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Blunted renal dopaminergic system activity in $HgCl_2$-induced membranous nephropathy

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Abstract

The present study evaluated the possible role of the renal dopaminergic system in the sodium retention of $HgCl_2$-induced nephrotic syndrome. The time courses of urinary excretion of sodium, protein, dopamine and the precursor L-3,4-dihydroxyphenylalanine (L-Dopa) were evaluated in $HgCl_2$-treated and control rats up to day 21. The renal aromatic L-amino acid decarboxylase (AADC) activity, the enzyme responsible for the synthesis of renal dopamine, was evaluated during negligible proteinuria accompanied with enhanced sodium retention (day 7), increased proteinuria accompanied with greatest sodium retention (day 14) as well as during increased proteinuria accompanied with negative sodium balance (day 21). Also, the influence of volume expansion (VE, 5% bw) and the effects of the D_1-like agonist fenoldopam (10 μg kg bw^{-1} min^{-1}) on natriuresis and on proximal tubular Na^+,K^+-ATPase activity were examined on day 14. The daily urinary dopamine output and urinary dopamine/L-Dopa ratios were reduced in $HgCl_2$-treated rats from day 2 and beyond. This was accompanied by a marked decrease in renal AADC throughout the study. During VE, the fenoldopam-induced inhibition of proximal tubular Na^+,K^+-ATPase activity was similar between $HgCl_2$-treated and control rats. However, the urinary sodium excretion during fenoldopam infusion was markedly increased by 60% to 120% in control rats but was not altered in $HgCl_2$-treated rats. It is concluded that $HgCl_2$ nephrosis is associated with a blunted renal dopaminergic system activity. However, the lack of renal dopamine in $HgCl_2$ nephrosis does not appear to be related with the overall renal sodium retention in a state of proteinuria.
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Keywords: Aromatic L-amino acid decarboxylase (AADC); Fenoldopam; $HgCl_2$; Na^+; K^+-ATPase; Nephrotic syndrome; Sodium handling; Renal dopamine; Volume expansion

Introduction

The features of the nephrotic syndrome in both man and experimental rat models are massive proteinuria and development of extra-cellular volume expansion due to enhanced renal sodium retention. Although the exact mechanisms involved in the enhanced sodium reabsortion in the nephrotic syndrome still remain to be fully elucidated, most of the available evidence implicates a primary distal renal sodium handling abnormality in this edema formation condition (Humphreys, 1994). It was suggested by Deschenes and Doucet (2000) 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) (Plum et al., 1996; Rabelink et al., 1987) and an enhanced Na^+,K^+-ATPase activity in the cortical collecting duct (Deschenes and Doucet, 2000; Zolty et al., 1999). Recently, a primary sodium handling abnormality has been also invoked in the proximal tubules with the observation in nephrotic animals of a shift of the Na^+/H^+ exchanger NHE3 from the inactive to an active pool (Besse-Eschmann et al., 2002). However, the proximal tubular Na^+,K^+-ATPase activity was not examined (Besse-Eschmann et al., 2002) and, therefore, the role of the proximal tubules in the enhanced sodium retention in the nephrotic syndrome still remains to be elucidated.

Renal dopamine behaves as an endogenous natriuretic hormone by activating D_1-like receptors as a paracrine/autocrine substance (Cuche et al., 1976; Debska-Slizien et al., 1994; Jose et al., 1998). The epithelial cells of proximal tubules, but not of distal segments of the nephron, are endowed

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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, 1994). This 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 (Soares-da-Silva, 1994; Hussain and Lokhandwala, 1998; Aperia, 2000; Carey, 2001). During moderate sodium surfeit, dopamine of renal origin accounts for ~50% of sodium excretion (Siragy et al., 1989; Pelayo et al., 1983). 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 (Siragy et al., 1989; Jose et al., 2000). Dopamine of renal origin can regulate sodium balance also by interaction with other natriuretic factors such as ANP (Aperia et al., 1996). In the late 1980s, several laboratories reported that the natriuretic response to ANP requires an intact renal dopaminergic system (Katoh et al., 1989; Rabelink et al., 1987). 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 (Holtback et al., 1999). Interestingly, a decreased AADC activity in the proximal tubules was observed in puromycin aminonucleoside-induced nephrotic syndrome rat model (Sampaio-Maia et al., 2004).

On the basis of these considerations this study was undertaken with the aim of evaluating the possible role of renal dopaminergic system in the sodium retention observed in $HgCl_2$-induced membranous nephropathy. For this purpose we examined the time courses of the urinary excretion of sodium, protein, dopamine and the precursor L-Dopa in $HgCl_2$-treated and control rats. The rats were sacrificed during negligible proteinuria accompanied with enhanced sodium retention (day 7), increased proteinuria accompanied with greatest sodium retention (day 14) as well as during increased proteinuria accompanied with negative sodium balance (day 21) for the evaluation of the renal AADC activity. Also, the influence of volume expansion and the effects of the dopamine D_1 receptor agonist fenoldopam on natriuresis and on the Na^+,K^+-ATPase activity in the renal proximal tubules were examined during the phase of greatest sodium retention and ascites accumulation (day 14).

