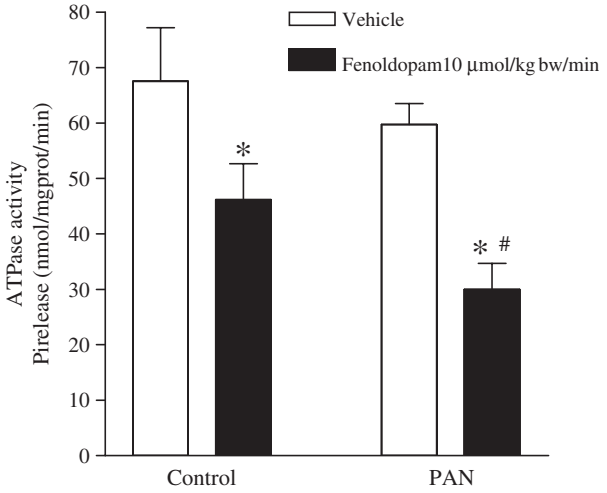

Fig. 7. Na^+,K^+-ATPase activity in proximal tubules of PAN and control rats after fenoldopam (10 µg/kg bw/min) or vehicle infusion, 7 days after injection, expressed as the rate of Pi release. Bars represent means of 6–10 experiments per group and error bars represent SE. $*P < 0.01$, significantly different from values in vehicle-treated rats; $\#P < 0.05$, significantly different from values in corresponding control rats.

sodium excretion accompanied with positive sodium balance and ascites accumulation. The second phase, from days 10 to 14, was marked by almost complete recovery of ascites, despite persistent high proteinuria. During this phase, urinary sodium excretion increased progressively towards higher than control levels from days 12 to 14, so that sodium balance was negative during this period. Thus, the appearance of proteinuria in PAN-treated rats did not influence the course of urinary sodium excretion. This dissociation between proteinuria and urinary sodium excretion was reported previously in PAN-treated and other nephrotic syndrome rat models [1] and supports the hypothesis that the reduction of urinary sodium excretion may be independent of the development of proteinuria and may be, instead, attributable to a tubular disorder independent of glomerular events.

The combined data of our study point to a marked decrease in renal dopaminergic system activity in both phases of PAN nephrosis. The reduced renal dopaminergic system activity in PAN-treated rats was evidenced by low AADC activity (on both days 7 and 14) and low urinary levels of dopamine, from day 5 and beyond. Renal dopamine synthesis is supposed to be mainly influenced by the: (1) total number of well functioning tubular units endowed with AADC; (2) renal delivery of sodium and L-Dopa; and (3) activity of AADC in proximal tubular cells [7]. Given that the creatinine clearance is a rough measure of the total number of well functioning nephrons, the reduced creatinine clearance observed in PAN-treated rats suggests that a decreased delivery of sodium to a reduced number of well-functioning tubular units may contribute to the decrease in urine dopamine excretion in PAN-treated rats. Taken together, our results suggest that the reduced urinary dopamine excretion in PAN nephrosis is mainly related to (1) reduced number of well-functioning nephrons; (2) reduced tubular delivery of sodium; and (3) decreased renal tubular decarboxylation of L-Dopa to dopamine.

Although puromycin is widely used to induce proteinuria, evidence has been gathered that PAN has no selective glomerular effect, but can also damage the proximal tubules before proteinuria develops [5,18]. Thus, one can hypothesize that the decreased renal dopamine synthesis and urinary excretion may be due, at least in part, to PAN tubulotoxicity. Indirect evidence of such an effect can be derived from Figure 3, which shows an initial increase in urinary dopamine excretion, suggesting a release of dopamine from damaged proximal tubule cells before the increase in urine protein excretion.

The reduced renal dopamine activity in PAN-treated rats was not accompanied by changes in the renal tissue levels of L-Dopa or dopamine. The explanation for this apparent discrepancy has to do with the nature of this non-neuronal dopaminergic system [7,8]. The amine storage structures normally present in monoaminergic neuronal systems and the classical mechanisms for the regulation of amine formation and release are not present or in operation; the basic mechanisms for the regulation of this system are thought to depend on the availability of L-Dopa, its fast decarboxylation into dopamine and in precise and accurate cell outward amine transfer mechanisms [7,8]. It is interesting to note that AADC in jejunal epithelial cells failed to change in PAN-treated rats suggesting that the decrease in enzyme activity observed in the renal parenchyma resulted from local effects.

The urinary excretion of DOPAC followed quite closely the urinary excretion of the parent amine throughout the study. This suggests that deamination of dopamine was not compromised in PAN-treated rats and further supports our previous suggestion that urinary DOPAC is a good marker of renal production of dopamine and simultaneously a good index of cell integrity and viability [19], since MAO is a mitochondrial enzyme and is quite sensitive to changes in tissue oxygen tension. By contrast, the urinary excretion of HVA did not follow the decrease in the daily urinary excretion of dopamine and DOPAC in PAN-treated rats. This agrees well with the suggestion that urinary HVA has its origin mainly in extrarenal tissues.

