Life Sciences 208 (2018) 72–78

Contents lists available at ScienceDirect

Life Sciences

journal homepage: www.elsevier.com/locate/lifescie

Altered expression of Aquaporin-2 in one-kidney, one-clip hypertension

Maria Florencia Albertoni Borghese[a,f], Sandra Hope[b,c], Maria del Carmen Ortiz[a], Magalí Barchuk[a], Camila Kessler[a], Carlos Davio[d,e], Marcelo Vatta[b,c], Mónica Majowicz[a,*]

[a] Universidad de Buenos Aires, Facultad de Farmacia y Bioquímica, Departamento de Ciencias Biológicas, Cátedra de Biología Celular y Molecular, Buenos Aires, Argentina
[b] Universidad de Buenos Aires, Facultad de Farmacia y Bioquímica, Departamento de Ciencias Biológicas, Cátedra de Fisiología, Buenos Aires, Argentina
[c] Universidad de Buenos Aires, CONICET, Facultad de Farmacia y Bioquímica, Instituto de Química y Metabolismo del Fármaco (IQUIMEFA), Buenos Aires, Argentina
[d] Universidad de Buenos Aires, Facultad de Farmacia y Bioquímica, Departamento de Farmacología, Buenos Aires, Argentina
[e] Universidad de Buenos Aires, CONICET, Facultad de Farmacia y Bioquímica, Instituto de Investigaciones Farmacológicas (ININFA), Buenos Aires, Argentina
[f] Universidad de Buenos Aires, CONICET, Facultad de Farmacia y Bioquímica, Departamento de Ciencias Biológicas, Cátedra de Biología Celular y Molecular, Buenos Aires, Argentina

ARTICLE INFO

Keywords:
Renovascular hypertension
Goldblatt 1-kidney-1-clip
Aquaporin-2
NFkB
Vasopressin V2 receptor

ABSTRACT

Aims: The aim of the present study was to evaluate the regulation of Aquaporin-2 (AQP2) water channel in the kidney of one-kidney, one-clip rats (Goldblatt-1 model). In addition, some mechanisms that underlie the role of AQP2 in the Goldblatt-1 model were evaluated.

Main methods: Sprague-Dawley rats were divided in three groups: control two-kidney, no clip (C, 2 K-NC); nephrectomized one-kidney, no clip (N, 1 K-NC) and Goldblatt one-kidney, one-clip (G, 1 K-1C). AQP2 expression (by westernblot, real time PCR, immunohistochemistry and immunofluorescence), vasopressin V2 receptor expression (by real time PCR), cAMP concentration, NFkB and TonEBP (cytosol to nucleus ratio) were evaluated in the renal medulla.

Key findings: AQP2 expression, V2 receptor expression and cAMP concentration were decreased in the renal medulla of 1 K-1C rats, NFkB translocation was favoured towards the nucleus suggesting its activation while TonEBP translocation was not altered in this model of hypertension.

Significance: In this model of hypertension the decrease of AQP2 expression could be a mechanism that counteracts the high blood pressure promoting water excretion and this may be consequence of decreased vasopressin sensitivity and/or the increased activity of NFkB at renomedullary collecting duct level. Given that renovascular hypertension is among the most common causes of secondary hypertension, it is important to elucidate all the relevant mechanisms involved in the generation or in the compensation of the hypertensive state in order to improve the diagnoses and treatment of the patients.

1. Introduction

The kidney plays a central role in the regulation of salt and water balance, contributing to the control of arterial blood pressure. This regulation is achieved through the activity of both sodium and water transporters. This work has a special focus on water transport mediated by the aquaporins and in particular aquaporin-2 (AQP2). AQP2 plays a central role in the final regulation of water reabsorption in the renal collecting duct, being vasopressin (AVP) the main regulator of this process [1]. AVP exerts its effects acting through three different receptors: V1a, V1b (or V3) and V2 [2]. V1b receptors are present at central nervous system level, being involved in cognitive functions in the brain. Both V1a and V2 receptors are present in the kidney. V1a is associated with the vasa recta and is probably present in the intercalated cells of the cortical collecting duct. V2 receptor, the unique AVP receptor that signals through cAMP, has the key function of controlling fluid homeostasis [3]. Binding of AVP to V2 receptors in the basolateral membrane of principal cells in the collecting ducts stimulates adenylate cyclase producing cAMP, whose effects are mainly mediated by protein kinase A (PKA) [4, 5].

