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Long-term food restriction attenuates age-related changes in the expression of renal aldosterone-sensitive sodium transporters in Wistar-Kyoto rats: A comparison with SHR

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ABSTRACT

In the present study we hypothesized that age-associated changes in the renal aldosterone/mineralocorticoid receptor (MR) system may differ between normotensive Wistar-Kyoto (WKY) and spontaneously hypertensive rats (SHR). In WKY, body mass index significantly increased with age. Fat mass may operate as a confounding factor; therefore, WKY (WKY-FR) was pair-fed with SHR. Pair-feeding resulted in a 14% body weight reduction at the age of 52 weeks in WKY-FR. Renal oxidative stress was increased in aged WKY and SHR. Aged WKY and SHR had increased MR functionality, which correlated positively with increased plasma aldosterone levels, nuclear MR content and abundance of aldosterone effectors in the renal medulla. In contrast, decreases in nuclear MR content were observed in the renal cortex of both strains with aging. When compared to aged SHR, aged WKY-FR had decreased plasma aldosterone levels and decreased activation of the aldosterone/MR system in the renal medulla. Increases in renal oxidative stress and plasma aldosterone in aged WKY, to levels observed in SHR, were not sufficient to result in sustained increases in blood pressure. In conclusion, activation of the aldosterone/MR system is intensified by aging in SHR, whereas increases in body fat mass in WKY associate with hyperaldosteronism and oxidative stress.

1. Introduction

A broad-spectrum of physiological, functional and morphological changes in the kidney is associated with age, resulting in an almost inevitable decline in renal function (Epstein, 1996; Kielstein et al., 2003). Previous studies have shown renal function decline to be associated with both structural (glomerulosclerosis, tubular atrophy and interstitial fibrosis) and functional (decreases in glomerular filtration rate (GFR), proteinuria, reduced ability to concentrate or dilute urine, impairment of electrolyte and ion transport, alteration in hormonal functions, reduced drug excretion) changes in the kidney (reviewed in (Martin and Sheaff, 2007; Zhou et al., 2008)). In the presence of age-related diseases, such as heart failure and hypertension, these changes can be accelerated (Fischer and O'Hare, 2010). The prevalence of hypertension increases with age (Mosterd et al., 1999).

Hypertension also correlates with altered kidney function and structure, which has been suggested to play a role in the development of this disorder (Mullins et al., 2006). This became particularly evident with renal cross-transplantation between normotensive and hypertensive strains; normotensive rats receiving one kidney from hypertensive rats developed hypertension (Bianchi et al., 1974). The spontaneously hypertensive rat (SHR) is a genetic model of hypertension characterized by the resistance to the natriuretic effect of dopamine and D1-like receptor agonists, as a result of a defective transduction of the D_1 receptor signal in renal proximal tubules (Jose et al., 2010). Recently, we reported that the activity of renal dopaminergic system in 91-week old SHR is greater than in aged-matched WKY, which may correspond to a compensatory mechanism activated by stimuli leading to sodium reabsorption (Pinto et al., 2011).

Aldosterone is a major regulator of extracellular fluid (ECF) volume and is the principal determinant of K^+ homeostasis (Bhargava et al., 2004). Acting on the mineralocorticoid receptors (MR) it stimulates Na^+ reabsorption, and K^+ and H^+ secretion by the distal nephron, particularly in the collecting duct (O'Neil, 1990). The MR is primarily localized in the cytosol of the cell in the absence of ligand (Nishi et al., 2001). Binding of aldosterone to the MR triggers its nuclear translocation and activity as a transcription factor (Fuller and

Abbreviations: αENaC, α-subunit of the epithelial sodium channel; ANOVA, one-way analysis of variance; BMI, body mass index; DBP, diastolic blood pressure; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; GFR, glomerular filtration rate; MDA, malondialdehyde; MR, mineralocorticoid receptor; NHE3, sodium/hydrogen exchanger type 3; SBP, systolic blood pressure; SDS-PAGE, sodium dodecyl sulfate polyacrylamide gel electrophoresis; SEM, standard error of the mean; SHR, spontaneously hypertensive rat; WKY, Wistar-Kyoto rat.

