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Nitric oxide and AQP2 in hypothyroid rats: A link between aging and water homeostasis

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ABSTRACT

Objective. Hypothyroid state and aging are associated with impairment in water reabsorption and changes in aquaporin water channel type 2 (AQP2). Nitric oxide (NO) is involved in AQP2 trafficking to the apical plasma membrane in medullary collecting duct cells. The purpose of this study was to investigate whether aging and hypothyroidism alter renal function, and whether medullary NO and AQP2 are implicated in maintaining water homeostasis.

Materials/Methods. Sprague–Dawley rats aged 2 and 18 months old were treated with 0.02% methimazole (w/v) during 28 days. Renal function was examined and NO synthase (NOS) activity ([^{14}C (U)]-L-arginine to [^{14}C (U)]-L-citrulline assays), NOS, caveolin-1 and -3 and AQP2 protein levels were determined in medullary tissue (Western blot). Plasma membrane fraction and intracellular vesicle fraction of AQP2 were evaluated by Western blot and immunohistochemistry.

Results. A divergent response was observed in hypothyroid rats: while young rats exhibited polyuria with decreased medullary NOS activity, adult rats exhibited a decrease in urine output with increased NOS activity. AQP2 was increased with hypothyroidism, but while young rats exhibited increased AQP2 in plasma membrane, adult rats did so in the cytosolic site.

Conclusions. Hypothyroidism contributes in a differential way to aging-induced changes in renal function, and medullary NO and AQP2 would be implicated in maintaining water homeostasis.

1. Introduction

Thyroid hormones regulate basic metabolism in many organs and cells, having relevant effects on the cardiovascular and renal functions [1–3]. It has been demonstrated that thyroid hormones would be one of the factors involved in the modulation of both cardiovascular nitric oxide (NO) production and negative regulators of NO synthase (NOS) such as caveolin (cav) 1 and 3, regardless of age [4,5]. With regard to renal tissue, it is well known that hypothyroidism is associ-

Abbreviations: cav, caveolins; cav-1, caveolin-1; cav-3, caveolin-3; eNOS, endothelial nitric oxide synthase; Eut, euthyroid; HR, heart rate; Hypo, hypothyroid; iNOS, inducible nitric oxide synthase; MAP, mean arterial pressure; nNOS, neuronal nitric oxide synthase; NO, nitric oxide; NOS, nitric oxide synthase; T_4, total thyroxine; TSH, thyroid-stimulating hormone; Ip, Intraperitoneal.

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ated with marked changes in renal hemodynamic and tubular function. Some studies have shown that the major cause of impaired water reabsorption in hypothyroidism is an alteration in renal perfusion, glomerular filtration rate (GFR) and sodium handling, secondary to systemic effects of thyroid hormone deficiency on cardiac output and peripheral vascular resistance [6,7]. However, the specific mechanism whereby hypothyroidism induces alterations in water handling is not yet fully understood. Water movement across cellular membranes is in part mediated by aquaporin water channel type 2 (AQP2), which mediates water permeability across the luminal membrane in late distal tubule and collecting duct [8]. A defective urinary dilution in the hypothyroid state due to alterations in water diffusion in the distal nephron has been reported. In this sense, several authors have shown that impaired urinary diluting capacity would be associated with an upregulation of arginine vasopressin (AVP)-mediated AQP2 expression and membrane trafficking in the inner medulla in the hypothyroid state [9–12]. On the other hand, studies conducted in both humans and experimental animals demonstrate that NO decreases renal vascular resistance and increases GFR, natriuresis and water excretion [13,14]. It is well known that AVP increases collecting duct water permeability by enhancing AQP2 channel insertion in the apical membrane of principal cells through the AVP/cAMP pathway [14]. Moreover, it has been shown that NO donors such as sodium nitroprusside and L-arginine, among others, increase membrane insertion of AQP2 in cultured cells and collecting duct *in vitro* [14,15].

An age-related decline in urinary concentrating ability has been documented in both experimental animals and elderly humans. Senescence processes may reduce the key transport proteins as AQP2 induces changes in water homeostasis [16,17].

