Eur J Nutr (2009) 48:437–445
DOI 10.1007/s00394-009-0032-z

ORIGINAL CONTRIBUTION

Chronic undernutrition alters renal active Na^+ transport in young rats: potential hidden basis for pathophysiological alterations in adulthood?

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Received: 3 December 2008 / Accepted: 27 April 2009 / Published online: 12 May 2009

Abstract
Background Epidemiological studies in the northeastern region of Brazil show an association between hypertension and malnutrition, especially in areas where protein-deficient diets are combined with high salt intake.
Aims of study We studied the consequences of a widely consumed deficient diet (basic regional diet, BRD), combined with high NaCl, on growth, renal Na^+ and water handling and activities of ATP-dependent Na^+ transporters in kidney proximal tubules.
Methods Young rats were fed after weaning with a low-protein and high-salt diet, which mimics that used in a vast region of Brazil. Body mass was evaluated from weaning up to the 19th week of age. Glomerular filtration rate, proximal Na^+ reabsorption, distal Na^+ delivery, urinary excretion of Na^+ and water, and urine concentration capacity were evaluated from serum and urine concentrations of creatinine, Na^+ and Li^+, and by measurement of urinary volume and density. The $(Na^+ + K^+)$ATPase and the ouabain-insensitive Na^+-ATPase were studied in vitro by measuring ATP hydrolysis. Expression of $(Na^+ + K^+)$ATPase was evaluated by immunodetection with the use of a specific antibody anti α1-catalytic subunit isoform.
Results Undernourished rats reached early adulthood (14 weeks) with body and renal masses that were 2.3 times lower than controls. These rats became hypertensive (mean arterial pressure 18.7 ± 0.6 kPa vs 15.5 ± 0.9 kPa in control group) and showed augmented fractional proximal Na^+ reabsorption ($61.0 \pm 0.3\%$ vs $81.8 \pm 2.2\%$) with a concomitant decrease in distal Na^+ delivery (9.5 ± 0.5 μmol/min vs 14.0 ± 0.2 μmol/min per 100 g body weight). Urinary Na^+ excretion was higher in BRD rats, (juvenile and adult) being however twice the increase in Na^+ intake. The ATP-dependent Na^+ transporters were affected in opposite ways. The $(Na^+ + K^+)$ATPase activity from undernourished rats fell by 30%, in parallel with a 20% decrease in its immunodetection, whereas the ouabain-insensitive Na^+-ATPase, which is responsible for the fine-tune control of Na^+ reabsorption, increased three-fold.
Conclusions We conclude that early alterations in proximal tubule Na^+ pumps, together with an abnormally augmented

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urinary Na^+ excretion, might be the link between undernutrition and late renal dysfunction.

Keywords Chronic undernutrition · $(Na^+ + K^+)$ATPase · Na^+-ATPase · Renal sodium handling · Alterations in renal transporters

Introduction

During the past decade, it has become apparent that the risk of disease in adult life is closely related to environmental factors that impair fetal growth. The most significant factor is malnutrition, a serious public health problem worldwide, principally in underdeveloped countries [2]. Human epidemiological studies have demonstrated a relationship between undernutrition and the risk of developing hypertension [10], coronary heart disease [25] and renal disease [15]; for a recent general review see Ref. [11]. These studies are supported by a broad range of experimental animal models showing that the renal system in the offspring is extremely sensitive to mild shifts in maternal nutrition [5].

In Brazil, especially in the northeast, many epidemiological studies have shown moderate to severe malnutrition [30, 35]. In contrast to intrauterine undernutrition, few if any studies have been performed on animal models to identify the cellular and molecular mechanisms that may be involved in the cardiovascular and renal alterations in chronic undernutrition associated with high salt intake. In this study, we induced malnutrition using a diet derived from the basic regional diet (BRD) available in the vast sugarcane cultivation areas in northeastern Brazil. This diet induces a high incidence of malnutrition of different degrees in the population of this region [30]. Essentially, BRD is deficient in proteins, and depending on the subregion of the Brazilian northeast, on the season and on cultural food preparation habits, the salt content of the diet is high [20] and so BRD could be rich in NaCl. This is the reason why we combined the protein-deficient diet with high NaCl content to promote undernutrition in the experimental group. The human epidemiological trends mentioned above strongly support the hypothesis that it could have led to metabolic and physiological changes in local children and young people. These modifications may have evolved to culminate in the establishment of cardiovascular and renal diseases in the adult population, in which the underlying cellular and molecular mechanisms are poorly understood [21].

