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Jejunal Dopamine and Na^+,K^+-ATPase Activity in Nephrotic Syndrome

B. Sampaio-Maia[a] M. Moreira-Rodrigues[b] P. Serrão[a] M. Pestana[b]

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

Key Words

Aromatic *L*-amino acid decarboxylase · Dopamine · $HgCl_2$ nephrosis · Jejunum · Na^+,K^+-ATPase · PAN nephrosis · Sodium homeostasis

Abstract

Background: The occurrence of complementary functions in sodium transport between the intestine and the kidney was suggested to occur when the renal function is immature or compromised and jejunal dopamine has been implicated in this renal-intestinal cross-talk. The jejunal sodium transport was not previously evaluated in the nephrotic syndrome. ***Methods:*** We examined the jejunal Na^+,K^+-ATPase activity and the role of dopamine in puromycin aminonucleoside (PAN) and $HgCl_2$-induced nephrotic syndrome rat models. ***Results:*** In both nephrotic syndrome rat models, the jejunal Na^+,K^+-ATPase activity was reduced during greatest sodium retention and ascites accumulation (PAN nephrosis, day 7; $HgCl_2$ nephrosis, day 14), whereas during enhanced sodium excretion and ascites mobilization the jejunal Na^+,K^+-ATPase activity was increased in $HgCl_2$ nephrosis (day 21) and was similar to controls in PAN nephrosis (day 14). In both PAN- and $HgCl_2$-induced nephrosis, the jejunal aromatic *L*-amino acid decarboxylase (AADC) activity, the enzyme responsible for the synthesis of jejunal dopamine, did not differ from controls. In addition, the jejunal Na^+,K^+-ATPase activity was not sensitive to inhibition by dopamine (1 μM) in both experimental groups throughout the study. ***Conclusions:*** In the nephrotic syndrome the jejunal Na^+,K^+-ATPase activity may respond in a compensatory way to changes in extracellular volume, through dopamine-independent mechanisms.

Introduction

In the mammalian small intestine the driving force for fluid absorption is the active transport of sodium and chloride, which may be electrically silent [1] or which may involve electrogenic sodium transport [2]. The primary mechanism responsible for the transepithelial sodium transport in the small intestine is the basolateral Na^+,K^+-ATPase [3] of both absorptive and secretory cell types [4, 5]. The transepithelial transport of other solutes and electrolytes such as glucose, amino acids, chloride and bicarbonate is indirectly coupled to sodium transport [4].

The basal activity of the intestinal Na^+,K^+-ATPase can be influenced or modulated by different factors including dopamine. In the intestine, the dopaminergic system has been characterized as a local non-neuronal system constituted by epithelial cells of intestinal mucosa rich in aromatic *L*-amino acid decarboxylase (AADC), the enzyme responsible for the synthesis of dopamine from circulating or luminal *L*-3,4-dihydroxyphenylalanine (*L*-Dopa) [6]. Dopamine is particularly abundant in the mucosal

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Manuel Pestana, MD
Unit of Research and Development of Nephrology, Faculty of Medicine
University of Porto, Alameda Prof. Hernani Monteiro
PT–4200-319 Porto (Portugal)
Tel. +351 91 9194907, Fax +351 22 5502023, E-Mail mvasconcelos@hsjoao.min-saude.pt

cell layer [7, 8] and the highest AADC activity is located in the jejunum [9], where dopamine activates dopamine receptors as a paracrine/autocrine substance [6].

Although the small intestine is responsible for most of the absorption of nutrients, water and electrolytes [10], under normal circumstances wide variations in salt intake are translated into parallel changes in renal salt excretion, so that the extra-cellular volume is maintained within narrow limits [11]. This suggests that the relative importance of the intestinal sodium absorption on the control of extracellular volume may assume particular relevance when the renal handling of sodium is compromised. Accordingly, wide variations in sodium intake did not change the jejunal Na^+,K^+-ATPase activity or the jejunal dopamine production in adult rats with mature and well-functioning kidneys whereas during early postnatal life when the kidney has a limited capacity to regulate fluid and electrolytes metabolism a high sodium intake was accompanied by a decreased jejunal Na^+,K^+-ATPase activity which was sensitive to inhibition by dopamine [12]. These results suggest the occurrence of complementary functions between the intestine and the kidney during development. Interestingly, it has been recently reported a significant reduction in jejunal Na^+,K^+-ATPase activity with recovered sensitivity to inhibition by dopamine in rats submitted to uninephrectomy [13]. This further reinforces the view that the influence of jejunal Na^+,K^+-ATPase activity on sodium homeostasis may assume particular importance when the renal function is compromised.

