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Natriuresis does not account for urinary concentration inability in the chronically undernourished rat

Variação nos teores de sódio em dieta multicarenciada: efeitos sobre alguns aspectos da função renal em roedores

Edinéia Goedert¹, Ana Durce Oliveira da Paixão¹, Carmen de Castro-Chaves^{*1}

Corresponding author: Carmen de Castro-Chaves, Departamento de Fisiologia e Farmacologia, Centro de Ciências Biológicas, Universidade Federal de Pernambuco, Av. Prof. Moraes Rego S/N, 50670-901, Recife, PE, Brazil. E-mail: cchaves@ufpe.br

Abstract

Through different concentrations of sodium in the diet, we investigated whether the natriuresis observed in the DBR rats since weaning resulted from the higher Na + intake or from its lower conservation by the kidney and its effect on the urinary concentration. For this purpose, we used a multicentre diet, locally called the regional basic diet (DBR) and 60 male Wistar rats, treated from weaning with: (i) control diet, by the American Institute of Nutrition (AIN); ii) DBR supplemented with low Na + content (0.06%), the DBR group; (iii) DBR supplemented with normal Na + content (0.3%), the DBRnorm group; iv) DBR supplemented with high Na + content (3.12%), the DBRhiper group. The excretion capacity in relation to sodium intake was similar between these two groups (99.01 ± 0.12 vs $99.02 \pm 0.17\%$). Malnutrition compromised the urinary concentration mechanism.

Keywords: Undernutrition, glomerular filtration rate, sodium balance, urinary concentration.

Resumo

Através de diferentes concentrações de sódio na dieta, investigamos se a natriurese observada nos ratos em DBR desde o desmame decorria do maior aporte de Na+ ou da sua menor conservação pelo rim e o seu efeito sobre a concentração urinária. Para isso utilizamos uma dieta multicarenciada, localmente denominada dieta básica regional (DBR) e 60 ratos machos Wistar, tratados a partir do desmame com: i) dieta controle, nos padrões da American Institute of Nutrition (AIN), o grupo controle (C); ii) DBR suplementada com baixo teor de Na+ (0,06%), o grupo DBRhipo; iii) DBR suplementada com teor normal de Na+ (0,3%), o grupo DBRnormo; iv) DBR suplementada com teor alto de Na+ (3,12%), o grupo DBRhiper. A capacidade de excreção em relação a ingestão de sódio apresentou-se similar entre estes dois grupos ($99,01 \pm 0,12$ vs $99,02 \pm 0,17\%$). A desnutrição comprometeu o mecanismo de concentração urinária.

Palavras-chave: Desnutrição, filtração glomerular em ratos, balanço de Na, concentração urinária.

Introduction

Natriuresis, defective water handling, impaired urine concentration, activation of renin-angiotensin-system, hypoproteinemia and metabolic alterations, were described in chronic undernutrition [1, 2]. Epidemiological studies in Pernambuco State, Brazil, found undernutrition mostly in children due to the diet consumed. These studies were the base for an experimental diet normal in carbohydrates but deficient in proteins, lipids, vitamins and minerals, and also in NaCl [3]. The effects of chronic undernutrition by this diet on renal function aspects have been addressed as follow. Adult rats with chronic undernutrition presented hypoproteinemia, renal vasodilatation and natriuresis leading to a negative balance, with unaltered GFR [4].

The intake of such a diet from weaning impaired renal Na⁺ and H₂O conservation, but increased GFR and proximal tubule re-absorption in adult rats with unaltered levels of nitric oxide in blood and urine [5]. Juvenile and adult undernourished rats urinary Na⁺ excretion was higher being twice the Na⁺ intake a in control group) and the adult undernourished showed augmented fractional proximal Na⁺ reabsorption ($61.0 \pm 0.3\%$ vs $81.8 \pm 2.2\%$) with a concomitant decrease in distal delivery (9.5 ± 0.5 l mol/min vs 14.0 ± 0.2 l mol/min per 100 g BW). At the molecular level, the lack angiotensin II sensibility could be due to Na⁺-ATPase hyperactivity and 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 threefold [5].

The present work was designed to verify if the natriuresis seen on rats on chronic undernutrition was due to increased dietary Na⁺ intake or to renal impairment to conserve this electrolyte. Additionally, the effects of variable dietary Na⁺ content and chronic undernutrition on renal concentrating mechanism were assessed. Plasma volume was measured in normal sodium content diets for its effect on effective circulatory volume.

Materials and methods

Materials

All reagents used here were of the highest purity available: creatinin kit Biosystem; Lithium chloride (Cloreto de lítio) P.A. VETEC, Sodium Chloride (Cloreto de sódio) P.A. VETEC.