Impaired global Na^+ and water handling in undernutrition, as well as metabolic alterations, were also described several years ago (for review see Ref. [4]). However, possible molecular alterations in the energy-driven renal Na^+ transporters, which could underlie the modifications described so far, have not been studied. The present work was undertaken to investigate the impact of undernutrition associated with chronic intake of a NaCl-enriched BRD on functional parameters in proximal tubules and, at a molecular level, on the two ATP-dependent Na^+-pumping activities found in the kidney [3, 7, 37].

Materials and methods

All the reagents used were of the highest purity available. Goat polyclonal antibody against the $(Na^+ + K^+)$ATPase α1-catalytic subunit (α1[N-15]) was from Sigma Chemical Co. and the anti-goat secondary antibody was from Santa Cruz Biotechnology.

Ethical considerations

All procedures used were performed in accordance with "The guide for care and use of laboratory animals" [DHHS Publication No. (NIH) 85-23] and also approved by the Committee for Experimental and Animal Ethics at the Federal University of Pernambuco and the Federal University of Rio de Janeiro.

Animals

Male Wistar rats were weaned at the end of the 3rd week and reared four per cage and, except when otherwise indicated, had free access to food and water in a room maintained at 25 ± 1 °C with a 12-h light–dark cycle. For the study of renal function parameters, the rats were kept in individual metabolic cages only during the experimental procedures (3 h) in order to avoid chronic social isolation and adrenal-dependent elevation of blood pressure. The four age-matched groups of control and undernourished rats were separated randomly for independent determinations, at 8, 14, 17 and 19 weeks after birth, of renal physiological parameters and in vitro $(Na^+ + K^+)$ATPase and ouabain-insensitive Na^+-ATPase activities, as well as for Western blotting analysis of the first protein in the membranes of proximal tubule cells.

Diets and induction of undernutrition

A balanced commercial rodent chow purchased from Purina-Agribrands was given to the control groups (CD), while BRD was supplied to the experimental rats. The protein-deficient BRD was prepared according to Ref. [30] with augmented NaCl content to allow the study of the influence of combined low protein/high salt on renal function. It had the following ingredients (in g%): manioc

flour (64.8), beans (18.3), sweet potatoes (12.8) and cured meat (3.7). The meat was partially desalted; each component was cooked, dehydrated at 60 °C, pulverized and mixed with water to obtain a consistent mass. Meat fat (0.3 g%) was then added and the mixture was shaped into squares that were dehydrated at 60 °C for 24 h. The components (in g%, wet weight) of CD and BRD diets were, respectively: protein 23.0 and 7.9, carbohydrates 44.5 and 69.2, fat 2.5 and 0.8, fiber 7.2 and 8, mineral mix (except NaCl) 7.8 and 5.3, NaCl 0.16 and 0.33. The NaCl content was assessed at the Department of Nutrition, Federal University of Pernambuco. The other values for BRD were as in Ref. [30]. The total kcal/100 g was 290 and 314 for CD and BRD, respectively. The rats were weighed weekly to obtain growth curves. Food and water intake were recorded at the last day of the corresponding week and Na^+ intake was estimated by the product of food consumption and dietary Na^+ content. Data correspond to the end of the linear and faster phase of growth (8th week of age, 5th week after weaning) and to the end of the growth trajectory (17th week of age, 14th week after weaning). At the 17th week of age, the proximal tubule physiological parameters were also measured.

Study of renal function in growing and adult rats

The renal index (left plus right kidney weight/body weight) was measured at the early adult stage (14th week) when one age-matched group was killed and the organs removed to study the two Na^+ pumps. Renal function parameters were investigated during growth (8th week) and in adult rats (17th week). These stages were also used for measuring total plasma protein concentration by refractometry. Glomerular filtration rate (GFR) was measured by creatinine clearance [18]. Values for Li^+ clearance (Cl_{Li}) were obtained as follows. At 12 h prior to urine collection, rats were given 0.06 mmol LiCl orally/100 g body weight, which was enough to achieve plasma Li^+ concentrations of 0.23 ± 0.07 (CD group) and 0.23 ± 0.02 μmol/ml (BRD group). This Li^+ administration is well below nephrotoxic levels and therefore does not promote alterations in GFR as observed in chronic treatments in humans [23]. Overnight, the rats were allowed water ad libitum, but not food. In order to suppress the effects of antidiuretic hormone on urinary volume, the rats received water by gavage, 3 ml/100 g of body weight 90 min before and 2 ml/100 g 30 min before being placed in the cages. Blood was collected in non-heparinized tubes. Urinary density was assessed by refractometry (Atago); Na^+, Li^+ and creatinine concentrations in serum and urine were measured with a Cobas Mira analyzer (Roche). Renal concentration capacity was assessed by the response to 12 h of water deprivation at two different times in the same group of rats. Urine density was measured in rats before (at the 17th week) and after a dietary supplement of 0.1 g urea/100 g of body weight for 7 days, as previously done in humans [13].