AVP regulates water permeability in the renal collecting duct by both short and long-term mechanisms. Short-term regulation occurs as a result of the modulation of trafficking of AQP2-containing membrane vesicles to and from the apical plasma membrane and long-term regulation occurs as a result of AQP2 expression regulation [6, 7].

Other proteins have been described as regulators of AQP2

* Corresponding author at: Cátedra de Biología Celular y Molecular, Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires, Piso 1 (C1113AAD), Junín 956, Argentina.
E-mail address: mmajow@ffyb.uba.ar (M. Majowicz).

https://doi.org/10.1016/j.lfs.2018.07.020
Received 4 April 2018; Received in revised form 4 July 2018; Accepted 12 July 2018
0024-3205/ © 2018 Elsevier Inc. All rights reserved.

expression, such as transcription factor tonicity responsive enhancer binding protein (TonEBP) [8] and the inflammatory mediator nuclear factor-kappaB (NFkB) [9]. TonEBP is a transcription factor activated under hypertonic conditions to protect renal cells from hypertonic stress stimulating the transcription of specific proteins [10]. Inflammation is a key feature in the initiation, progression and clinical implication of several cardiovascular diseases and the inflammatory response is essential in the pathogenesis of hypertension [11–13].

In this pathology, focus has been made on sodium transport but not on water transport, despite the importance of renal water handling in this pathological condition. There are no conclusive studies about the state of AQP2 during hypertension. However, some studies demonstrated changes in AQP2 targeting and expression in different experimental models of hypertension.

An increased expression and apical targeting of AQP2 in association with enhanced activity of the AVP/cAMP pathway has been reported in spontaneously hypertensive rats (SHR) [14–16]. On the other hand, a decrease in AQP2 expression was described in a Goldblatt-2 model of hypertension (two-kidney, one-clip; 2 K-1C) [17, 18]. However, there are no reports on the status of AQP2 in the Goldblatt-1 model of hypertension (one-kidney, one-clip; 1 K-1C). This renovascular model of hypertension described by Goldblatt is appropriate for studying the role of volume expansion in the development of hypertension [19, 20]. Since in the model of 1 K-1C there is no other kidney, there is no pressure diuresis and natriuresis, there is rapid salt and water retention and hypertension soon becomes volume dependent and plasma renin activity usually remains normal after a short increase following the surgery [21, 22].

On these bases, the aim of the present study was to evaluate the regulation of AQP2 water channels in the kidney of one-kidney-one-clip rats. In addition, some mechanisms that underlie the role of AQP2 in the Goldblatt-1 model were evaluated.

2. Materials and methods

2.1. Animals and hypertension model

Male Sprague Dawley rats were purchased from Facultad de Ciencias Veterinarias -Universidad de Buenos Aires. Protocols were in compliance with ARRIVE guidelines, were designed according to the National Institutes of Health Guide for the Care and Use of Laboratory Animals, and were approved by the Institutional Committee for Use and Care of Laboratory Animals (CICUAL) from Facultad de Farmacia y Bioquímica. Forty-five-day-old rats weighing 150 g were anesthetized with Ketamine/Xylazine (50–10, mg/Kg of body weight), the left kidney was exposed and a 0.25 mm gap silver clip was placed around the renal artery. For right nephrectomy, two ligatures were made around the renal vascular pedicle and the ureter. The kidney was then removed without the adrenal gland (Goldblatt rats). In another group of rats only the right kidney was extracted (nephrectomized rats) and a third group was subjected to a sham surgery procedure, with the exception of the artery clip and the nephrectomy (Control rats). All rats received 0.1 ml (40,000 IU) of amoxicillin at the end of the surgery.