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Young, 2005; Loffing et al., 2001). Inappropriate aldosterone secretion in relation to sodium balance leads to hypertension in the case of hyperaldosteronism, or to hypotension in the case of hypoaldosteronism (Epstein, 2001).

The available data on the relationship between age and the function of the renin–angiotensin–aldosterone system in normotensive healthy adults is conflicting. Some authors noted diminished urinary aldosterone or plasma renin and aldosterone values (Weidmann et al., 1975), but others reported that plasma aldosterone remains unchanged with aging (Abd-Allah et al., 2004). Recently, plasma aldosterone levels were shown to be increased in aged WKY (Pinto et al., 2011). In addition, aging (from 13- to 91-weeks of age) was also reported to be accompanied by increases in Na^+,K^+-ATPase expression and activity in the renal medulla of WKY (Silva et al., 2010). Furthermore, increased oxidative stress was observed in renal tissues of aged WKY and SHR (Simao et al., 2011). In the present study age-related changes in the renal regulation of the aldosterone/MR system in normotensive Wistar-Kyoto (WKY) and SHR were investigated. In comparison with SHR, aging in WKY was accompanied by marked increases in body weight, renal oxidative stress and a relative greater increase in plasma aldosterone levels. In WKY, after long-term food-restriction, plasma aldosterone and oxidative stress were found to be markedly reduced.

2. Materials and methods

2.1. Animal preparation and experimental design

All rat interventions were performed in accordance with the European Directive number 86/609, and the rules of the "Guide for the Care and Use of Laboratory Animals", 7th edition, 1996, Institute for Laboratory Animal Research (ILAR), Washington, DC. Five-week old male WKY and SHR rats were obtained from Harlan-Interfauna Ibérica (Barcelona, Spain) and carefully maintained and monitored until 13 and/or 52 weeks of age.

WKY and SHR were fed ad-libitum from 5 until 13 or 52 weeks of age. Another group of WKY (WKY-FR) was pair-fed with SHR from 5 until 13 or 52 weeks of age. WKY-FR received 85% of the total food consumed by the WKY ad-libitum group. The rats were housed under controlled conditions (12 h light/dark cycle and room temperature at 22 ± 2 °C) and had free access to tap water and fed standard rat chow (A04 PANLAB, Barcelona, Spain). Blood pressure (systolic and diastolic) was measured using a photoelectric tail-cuff detector (LE 5000, Letica, Barcelona, Spain). Body mass index (BMI) was determined in all animals as reported previously by other authors (Novelli et al., 2007), using the formula: BMI = body weight (g)/length^2 (cm^2), where "length" corresponds to the "nose-to-anus" length.

2.2. Metabolic study

Forty-eight hours before the experiments, 13- or 52-week old rats were placed in metabolic cages (Tecniplast, Buguggiate, Italy) for a 24 h urine collection. The urine samples were collected in vials that were subsequently stored at −80 °C until assayed. After completion of this protocol, rats were anesthetized with sodium pentobarbital (60 mg/kg, i.p.). The animals were then sacrificed by exsanguination using cardiac puncture and the blood were collected into tubes containing K_3 EDTA for later determination of plasma biochemical parameters. Before excising their kidneys, a cannula was inserted in the right ventricle of the heart and animals were perfused with ice-cold saline (0.9% NaCl) to remove all blood from the kidneys. The kidneys were then excised, weighed, decapsulated, and the renal cortex and medulla rapidly were separated by fine dissection.

2.3. Plasma and urine biochemistry

All biochemical assays were performed by Cobas Mira Plus analyzer (ABX Diagnostics for Cobas Mira, Switzerland). Plasma aldosterone was assayed by radioimmunoassay (Diagnostic Products Corporation; Los Angeles, CA). Creatinine clearance was calculated using 24 h urine creatinine excretion in absolute values (ml/min).