Materials and methods

In vivo studies

Mercury chloride-induced membranous nephropathy

Normotensive male Brown–Norway rats (Harlan, Barcelona, Spain), weighing 150–160 g, were selected after a 7-day period of stabilization and adaptation to blood pressure measurements. The animals received subcutaneous injections of 1 ml kg bw^{-1} of $HgCl_2$ (1 mg kg bw^{-1}) or the vehicle (NaCl 0.9%) on days 0, 2, 4, 7, 9 and 11.

Metabolic studies

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. Two days before the first $HgCl_2$ or vehicle injection, the rats were placed in metabolic cages (Techniplast, Buguggiate-VA, Italy). The $HgCl_2$-treated and control rats had free access to tap water. The $HgCl_2$-treated rats were fed ad libitum throughout the study with ordinary rat chow (Panlab, Barcelona, Spain) containing 1.9 g kg^{-1} 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 $HgCl_2$-treated animals. Twenty-four hours urine were collected on uneven days in empty vials, for later determinations of protein, creatinine and sodium, and on even days in vials containing 1 ml hydrochloric acid 6 M (to avoid the spontaneous oxidation of the amines) 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). Four determinations were made each time and the means were used for further calculation.

Animals were sacrificed during negligible proteinuria accompanied with enhanced sodium retention (day 7: $HgCl_2$, $n=6$; vehicle, $n=6$), increased proteinuria accompanied with greatest sodium retention (day 14: $HgCl_2$, $n=12$; vehicle, $n=10$) as well as during increased proteinuria accompanied with negative sodium balance (day 21: $HgCl_2$, $n=9$; vehicle, $n=7$). On the days of sacrifice after the 24 h urine collection for determination of sodium and creatinine, the animals were anaesthetized with pentobarbital sodium (50 mg kg bw^{-1}; ip) 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. Thereafter, the kidneys were rapidly removed, weighed and the outer cortex isolated. Fragments of renal cortex were used later for determination of AADC and Na^+,K^+-ATPase activity in proximal tubular cells. Other fragments of renal cortex weighing around 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, fourteen days after first $HgCl_2$ or vehicle injection, the animals were anaesthetized with pentobarbital sodium (50 mg kg bw^{-1} followed by 20 mg kg

bw^{-1} h^{-1}; ip) 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 with a PE50 tube (Becton Dickson, Lisboa, Portugal) for volume expansion (VE) and infusion of fenoldopam (10 μg kg bw^{-1} min^{-1}) or the vehicle (0.9% NaCl). 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 fenoldopam or the vehicle started at a rate of 5 ml kg bw^{-1} h^{-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 changing the infusion to a rate of 100 ml kg bw^{-1} h^{-1} (5% body weight) during 30 min (t=120–150 min, VE). Thereafter, the infusion was again reduced to 5 ml kg bw^{-1} h^{-1} 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). 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

AADC activity was determined in homogenates of renal tissues and jejunal mucosa, using L-Dopa (0.1 to 10 mM) as substrate (Sampaio-Maia et al., 2005). The assay of dopamine was performed by HPLC with electrochemical detection. The protein content in cell suspension (1.5 mg ml^{-1}) was determined by the Bradford method (Bradford, 1976).

Na^+,K^+-ATPase activity

Na^+,K^+-ATPase activity was measured by the method of Quigley and Gotterer (1969) adapted in our laboratory with slight modifications. The rat renal proximal tubules were isolated as previously described (Guimaraes et al., 1997). In brief, the kidneys were decapsulated, the outer cortex was cut out with fine scissors and placed in ice-cold Collins solution containing, in mM, 15 KH_2PO_4, 50 K_2HPO_4, 15 KCl, 15 $NaHCO_3$, 60 $MgSO_4$ and 140 glucose, pH 7.4. Thereafter, the outer cortex was minced with a scalpel into a fine paste. This cortical paste was filtered sequentially through a series of Nybolt nylon sieves, first 180 μm and then 75 μm. Unseparated cortex remained on the upper (180 μm) sieve, while the lower one (75 μm) retained predominantly proximal nephron segments. The sieves were rinsed continuously with cold Collins solution throughout. The retained tubules 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 containing, in mM, 137 NaCl, 5 KCl, 0.8 $MgSO_4$, 0.33 Na_2HPO_4, 0.44 KH_2PO_4, 0.25 $CaCl_2$, 1 $MgCl_4$, 0.15 Tris–HCl and 1 sodium butyrate, pH 7.4. 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 MgCl2, 75 NaN_3, 75 tris(hydroxymethyl)aminomethane(tris) hydrochloride and 100 μl cell suspension. For 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 milligram 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^{-1}) was determined by the Bradford method (Bradford, 1976) using bovine serum albumin as standard. 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^{-1}.