Nephrotic syndromes may develop in human patients as a result of primary diseases as well as in laboratory animals in response to toxic substances such as puromycin aminonucleoside (PAN) and mercury chloride ($HgCl_2$) [14]. The features of the nephrotic syndrome in both man and experimental rat models are massive proteinuria and development of edema and extra-cellular volume expansion secondary to abnormal renal sodium retention. Although the exact mechanisms involved in the enhanced sodium reabsorption in the nephrotic syndrome still remain to be fully elucidated, most of the available evidences implicate a primary renal sodium handling abnormality in the cortical collecting ducts [15].

Because the extracellular volume expansion in the nephrotic syndrome is related to disruption of the normal renal sodium handling mechanisms, we found it was worthwhile to study the jejunal Na^+,K^+-ATPase and dopaminergic activities in both PAN nephrosis and $HgCl_2$-induced membranous nephropathy.

Materials and Methods

In vivo Studies

PAN 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^{-1} of PAN (150 mg·kg BW^{-1}) or the vehicle (NaCl 0.9%) on the day 0.

$HgCl_2$ Nephrosis

Normotensive male Brown-Norway rats (Harlan), 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.

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 had free access to tap water. The PAN-treated and $HgCl_2$-treated animals 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 groups, the vehicle-treated rats had only access to the mean daily rat chow intake of the respective nephrotic animals. The rats were kept in metabolic cages (Techniplast, Buguggiate, VA, Italy) for the collection of 24-hour urine. Blood pressure (systolic and diastolic) was measured weekly throughout the study in conscious restrained animals 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. On the days of sacrifice (PAN, days 7 and 14; $HgCl_2$, days 7, 14 and 21), the animals were anesthetized with pentobarbital sodium (50 mg·kg BW^{-1} i.p.). The ascites weight was determined through moistening and weighing an absorbent paper. Blood was collected from the heart in tubes containing lithium/heparin for later determination of sodium and creatinine. Segments of jejunum with ~10 cm in length were removed, opened longitudinally with fine scissors and rinsed free from blood and intestinal contents with cold saline. One segment was used for the assay of Na^+,K^+-ATPase activity in jejunal epithelial cells. On a different segment, the jejunal mucosa was removed with a scalpel. Fragments of jejunal mucosa were used for later determination of AADC activity, whereas other fragments weighing around 200 mg were placed in vials containing 1 ml of 0.2 *M* perchloric acid and stored at –80°C until quantification of catecholamines by HPLC with electrochemical detection.

In vitro Studies

AADC Activity

AADC activity was determined in homogenates of jejunal mucosa, using *L*-Dopa (0.1–10 m*M*) as substrate [16, 17]. 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 method of Bradford [18].

Na^+,K^+-ATPase Activity

Na^+, K^+-ATPase activity was measured by the method of Quigley and Gotterer [19] adapted in our laboratory with slight modification [20]. The rat jejunal epithelial cells were isolated as described

by Vieira-Coelho et al. [12]. The Na^+,K^+-ATPase activity was determined in conditions of saturating sodium and Tris salt adenosine 5′-triphosphate (ATP) concentration. The isolated jejunal epithelial cells 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 m*M*): 37.5 imidazole buffer, 75 NaCl, 5 KCl, 1 sodium EGTA, 5 $MgCl_2$, 75 NaN_3, 75 tris(hydroxymethyl) amino methane(tris) hydrochloride and 100 μl cell suspension. For determination of ouabain-resistant ATPase, NaCl and KCl were omitted, and Tris-HCl (150 m*M*) and ouabain (1 m*M*) were added to the assay. The reaction was initiated by addition of 4 m*M* ATP for all assays except for the determination of the kinetic parameters of Na^+,K^+-ATPase activity, where increasing concentrations of ATP (0.025–4 m*M*) were used. 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 (4,000 rpm) and liberated P_i in the supernatant was measured as the result of ATPase activity. The assay of P_i was performed by spectrophotometry. Ouabain-sensitive ATPase activity is expressed as nanomoles of P_i 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 $mg \cdot ml^{-1}$) was determined by the method described by Bradford [18] using bovine serum albumin as standard. For both Sprague-Dawley and Brown-Norway rat strains, 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 $mg \cdot ml^{-1}$.

Assay of Catecholamines

The assays of dopamine and *L*-Dopa in urine, norepinephrine in jejunal mucosa and dopamine in samples from AADC studies were performed by HPLC with electrochemical detection, as previously described [21]. In our laboratory, the lower limit of detection of dopamine ranged from 350 to 1,000 fmol.

Plasma and Urine Ionogram and Biochemistry

Ion-selective electrodes performed the quantifications of sodium. Creatinine was measured by the Jaffé method. Total proteins were determined by a colorimetric test, the biuret reaction. The assays were performed through Cobas Mira Plus analyser (ABX Diagnostics, Switzerland). Creatinine clearance was calculated using 24-hour urine creatinine excretion. Fractional excretion of sodium (FE_{Na+}) was calculated as previously reported [16]. Sodium balance was determined subtracting the absolute daily urinary sodium excretion ($mmol \cdot 24\ h^{-1}$) to daily sodium intake ($mmol \cdot 24\ h^{-1}$).