Animals

Experiments were conducted in accordance with the Guide for the Care and Use for Laboratory Animals – EUA and were approved by the Ethics Committee for Animal Experimentation from the Federal University of Pernambuco, Brazil by report number N 029/06. Wistar rats were maintained in a room at 22 ± 2 °C, with 12-h light–dark cycle, 50% humidity, and reared at four per collective cage with free access to food and water, except when indicated. Only male rats were used in all experiments. All rats were weighed weekly from weaning, and had tail

blood collected at weaning and at three week intervals to measure hematocrit. 40 rats were previously adapted to individual metabolic cages, remaining there only during the experimental procedures [7]. At the completion of the 24h balance studies, renal concentration was assessed. At least 72 h later, the rats were treated with LiCl, as described below, to evaluate GFR and proximal tubule Na⁺ re-absorption.

Diets and experimental groups

Control diet (CD) was AIN 93 M [8], modified by adding NaCl to obtain a Na⁺ 0.3 g/100 g diet. The deficient diet (DD) was prepared according to Teodósio et al, 1990 [3] with the following ingredients (g/100g): manioc flour (64.9), beans (18.5), sweet potatoes (12.9) and cured meat (3.7). All dietary components but manioc, were cooked, dehydrated at 60°C, pulverized, weighed, mixed and had meat fat added (0.3 g/100g). The mixture was hydrated into a paste and dehydrated at 60°C for 24 h to obtain pellets. Dietary modifications: i) the cured meat was repeatedly washed, the diet was prepared as above and the diet final sodium was 0,049 mg/100 g; ii) the diet was prepared as above employing cured meat washed as above, NaCl was added at the end, to obtain diets with low, normal and high Na⁺ levels, respectively: 0.15 (LSDD), 0.3 (NSDD) or 0.6 (HSDD) g/100g [9].

Dietary composition was held at the Federal University of Pernambuco Nutrition Department. At the day 22nd day of life, age-matched rats were weaned and randomly assigned into CD or to one of the deficient diets LSDD, NSDD or HSDD until 18th week of age.

Sodium and water balances

Rats were housed in individual metabolic cages. As being previously adapted to the cages, after 1 day of acclimatization, diet and H₂O intake plus urine volume were measured for two 24 h periods at 8th, 13th and 18th weeks of age. Na⁺ intake was calculated by the product of food intake and Na⁺ dietary content. Data is a mean of the two measurements.

Renal concentration capacity

Renal concentration capacity was assessed by a 12 h overnight H₂O deprivation in rats from all groups but HSDD group, as previously done in humans [1] and in rats [10]. Urine volume, density and Na⁺ concentrations were determined but Na⁺ excretion was calculated. All values were corrected for 100g BW.

GFR and proximal tubule Na⁺ re-absorption

GFR and proximal tubule Na⁺ re-absorption were measured respectively by creatinine (Cl_{cr}) and lithium (Cl_{Li}) clearances while Na⁺ tubular transport was calculated [11, 12]. General clearance formula (C_x) is: $C_x = U_x \times V / P_x$ (1) Where, U_x is x concentration in urine, V is urine volume and P_x is plasma concentration of x. The rats were given LiCl (0.06 mmol/100 g BW) by gavage and were maintained with H₂O but no food overnight (12 h). Then, they received a H₂O overload (5 mL/100 g BW) by gavage in two steps (3 and 2 mL/100 g BW), respectively 90 and 30

min before being housed in metabolic cages. At the end, blood was collected by decapitation in non-heparinized tubes. Then, major organs were removed and weighed. All values were corrected for 100 g of BW. Na tubular transport was assessed by the following formula:

Na⁺ filtered load (FLNa⁺) = Cl_{cr} x [Na⁺]_{plasma}, in μEq/min/100g (2);

Na⁺ distal delivery (DDNa⁺) = C_{Li+} x [Na⁺]_{plasma}, in μEq/min/100g (3);

Na⁺ proximal tubule fractional reabsorption (PTFrNa⁺R) = [(FLNa⁺- DDNa⁺)/FLNa⁺] x 100(4);

Na⁺ distal tubule fractional reabsorption (DTFrNa⁺R) = [(DDNa⁺)-(UNa⁺V)/DDNa⁺] x 100 (5).

