Original Article

Jejunal dopamine and Na\(^+\),K\(^+\)-ATPase activity in early chronic renal insufficiency

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SUMMARY:

Aim: The uninephrectomised and three-quarter nephrectomised (3/4nx) rats present dopamine-sensitive enhanced natriuresis. This is accompanied in uninephrectomised rats by a reduced jejunal Na\(^+\),K\(^+\)-ATPase activity with recovered sensitivity to inhibition by dopamine. The present study examined the jejunal Na\(^+\),K\(^+\)-ATPase activity and the role of dopamine in 3/4nx animals.

Methods: Fourteen days after surgery, the \(\text{L}\)-amino acid decarboxylase activity (AADC) activity, the enzyme responsible for the synthesis of dopamine, and the Na\(^+\),K\(^+\)-ATPase activity, were determined in jejunal epithelial cells from 3/4nx and Sham rats. In addition, the effect of dopamine (1 \(\mu\)mol/L) on jejunal Na\(^+\),K\(^+\)-ATPase activity was evaluated in both groups.

Results: The 3/4nx rats presented a reduced AADC activity in jejunal epithelial cells (\(V_{\text{max}}\) in nmol/mg prot/15 min, \(142 \pm 6\) vs \(190 \pm 10\), \(P < 0.05\)). In addition, the jejunal Na\(^+\),K\(^+\)-ATPase activity was increased in 3/4nx rats (Pi release in nmol/mg prot/min, \(137 \pm 1\) vs \(122 \pm 2\), \(P < 0.05\)). However, dopamine was unable to inhibit the Na\(^+\),K\(^+\)-ATPase activity in jejunal epithelial cells from both 3/4nx and Sham animals.

Conclusions: In contrast to uninephrectomy, the jejunal Na\(^+\),K\(^+\)-ATPase activity is increased in 3/4nx rats and is not sensitive to inhibition by dopamine.

KEY WORDS: aromatic L-amino acid decarboxylase, jejunal dopamine, Na\(^+\),K\(^+\)-ATPase, remnant kidney, sodium handling.

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 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 characterised has 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 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 extracellular volume is maintained within narrow limits.\(^11\) This suggests that the relative importance of the intestinal sodium absorption on the control of sodium homeostasis 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 electrolyte metabolism, a high sodium intake was accompanied by decreased jejunal Na\(^+\),K\(^+\)-ATPase activity which was sensitive to inhibition by dopamine.\(^12\) These results suggested the occur-
rence of complementary functions between the intestine and the kidney during development. More recently, a significant reduction in jejunal Na\(^+\),K\(^+\)-ATPase activity with recovered sensitivity to inhibition by dopamine in rats submitted to uninephrectomy has been reported.\(^{13}\) The finding that blood pressure was not increased in uninephrectomised rats\(^{13}\) suggests that the changes in jejunal Na\(^+\),K\(^+\)-ATPase activity in uninephrectomised rats might contribute to the maintenance of sodium homeostasis in conditions of slightly compromised renal function.

Rats submitted to 3/4 nephrectomy, a model of chronic renal insufficiency, present the known consequences of partial renal ablation, namely compensatory renal growth, significant increase in fractional excretion of sodium and consistent increase in systemic blood pressure.\(^{14}\) Because the extracellular volume expansion in chronic renal insufficiency is related to disruption of the normal renal sodium handling mechanisms, we found that it was worthwhile to evaluate the jejunal Na\(^+\),K\(^+\)-ATPase activity and the role of dopamine on jejunal sodium transport, in 3/4 nephrectomised animals.

**METHODS**

**In vivo studies**

Normotensive male Wistar-Han rats (Harlan, Barcelona, Spain), weighing 190–210 g, were selected after a 7-day period of stabilisation and adaptation to blood pressure measurements. The animals were kept under controlled environmental conditions (12:12 h light/dark cycle and room temperature 22 ± 2°C); fluid intake and food consumption were monitored daily throughout the study. All animals had free access to tap water and were fed ad libitum throughout the study with ordinary rat chow (Panlab, Barcelona, Spain) containing 1.9 g/kg of sodium. Blood pressure (systolic and diastolic) and heart rate were measured weekly in conscious restrained animals, between 07:00–10:00, 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. All in vivo investigation was performed in accordance with the European Directive number 86/609, transposed to the Portuguese Law by DL 129/92 and by Portaria 1003/92.