Drugs

The compounds ATP; dopamine hydrochloride; $HgCl_2$; *L*-Dopa; ouabain and PAN 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 Michaelis-Menten coefficient (K_m) for AADC and Na^+,K^+-ATPase enzymatic assay were calculated from nonlinear regression analysis using GraphPad Prism statistics software package [22] 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

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 (fig. 1). In parallel, the PAN-treated rats showed a marked decrease in urinary sodium excretion compared with control animals from days 2 to 8, followed by an increase in the urinary excretion of sodium from days 12 to 14 (fig. 1). On day 7, the PAN-treated rats exhibited a positive sodium balance and a reduced FE_{Na+} accompanied by marked ascites accumulation, whereas on day 14 the PAN-treated rats presented a negative sodium balance and an increased FE_{Na+} accompanied by ascites of much smaller magnitude (table 1). The creatinine clearance was lower in PAN-treated than in control rats either on day 7 or on day 14 (table 1). Mean arterial pressure (MAP) did not differ between PAN-treated and control rats throughout the study (in mm Hg, day 7, 110 ± 8 vs. 123 ± 8, day 14, 107 ± 6 vs. 103 ± 3).

The jejunal Na^+,K^+-ATPase and dopaminergic activities were evaluated in PAN-treated rats in conditions of greatest sodium retention and ascites accumulation (day 7), as well as during enhanced sodium excretion and negative sodium balance (day 14) (fig. 1; table 1).

The kinetic parameters of Na^+,K^+-ATPase activity in jejunal epithelial cells from Sprague-Dawley rats in basal conditions were: V_{max}, 210 ± 23 nmol $P_i \cdot mg\ prot^{-1} \cdot min^{-1}$ and K_m, 1.2 ± 0.4 m*M*. In experiments performed at V_{max} conditions, the Na^+,K^+-ATPase activity in the jejunal epithelial cells from PAN-treated rats was reduced on day 7 the but not on day 14 (fig. 2).

The activity of AADC in homogenates of jejunal mucosa from PAN-treated and control rats is depicted in table 2. As can be observed, the V_{max} and K_m values for AADC activity in the jejunal mucosa did not differ between PAN-treated and control rats, either on day 7 or day 14 (table 2). In addition, dopamine (1 μ*M*) did not change the jejunal Na^+,K^+-ATPase activity in either PAN-treated or control rats on days 7 and 14 (table 3).

In PAN-treated rats the urinary levels of dopamine were significantly reduced on days 7 and 14 (table 4). By contrast, the urinary excretion of the dopamine precursor, *L*-Dopa, did not differ between PAN-treated and control animals (table 4). This resulted in markedly reduced urinary dopamine/*L*-Dopa ratios in PAN-treated rats throughout the study (table 4).

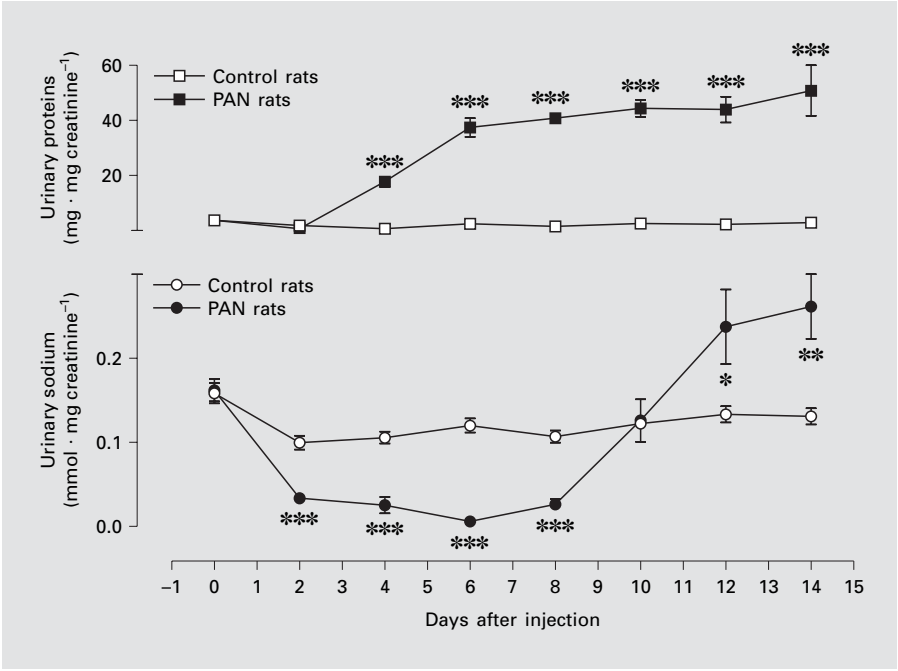

Fig. 1. Urinary excretion of proteins and sodium in PAN and control rats throughout the study. Symbols represent means of 12 rats per group and error bars represent SE. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, significantly different from values in control rats.