Plasma volume measurement

Plasma volume was assessed by Evans Blue dye, after anesthesia with sodium pentobarbital (60 mg/kg BW, i.p.). Briefly, a femoral artery was catheterized one 1 mL basal blood sample was collected in heparinized syringe to obtain plasma, after centrifugation and the catheter was filled with physiological saline. The dye (0.1% in physiological saline) was administered (100 μg/100 g BW) and the catheter was flushed with 200 μL of physiological saline. After 7.5 min, physiological saline in the catheter was discarded and another 1 mL blood sample was collected as above. The plasmatic dye concentration was determined spectrophotometrically at 610 nm and compared to a standard curve constructed with known concentrations of Evans Blue dye and samples of basal plasma [13].

Analytic technique

Urine density was assessed by refractometry (Atago), showing upper limit of 1.050. Na⁺ and creatinine concentrations in serum and urine were determined respectively by a selective ion analyser 9180 (Roche) and colorimetry on a Beckman Coulter, CX9 ALX.

Statistical analysis

Data is presented as mean±SD. The statistical analysis was by Student-Newman-Keuls test. Significance values were set at 95% (p<0.05)

Results

Although at weaning all offspring had similar BW, the weight gain during development was decreased on the DD groups at all Na⁺ levels. The adult LSDD rats weighed less than NSDD rats only at 18th week of age, but the HSDD rats had the lowest weights at all weeks either compared to CD or to NSDD. The hematocrit increased with age similarly among groups: at weaning the value was 34±35 for all; at 8th week (CD: 40±4, LSDD: 45±2, NSDD: 44±2, HSDD: 45 ± 2), at 13th week (CD: 45±3, LSDD: 48±2, NSDD: 47±1, HSDD: 47±2) and at 18th week of age (CD: 47±2, LSDD: 50±2, NSDD: 49±1, HSDD: 49±1). The ratio liver/body weight was higher only at HSDD compared to NSDD, while the relative heart weight (heart/body weight) was increased in HSDD either compared to CD or NSDD groups. The ratio testicles/body weight was augmented in all DD

groups compared to CD, and in HSDD also compared to NSDD. Undernutrition induced kidney atrophy as can be seen in groups LSDD and NSDD, but the high dietary sodium led to kidney hypertrophy in the HSDD group. The renal index was similar between HSDD and CD.

All rats on the DD from weaning had a higher diet intake throughout life than rats on CD at all study weeks but rats on LSDD and HSDD diet intake was greater than NSDD. The values of percent Na⁺ excretion over intake were similar among NSDD, HSDD and CD. Due to the low sodium intake, the levels of Na⁺ urinary sodium concentration shown by LSDD were undetectable in the sodium electrolyte analyzer.

At 8th of age, HSDD exhibited H₂O intake 5 times all other groups but at 13th and 18th weeks of age, the difference was 3 times. The values of percent H₂O excretion in urine over intake decreased with age. At 8th week the mean values were 40% for CD, LSDD and NSDD but 77% for HSDD; at 13th week mean values were 31% for CD, LSDD and NSDD but 56% for HSDD; and at the 18th week of age CD, LSDD and NSDD mean values were 23% and the rats from HSDD group excreted 55% percent of the H₂O intake. Dietary Na⁺ influenced H₂O intake as expected, but H₂O balance was maintained independently of nutrition condition and Na⁺ intake.

After 12 h overnight H₂O deprivation, the decrement in urine volume was less than CD and lower urinary density on NSDD group points to incapacity to conserve H₂O.

Creatinin and lithium clearances were similar among rats from all groups. The mean value of plasma lithium in all experimental groups was 0.30 ± 0.09 $\mu\text{Eq/mL}$, varying from 0.26 to 0.33, $\mu\text{Eq/mL}$. Besides being measured after an acute administration, these values were well below the nephrotoxic levels observed in humans in chronic treatments [14]. Sodium tubular transport values, assessed by filtered load, distal delivery plus proximal tubule fractional re-absorption were similar among groups. However, fractional distal re-absorption was increased in LSDD and decreased in HSDD compared to CD and NSDD, due to Reinforcing data on maintained Na⁺ balance in undernourished rats, the plasma volume was similar (3.4 ± 0.41 vs $3.3 \pm 0.37\text{mL}$) between NSDD and CD groups.

Discussion

The organ weight relative to body weight was adopted here due to great difference in rat weights from CD and the DD groups at 18th week of age. Rats on DD at different Na⁺ levels weighed less than the ones on CD, due to deficient quality and quantity of nutrients but carbohydrates. Na⁺ levels were the only difference among the DD, and NSDD rats weighed more than LSDD at 18th week but rats on HSDD group weighed less throughout the study, compared to CD and NSDD rats. The results on LSDD are different from the weight pattern seen on 12 week old rats, from weaning on normal protein diets but with low, normal or high s Na⁺: the rats on low Na⁺ diet weighed more than controls, and rats on the high Na⁺ weighed less from the 8th week until adults [9], in agreement with the NSDD data here.