Assay of catecholamines

The assay of catecholamines in urine, plasma samples, renal tissues and in samples from AADC studies was performed by HPLC with electrochemical detection, as previously described (Soares-da-Silva et al., 1993). In our laboratory, the lower limit of detection of dopamine and L-Dopa ranged from 350 to 1000 fmol.

Plasma and urine ionogram and biochemistry

Ion-selective electrodes performed the quantifications of sodium. 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 urine creatinine excretion. Fractional excretion of sodium (FE_{Na+}) was calculated as previously reported (Sampaio-Maia et al., 2005). Sodium balance was determined by subtracting the absolute daily urinary sodium excretion (mmol 24 h^{-1}) from daily sodium intake (mmol 24 h^{-1}).

Drugs

The compounds ATP; dopamine hydrochloride; $HgCl_2$; L-Dopa; ouabain and fenoldopam were obtained from Sigma (St. Louis, MO, USA).

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 (Motulsky et al., 1994) 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

$HgCl_2$ membranous nephropathy — renal function and sodium handling

The sodium intake was similar in both $HgCl_2$-treated and control animals throughout the study since the control animals had the same food intake as $HgCl_2$-treated rats (Table 1). As shown in Fig. 1, proteinuria in $HgCl_2$-treated rats remained undetectable or very low until day 9 and increased from day 11 and beyond reaching the highest levels between days 15 and 17. The urinary sodium excretion was decreased in $HgCl_2$-treated rats from days 3 to 14, followed by an increase in urinary sodium excretion from days 17 to 21 (Fig. 1). Consequently, the $HgCl_2$-treated rats presented a positive sodium balance from days 3 to 14, this being statistically significant on day 14, when the $HgCl_2$-treated rats exhibited a reduced FE_{Na+} and ascites accumulation (Table 1). On day 21, the $HgCl_2$-treated rats presented a negative sodium balance and no ascites accumulation (Table 1). The creatinine clearance did not differ between $HgCl_2$-treated and control rats on days 7, 14 or 21 (Table 1). Systolic and diastolic blood pressure did not differ between $HgCl_2$-treated and control rats throughout the study (Table 1). Plasma levels of sodium were similar between the two groups on days 7, 14 or 21 (Table 1). Plasma levels of urea nitrogen were increased in $HgCl_2$-treated rats on day 14 whereas plasma levels of creatinine did not differ between $HgCl_2$-treated and control rats on days 7, 14 or 21 (Table 1).

Renal dopaminergic activity

In $HgCl_2$-treated rats the urinary levels of dopamine were significantly reduced from day 2 to day 4 as well as from days 12 to 18 (Fig. 2A). By contrast, the urinary excretion of the dopamine precursor, L-Dopa, was increased from day 2 to day 4 as well as from days 14 to 18 (Fig. 2B). This resulted in markedly reduced urinary dopamine/L-Dopa ratios in $HgCl_2$-treated rats throughout the study (Fig. 2C). The urinary dopamine/L-Dopa ratios are used as an indirect measure of L-Dopa uptake/decarboxylation in renal tubular cells. Thus, these data suggest that the $HgCl_2$-treated rats might have a reduced ability to synthesize dopamine in renal proximal tubules. In agreement with this view are the results of 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 (Fig. 3). The V_{max} values for AADC activity in renal cortex were found to be significantly lower in $HgCl_2$-treated rats than in control animals on days 7, 14 and 21; 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 $HgCl_2$-treated and control rats (Table 2).

Notwithstanding the differences observed in urinary levels of dopamine as well as in renal AADC activity between the two experimental groups, the plasma and renal tissue levels of dopamine and the precursor L-Dopa did not differ between the $HgCl_2$-treated and control animals on days 7, 14 or 21 (Table 3).

Volume expansion and assessment of D_1-like receptor-mediated natriuresis

Because the $HgCl_2$-treated rats presented a reduced renal dopamine synthesis and the decrease in urine dopamine output preceded the decrease in urinary sodium excretion we found that it was worthwhile to study the effect of a D_1 receptor agonist (fenoldopam) on natriuresis, in conditions of sodium retention (reduced FE_{Na+}) and ascites accumulation (day 14). Since the natriuretic effect of dopamine is evident in euvolemic and volume-expanded states but not in sodium-depleted states (Agnoli et al., 1987; Ragsdale et al., 1990), the experiments

Table 1
Body weight, metabolic balance, renal function and blood pressure in $HgCl_2$-treated and control rats on days 7, 14 and 21 after first injection