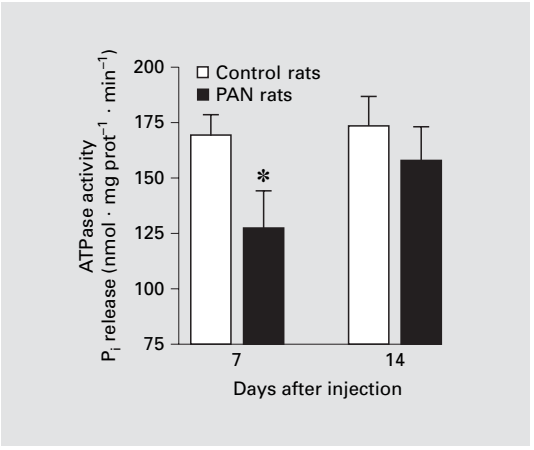

Fig. 2. Na^+,K^+-ATPase activity in isolated jejunal epithelial cells from PAN-treated and control rats 7 and 14 days after injection, expressed as the rate of P_i release. Bars represent means of 4–7 experiments per group and error bars represent SE. * $p < 0.05$, significantly different from values from corresponding control rats.

Table 1. Body weight, creatinine clearance (C_{cr}), sodium balance, fractional excretion of sodium (FE_{Na+}) and ascites weight in PAN-treated and control rats on days 7 and 14 after injection

	Day 7		Day 14	
	control	PAN	control	PAN
Body weight, g	221 ± 5	248 ± 4*	241 ± 2	234 ± 5
C_{cr}, ml·min^{-1}	5.65 ± 1.38	2.24 ± 0.81*	4.51 ± 1.08	2.24 ± 0.32*
Na^+ balance, mmol·24 h^{-1}	–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*

Values are means ± SE; n = 5–10 experiments per group. * $p < 0.05$, significantly different from corresponding values in control rats.

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

	Day 7		Day 14	
	control	PAN	control	PAN
V_{max}, nmol·mg prot^{-1}·15 min^{-1}	105 ± 10	105 ± 24	103 ± 5	83 ± 11
K_m, m*M*	2.5 ± 0.1	2.2 ± 0.2	2.9 ± 0.2	2.5 ± 0.3

Values are means ± SE; n = 8–10 experiments per group.

Table 3. Effect of dopamine on Na^+,K^+-ATPase activity in jejunal epithelial cells from PAN-treated and control rats on days 7 and 14 after injection

	Day 7		Day 14	
	control	PAN	control	PAN
Basal	100 ± 6	100 ± 13	100 ± 8	100 ± 10
Dopamine 1 μ*M*	93 ± 4	100 ± 15	98 ± 6	94 ± 5

Values are means ± SE; n = 4–7 experiments per group. Na^+,K^+-ATPase activity is expressed as percentage of control.

Table 4. Urinary dopamine, *L*-Dopa and urinary dopamine/*L*-Dopa ratios in PAN-treated and control rats 7 and 14 days after injection

	Day 7		Day 14	
	control	PAN	control	PAN
Dopamine, nmol·24 h^{-1}	11.1 ± 0.9	4.7 ± 0.4*	14.5 ± 0.6	5.7 ± 0.7*
L-Dopa, nmol·24 h^{-1}	0.54 ± 0.11	0.73 ± 0.09	0.73 ± 0.23	0.77 ± 0.29
Dopamine/*L*-Dopa	30.7 ± 8.1	6.7 ± 0.5*	27.4 ± 8.4	12.7 ± 5.9*

Values are means ± SE; n = 4–8 experiments per group. * $p < 0.05$, significantly different from corresponding values in control rats.

The tissue levels of norepinephrine in the jejunal mucosa were increased in PAN-treated rats on day 7, whereas on day 14 the jejunal tissue levels of norepinephrine were similar between PAN-treated and control rats (table 5).

$HgCl_2$ Nephrosis

In $HgCl_2$-treated rats, the proteinuria remained undetectable or very low until day 9 and increased from day 11 and beyond reaching the highest levels between days 15 and 17 (fig. 3). The urinary sodium excretion was decreased in $HgCl_2$-treated rats compared with control animals from days 3 to 14 followed by an increase in urinary sodium excretion from days 17 to 21 (fig. 3). Consequently, the $HgCl_2$-treated rats presented a positive sodium balance on both days 7 and 14, this being statistically significant on

Table 5. Norepinephrine tissue levels in jejunal mucosa from PAN-treated and $HgCl_2$-treated rats and respective control rats 7, 14 and 21 days after injection

	Day 7	Day 14	Day 21
Control	327 ± 72	290 ± 54	–
PAN	750 ± 175*	302 ± 54	–
Control	148 ± 14	160 ± 34	150 ± 34
$HgCl_2$	127 ± 25	136 ± 29	92 ± 28

Values are means ± SE; n = 4–6 experiments per group, values are expressed in pmol/g. * $p < 0.05$, significantly different from corresponding values in control rats.