Renal dopamine decreases proximal tubular sodium reabsorption by inhibition of Na^+,K^+-ATPase activity directly or in response to the decrease in intracellular sodium following inhibition of Na^+/H^+ exchanger NHE3 [9,10]. Thus, one can hypothesize that the observed reduced renal dopamine tonus in rats with PAN nephrosis may contribute to proximal sodium retention by increasing the proximal Na^+,K^+-ATPase activity directly or through the increase in the apical membrane Na^+/H^+ exchanger NHE3 protein.

On the basis of these considerations we decided to study the relative importance of the lack of renal dopamine in PAN-treated rats on the proximal tubular Na^+,K^+-ATPase activity and on the enhanced sodium

retention. Since the natriuresis due to the direct inhibitory effect of tubular transport by dopamine is mainly mediated by D_1-like receptors and is evident in volume-expanded states but not in sodium-depleted states [8,9], we decided to evaluate the effect of the infusion of the D_1-like agonist fenoldopam during an acute VE with saline, in conditions of greatest sodium retention and ascites accumulation (day 7). When the PAN-treated and control rats were submitted to VE on day 7, we found that the proximal tubular Na^+,K^+-ATPase activity was similar between the two groups. It should be mentioned, however, that the PAN-treated rats had a decreased whole-animal glomerular filtration rate (GFR), as revealed by the significantly lower creatinine clearance compared with control animals. Given the reduced total kidney GFR, the 'normal' proximal tubular Na^+,K^+-ATPase activity suggests a heightened level of proximal sodium absorption in PAN-treated rats reflecting a reset level of glomerulotubular balance. Our results of increased and insufficiently suppressible proximal tubular sodium reabsorption by volume loading in PAN nephrosis agree well with the findings of others [20]. As the inhibition of proximal tubular Na^+,K^+-ATPase activity by fenoldopam was more pronounced in PAN-treated rats than in control animals, it seems reasonable to postulate that the reduced renal dopamine tonus may contribute, at least in part, to proximal sodium retention during PAN nephrosis.

One should recall that the relative increase of sodium reabsorption in the proximal tubules and the reset of the glomerulotubular balance remain controversial in PAN nephrosis. Actually, other investigators showed that sodium reabsorption was decreased in the proximal tubules of experimental nephrotic syndrome and that the correction of the GFR following saralazin infusion did not change the renal sodium retention and urinary sodium excretion [21]. It should be mentioned, however, that those studies were performed with the unilateral model of PAN-induced nephrosis using a different rat strain and only evaluated superficial nephrons. Moreover, the plasma protein concentration remained unaltered in the study from Ichikawa *et al.*, whereas in the present study the plasma protein concentration was reduced on day 7 in the PAN-treated rats, suggesting that alterations in plasma composition may also contribute to increase proximal sodium reabsorption.

Besides the proximal tubule, dopamine can also inhibit sodium reabsorption in both the thick ascending limb and the cortical collecting duct [8]. However, despite the enhanced sensitivity of proximal tubular Na^+,K^+-ATPase activity to inhibition by fenoldopam, the urinary sodium excretion was not significantly altered in PAN-treated rats during the administration of the D_1 agonist whereas the natriuresis in control rats increased by 20–80%. Thus, one can hypothesize that the fenoldopam-induced increase in sodium delivery from the proximal tubules was subjected to an enhanced reabsorption in distal nephron segments by dopamine-independent mechanisms. This suggestion fits well with the observations showing that the cortical collecting duct is the primary site of salt retention in nephrotic syndrome probably due to enhanced Na^+,K^+-ATPase activity and to ANP resistance [1].

There is an abundance of evidence suggesting that the natriuretic effects of ANP may be to a large extent mediated via renal D_1-like dopamine receptors [8]. Recently, it has been shown that ANP and cGMP may recruit silent D_1 dopamine receptors from the interior of the cells towards the plasma membrane [11]. Although our study was not designed to evaluate the relationship between ANP resistance and dopamine in the nephrotic state, one cannot exclude that the ANP resistance in the cortical collecting duct may be accompanied by a decreased number of D_1 receptors available for dopamine binding in the distal tubules which may contribute to the blunted natriuretic response to fenoldopam observed in PAN-treated rats. For the purpose of establishing a link between blunted renal dopaminergic system and edema development in PAN nephrosis, longer periods of time will be required to address this issue in a more complete way.

In summary, PAN-induced nephrotic syndrome is associated with a blunted renal dopaminergic system activity which may contribute to the relative increase of the proximal tubular Na^+,K^+-ATPase activity, irrespective of the decrease in GFR. However, the lack of renal dopamine appears not to be related to the overall renal sodium retention in a state of proteinuria.

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