	Day 7		Day 14		Day 21	
	Control	$HgCl_2$	Control	$HgCl_2$	Control	$HgCl_2$
Body weight, g	176±3	182±2	184±3	184±6	192±4	153±4*
Ccreatinine, ml min^{-1}	1.16±0.08	1.04±0.10	1.61±0.22	1.68±0.15	1.96±0.16	1.81±0.17
P creatinine, mg dl^{-1}	0.41±0.03	0.43±0.03	0.27±0.03	0.30±0.02	0.29±0.03	0.21±0.03
P urea, mg dl^{-1}	36±3	38±2	30±3	55±5*	39±4	44±4
P Na^+, mmol l^{-1}	137±1	137±1	140±2	142±1	141±1	141±1
Na^+ intake, mmol 24 h^{-1}	1.35±0.02	1.29±0.06	1.07±0.02	1.04±0.09	1.25±0.03	1.06±0.18
Na^+ excretion, mmol 24 h^{-1}	1.10±0.04	0.88±0.04*	1.03±0.05	0.54±0.07*	1.09±0.07	1.52±0.49
Na^+ balance, mmol 24 h^{-1}	0.25±0.04	0.41±0.06	0.04±0.06	0.50±0.08*	0.16±0.08	−0.76±0.48*
FE_{Na+}, %	0.39±0.03	0.33±0.03	0.30±0.05	0.16±0.02*	0.28±0.03	0.26±0.03
Ascite, g	0.5±0.1	1.2±0.1*	0.4±0.1	1.6±0.2*	0.8±0.1	0.6±0.1
Systolic BP, mm Hg	123±2	123±3	120±4	107±5	123±3	150±10
Diastolic BP, mm Hg	77±9	69±4	67±3	61±8	74±3	81±11
Heart rate, bpm	373±14	407±12	359±10	344±9	351±7	343±33

Values are means±SE; $n=6$ to 12 experiments per group. Ccreatinine=creatinine clearance, P=plasma and FE=fractional excretion. *$p<0.05$, significantly different from corresponding values in control rats.

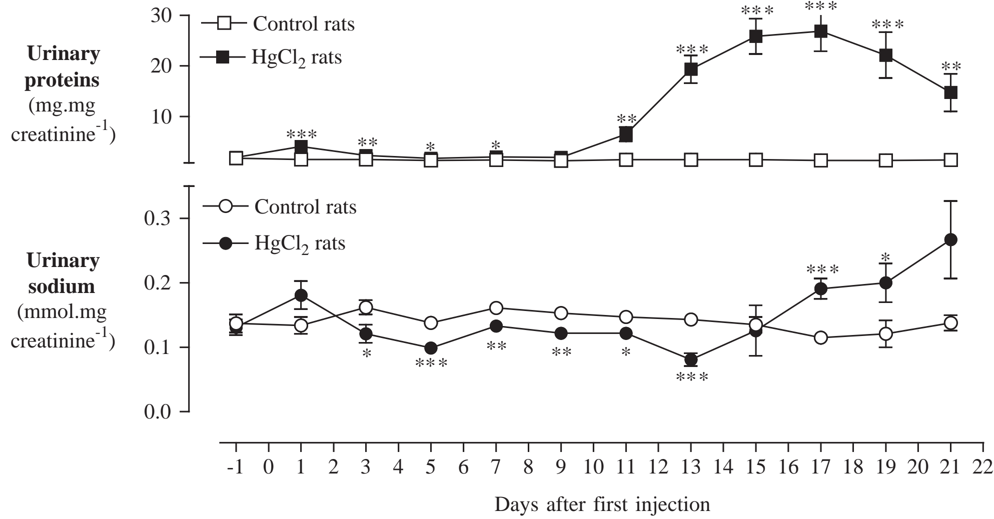

Fig. 1. Urinary excretion of proteins and sodium in $HgCl_2$ and control rats throughout the study. Symbols represent means of 5 to 13 rats per group and error bars represent SE. *$p<0.05$, **$p<0.01$, ***$p<0.001$, significantly different from values in control rats.

Fig. 2. Urinary levels of dopamine (A), L-Dopa (B) and urinary dopamine/L-Dopa ratios (C) in $HgCl_2$ and control rats throughout the study. Symbols represent means of 5 to 13 rats per group and error bars represent SE. *$p<0.05$, **$p<0.01$, ***$p<0.001$, significantly different from values in control rats.