Blood pressure determinations

Arterial pressure was assessed in adult rats by the tail-cuff method using a Letica LE 5000 apparatus, which allows repeated and reliable measurements in a short period in conscious animals [12]. For this purpose, the rats were kept at 30–32 °C for 30 min and three successive determinations were performed on each animal. This procedure was repeated on 3 consecutive days during the 14th week of life.

Preparation of membrane-enriched fraction

The microsomal fraction was obtained using a previous method, with several and random controls for contamination of intracellular structures in the cell membrane fraction [36]. The membranes were prepared from the outermost cortex (*cortex corticis*), a region of the renal tissue in which more than 90% of the cell population correspond to proximal tubules [37]. The kidneys were collected and placed in a cold solution containing 250 mM sucrose, 10 mM Hepes–Tris (pH 7.4), 2 mM EDTA and 0.15 mg/ml trypsin inhibitor (Sigma-Aldrich; type II-S). Thin transverse slices of the *cortex corticis* were removed using a Stadie-Riggs microtome and carefully dissected using iridectomy scissors to avoid contamination with the rest of the tissue. The suspension of fragments was homogenized in the same cold solution (4 ml/g) using a Teflon/glass homogenizer. The homogenate was processed as described in Ref. [36] and the final pellet was resuspended in 250 mmol/l sucrose at a final concentration of 5–15 mg protein/ml and stored at −4 °C. Protein determination was as described in Ref. [17].

$(Na^+ + K^+)$ATPase and ouabain-insensitive Na^+-ATPase in proximal tubule cells

$(Na^+ + K^+)$ATPase activity was measured using a colorimetric method [29]. Ouabain-insensitive Na^+-ATPase was measured by the difference in P_i release in the absence and presence of 2 mM furosemide [16]. The reaction conditions are described in Fig. 3.

SDS-PAGE and imumunoblotting

Electrophoresis of membrane proteins, followed by immunodetection of proximal tubule $(Na^+ + K^+)$ATPase, was carried out as described elsewhere [34], using goat polyclonal antibody against the $(Na^+ + K^+)$ATPase α1-subunit (α1[N-15]). Each gel was stained with Ponceau

Red to evaluate the amount of protein loaded on each lane cand to normalize the levels of expression to the total protein loaded. The protein band at 104 kDa detected by immunoblotting was quantified using Scion Image software.

Results

Figure 1 shows the growth curves for CD (filled circles) and BRD (open circles) rats. The latter attained a maximum adult body weight (BW_{max}), the asymptotic endpoint of growth, which was only 30% of the CD group (146 ± 4.3 g vs 481 ± 20.6 g). However, the BRD group grew significantly faster: its growth rate constant was higher and its half-time ($T_{1/2}$) for maximal growth decreased significantly when compared with the CD group. The renal index (inset to Fig. 1) was similar in both groups, indicating that malnutrition affects the trajectories of both body and kidney growth to the same extent.

Figure 2 shows urinary Na^+ and water excretion and urine density in CD and BRD rats at 8 and 17 weeks after birth. These stages of development were chosen because kidney development in rats (organ growth associated with both hyperplasia and hypertrophy) is considered complete just before 8 weeks [6, 28], while body weight approaches the asymptotical B_{max} value (Fig. 1) at 14 weeks after weaning (17 weeks of age). The evolution of the ability to retain water in BRD rats does not parallel the development of Na^+ retention (Fig. 2a), since the water excretion rate is the same at 8 and 17 weeks (Fig. 2b). These latter results match those shown in Fig. 2c: while urine density increases in the CD group when the rats reach adulthood, the urine remains dilute in the BRD rats despite the higher dietary Na^+ content and even when they are challenged by water deprivation (Fig. 2d). Table 1 presents the data of food and water intake at the end of the 8th and 17th weeks of age when the determinations of Na^+ and water excretion were carried out. When the dietary Na^+ intake is compared with the urinary Na^+ excretion (Fig. 2), it becomes clear that the rise in urinary Na^+ loss in the BRD group is twice the increase in its Na^+ ingestion.