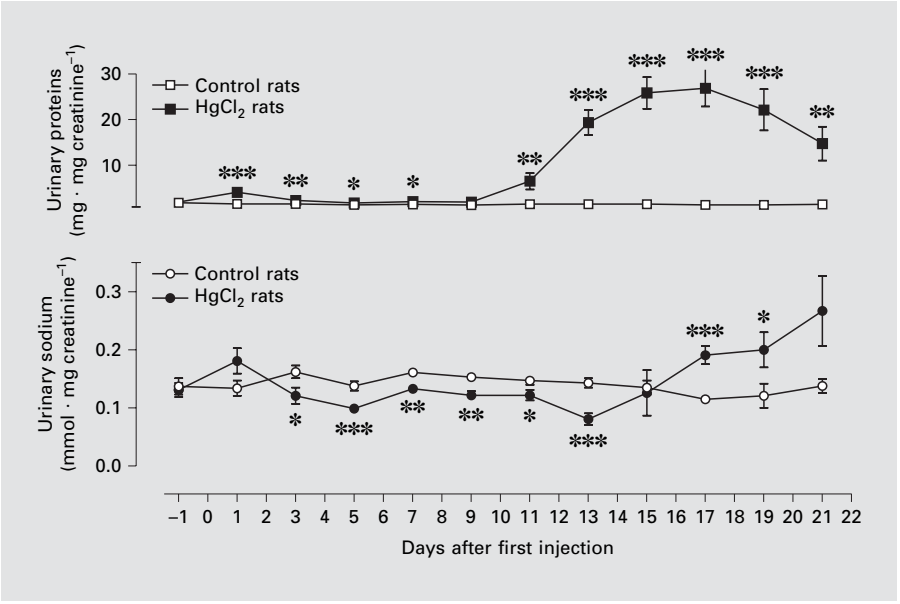

Fig. 3. Urinary excretion of proteins and sodium in $HgCl_2$ and control rats throughout the study. Symbols represent means of 5–13 rats per group and error bars represent SE. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, significantly different from values in control rats.

Table 6. Body weight, creatinine clearance (C_{cr}), sodium balance, fractional excretion of sodium (FE_{Na+}) and ascites weight 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*
C_{cr}, 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
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
Ascites, 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

Values are means ± SE; n = 5–10 experiments per group. * $p < 0.05$, significantly different from corresponding values in control rats.

day 14 when the $HgCl_2$-treated rats exhibited a reduced FE_{Na+} and greatest ascites accumulation (table 6). On day 21, the $HgCl_2$-treated rats presented a negative sodium balance and ascites mobilization (table 6). The creatinine clearance did not differ between $HgCl_2$-treated and control rats on days 7, 14 or 21 (table 6). MAP did not differ between $HgCl_2$-treated and control rats throughout the study (in mm Hg; day 7, 87 ± 3 vs. 93 ± 6, day 14, 76 ± 7 vs. 85 ± 1, day 21, 104 ± 10 vs. 90 ± 2).

Based on these findings the jejunal Na^+,K^+-ATPase and dopaminergic activities were evaluated in $HgCl_2$-treated rats in conditions of sodium retention and negligible proteinuria (day 7), during greatest sodium retention and increased proteinuria (day 14) as well as during enhanced sodium excretion and negative sodium balance (day 21) (fig. 3; table 6).

The kinetic parameters of Na^+,K^+-ATPase activity in jejunal epithelial cells from Brown-Norway rats in basal condition were: V_{max}, 98 ± 16 nmol P_i·mg prot^{-1}·min^{-1} and K_m, 0.33 ± 0.10 m*M*. The Na^+, K^+-ATPase activity in the jejunal epithelial cells from $HgCl_2$-treated and control rats is depicted in figure 4 at V_{max} condition. As can be observed, during the phase of sodium retention the $HgCl_2$-treated rats presented a reduced jejunal Na^+,K^+-ATPase activity on day 14 but not on day 7 (fig. 4), whereas during the phase of sodium excretion the jejunal

Na^+,K^+-ATPase activity was markedly increased in $HgCl_2$-treated animals on day 21 (fig. 4).

The activity of AADC in homogenates of jejunal mucosa from $HgCl_2$-treated and control rats is depicted in table 7. As can be observed, the V_{max} and K_m values for AADC activity in the jejunal mucosa did not differ between $HgCl_2$-treated and control rats on days 7, 14 or 21 (table 7). In addition, dopamine (1 μ*M*) did not change the Na^+,K^+-ATPase activity in jejunal epithelial cells from both $HgCl_2$-treated and control rats throughout the study (table 8).