Interestingly, the weight gain stabilized differently among the experimental groups: at the 13th week for the CD, at the 18th week for HSDD and LSDD but rats of NSDD group were still growing at the week 18th, since protein-malnourished rats grow more slowly but for longer durations [15], although here the rats did not reach normal final size. Organ weights were more affected in the HSDD group. The higher testicles weights on all DD groups could be due to

increased angiogenesis as previously been seen [16]. Higher heart weight in the HSDD rats might be by increased oxidative stress, as has been shown that a high-salt diet leads to increased generation of reactive oxygen species in striated muscle micro-vessels, which are responsible for decreased endothelium-dependent dilation [17]. This could lead to cardiac hypertrophy by hemodynamic mechanisms. Furthermore, increased oxidative stress could lead also to hypertrophy in some tissue [18].

Regarding the sodium balance, the values of percent Na^+ excretion over ingestion shows that rats from all groups maintained their balances. However, previous results from this laboratory found natriuresis in DD rats from weaning: i) in adult rats in a high Na^+ control diet (807.5 mg/100g) or on a low Na^+ deficient diet (207.5 mg/100 g), although Na^+ intake was higher in control rats, Na^+ excretion were similar but the percent excreted over the ingested was 3 times higher in the low sodium deficient diet rat, thus indicating a difficulty on sodium conservation [19]; ii) Urinary Na^+ excretion was increased and almost twice the Na^+ intake in juvenile and adult DD rats as to controls. At the molecular level, 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 threefold. Then, that early alterations in proximal tubule Na^+ pumps, together with an abnormally augmented urinary Na^+ excretion, might be the link between undernutrition and late renal dysfunction [6].

Also, anesthetized 3-month day old rats assigned from weaning to a multideficient diet, which was low in sodium, presented a high urinary sodium excretion, a negative sodium balance and renal vasodilatation as well [4]. In spite of the diverse dietary Na^+ , balance was maintained at all weeks with Na^+ excretion paralleling ingestion in this study.

The present work was designed to shed some light on the nature of the natriuresis seen on rats on a DD since weaning. There were three major possibilities: increased ingestion, renal impairment to conserve Na^+ and comparison to a control diet low in Na^+ . All rats on the DD from weaning had higher diet intake throughout life than rats on control. Na^+ intake is a product of diet intake and Na^+ dietary content, which was respectively low, normal and high on DD rats. The excretion relative to intake indicates that rats on DD at diverse Na^+ levels were able to maintain their respective Na^+ balances at all study weeks.

The capacity to concentrate urine is the finest and most complex of the renal functions, the last to be acquired in normal life and the first to be lost when functioning renal mass starts to decrease. Then, NSDD were unable to concentrate urine, corroborating literature data in humans [1, 2], in rats [2, 10] and our previous results [5, 6, 19]. The impairment of urinary concentration mechanisms was not correlated with increment in GFR, i. e, medullar washout seem unlikely in these undernourished animals, since plasma volume was unchanged in the normal sodium levels.

GFR is reduced both in humans with caloric protein malnutrition and experimentally in rats on low protein diets [2] contributing to the hydro-electrolytic alterations. Here, GFR was not different in adult conscious on DD, independently of dietary Na^+ content. Since plasma volume was similar between CD and DD rats, it may be suggested that effective circulatory volume might be unchanged. Measurement of GFR by creatinine clearance is a valid method in conscious animals, since GFR measured simultaneously by creatinine and by $[3\text{H}]\text{inulin}$ clearances, a significant correlation was shown between them [20] The increased urinary volume in NSDD rats could not be

attributed to reduction in Na⁺ re-absorption in the proximal and distal tubules. Kudo and others [21] have shown that Na⁺ re-absorption is reduced in the thick ascendant limb of Henle loop in undernourished rats and here, we cannot discard a reduced Na⁺ re-absorption in the ascending limb of Henle's loop. Furthermore, the synthesis of urea is reduced in the liver of undernourished rats, consequently diminishing medullar osmolarity [22]. This disturbance of urinary concentration leads to an increment of urea transporters in the inner medullary collecting ducts [23].

Conclusions

Malnutrition and sodium content did not influence: glomerular filtration; proximal tubular sodium transport; sodium balance; plasma volume; pressure levels. Malnutrition compromised the urinary concentration mechanism, however, sodium excretion does not seem to contribute to this disorder, since it remained closely correlated with the concentration of this electrolyte in the diet.

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