Table 2 shows the BRD-induced modifications in physiological parameters concerning Na^+ and water handling by the proximal tubules. The GFR increases in BRD rats, probably because of the hypoproteinemia (5.7 ± 0.3 g% vs 7.2 ± 0.5 g% in the CD group), which decreases the resistance to ultrafiltration [26]. Table 2 also demonstrates an increase in fractional proximal tubule Na^+ reabsorption in the BRD group. This rise cannot be taken as a physiological response to an increased GFR because the hypoproteinemia in BRD rats must disrupt the normal glomerulo-tubular balance [27]. Accordingly, the distal

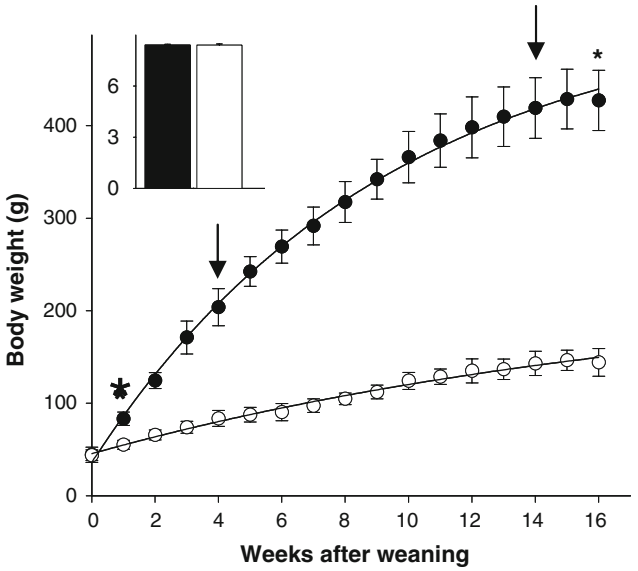

Fig. 1 Time course of body weight (BW) gain. After weaning (end of the 3rd week of age), rats fed with the control diet (CD, *filled circles*) or basic regional diet (BRD, *empty circles*) were weighed regularly until the 16th week (19th after birth). In the chronic undernutrition group, the end point of growth was significantly different from that in the normal diet controls. Therefore, a simplified equation that fitted adequately to the experimental points was obtained (body weight BW_t was used to obtain a rate constant (k) that describes the growth trajectories along the time after weaning (t), as well as the adult body weight (BW_{max}): $BW_t = BW_o + BW_{max}\ (1 - e^{-kt})$ where BW_o is the weight at the moment of weaning. $^*P < 0.001$ (t test; only two symbols are included for simplicity); values are mean ± SEM, $n = 11$ (each diet-matched group). The rate constants of growth were: $k = 0.15 \pm 0.01$ and 0.21 ± 0.01/week ($P < 0.001$, t-test) for CD and BRD groups, respectively. The half-times for maximal growth were $T_{1/2} = 7.9 \pm 0.3$ and 6.5 ± 0.2 week after birth ($P < 0.01$, t-test) for CD and BRD, respectively. To calculate $T_{1/2}$ ln 2 was divided by each k value and 3 weeks (weaning time) was added. *Inset*: renal index measured by the ratio between the total kidney weight (in mg) and the body weight (in g) for CD (*filled bar*) and BRD (*open bar*). *Arrows* indicate the weeks chosen for measurements of renal functional parameters. Food, Na^+ and water intake were also registered in these weeks

delivery of Na^+ in BRD rats is lower by a similar amount than that in normal animals. Li^+ clearance also decreases in the undernourished rats, thus confirming that fractional reabsorption of Na^+ in proximal tubules is enhanced.

The following experiments were performed to investigate whether modifications in the ATP-dependent Na^+ transporters of the proximal tubule could explain the increased fractional Na^+ reabsorption demonstrated in Table 2. Figure 3 shows that the $(Na^+ + K^+)$ATPase activity is significantly decreased in membranes from BRD rats. Since the pumping activities in whole cell homogenates of CD and BRD rats are identical (Fig. 3a), it is clear that undernutrition leads to misrouting of the ouabain-sensitive Na^+ pumps and lower-than-normal insertion into the basolateral membranes. Western blotting for the α1-catalytic subunit of $(Na^+ + K^+)$ATPase (Fig. 3b) in