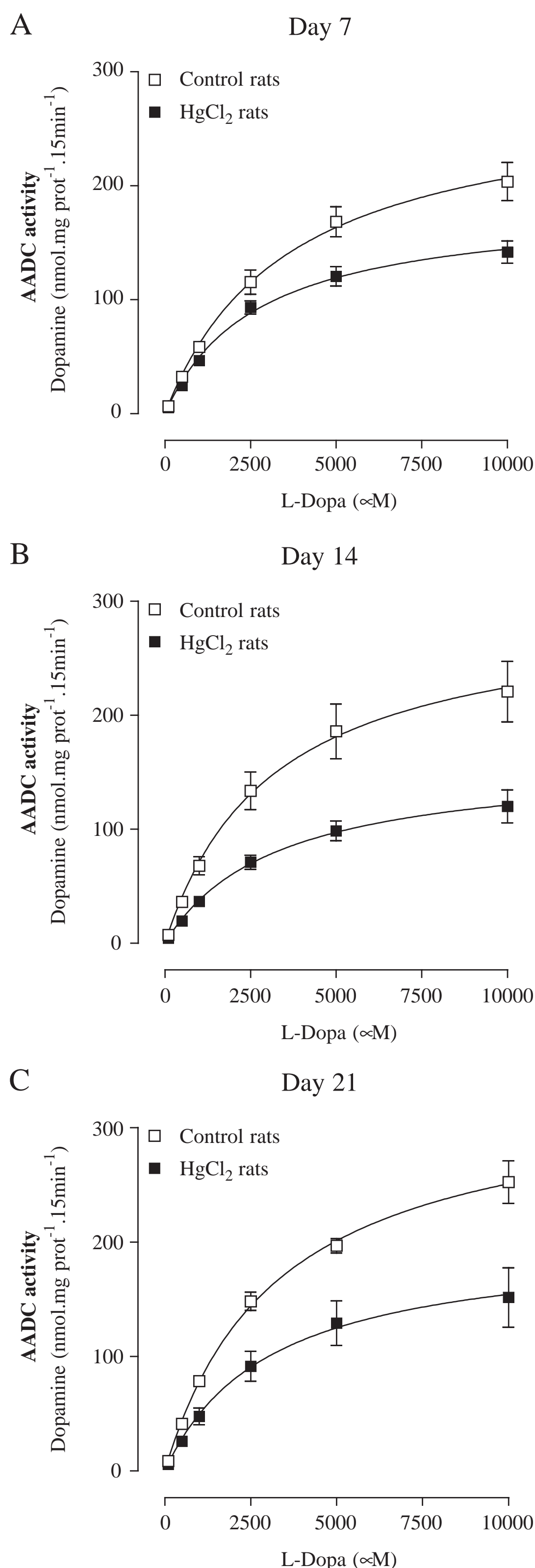

Fig. 3. Aromatic L-amino acid decarboxylase (AADC) activity in homogenates of renal cortex obtained from $HgCl_2$ and control rats on days seven (A), fourteen (B) and twenty-one (C) after first injection. AADC activity is expressed as the rate of formation of dopamine vs. concentration of L-Dopa. Symbols represent means of 5 to 12 experiments per group and error bars represent SE.

were performed during an acute VE with saline. The urinary sodium excretion before (t=0–120 min), during (t=120–150 min) and after (t=150–240 min) VE with isotonic saline in $HgCl_2$-treated and control rats on day 14 is depicted in Fig. 4. The urinary sodium excretion was markedly lower in $HgCl_2$-treated than in control rats both during and after VE. The accumulated urinary sodium excretion during infusion of fenoldopam or the vehicle in both groups is depicted in Fig. 5. As can be observed, fenoldopam induced a 60% to 120% increase in the accumulated urinary sodium excretion in control rats whereas the D_1 receptor agonist did not change the urinary sodium excretion in $HgCl_2$-treated animals throughout the study (Fig. 5).

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

The Na^+,K^+-ATPase activity in renal proximal tubules was determined in $HgCl_2$-treated and control rats both in basal conditions and after VE with the infusion of fenoldopam 10 μg kg bw^{-1} min^{-1} or the vehicle (NaCl 0.9%). The Na^+,K^+-ATPase activity in basal conditions did not differ between $HgCl_2$-treated and control animals (in nmol mg prot^{-1} min^{-1}, 61±6 vs. 64±6). Also, the Na^+,K^+-ATPase activity after volume expansion was similar between $HgCl_2$-treated and control rats (Fig. 6). The effect of the dopamine D_1 receptor agonist fenoldopam was an ~40% decrease in proximal tubular Na^+,K^+-ATPase activity in both $HgCl_2$-treated and control animals (Fig. 6).

Discussion

In the present study we found that the $HgCl_2$-treated rats presented a markedly reduced renal dopaminergic system activity throughout the study. Since dopamine of renal origin may be responsible for up to 50% of urinary sodium excretion and the decrease in urine dopamine output preceded the decrease in urinary sodium excretion in $HgCl_2$-treated rats, one could hypothesize that the lack of renal dopamine may contribute, at least in part, to enhance renal sodium retention in $HgCl_2$ nephrosis. However, the proximal tubular Na^+,K^+-ATPase activity did not differ between $HgCl_2$-treated and control rats either during vehicle or fenoldopam infusion and the D_1 agonist did not increase the urinary sodium excretion in nephrotic rats. Taken together, our findings suggest that the reduced renal dopamine tonus appears not be of major importance in the overall renal sodium retention in a state of proteinuria.