2.4. H_2O_2 production by renal medulla and cortex

H_2O_2 was measured fluorometrically using the Amplex Red Hydrogen Peroxide Assay kit (Molecular Probes Inc., Eugene, OR, USA). Amplex Red is a fluorogenic substrate with very low background fluorescence that reacts with H_2O_2 with a 1:1 stoichiometry to produce a highly fluorescent reagent. Renal cortex and medulla were cut into square pieces and incubated at 37 °C in Krebs–HEPES buffer (in mM: NaCl 118, KCl 4.5, $CaCl_2$ 2.5, $MgCl_2$ 1.20, K_2HPO_4 1.2, $NaHCO_3$ 25.0, Na-HEPES 25.0, and glucose 5; pH 7.4) for 90 min. H_2O_2 released from the tissue was detected using the Amplex Red Hydrogen Peroxide Assay kit. Fluorescence intensity was measured in a multiplate reader (Spectromax Gemini Molecular Devices) at an excitation wavelength of 530 nm and emission wavelength of 590 nm at room temperature. After subtracting background fluorescence, the concentrations of renal cortical and medullary H_2O_2 (in pmol/mg) were calculated using a resorufin–H_2O_2 standard calibration curve generated from experiments using H_2O_2 and Amplex Red.

2.5. Malondialdehyde (MDA) determination

Briefly, urine samples were combined with 8.1% SDS for 10 min. Equal volumes of 28% trichloroacetic acid (TCA) and 0.6% thiobarbituric acid (TBA) were added and heated at 95 °C during 1 h. After cooling at room temperature, a mixture of chloroform/methanol (2:1) was added and centrifuged at 5000 rpm for 10 min. Supernatant absorbance was measured at 532 nm. The content of urinary malondialdehyde MDA was calculated using a MDA standard calibration curve and results were expressed as nanomoles of MDA per 24 h urine volume.

2.6. Western blotting

Isolated renal cortex and medulla of 13- and 52-week old WKY, WKY-FR and SHR were sliced very thinly and lysed by the addition of RIPA buffer containing 150 mM NaCl, 50 mM Tris–HCl, pH 7.4, 5 mM EDTA, 1% Triton X-100, 0.5% sodium deoxycholate, 0.1% SDS, 100 μg/ml phenylmethylsulfonyl fluoride (PMSF), 2 μg/ml leupeptin and 2 μg/ml aprotinin, as described previously (Amaral et al., 2009). Nuclear protein was prepared as described by other authors (Kanematsu et al.). Briefly, renal medullary and cortical tissues were homogenized in ice-cold buffer (A) containing 10 mM HEPES (pH 7.9), 1.5 mM $MgCl_2$, 10 mM KCl, 0.5 mM dithiothreitol (DTT), 0.5 mM PMSF, 2 μg/ml leupeptin, 2 μg/ml aprotinin and 10% Nonidet P-40. After centrifugation of the homogenate at 1000 × *g* for 5 min at 4 °C, the supernatants and pellets were collected separately. The supernatants were centrifuged again at 6000 × *g* for 10 min. For nuclear fraction isolation, the pellets from the first centrifugation, which contain cell nuclei, were washed with buffer A and then incubated with ice-cold buffer (B) containing 5 mM HEPES (pH 7.9), 1.5 mM $MgCl_2$, 300 mM NaCl, 400 mM KCl, 0.2 mM EDTA, 0.5 mM DTT, 0.5 mM PMSF, 2 μg/ml leupeptin, 2 μg/ml aprotinin, and 26% glycerol for 30 min to release nuclear proteins. Next, the reaction mixtures were centrifuged at 24,000 × *g* for 30 min, and the supernatant (containing nuclear-enriched protein fraction) was collected and frozen in liquid nitrogen until use as nuclear extracts for western blot analysis. Protein concentrations in nuclear protein enriched fractions and homogenates of total protein were determined by the Bradford assay. Proteins were subjected to SDS-10%PAGE and then electroblotted