Biochemical and Biophysical Research Communications 461 (2015) 254–259

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

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

High salt-diet reduces SLC14A1 gene expression in the choroid plexus of Dahl salt sensitive rats

Lirong Guo [a, b, **], Jie Meng [a], Chengluan Xuan [b], Jingyan Ge [b], Wenzhu Sun [b], Stephen T. O'Rourke [b], Chengwen Sun [b, *]

[a] Department of Pathophysiology, College of Basic Medical Sciences, and Second Hospital of Jilin University, Jilin University, Changchun, Jilin 130021, China
[b] Department of Pharmaceutical Sciences, North Dakota State University, Fargo, ND 58105, USA

ARTICLE INFO

Article history:
Received 20 March 2015
Available online 11 April 2015

Keywords:
Gene regulation
Salt-sensitive hypertension
UT-B
Choroid plexus

ABSTRACT

Elevated Na^+ concentration ($[Na^+]$) in the cerebrospinal fluid (CSF) contributes to the development of salt-sensitive hypertension. CSF is formed by the choroid plexus (CP) in cerebral ventricles, and $[Na^+]$ in CSF is controlled by transporters in CP. Here, we examined the effect of high salt diet on the expression of urea transporters (UTs) in the CP of Dahl S vs Dahl R rats using real time PCR. High salt intake (8%, for 2 weeks) did not alter the mRNA levels of UT-A (encoded by SLC14A2 gene) in the CP of either Dahl S or Dahl R rats. In contrast, the mRNA levels of UT-B (encoded by SLC14A1 gene) were significantly reduced in the CP of Dahl S rats on high salt diet as compared with Dahl R rats or Dahl S rats on normal salt diet. Reduced UT-B expression was associated with increased $[Na^+]$ in the CSF and elevated mean arterial pressure (MAP) in Dahl S rats treated with high salt diet, as measured by radiotelemetry. High salt diet-induced reduction in UT-B protein expression in the CP of Dahl S rats was confirmed by Western blot. Immunohistochemistry using UT-B specific antibodies demonstrated that UT-B protein was expressed on the epithelial cells in the CP. These data indicate that high salt diet induces elevations in CSF $[Na^+]$ and in MAP, both of which are associated with reduced UT-B expression in the CP of Dahl S rats, as compared with Dahl R rats. The results suggest that altered UT-B expression in the CP may contribute to an imbalance of water and electrolytes in the CSF of Dahl S rats on high salt diet, thereby leading to alterations in MAP.

1. Introduction

Hypertension and its related cardiovascular diseases are the most prevalent cause of death and disability in the world. Epidemiological, migration, intervention, and genetic studies in humans provide very strong evidence of a causal link between high salt intake and hypertension [1]. A positive link has been established between salt intake and elevated blood pressure in 30% and 50% of hypertensive whites and blacks, respectively [2]. Enhanced sympathetic nervous activity plays a major role in the development of salt-induced hypertension both in humans [3] and in genetic animal models, such as Dahl salt-sensitive (Dahl S) rats [4]. Blockade of the neural pathways in the central nervous system (CNS) mediating sympathetic hyperactivity prevent or reverse the hypertension in Dahl S rats [4,5]. However, the molecular mechanisms in the central nervous system (CNS) underlying the high salt intake-induced sympathoexcitation and hypertension are not yet fully clear.

High salt intake increases sodium concentration $[Na^+]$ in the cerebrospinal fluid (CSF) in Dahl S rats and spontaneously hypertensive rats (SHR), whereas CSF sodium shows minimal changes in Dahl salt-resistant (Dahl R) and Wistar-Kyoto (WKY) rats [6,7]. Elevated CSF Na^+ may increase neuronal activity in the CNS, sequentially leading to over-activation of the sympathetic nervous system. This hypothesis is supported by the observation that acute and chronic increases in CSF Na^+ by intracerebroventricular (ICV) infusion of hypertonic saline cause sympathetic hyperactivity and hypertension in normotensive Lewis rats as well as in Dahl S rats [8]. CSF is produced by the choroid plexus (CP), a tissue with

* Corresponding author. Department of Pharmaceutical Sciences, North Dakota State University, 1401 Albrecht Blvd, Fargo, ND 58105, USA.
** Corresponding author. Department of Pathophysiology College of Basic Medical Sciences Jilin University Changchun, Jilin 130021, China.
E-mail addresses: Guolr@jlu.edu.cn (L. Guo), chengwen.sun@ndsu.edu (C. Sun).

http://dx.doi.org/10.1016/j.bbrc.2015.04.010
0006-291X/

characteristics similar to kidney, located within the cerebral ventricles. The CSF electrolyte concentration, water secretion, and osmolality balance between CSF and blood are precisely regulated by ion channels, ion exchangers, and transporters located on epithelial cells of the CP. However, transporters that may be altered in the CP of Dahl S rats on high salt diet have not been fully identified.