**Three-quarter nephrectomy**

Rats were anaesthetised with pentobarbital sodium (60 mg/kg; intraperitoneally, i.p.); the right kidney was removed and a surgical ablation of both poles of the left kidney was performed, according to what was previously described,\(^{14}\) providing six three-quarter nephrectomised (3/4nx) rats. The percentage of remnant renal mass was obtained assuming that both the right and the left kidneys were of the same weight.\(^{15}\) The remnant renal mass was calculated by subtracting the weight of the left poles from the weight of the right kidney removed. The mean percentage of remnant renal mass in 3/4nx rats was 28 ± 3%. Control animals were rats submitted to sham surgery under similar conditions; however, their kidneys remained intact, providing five sham-operated (Sham) rats. After total recovery from surgery (4–6 h), both groups of rats were placed in an animal facility where they had free access to food and water. Survival rate was 100%.

Twelve days after surgery, the rats were placed in metabolic cages (Tecniplast, Buguggiate-VA, Italy) for the collection of 24 h urine. Fourteen days after the surgery, the animals were anaesthetised with pentobarbital sodium (60 mg/kg; i.p.). Blood was collected from the heart in tubes containing lithium/heparin for later determination of sodium and creatinine. Thereafter, segments of jejunum with approximately 10 cm in length were removed, opened longitudinally with fine scissors and rinsed free from blood and intestinal contents with cold saline. Some segments were used for the assay of Na\(^+\),K\(^+\)-ATPase activity in jejunal epithelial cells. In other segments, the mucosa was removed with a scalpel for later determination of AADC activity. Other fragments of mucosa, weighing around 100 mg, were placed in vials containing 1 mL of 0.2 mol/L perchloric acid and stored at −80°C until quantification of dopamine, L-Dopa and norepinephrine by high-performance liquid chromatography (HPLC) with electrochemical detection.

**In vitro studies**

**L-amino acid decarboxylase activity**

L-amino acid decarboxylase activity was determined in homogenates of jejunal mucosa, using L-Dopa (0.1–10 mmol/L) as substrate.\(^{14,16}\) 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 method of Bradford.\(^{17}\)

**Na\(^+\),K\(^+\)-ATPase activity**

Na\(^+\),K\(^+\)-ATPase activity was measured by the method of Quigley & Gotterer\(^{19}\) adapted in our laboratory with slight modification.\(^{19}\) The rat jejunal epithelial cells were isolated as described by Vieira-Coelho.\(^{12}\) Thereafter, 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 permeabilisation. The reaction mixture, in the final volume of 1.025 mL, contained: 37.5 mmol/L imidazole buffer, 75 mmol/L NaCl, 5 mmol/L KCl, 1 mmol/L sodium ethyleneglycolctetaecetic acid (EGTA), 5 mmol/L MgCl\(_2\), 75 mmol/L Na\(_2\)SO\(_4\), 75 mmol/L L-tris(hydroxymethyl)aminomethane(tris) hydrochloride and 100 µL cell suspension. For determination of ouabain-insensitive ATPase, NaCl and KCl were omitted, and Tris-HCl (150 mmol/L) and ouabain (1 mmol/L) were added to the assay. The reaction was initiated by addition of 4 mmol/L tris salt adenosine 5′-triphosphate (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 (2665 g) 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 was expressed as nanomoles of Pi per milligram of protein per minute and determined as the difference between total and ouabain-insensitive ATPase. The protein content in cell suspension (1.1 mg/mL) was determined by the method of Bradford.\(^{17}\)

**Assay of catecholamines**

The assay of dopamine in samples from AADC studies and the assay of dopamine, L-Dopa and norepinephrine in jejunal mucosa was performed by HPLC with electrochemical detection, as previously described.\(^{20}\) In our laboratory, the lower limit of detection ranged 350–1000 fmol.