Mercury chloride induces a systemic autoimmune disease that includes membranous nephropathy with IgG deposits (Deschenes and Doucet, 2000). This nephropathy is responsible for the development of high-range proteinuria and full-blown nephrotic syndrome (Druet et al., 1978). In order to evaluate the relationship between urinary sodium excretion and proteinuria of $HgCl_2$-treated rats we performed a systematic study of the time courses of these parameters. Similar to the findings of others (Deschenes and Doucet, 2000), our results indicate that up to day 21 the $HgCl_2$ nephrosis can be divided

Table 2
Kinetic parameters (V_{max} and K_m) of AADC activities in homogenates of renal cortex and jejunal mucosa from $HgCl_2$-treated and control rats on days 7, 14 and 21 after first injection

	Day 7		Day 14		Day 21	
	Control	$HgCl_2$	Control	$HgCl_2$	Control	$HgCl_2$
Renal cortex						
V_{max}, pmol mg prot^{-1} 15 min^{-1}	281±9	183±8*	294±10	161±3**	333±8	202±7*
K_m, mM	3.6±0.3	3.2±0.4	3.1±0.3	3.2±0.2	3.3±0.2	3.1±0.3
Jejunal mucosa						
V_{max}, pmol mg prot^{-1} 15 min^{-1}	125±26	126±19	191±20	170±20	191±35	212±34
K_m, mM	3.1±0.2	4.0±0.5	3.8±0.3	3.3±0.4	3.4±0.5	3.0±0.5

Values are means±SE; n=6 to 12 experiments per group. *$p<0.05$, **$p<0.01$, significantly different from corresponding values in control rats.

in three phases, namely: 1) negligible proteinuria accompanied with significant sodium retention (from day 3 to day 9); 2) increased proteinuria accompanied with greatest sodium retention (from day 11 to day 14) and 3) increased proteinuria accompanied with enhanced renal sodium excretion (from day 17 to day 21).

The combined data of our study point toward a marked decrease in renal dopaminergic system activity in all the three phases of $HgCl_2$ membranous nephropathy. The markedly reduced renal tubular AADC activity (on days 7, 14 and 21), the low urinary levels of dopamine and the low urinary dopamine/L-Dopa ratios throughout the study evidenced the reduced renal dopaminergic activity in $HgCl_2$-treated rats. It is worth to note that AADC in jejunal epithelial cells failed to change in $HgCl_2$-treated rats suggesting that the decrease in AADC activity in the renal parenchyma is the result of local effects. This is in agreement with the observations suggesting that the main controlling factors for renal dopamine production appear to be the local delivery of sodium and/or chloride ions, protein and the intracellular electrolyte and hormonal medium (Lee, 1993).

The reduced renal dopamine activity in $HgCl_2$-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 (Soares-da-Silva, 1994; Hussain and Lokhandwala, 1998; Aperia, 2000; Carey, 2001). 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 (Soares-da-Silva, 1994; Hussain and Lokhandwala, 1998; Aperia, 2000; Carey, 2001).

Although Hg is known to induce toxic effects in different body organs and cellular components including renal proximal tubules and jejunal epithelial cells (Lash and Zalups, 1992; Pritchard, 1979; Bigazzi, 1999), non-existing data is available regarding the effect of Hg on renal or jejunal AADC activity. The results of the present study in $HgCl_2$-treated rats showing a reduced renal AADC activity with no changes in jejunal AADC activity suggest that the decrease in enzyme activity in the renal parenchyma may result from Hg-independent mechanisms and further agree with the observations suggesting that the main controlling factors for renal dopamine production are related to the local delivery of sodium as well as the intracellular electrolyte and hormonal medium (Lee, 1993). The finding of a similar decrease of AADC activity in the proximal tubules of puromycin aminonucleoside-induced nephrosis (Sampaio-Maia et al., 2004), a nephrotic syndrome rat model involving a different histologic type of glomerular lesions, agrees with this view and further suggests that the blunted renal dopaminergic system may be a feature of nephrotic syndrome.

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 (Jose et al., 2000). Indeed, inhibition of renal dopamine synthesis is accompanied with increases in renal proximal NHE3 activity followed by a stimulatory effect on Na^+,K^+-ATPase activity (Debska-Slizien

Table 3
Levels of dopamine and L-Dopa in plasma and in renal cortex of $HgCl_2$-treated rats and control rats on days 7, 14 and 21 after first injection

	Day 7		Day 14		Day 21	
	Control	$HgCl_2$	Control	$HgCl_2$	Control	$HgCl_2$
Plasma levels						
Dopamine	0.92±0.10	0.83±0.15	0.71±0.13	0.97±0.14	0.80±0.18	1.0±0.10
L-Dopa	7.3±0.5	6.0±0.7	5.4±0.9	6.4±1.1	5.4±1.2	5.8±1.6
Renal cortex levels						
Dopamine	45.9±2.4	44.7±2.2	40.6±6.4	30.1±4.8	47.1±13.7	40.0±10.7
L-Dopa	54.3±9.0	59.3±2.8	58.2±8.93	69.2±7.0	56.9±5.5	64.4±4.1

Values are means±SE; n=4 to 7 experiments per group. Values are expressed for plasma in picomoles per millilitre and for renal cortex in picomoles per gram.