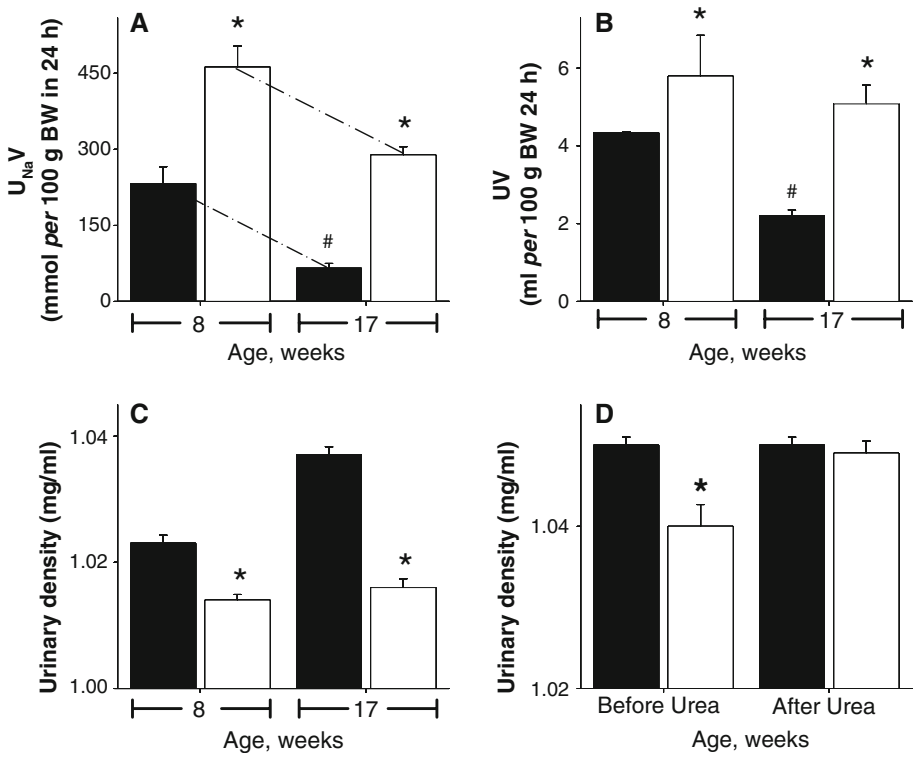

Fig. 2 Alterations of urinary parameters and distal water handling in chronic undernutrition. **a** Na^+ excretion, **b** urine volume and, **c** urinary density in CD (*filled bars*) and BRD rats (*open bars*). The renal parameters shown were determined at the ages indicated on the *abscissa*, as described under "Materials and methods". $^*P < 0.001$ with respect to the diet-matched group; $^{\#}P < 0.001$ with respect to the age-matched group; values are mean ± SEM, $n = 11$ (each group). The *dotted–dashed lines* indicate that the trajectories of the evolution of Na^+ excretion are parallel between 8th and 17th weeks in the age-matched groups. **d** Urine density was measured in rats fed with CD (*filled bars*) or BRD (*empty bars*) before (at the 17th week) and after a 7-day dietary urea supplementation, as indicated on the *abscissa*. $^*P < 0.05$ with respect to the other three groups; $n = 8$ (each group)

Table 1 Food, Na^+ and water intake in BRD rats at the juvenile (8th week) and adult (17th week) phases of growth

Intake (per 100 g BW)	8th Week		17th Week	
	CD rats	BRD rats	CD rats	BRD rats
Food (g)	6.2 ± 0.1 (8)	4.5 ± 0.3 (8)*	5.2 ± 0.2 (12)	7.6 ± 0.1 (12)*
Na^+ (mg)	9.9 ± 0.2 (8)	14.8 ± 0.9 (8)*	8.2 ± 0.3 (12)	25.2 ± 0.3 (12)*
Water (ml)	9.0 ± 0.5 (11)	5.9 ± 0.6 (9)*	2.6 ± 0.1 (10)	4.9 ± 0.3 (11)*

Food, Na^+ and water ingestion were recorded on the last day of the corresponding week. The weeks chosen for the register of food, Na^+ and water ingestion correspond to those in which renal function parameters were measured (Fig. 2). Data collections correspond to the end of the linear and faster phase of growth (8th week of age, 5th week after weaning) and to the end of the growth trajectory (17th week of age, 14th week after weaning; arrows in Fig. 1). Values are mean ± SEM

n Numbers in parentheses

$^*P < 0.001$ with respect to CD group

the basolateral membrane-enriched fraction also shows a significant decrease in the specific signal. This agrees quantitatively well with the diminished activity shown in Fig. 3a, confirming that chronic administration of BRD affects the targeting of this pump to the membranes. In contrast, the ouabain-insensitive Na^+-ATPase activity is increased in young adult (14 weeks old) BRD rats (Fig. 3c) and a further relative increase over the control value becomes apparent when this activity is corrected for the renal weight (Fig. 3d), indicating that the smaller the kidney, the higher the specific activity of this Na^+ pump. Successive (3 days) measurements of mean arterial pressure at the same age reveal a significant increase from 15.5 ± 0.9 (CD rats) to 18.7 ± 0.6 kPa (BRD rats).