**Plasma and urine ionogram and biochemistry**

Ion-selective electrodes performed the quantifications of sodium whereas creatinine was measured by the Jaffé method. The assays were...
performed in Cobas Mira Plus analyser (ABX Diagnostics, Kaiseraugst, Switzerland). Creatinine clearance was calculated using 24 h urine creatinine excretion. Fractional excretion of sodium (FE_{Na\text{c}}) was calculated as previously reported.\textsuperscript{14}

### Drugs

The compounds ATP; dopamine hydrochloride; L-Dopa, norepinephrine and ouabain 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 enzymatic assay were calculated from non-linear regression analysis using GraphPad Prism statistics software package\textsuperscript{21} 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

Ablation of renal mass had no effects on body growth, as 3/4nx rats attained the same weight 14 days after surgery as Sham rats did (Table 1). Kidney growth, however, was significantly altered in 3/4nx rats; 14 days after surgery the 3/4nx rats presented a hypertrophied remnant renal mass, weighing 95 ± 5% more than on the day of surgery. The 3/4nx rats presented a 55% reduction in creatinine clearance values accompanied by a 146% increase in fractional excretion of sodium (Table 1). Both systolic and diastolic blood pressures were higher in 3/4nx rats than in Sham animals (Table 1). Heart rate did not differ between the two groups.

The activity of AADC was determined in homogenates of jejunal mucosa with L-Dopa as substrate, which resulted in a concentration-dependent formation of dopamine (Fig. 1). The V_{max} values for AADC activity in jejunal mucosa were found to be significantly lower in 3/4nx rats than in Sham animals (Table 2). The decarboxylation reaction was a saturable process, with K_{m} values of the same magnitude in the two groups (Table 2). The tissue levels (in pmol/g) of L-Dopa (23.7 ± 1.4 vs 23.5 ± 3.0), dopamine (11.9 ± 1.7 vs 10.2 ± 2.0) and norepinephrine (121 ± 8 vs 111 ± 11) in fragments of jejunal mucosa did not differ between 3/4nx and Sham rats.

The Na\textsuperscript{+},K\textsuperscript{+}-ATPase activity in the jejunal epithelial cells from 3/4nx and Sham rats is depicted in Figure 2. As can be observed, the jejunal epithelial cells from 3/4nx animals presented an increased Na\textsuperscript{+},K\textsuperscript{+}-ATPase activity 14 days after surgery. In addition, dopamine (1 \text{µmol/L}) failed to change the jejunal Na\textsuperscript{+},K\textsuperscript{+}-ATPase activity in either 3/4nx or Sham rats (Fig. 2).

### DISCUSSION

The present study was undertaken with the aim of clarifying the possible role of the jejunal Na\textsuperscript{+},K\textsuperscript{+}-ATPase and dopaminergic activities in the control of sodium homeostasis in 3/4nx rats, an animal model of chronic renal insufficiency. We found increased jejunal Na\textsuperscript{+},K\textsuperscript{+}-ATPase activity in 3/4nx rats. In addition, the jejunal dopaminergic activity was

![Fig. 1 Aromatic L-amino acid decarboxylase (AADC) activity in homogenates of jejunal mucosa obtained from sham-operated (Sham, □) and 3/4 nephrectomised (3/4nx, ■) rats 14 days after surgery. AADC activity is expressed as the rate of formation of dopamine versus the concentration of L-Dopa. Symbols represent the means of 4–6 experiments per group and error bars represent SE.](image)

### Table 1

<table>
<thead>
<tr>
<th></th>
<th>Sham</th>
<th>3/4nx</th>
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<tbody>
<tr>
<td>Body weight (g)</td>
<td>258 ± 5</td>
<td>253 ± 2</td>
</tr>
<tr>
<td>Ccreatinine (mL/min)</td>
<td>1.95 ± 0.17</td>
<td>0.88 ± 0.07*</td>
</tr>
<tr>
<td>FE_{Na\text{c}} (%)</td>
<td>0.41 ± 0.04</td>
<td>1.01 ± 0.07*</td>
</tr>
<tr>
<td>Systolic BP (mmHg)</td>
<td>123 ± 3</td>
<td>139 ± 4*</td>
</tr>
<tr>
<td>Diastolic BP (mmHg)</td>
<td>80 ± 3</td>
<td>106 ± 4*</td>
</tr>
<tr>
<td>Heart rate (beats/min)</td>
<td>415 ± 12</td>
<td>473 ± 32</td>
</tr>
</tbody>
</table>

*Significantly different from corresponding values in sham-operated rats (\( P < 0.05 \)). Values are means ± SE; \( n = 4–6 \) experiments per group.