Therefore we worked with three groups: control two-kidney-no clip (C, 2 K-NC, $n = 12$); nephrectomized one-kidney-no clip (N, 1 K-NC n = 12) and Goldblatt hypertensive group one-kidney-one-clip (G, 1 K-1C, $n = 15$), which were studied four weeks after surgery.

2.2. Determination of systolic blood pressure

Systolic BP was recorded weekly in conscious rats by tail plethysmography (ADInstruments PowerLab 8/30 and NIBP Controller ML125).

2.3. Determinations in the 24-h metabolic cage studies

Animals were acclimatized to metabolic cages for 2 days before 24 h urine collection. Urine samples were analyzed for total protein concentration (Proti U/LCR Kit; Wiener Lab., Rosario, Argentina) and urine osmolality (OSMETTE, Precision Systems Inc., MA, USA). Urine volume was measured gravimetrically. Kinetic determinations of serum and urinary creatinine concentration were evaluated using a kit provided by Wiener. Urinary sodium was measured using an ion analyzer (Tecnolab; Mod. T-412, Argentina).

Blood collection was performed with the rats under urethane anesthesia (1 g/Kg via i.p) by cardiac puncture; animals were killed by exsanguination, then both kidneys or the remaining kidney were immediately removed and weighed.

2.4. Tissue processing for Western blot analysis in medulla homogenates

Immediately after the animals were sacrificed, their kidneys were isolated and the renal medulla was dissected and homogenized in an appropriate homogenization buffer (250 mmol/l sucrose, 1 mmol/l EDTA, 0.1 mmol/l PMSF and 10 mmol/l Tris-ClH), pH 7.6. Large tissue debris and nuclear fragments were removed by a low-speed spin (1000 g, 10 min, 4 °C). Protein concentration was measured using BCA TM Protein Assay Kit (Pierce, Rockford, IL, USA).

2.5. Western blot for AQP2

Immunoblotting analysis was used to identify AQP2. AQP2 antibody (rabbit anti-rat AQP2; Santa Cruz Biotechnology, Inc., CA, USA) was followed by a donkey anti-rabbit IgG (HRP) secondary antibody (Abcam Inc., Cambridge, MA, USA) and revealed using Bio-Lumina kit (Kalium Technologies; Buenos Aires, Argentina). Beta-tubulin was used as loading control (Abcam). AQP2 antibody recognizes at least two bands of 29 and 35–40 kDa corresponding to unglycosylated and glycosylated AQP2, respectively.

The relative protein levels were analyzed with Gel Pro Analyzer 3.1 for Windows and the ratio of AQP2 to beta-tubulin was calculated.

2.6. Tissue processing for Western blot analysis in cytosolic fraction and purified nuclei

After homogenization of the renal medulla, the homogenate was centrifuged for 10 min at 800 $\times g$ to obtain the nuclear fraction in the pellet and the cytosolic fraction in the supernatant. The pellet was washed with homogenization buffer and suspended in a solution of 2.4 M sucrose followed by a centrifugation at 50000 $\times g$ for 1 h to obtain the purified nuclei in the pellet.

2.7. Western blots for NFkB and TonEBP

Immunoblotting analysis was used to identify NFkB or TonEBP in the cytosolic fraction and in purified nuclei. The appropriate primary antibody (mouse anti-rat NFkB, Santa Cruz Biotechnology, or rabbit anti-rat TonEBP; Santa Cruz Biotechnology) was followed by a secondary antibody (Horse anti mouse HRP, GE Healthcare, UK or donkey anti-rabbit IgG HRP Abcam) and revealed as described above. NFkB antibody recognizes a 65-kDa band corresponding to p65 subunit and TonEBP antibody recognizes a 170-KDa band.

Beta-tubulin was used as loading control for the cytosolic fraction and Lamina (Mouse anti rat Millipore MA, USA) was used as loading control for the purified nuclei. The relative protein levels were analyzed with Gel Pro Analyzer 3.1 for Windows and the ratio of cytosolic to nuclei expression was used as an indicator of transcription factor activation.

Download English Version:

https://daneshyari.com/en/article/8534477

Download Persian Version:

https://daneshyari.com/article/8534477

Daneshyari.com