Table 2 Alterations in proximal tubule physiological parameters in BRD rats (17th week)

Parameters	CD rats	BRD rats
Cl_{cr} (μl/min/100 g BW)	259.3 ± 22.7	380.9 ± 43.6*
P_{Na} (μmol/ml)	140.8 ± 2.1	139.2 ± 1.6 (NS)
FL_{Na} (μmol/min/100 g BW)	36.3 ± 3.0	52.6 ± 6.0*
Cl_{Li} (μl/min/100 g BW)	99.3 ± 15.3	75.1 ± 8.8*
DD_{Na} (μmol/min/100 g BW)	14.0 ± 0.2	9.5 ± 0.5*
$FPT_{Na}R$ (%)	61.0 ± 0.3	81.8 ± 2.2*

Values are mean ± SEM, $n = 8$ (each group). The following expressions were employed to calculate the renal physiological parameters. Glomerular filtration rate from the clearance of endogenous creatinine: $Cl_{cr} = U_{cr} \times V/P_{cr}$, where V is the urinary volume (in ml/24 h) and U_{cr} and P_{cr} are the urinary and plasma creatinine concentrations, respectively (in μmol/ml); urinary Na^+ excretion (in μmol/24 h) $= U_{Na} \times V$, where U_{Na} is the urine concentration of Na^+ (in μmol/ml) and V is the urinary volume (in ml/24 h); Na^+ filtered load (FL_{Na}, in μmol/min) = GFR (in μl/min) × P_{Na} (Na^+ plasma concentration in μmol/ml); Li^+ clearance (Cl_{Li}, in μl/min) = $U_{Li} \times V/P_{Li}$, where V is the urinary volume and U_{Li} and P_{Li} are the urinary and plasma Li^+ concentrations, respectively (in μmol/ml); Na^+ distal delivery (in μmol/min) estimated by: $DD_{Na} = Cl_{Li} \times P_{Na}$, because Cl_{Li} reflects the delivery of fluid from the proximal tubules to the distal regions and P_{Na} is considered to be similar to luminal Na^+ concentration (adapted from Ref. [27, 31]); fractional proximal tubule Na^+ reabsorption: $FPT_{Na}R$ (%) = $[(FL_{Na} - DD_{Na})/FL_{Na}] \times 100$. The physiological parameters were normalized to 100 g of body weight (BW) when appropriate

NS not significant

*$P < 0.001$ (t-test) in all cases

Discussion

In this study, we investigated the effects of simultaneous low protein and high sodium intake in rats in a combined model diet that mimics the diet of an impoverished regional Brazilian population. The aim was to study its impact on active Na^+ transporters in rat proximal tubule cells at a molecular level. Epidemiological data show a high prevalence of arterial hypertension in individuals with history of undernutrition [9], especially with high dietary Na^+ [20]. This suggests the possibility that alterations in renal Na^+ handling could be a key point in the establishment of hypertension associated with undernutrition in these Brazilian populations together with other environmental and genetic factors. For this reason, the $(Na^+ + K^+)$ATPase and the ouabain-insensitive Na^+-ATPase activities were investigated in the middle of the growth trajectory (14 weeks) when the onset of hypertension was detected.

It is noteworthy that young rats exposed to a low-protein diet in the uterus display a 36% lower $(Na^+ + K^+)$ATPase activity than controls 4 weeks after birth [1], indicating that the lower $(Na^+ + K^+)$ATPase activity could be a consequence of protein deprivation at different stages of renal development. In contrast, in young rats from undernourished dams, several Na^+ transporters are highly expressed in luminal membranes [19], so apical Na^+ fluxes are augmented and more Na^+ reaches the pumps located in the opposite side of the cells. Recent results obtained with a normosodic hypoproteic diet (8–9% protein) also show a 100% increase in Na^+-ATPase, as in the case of BRD (unpublished observations). The results from Ref. [19] and ours, especially the contrasting observations presented in Fig. 3a–d, clearly indicate that undernutrition before and after birth can selectively alter different Na^+ transporters in opposing ways.

This selectivity clearly emerges when the results concerning $(Na^+ + K^+)$ATPase and Na^+-ATPase are compared. One of the main findings is the huge increase in the Na^+-ATPase activity despite the higher dietary Na^+ content, which correlates with the observed increase in Na^+ proximal reabsorption. The augmented turnover of this pump, which is considered to be the molecular machinery responsible for the fine-tune control of Na^+ fluxes across the proximal tubule cells [3, 16, 24], reveals an unexpected response toward a higher salt intake that, if sustained in adult life, will lead to altered and worsening renal and cardiovascular responses [2, 15]. Absolute proximal tubule Na^+ reabsorption increases in rats fed with high Na^+ and this was associated with changes in local Ang II formation [22]. Increased local Ang II under conditions of high Na^+ intake can alter the complex proximal balance involved in Na^+ homeostasis. We demonstrated that Ang II is one of the most important activators of the ouabain-insensitive Na^+ pump in a process in which AT_1 receptors are involved [24], so local modifications in Ang II content may also be associated with stimulation of both the ouabain-insensitive Na^+-ATPase (Fig. 3c, d) and the macula densa responses to a diluted distal fluid under low-protein diet [8].