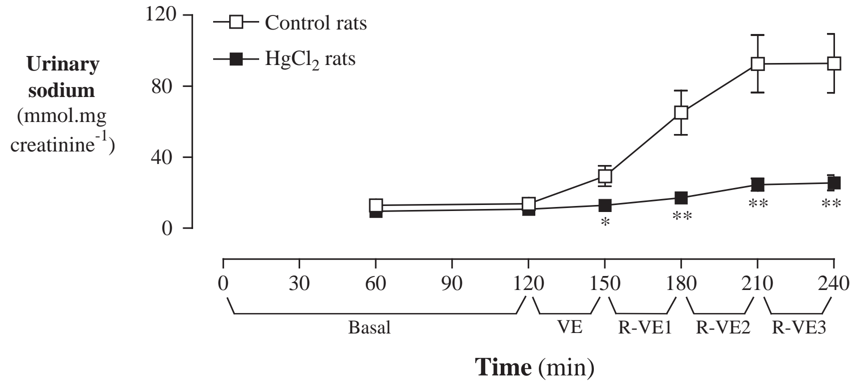

Fig. 4. Urinary sodium excretion in $HgCl_2$ and control rats before ($t=0–120$ min, basal), during ($t=120–150$ min, VE) and after ($t=150–180$ min, R-VE1; $t=180–210$ min, R-VE2; $t=210–240$, R-VE3) 5% volume expansion with isotonic saline, fourteen days after first injection. Symbols represent means of 6 to 9 experiments per group, and error bars represent SE. *$p<0.05$, **$p<0.01$, significantly different from values in control rats.

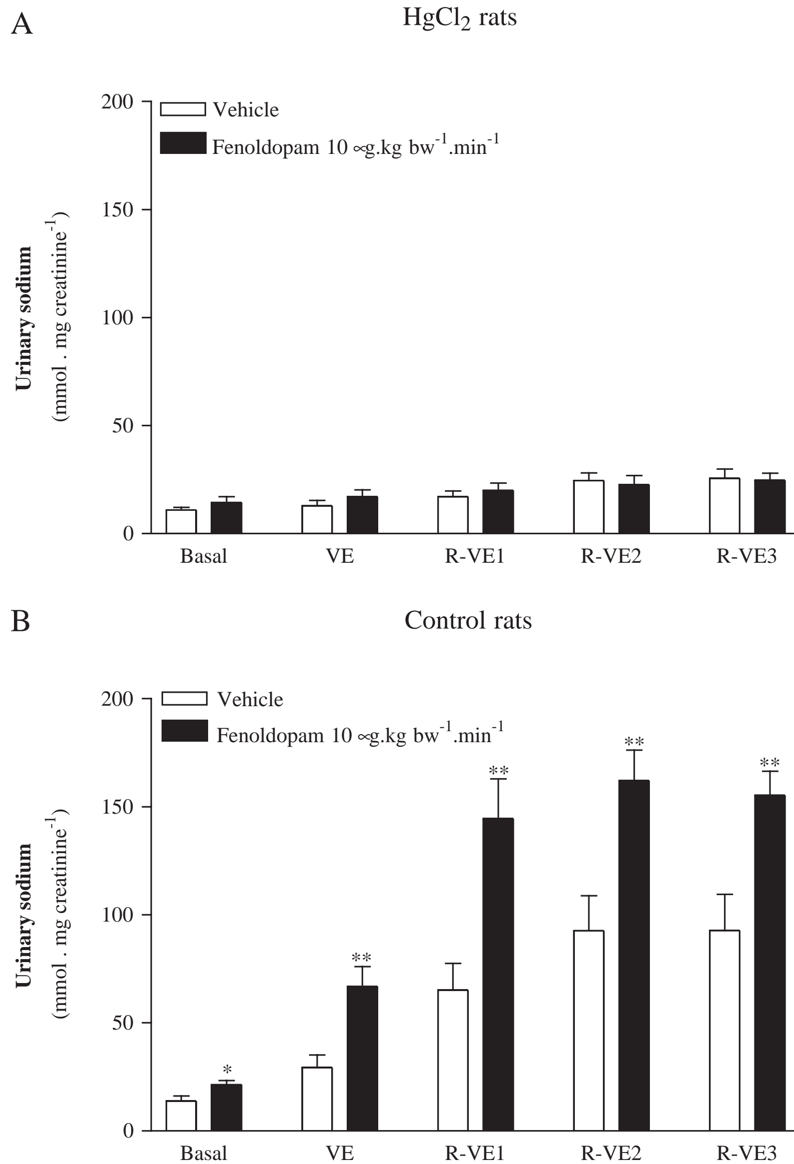

Fig. 5. Accumulated urinary sodium excretion in vehicle-treated and fenoldopam-treated (10 μg kg bw^{-1}min^{-1}) $HgCl_2$ (A) and control (B) rats before ($t=0–120$ min, basal), during ($t=120–150$ min, VE) and after ($t=150–180$ min, R-VE1; $t=180–210$ min, R-VE2; $t=210–240$, R-VE3) 5% volume expansion, fourteen days after first injection. Bars represent means of 6 to 13 experiments per group and error bars represent SE. *$p<0.05$, **$p<0.01$, significantly different from values in vehicle-treated rats.

