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Original article

Involvement of nitric oxide and caveolins in the age-associated functional and structural changes in a heart under osmotic stress

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ABSTRACT

Previous work done in our laboratory showed that water restriction during 24 and 72 h induced changes in cardiovascular NOS activity without altering NOS protein levels in young and adult animals. These findings indicate that the involvement of NO in the regulatory mechanisms during dehydration depends on the magnitude of the water restriction and on age. Our aim was to study whether a controlled water restriction of 1 month affects cardiac function, NO synthase (NOS) activity and NOS, and cav-1 and -3 protein levels in rats during aging. Male Sprague-Dawley rats aged 2 and 16 months were divided into 2 groups: (CR) control restriction (WR) water restriction. Measurements of arterial blood pressure, heart rate, oxidative stress, NOS activity and NOS/cav-1 and -3 protein levels were performed. Cardiac function was evaluated by echocardiography. The results showed that adult rats have greater ESV, EDV and SV than young rats with similar SBP. Decreased atria NOS activity was caused by a reduction in NOS protein levels. Adult animals showed increased cav-1. Water restriction decreased NOS activity in young and adult rats associated to an increased cav-1. TBARS levels increased in adult animals. Higher ventricular NOS activity in adulthood would be caused by a reduction in both cav. Water restriction reduced NOS activity and increased cav in both age groups. In conclusion, our results indicated that dehydration modifies cardiac NO system activity and its regulatory proteins cav in order to maintain physiological cardiac function. Functional alterations are induced by the aging process as well as hypovolemic state.

1. Introduction

Healthy humans and animals maintain homeostatic control of the balance of body fluids by physiologic and behavioral adaptations [1]. When fluids are limited, such as osmotic stress induced by water restriction, this fluid balance is disturbed, leading to activation of a number of physiological mechanisms to promote the reestablishment of vascular volume and blood pressure, including activation of several neurohormonal factors, such as nitric oxide (NO), catecholamines, endothelins, arginine vasopressin, and renin–angiotensin system, among others [2,3]. Aging is more vulnerable to water balance disorders that could result in a progressive functional and structural decline in multiple organs and, in particular, it has profound effects on heart that is associated with significant mechanical remodeling that includes fibrosis, or accumulation of collagen and other extracellular matrix proteins [4,5].

It is known that NO is one of the major regulators of water and electrolyte homeostasis, with multiple functions influencing cardiovascular system [6]. It was reported that the endothelial isoform of NO synthase (eNOS) is expressed in vascular endothelium and cardiac myocytes, the cellular regulation of eNOS being an important determinant of cardiovascular homeostasis [7]. This isoform is targeted to specialized invaginations of the plasmalemma termed caveolae, which serve as sites for sequestration of signaling proteins, being characterized by the presence of the intrinsic membrane proteins called caveolins (cav) [8]. As cardiovascular function is closely linked to NO system, changes in NO production may affect cardiovascular system regulation during aging process. Synthesis and release of NO decline with age in endothelial cells and this has been implicated in the development

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of several age-dependent cardiovascular diseases [9]. Interestingly, a few studies suggest that changes in cav expression could alter cell function with aging [10]. We provided evidence using an experimental model of hypovolemic state that specific expression patterns of ventricular NOS isoforms, alterations for interaction, are involved in age-related adjustment to acute blood loss [11]. Considering that older adults are a high-risk population for hypovolemic state induced by a dehydration, we hypothesize that the NO pathways are involved in cardiac function regulation after long controlled water restriction, this response being different with aging. Thus, our objective was to evaluate the effect of water restriction on hemodynamics parameters, NOS activity and NOS/cav interaction as well as the structural and functional changes in the heart of young (2 months old) and adult (16 months old) rats.

2. Methods and materials

Male Sprague-Dawley rats obtained through the breeding laboratories of the School of Veterinary (University of Buenos Aires) were received at ages of 2 (200–220 g body weight) and 16 months (500–550 g body weight). Ethical approval for animal experimentation was approved by the ethics committee of the School of Pharmacy and Biochemistry (CICUAL, No. 0031028/2014), University of Buenos Aires. Animals were allowed food and deionized water ad libitum. All the laboratory material was previously washed with nitric acid (20%) and water. Rats were housed separately in plastic cages in a humidity- and temperature-controlled environment, illuminated with a 12:12 light–dark cycle.

2.1. Experimental Protocols (see Fig. 1 for more details)

The rats at 2 and 16 months rats were randomly assigned to:

(1) *Group WR (water restriction)*: Animals were subjected to dehydration, which consisted on depriving them of water for 3 out of 4 days (1 cycle), repeating this for 8 successive cycles (32 days).

This model has been adapted from Gharbi et al., as it represents a true state of dehydration [2]. Rats had continuous access to food.