The volume of luminal fluid leaving the proximal tubule is lower in BRD than in CD rats as indicated by the reduced Cl_{Li} (Table 2). Despite the moderate decrease in $(Na^+ + K^+)$ATPase, this may be due to the enormously increased proximal Na^+ reabsorption mediated by the ouabain-insensitive Na^+-ATPase followed by passive water fluxes. After delivery of the fluid to the descending branch of Henle's loop, it is reasonable that water reabsorption is compromised. Urea concentration in the internal medullar interstitium diminishes in a protein-deficient diet, while urea supplementation in the diet restores the capacity to concentrate the urine [22], as also found in BRD rats. Urine density increased significantly in the CD rats during the trajectory to adulthood, whereas in the BRD group, despite the greater loss of Na^+ (Fig. 2a), it remained lower and constant, indicating that the countercurrent system had failed.

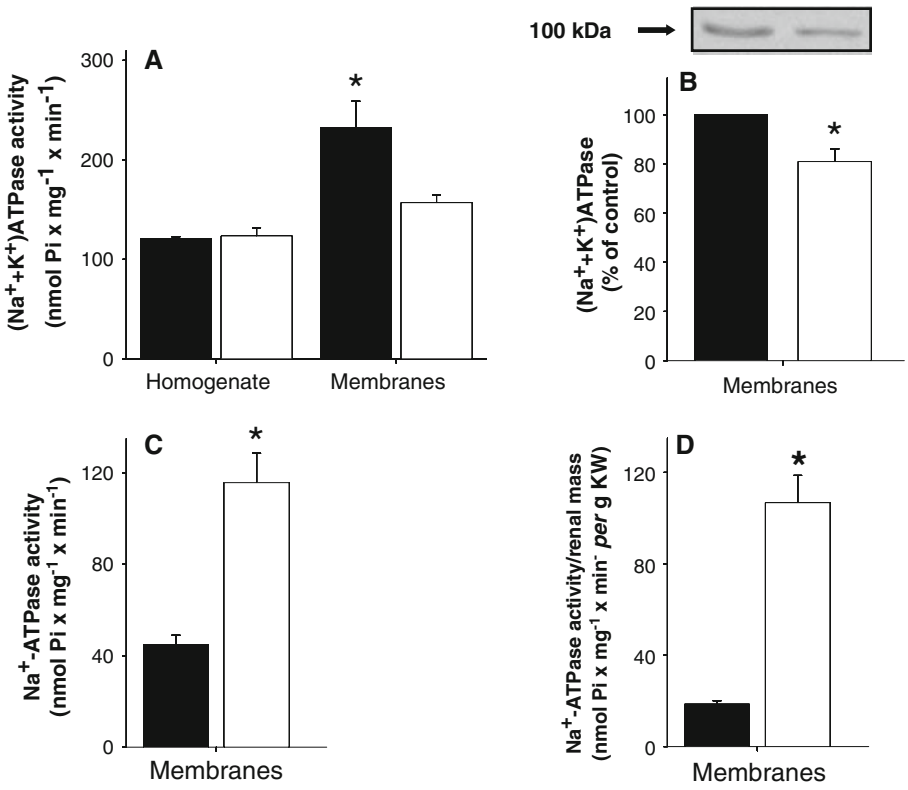

Fig. 3 Undernutrition decreases activity and targeting of $(Na^+ + K^+)$ATPase to the basolateral membranes in kidney proximal tubule cells and increases the activity of the ouabain-insensitive Na^+-ATPase. **a** $(Na^+ + K^+)$ATPase was measured in the whole homogenate and in the membrane-enriched fractions of CD (*filled bars*) and BRD rats (*empty bars*), as indicated on the *abscissa*. The reaction medium was (in mmol/l) 5 $MgCl_2$, 50 bis-Tris-propane (pH 7.4), 120 NaCl and 0.2 mg protein/ml (final concentration). The fractions were preincubated at 37 °C in the above medium in the absence or presence of 1 mmol/l ouabain for 10 min. Reactions (0.5 ml) were started by adding ATP and KCl (5 and 24 mmol/l, respectively, final concentrations) and stopped 10 min later by adding 1.5 ml activated charcoal in 0.1 mol/l HCl. After centrifugation (600 × *g*), aliquots of the supernatants (0.5 ml) were collected to measure the amount of P_i released. The $(Na^+ + K^+)$ATPase activity was calculated as the difference between P_i released in the absence and the presence of ouabain. Values are mean ± SEM, $n = 10$; $^*P < 0.001$ (*t*-test) with respect to the corresponding membrane preparation from diet-matched rats (**a**). **b** Representative immunodetection of $(Na^+ + K^+)$ATPase α1-catalytic subunit (*arrow* at 110 kDa) in the membrane fractions from the experimental groups shown in **a** and densitometric analysis of the immunodetection patterns, calculated by the ratio between the luminescence intensity (western blotting) and the staining intensity of the corresponding protein band. Values are mean ± SEM, $n = 3$; $^*P < 0.05$ (*t* test) with respect to the corresponding diet-matched membrane preparation. **c** Absolute activity values of ouabain-insensitive Na^+-ATPase activity measured in the membrane-enriched fraction from CD (*filled bars*) and BRD (*empty bars*) rats. The membranes were preincubated with 2 mmol/l ouabain at 37 °C for 10 min and then added to the assay medium (0.5 ml; 0.2 mg protein/ml, final concentration) containing (in mmol/l) 10 $MgCl_2$, 20 Hepes–Tris (pH 7.0), 120 NaCl and 5 ATP (3–5 Bq/nmol). The reaction was stopped after 15 min by adding 1.5 ml of 0.1 mol/l HCl-activated charcoal. After centrifugation (600 × *g*), aliquots of the supernatants (0.5 ml) were counted in a liquid scintillation counter to measure the $^{32}P_i$ released. The Na^+-ATPase activity was calculated from the difference between the $^{32}P_i$ released in the absence and in the presence of 2 mmol/l furosemide. **d** Na^+-ATPase activity normalized for renal weight (KW). Values are mean ± SEM, $n = 13$; $^*P < 0.001$ (*t* test) with respect to the corresponding diet-matched group