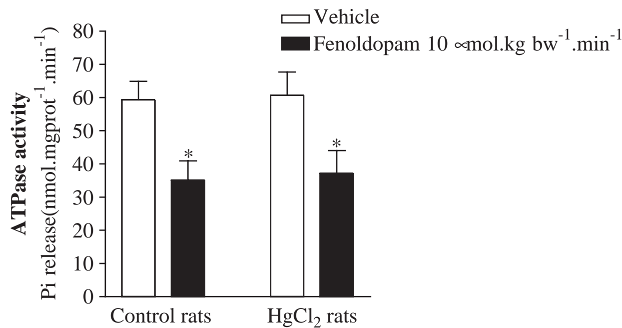

Fig. 6. Na^+,K^+-ATPase activity in proximal tubules of $HgCl_2$ and control rats after fenoldopam (10 μg kg $bw^{-1}min^{-1}$) or vehicle infusion, fourteen days after first injection, expressed as the rate of Pi release. Bars represent means of 5 to 7 experiments per group and error bars represent SE. *$p<0.05$, significantly different from values in vehicle-treated rats.

et al., 1994). Recently, an enhanced proximal NHE3 activity was observed in nephrotic animals (Besse-Eschmann et al., 2002). Thus, one can hypothesize that the reduced renal dopamine tonus observed in rats with $HgCl_2$ 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. This suggestion fits well with the finding that the decrease in urinary dopamine output in $HgCl_2$-treated rats preceded the decrease in urinary sodium excretion as well as with the finding that the reduction in proximal tubular AADC activity was more pronounced during the phase of greatest sodium retention (day 14) than during either day 7 or day 21.

Because the natriuresis resulting from the inhibitory effect of tubular transport by dopamine is mainly evident in volume-expanded states but not in sodium-depleted states (Agnoli et al., 1987; Ragsdale et al., 1990) we decided to evaluate the significance of the lack of renal dopamine in $HgCl_2$-treated rats during an acute volume expansion, in conditions of greatest sodium retention and ascites accumulation (day 14). Since it is generally agreed that the natriuretic effects of dopamine are mainly mediated by D_1-like receptors (Baines et al., 1992; Chen and Lokhandwala, 1993; Narkar et al., 2002; Felder et al., 1993), we decided to evaluate the proximal tubular Na^+,K^+-ATPase activity during volume expansion and the effects of the D_1-like agonist fenoldopam on both proximal sodium transport and urinary sodium excretion. We found that the proximal tubular Na^+,K^+-ATPase activity was similar between $HgCl_2$-treated and control rats and that the fenoldopam-induced inhibition of proximal tubular Na^+,K^+-ATPase activity did not differ between the two groups. Thus, our results suggest that, despite the reduced renal dopaminergic tonus, the proximal tubular sodium transport is not increased in $HgCl_2$ nephrosis and further provide evidence that the Na^+,K^+-ATPase sensitivity to inhibition by the D_1 agonist is not enhanced in $HgCl_2$-treated rats.

Besides the proximal tubule, dopamine can also inhibit sodium reabsorption in both the thick ascending limb (Grider et al., 1998) and the cortical collecting duct (Takemoto et al., 1992). However, the urinary sodium excretion was not significantly altered in $HgCl_2$-treated rats during the administration of the D_1 agonist whereas the natriuresis in control rats was increased by 60% to 120%. Thus, one can hypothesize that the fenoldopam-induced increase in sodium delivery from the proximal tubules of $HgCl_2$-treated rats 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 including $HgCl_2$ nephrosis (Deschenes and Doucet, 2000).

The enhanced distal sodium retention in the nephrotic syndrome was associated with enhanced Na^+,K^+-ATPase activity in the cortical collecting duct and to ANP resistance, a disturbance that was suggested to be related with accelerated breakdown of its second messenger, cGMP (Valentin et al., 1992, 1996). Also, 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 (Katoh et al., 1989; Marin-Grez et al., 1985). 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 (Holtback et al., 1999). Although our study was not designed to evaluate the relationship between ANP and renal dopamine in the nephrotic state, one cannot exclude that the ANP resistance in the cortical collecting duct may be accompanied with 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 infusion, observed in $HgCl_2$-treated rats.

Conclusion

$HgCl_2$-induced membranous nephropathy is associated with a blunted renal dopaminergic system activity as evidenced by a reduced renal dopamine output and a reduced ability of the renal proximal tubules to synthesize dopamine. However, the lack of renal dopamine appears not to be of major importance in the overall renal sodium retention in a state of proteinuria.

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