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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.6,12,22 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.23,24 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 early postnatally.25 The lack of effect of dopamine on jejunal Na⁺,K⁺-ATPase activity in adult animal coincided with the period in which renal function reached maturation.12,22 Recently, a reduction in jejunal Na⁺,K⁺-ATPase activity with recovered sensitivity to inhibition by dopamine was reported in rats submitted to uninephrectomy.13 Because the driving force that energises all mechanisms of jejunal sodium absorption is the hydrolysis of ATP catalysed by Na⁺,K⁺-ATPase located at the basolateral membrane of intestinal epithelial cells,26 those findings suggested that a decreased jejunal sodium absorption may have contributed to the maintenance of sodium homeostasis in uninephrectomised animals and further reinforced the view that the influence of dopamine on jejunal Na⁺,K⁺-ATPase activity may assume particular importance when the renal function is compromised.

In the present study, we found reduced AADC activity in jejunal epithelial cells from 3/4nx animals. Because the jejunal Na⁺,K⁺-ATPase activity was increased in 3/4nx rats, one could hypothesise that the lack of jejunal dopamine might contribute to the enhanced jejunal sodium absorption in 3/4nx rats. However, the jejunal Na⁺,K⁺-ATPase activity in 3/4nx animals was not sensitive to inhibition by dopamine. One can conclude therefore that the jejunal dopaminergic activity may respond differently depending on the percentage of reduction in renal mass. In uninephrectomised rats, dopamine contributes to the decrease in jejunal sodium transport and might be of importance in the maintenance of sodium homeostasis whereas in the presence of a more severe reduction in renal mass (3/4nx) dopamine fails to inhibit the enhanced jejunal sodium transport and this may contribute to the compromising of sodium homeostasis during early chronic renal insufficiency. The finding that the blood pressure values were increased in 3/4nx animals but not in uninephrectomised rats would be in agreement with this suggestion. It should be mentioned, however, that Pacha found no relationship between intestinal sodium absorption and experimental salt hypertension in either salt-sensitive or salt-resistant Dahl rats.27

The reduced jejunal AADC activity in 3/4nx rats was not accompanied by changes in the jejunal tissue levels of either L-Dopa or dopamine. The explanation for this apparent discrepancy may have to do with the fact that the jejunal tissue levels of L-Dopa and dopamine may reflect not only their concentrations in jejunal epithelial cells but also the concentrations of L-Dopa and dopamine in sympathetic neurons where dopamine is the precursor of norepinephrine.

Because our results were negative in terms of the role of the dopaminergic system in the jejunum, one can hypothesise that other mechanisms involved in sodium transport can account for the increase in jejunal Na⁺,K⁺-ATPase in chronic renal insufficiency. Norepinephrine is well-recognised to stimulate sodium transport by increasing the jejunal Na⁺,K⁺-ATPase activity. However, the jejunal tissue levels of norepinephrine did not differ between the 3/4nx and Sham rats, suggesting that the increase in jejunal sodium transport in 3/4nx rats is not related to enhanced sympathetic activity. It is recognised that the colonic potassium secretion is enhanced in chronic renal insufficiency by aldosterone and angiotensin-dependent mechanisms.28 In addition, jejunal Na⁺,K⁺-ATPase activity was found to be increased in rats with acute renal failure, and this was suggested to be related to serum aldosterone elevation.29 Although we did not measure the plasma levels of aldosterone in 3/4nx rats, it is worth mentioning that an increase in plasma aldosterone levels, due to enhanced adrenal production, was reported in a chronic renal failure rat model similar to ours (5/6nx rats).30

Besides aldosterone, other mediator mechanisms, such as angiotensin, ANP, nitric oxide and 5-hydroxytryptamine, can modulate the intestinal sodium transport and deserve further study.
We conclude that, in contrast to uninephrectomised rats, the 3/4nx animals present a reduced jejunal dopamine synthesis going along with a dopamine insensitive enhanced jejunal Na\(^+\)-K\(^+\)-ATPase activity.

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