Considering that hypothyroidism is one of the major endocrine diseases in adulthood and that it is associated with alterations in renal function, the aim of the present study was to examine both the effects of aging on renal function in hypothyroid rats and the involvement of NO and AQP2 in aging-related renal disorders induced by hypothyroidism.

2. Methods

2.1. Animals

All procedures were reviewed and approved by the National Food, Drug and Medical Technology Administration, Department of Health and Environment, Argentina (No. 6344-96). Male Sprague–Dawley rats aged 2 (referred to as young) and 18 months old (referred to as adult) from the breeding laboratories of "Facultad de Farmacia y Bioquimica" (Universidad de Buenos Aires, Argentina) were used throughout the study. Rats were housed two per cage under controlled humidity and temperature conditions, with an automatic 12-h light/dark cycle. Rats were randomly assigned to one of the two groups: euthyroid (Eut) and hypothyroid (Hypo). Rats were fed standard rat chow from Nutrimentos Purina (Buenos Aires, Argentina) and received water *ad-libitum*.

2.2. Study design

Rats were rendered hypothyroid after 28 days of treatment with 0.02% methimazole (w/v) in the drinking water [18]. In order to confirm hypothyroidism, serum thyroid-stimulating hormone (TSH), total triiodothyronine (T_3) and thyroxin (T_4) (TSH kit, National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health, Bethesda, USA) were measured by radioimmunoassay at the end of the experiment [19]. Intra and inter-assay coefficients of variation for TSH were 8.7% and 13.4%, respectively. The same rats were used in an earlier study on the cardiovascular system in hypothyroid rats [4].

2.3. Surgical procedures

Animals were placed in metabolic cages for adaptation to the environment two days before the beginning of the experiments. After the adaptation period, body weight (BW, g), urine volume ($ml \cdot day^{-1} \cdot 100\ g\ BW^{-1}$), water intake ($ml \cdot day^{-1} \cdot 100\ g\ BW^{-1}$), serum and urinary Na^+ ($mEq \cdot L^{-1}$), creatinine ($g \cdot dl^{-1}$) and osmolarity (osm) were measured by standard methods using an autoanalyzer. Creatinine clearance was assessed in order to determine GFR and fractional excretion of sodium (FENa). GFR, FENa, osmolar clearance (Closm) and tubular reabsorption of water (TcH2O) were calculated according to a standard formula. Each rat was then instrumented with a catheter inserted in the right femoral artery to measure mean arterial pressure (MAP). Cannulae were connected to a pressure transducer (Statham P23 ID, Gould Inst. Cleveland, OH) and MAP was recorded with a polygraph (Physiograph E & M, Houston, TX) during 30 min. Heart rate (HR) was derived from the pulsatile pressor signal via tachographic beat-to-beat conversion with a tachograph preamplifier (Coulbourn Instruments, Inc., tachometer S77-26, PA, USA). The Labtech Notebook program (Laboratory Tech., Wilmington, MD) was used for data acquisition. All surgical procedures were performed under aseptic conditions and urethane anesthesia (1.0 g/kg, ip). Throughout the experiment animals were kept under anesthesia by additional small doses of urethane and body temperature was monitored with a rectal probe and maintained at 37.0 ± 0.5 °C with heating lamps to avoid the influence of temperature on cardiovascular parameters.

2.4. Experimental procedures

Hemodynamic parameters were recorded for 30 min to allow stabilization of MAP and HR. Rats were sacrificed by overdose of anesthesia and both kidneys were removed. Western blotting was performed on this tissue for AQP2, NOS, and cav-1 and -3 proteins, and NOS activity was measured according to the method of the conversion of [^{14}C (U)]-L-arginine to [^{14}C (U)]-L-citrulline (n = 7 rats from each group). Determination of NOS activity and Western blotting were performed as reported previously [4]. Pooled samples (7 rats from each group) were revealed by chemiluminescence using ECL reagent for 2–4 min. Density of the respective bands was quantified by densitometric scanning of Western blots using a Hewlett-Packard scanner and Totallab analyzer software (Biodynamics, Seattle, WA, USA). Histograms of the ratio between optical densities of NOS,