If removal of water along the descending portion of Henle's loop is compromised, passive NaCl efflux along the thin ascending limb is also decreased because the intraluminal fluid remains dilute. So, more water and Na^+ (in dilute solution) will reach the final segments of the nephron than under normal conditions [14]. In the collecting duct, the increased tubular fluid flow rate will impair Na^+ (and water) reabsorption, even in the case of increased Ang II and aldosterone plasma levels. BRD rats develop hypertension from 14 weeks of age, a clear signal that cardiovascular alterations occur in young rats and this could be associated with hyperactivity of the systemic renin/Ang II/aldosterone axis. The impaired distal handling of fluid would be aggravated by the lower interstitial osmolality [22], finally leading to increased excretion of a diluted urine, as shown in Fig. 2c, d, despite the increased loss of Na^+ (Fig. 2a). In addition, arrival of a more diluted fluid than usual at the macula densa region will evoke renin release and consequently activate the renin–angiotensin system and aldosterone release, further increasing the

blood pressure, as found in BRD rats. Impaired distal Na^+ handling is confirmed by the observation that besides the huge increase in proximal Na^+ reabsorption, the Na^+ excretion in BRD rats is augmented by 100 and 350% at 8 and 17 weeks of age, respectively, with respect to CD animals (Fig. 2a). Whereas, at the same ages, the dietary Na^+ intake in the malnourished group was 50 and 200% higher than that for the CD group.

Glomerular filtration rate increases in normal rats fed with a high-salt diet [32], whereas the opposite was found in diabetic animals in association with disturbed tubuloglomerular feedback [33], an indication that renal handling of water and salt is affected by metabolic imbalances. These alterations may later contribute to rendering the kidney more susceptible to prolonged injury. This proposal also emerged in a recent paper [38] on the effects of intrauterine growth restriction: altered nephrogenesis with normal behavior of the renin–angiotensin system would evolve toward impaired renal responses in postnatal life.

From the results described in the present work, we conclude that the alterations in proximal Na^+ pumps and in distal Na^+ handling may constitute the molecular link between undernutrition and adult metabolic, renal and cardiovascular dysfunctions.

Acknowledgments The skillful technical assistance of Glória Costa-Sarmento, José Paulo Soares and Gilsandro J. Barbosa da Silva is acknowledged. This research was supported by grants from the Brazilian Research Council (CNPq), the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), the Rio de Janeiro State Research Foundation (to Marcelo Einicker-Lamas and Adalberto Vieyra) and the José Bonifácio Foundation (to Lucienne S. Lara). João H. Costa-Silva and Nadir Pedi were recipients of a fellowship from CNPq; Paulo A. Silva and Ricardo Luzardo were recipients of a fellowship from CAPES.

Conflict of interest None of the authors have any conflict of interest.

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