In $HgCl_2$-treated rats the urinary levels of dopamine were significantly decreased on days 7, 14 and 21 (table 9). By contrast, the urinary excretion of the dopamine precursor, *L*-Dopa, was increased in $HgCl_2$-treated animals throughout the study (table 9). This resulted in markedly reduced urinary dopamine/*L*-Dopa ratios in $HgCl_2$-treated rats throughout the study (table 9).

The tissue levels of norepinephrine were similar between $HgCl_2$-treated and control rats on days 7, 14 and 21 after surgery (table 5).

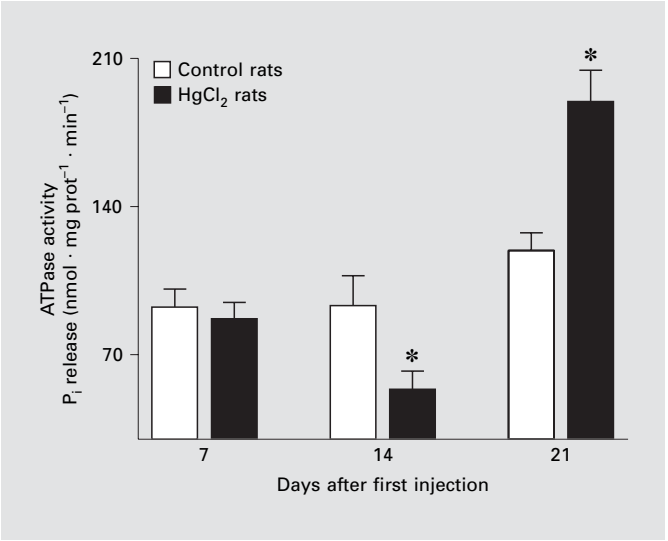

Fig. 4. Na^+,K^+-ATPase activity in isolated jejunal epithelial cells from $HgCl_2$-treated and control rats 7, 14 and 21 days after first injection, expressed as the rate of P_i release. Bars represent means of 4–7 experiments per group and error bars represent SE. * $p < 0.05$, significantly different from values from corresponding control rats.

Table 7. Kinetic parameters (V_{max} and K_m) of AADC activities in homogenates of 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	$HgCl_2$
V_{max}, pmol·mg prot^{-1}·15 min^{-1}	125 ± 26	126 ± 19	191 ± 20	170 ± 20	191 ± 35	212 ± 34
K_m, m*M*	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 = 6–10 experiments per group.

Table 8. Effect of dopamine on Na^+,K^+-ATPase activity in jejunal epithelial cells 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	$HgCl_2$
Basal	100 ± 9	100 ± 9	100 ± 15	100 ± 16	100 ± 7	100 ± 8
Dopamine 1 μ*M*	99 ± 10	97 ± 9	90 ± 15	90 ± 17	97 ± 8	96 ± 8

Values are means ± SE; n = 5–9 experiments per group. Na^+,K^+-ATPase activity is expressed as percentage of control.

Table 9. Urinary dopamine, *L*-Dopa and urinary dopamine/*L*-Dopa ratios 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	$HgCl_2$
Dopamine, nmol·24 h^{-1}	11.8 ± 1.4	8.6 ± 0.6*	11.0 ± 1.3	7.4 ± 1.5*	9.5 ± 1.5	5.1 ± 1.6*
L-Dopa, nmol·24 h^{-1}	0.47 ± 0.16	2.23 ± 0.82*	0.20 ± 0.04	0.77 ± 0.17*	0.32 ± 0.07	0.80 ± 0.45
Dopamine/*L*-Dopa	32.0 ± 7.0	6.44 ± 1.0*	63.0 ± 10.7	12.8 ± 3.6*	35.5 ± 9.6	14.0 ± 6.1*

Values are means ± SE; n = 5–13 experiments per group. * $p < 0.05$, significantly different from corresponding values in control rats.

Discussion

The present study was undertaken with the aim of clarifying the possible role of the jejunal Na^+,K^+-ATPase and dopaminergic activities in the control of sodium homeostasis in both PAN- and $HgCl_2$-induced nephrosis. We found a decreased jejunal Na^+,K^+-ATPase activity in both nephrotic syndrome rat models during the period of greatest sodium retention and ascites accumulation and an increased jejunal Na^+,K^+-ATPase activity in $HgCl_2$ nephrosis during the period of enhanced sodium excretion and ascites mobilization. However, the jejunal dopaminergic activity was not altered in either PAN or $HgCl_2$-induced nephrosis and dopamine was unable to modulate jejunal Na^+,K^+-ATPase activity in both nephrotic syndrome rat models. Taken together, our results suggest that in the nephrotic syndrome the jejunal Na^+,K^+-ATPase activity may respond in a compensatory way to changes in extracellular volume by dopamine-independent mechanisms.