(2) *Group CR (control restriction)*: Rats had continuous access to both food and water, during 32 days, representing a normal hydration status.

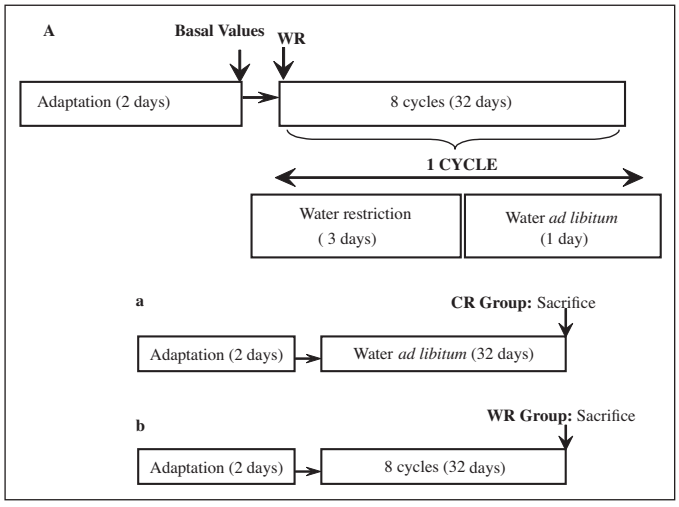

Fig. 1. (A) General design adapted from Gharbi et al. (B) Representation of the 2 groups for young and adult rats in (a) the control groups and (b) experimental groups.

The animals were placed in the metabolic cages for the adaptation to the environment for two days before the beginning of the experiments. Body weight and food intake as well as biochemical, urinary and hemodynamic parameters were evaluated after the adaptation period and at the end of the experimental time.

2.2. Biochemical and urinary parameters

Blood collections from caudal artery were made to determinate hematocrit, plasma osmolarity and urinary collections for urinary osmolarity. Serum Na^+ (mEq) was measured using ion selective analyzer (Tecno-lab t-140). Plasma and urinary osmolarity (mOSM) were measured by microosmometer (μosmetteTM Micro Osmometer). Hematocrit (%) was determinated from duplicate blood-filled hematocrit tubes. Urine volume (ml/min 100 g body weight) was determined gravimetrically.

2.3. Hemodynamic parameters

Systolic blood pressure (SBP) was indirectly measured in the awaken animals by the tail-cuff method using a PowerLab data acquisition system device and LabChart software (AD Instruments). Prior to measuring SBP, rats were warmed in a thermostated and silent room for 30 min. The SBP value for each rat was calculated as the average of five separate measurements at each session. Heart rate (HR) was also calculated from the pulse pressure signals by using LabChart software.

2.4. Echocardiography

At the end of the experimental protocol, another group of animals from each experimental group from both, 2-month-old and 16-month-old rats, were anesthetized with urethane (1.0 g/kg, intraperitoneally), their chests were shaved under aseptic conditions and echocardiographic measurements were performed in the left lateral decubitus position. Two-dimensional directed M-mode images were obtained using a Sonoscape (A6 Vet) system with a 9–4 MHz transducer. Measurements were taken in the right parasternal short axis plane at the level of the mitral valve leaflets. End-diastolic volume (EDV), end-systolic volume (ESV), ejection fraction (EF) and systolic volume (SV) were measured from left ventricular by the echocardiography system. All determinations were made according to the guidelines of the American Society of Echocardiography.

2.5. NOS activity

Capacity for left ventricular and right atria NO production was assessed determining NOS activity from different groups of animals by measuring the conversion of [^{14}C (U)]-L-arginine to [^{14}C (U)]-L-citrulline. Tissue homogenates (50 μg protein) were incubated in Tris–HCl buffer (pH 7.4) containing 1 μg/ml L-arginine, [^{14}C (U)]-L-arginine (346 μCi/ml), L-valine (67 mM), NADPH (1 mM), calmodulin (30 nM), tetrahydrobiopterin (5 μM) and $CaCl_2$ (2 mM) for 60 min at room temperature. At the end of the incubation period, NOS reaction was arrested by addition of a buffer solution in ice containing 20 mM HEPES buffer and 20 mM EDTA, pH 5.5. Reaction mixtures were loaded onto cation exchange columns (Dowex AG 50W-X8, Na^+ form; Bio-Rad) and [^{14}C (U)]-L-citrulline was eluted from columns with 0–50 ml ddH_2O. The amount of [^{14}C (U)]-L-citrulline eluted was quantified using a liquid scintillation counter (Wallac 1414 WinSpectral; EG&G Company, Turku, Finland). All compounds, except [^{14}C]-L-arginine monohydrochloride (346 mCi/mmol, Amersham Life Science), were purchased from Sigma Chemicals. Protein determination