PAN is a toxic substance that induces a nephrotic syndrome without glomerular inflammatory lesions or Ig deposits [23], whereas $HgCl_2$ induces a systemic autoimmune disease that includes membranous nephropathy with IgG deposits [24]. These drug-induced nephropathies develop high-range proteinuria and full-blown nephrotic syndrome [14]. Because the time courses of urinary sodium excretion and the relationship between excretion of proteinuria and ascites differed in these two nephrotic syndrome rat models we performed a systematic study of the time courses of these parameters and evaluated the jejunal Na^+,K^+-ATPase and dopaminergic activities in both PAN and $HgCl_2$ nephrosis in different temporal conditions including: (1) negligible proteinuria accompanied by significant sodium retention ($HgCl_2$ nephrosis, day 7); (2) increased proteinuria accompanied by greatest sodium retention and ascites accumulation (PAN nephrosis, day 7; $HgCl_2$ nephrosis, day 14), and (3) increased proteinuria accompanied by enhanced renal sodium excretion and ascites mobilization (PAN nephrosis, day 14; $HgCl_2$ nephrosis, day 21).

Interestingly, we found in the two nephrotic syndrome rat models that in the presence of increased proteinuria, the jejunal Na^+,K^+-ATPase activity was reduced during the phase of greatest sodium retention and positive sodium balance but not during enhanced sodium excretion and ascites mobilization. Because the driving force that energizes all mechanisms of jejunal sodium absorption is the hydrolysis of ATP catalyzed by Na^+,K^+-ATPase located at the basolateral membrane of intestinal epithelial cells [25], these findings provide evidence for a decreased jejunal sodium absorption in the nephrotic syndrome when the extracellular volume is expanded due to abnormal renal sodium retention. Our results fit well with the previous findings suggesting that extracellular volume expansion with isotonic saline, 5% albumin or during ascitic cirrhosis may be accompanied by decreases in jejunal ion and fluid absorption [26–32]. The finding of reduced Na^+,K^+-ATPase activity in jejunal epithelial cells during greatest sodium retention in both nephrotic syndrome rat models, involving different toxic agents and different histologic types of glomerular lesions, suggests that the modulation of jejunal sodium absorption may take place in response to changes in sodium metabolism in other nephrotic syndrome conditions, including minimal change disease and membranous nephropathy in human patients.

The phase of sodium retention with negligible proteinuria was only observed in $HgCl_2$-treated rats because in PAN nephrosis proteinuria was always profuse. We found in $HgCl_2$-treated rats that the jejunal Na^+,K^+-ATPase ac-

tivity on day 7 did not differ from controls. Given that in all experimental models of nephrotic syndrome, the expansion of extra-cellular volume and formation of ascites requires the association of proteinuria and marked decrease in urinary sodium excretion [14], one can speculate that on day 7 the $HgCl_2$-treated rats were not sufficiently volume expanded to induce a jejunal response.

During the phase of increased renal sodium excretion and ascites mobilization, the jejunal Na^+,K^+-ATPase activity was found to be significantly increased in $HgCl_2$-treated but not in PAN-treated rats. Because the $HgCl_2$-treated rats exhibited a negative sodium balance going along with a decrease in body weight, one can hypothesize that the enhanced jejunal Na^+,K^+-ATPase activity on day 21 may have resulted from the decrease in extracellular volume. In agreement with this view are the results showing that the decrease in extracellular volume following sodium depletion, dehydration and hemorrhage is associated with a substantial increase in the rate of ion and water absorption from small intestine [33–36]. The finding that the jejunal Na^+,K^+-ATPase activity on day 14 was similar between PAN-treated and control rats may be explained on the basis that the negative sodium balance in PAN-treated rats was not accompanied by a decrease in the extracellular volume. In agreement with this view is the similar body weight between PAN-treated and control rats as well as the presence of residual ascites in PAN-treated rats on day 14.

The use of drug-induced nephrotic syndrome rat models could be associated with direct toxicity of both PAN and Hg on jejunal epithelial cells as well as on Na^+,K^+-ATPase activity. However, a previous study on the jejunal transport in PAN-induced nephrotic syndrome suggested that the decrease in the intestinal absorption of glucose, phenylalanine, histidine, water and sodium is not related to acute PAN toxicity being instead associated with the metabolic consequences of the nephrotic syndrome [37]. Although Hg is known to induce toxic effects in different body organs and cellular components including jejunal epithelial cells [38, 39], limited or non existing data is available regarding the jejunal Na^+,K^+-ATPase. In jejunum, Hg was associated with inhibition of amino acid and sugar transport [40–42] and the sites of Hg action were suggested to be located in the mucosal surface of jejunal slices but not in the basolateral cell border [43]. The results of the present study in $HgCl_2$-treated rats showing that the jejunal Na^+,K^+-ATPase activity changed from normal to low and to high values in parallel with changes in sodium balance further suggest that in jejunal epithelial cells the basolateral Na^+,K^+-ATPase may escape Hg toxicity.

At the intestinal level, previous studies have shown that the inhibitory effects of dopamine on jejunal sodium absorption and Na^+,K^+-ATPase activity in rat jejunal epithelial cells are limited to animals under 20 days of age, adult animals being insensitive to the inhibitory effects of dopamine [6, 12, 44]. Intestinal function has a great impact during early postnatal life, not only on the uptake of nutrients but also on the maintenance of electrolytes and water metabolism [45, 46]. In fact, although nephrogenesis is complete at birth, renal tubular function continues to develop postnatally, and the kidney has a limited capacity to regulate fluids and electrolyte homeostasis [47]. The lack of effect of dopamine on jejunal Na^+,K^+-ATPase activity in adult animal coincided with the period in which renal function has reached maturation [12, 44]. Recently, a reduction in jejunal Na^+,K^+-ATPase activity with recovered sensitivity to inhibition by dopamine was reported in rats submitted to uninephrectomy presenting an enhanced dopamine synthesis per nephron [13]. This further suggested the occurrence of complementary functions between the intestine and the kidney with dopaminergic system playing a role in renal-intestinal cross-talk. In the present study, the jejunal AADC activity was not altered in both nephrotic syndrome rat models and the jejunal Na^+,K^+-ATPase activity was not sensitive to inhibition by dopamine throughout the study. This was accompanied in both nephrotic syndrome rat models by a decreased renal dopamine synthesis as evidenced by a reduced urinary dopamine excretion accompanied by decreased urinary dopamine/*L*-Dopa ratios throughout the study. Since the urinary dopamine/*L*-Dopa ratios are used as rough measure of tubular uptake and/or decarboxylation of *L*-Dopa to dopamine, our findings suggest that the decreases in urinary dopamine excretion observed in both nephrotic syndrome rat models are related to a reduced tubular uptake/decarboxylation of *L*-Dopa to dopamine. In addition, the PAN-treated rats presented a decreased creatinine clearance suggesting that a reduced number of well functioning tubular units may also contribute to the decrease in urine dopamine excretion in PAN-treated but not in $HgCl_2$-treated animals. Taken together, our results suggest that in either PAN- or $HgCl_2$-induced nephrosis the observed changes in jejunal Na^+,K^+-ATPase activity are mediated by dopamine-independent mechanisms despite the decreased renal dopaminergic activity and do not support the existence of dopamine-mediated complementary functions in sodium transport between the kidney and the intestine, in the nephrotic syndrome.

Since our results were negative in terms of the role of dopaminergic system in the jejunum, one can hypothesize that other mechanisms involved in sodium transport can account for the changes in jejunal Na^+,K^+-ATPase in the nephrotic syndrome. The changes observed in jejunal Na^+,K^+-ATPase in both nephrotic syndrome rat models could be influenced by blood pressure values. However, no significant changes were observed in MAP in either PAN or $HgCl_2$ nephrosis. Norepinephrine is well-recognized to stimulate sodium transport by increasing the jejunal Na^+,K^+-ATPase activity. However, the jejunal tissue levels of norepinephrine did not differ between the nephrotic and control rats throughout the study with the exception of PAN-treated rats on day 7 where a decreased jejunal Na^+,K^+-ATPase activity was accompanied by increased jejunal tissue levels of norepinephrine. Thus one can conclude that, similarly to dopamine, jejunal norepinephrine cannot account for the observed changes in jejunal Na^+,K^+-ATPase activity in both nephrotic syndrome rat models. Other mediator mechanisms can modulate the intestinal sodium transport in response to changes in extracellular volume and deserve further study in a state of proteinuria. Evidence has been gathered showing that ANP reduces fluid absorption in response to acute volume expansion [29], whereas both angiotensin II and angiotensin III can enhance intestinal electrolyte and fluid absorption in response to volume depletion [35]. In addition, angiotensin II was shown to modulate the intestinal Na^+,K^+-ATPase activity via calcium mobilization and PKC activation [48]. In acute renal failure, an increase in serum aldosterone was suggested to stimulate the jejunal Na^+,K^+-ATPase activity [49], whereas 5-hydroxytryptamine was found to increase Na^+,K^+-ATPase activity in jejunal epithelial cells from young rats [50]. On the other hand, NO donor (s-nitroso-n-acetylpenicillamine) was shown to inhibit Na^+,K^+-ATPase activity in an intestinal cell line (IEC-6) [51].

It is concluded that in the nephrotic syndrome the jejunal Na^+,K^+-ATPase activity appears to respond in a compensatory way to changes in extracellular volume by dopamine-independent mechanisms.

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