It is well known that urea transporters (UTs) are expressed in the kidney, and play an important role in water excretion and in the regulation of Na^+ concentrating in urine [9]. However, whether UTs are also expressed in the CP and are regulated by high salt diet is unknown. Two genes encode for UTs in mammals: SLC14A1 and SLC14A2, which encode UT-B and UT-A, respectively. Both UT-A and UT-B are expressed in the kidney, where they generate a urea gradient to concentrate urine [10]. UT-B knockout mice have a reduced ability to concentrate urine [11]. UT-B protein is also expressed in erythrocytes as the Kidd (or Jk) antigen, one of the minor blood group antigens. UT-B in erythrocytes also plays an important role in controlling the balance of osmolality across the cytoplasmic membrane, keeping the special shape of erythrocytes [12]. The SLC14A1 gene is located in the chromosome 18, 18q125 region, sitting in a QTL of blood pressure regulatory region in human chromosomes. Thus, the aims of present study were two fold: 1) to determine whether UTs are expressed in the CP; 2) whether their expression is altered by high salt diet in Dahl S versus Dahl R rats.

2. Materials and methods

2.1. Animals and materials

Adult male Dahl S and Dahl R rats (9–10 weeks old) were obtained from Charles River Farms (Wilmington, MA). Rats were housed on a 12:12-h light/dark cycle in a climate controlled room. Regular rat chow (0.4% Na^+) or high salt diet (8% Na^+) purchased from Harlan Tekland (Madison, WI) and water were provided ad libitum. All experimental procedures were approved by the North Dakota State University Institutional Animal Care and Use Committee and the Jilin University Institutional Animal Care and Use Committee.

2.2. Chronic blood pressure (BP) measurement

Chronic BP was measured with radiotelemetry in free-moving rats, as detailed in our previous publication [13]. After 5-day recovery from telemetry probe implantation surgery, the basal BP and HR were recorded for 3 days. The regular diet was then switched to the high salt diet in 8 Dahl R rats and 8 Dahl S rats; another 8 Dahl R and Dahl S rats were kept on the regular diet. The BP and HR were recorded continuously for 14 days. At the end of the experiment, the brain tissues were harvested and used for assessment of UT expression with Western blots and real-time RT-PCR. The CSF and blood were collected for measurement of $[Na^+]$ and $[K^+]$ using ion-specific electrodes (Lazar Research Laboratories, Los Angeles, CA).

2.3. Western blot analysis of UT-B protein levels

Animals were euthanized with an excessive dose of pentobarbital sodium. Brains were then removed; the choroid plexus and brain tissues were collected; and Western blots were performed as described in our previous publication [14]. The primary antibody (UT-B rabbit polyclonal antibody, Santa Cruz, 1:500) and secondary antibody (goat anti-rabbit IgG horseradish peroxidase-conjugated antibody, Bio-Bad, 1:3000) were used in current study to detect UT-B protein levels.

2.4. Real time PCR measurement of UTs mRNA levels

UT mRNA levels in the choroid plexus of rats were determined by real-time RT-PCR, as described in our previous publication [13]. TaqMan probes specific for rat UT-A and UT-B were purchased from Applied Biosystems Inc (Foster City, CA). Real-time RT-PCR was performed in an Applied Biosystems PRISM 7000 sequence detection system according to the protocol from the manufacturer. Data were normalized to 18S RNA. In each experiment, samples were analyzed in triplicate.

2.5. Immunohistochemistry

Immunofluorescence staining of choroid plexus brain sections was performed as described previously [13]. The brain sections containing choroid plexus were incubated with PBS plus 0.5% Tween 20 (PBS-T) containing 5% goat serum. Slices were incubated with primary antibodies (rabbit anti-UT-B 1:500) overnight at 4 °C. After being washed with PBS-T, the sections were incubated with secondary antibodies (Alexa Fluor 488 goat anti-rabbit IgG, 1:1000) for 2 h. The sections were then washed with PBS-T, and examined under a confocal fluorescent microscope (Olympus, Fluoview FV300). The fluorescent images were collected and analyzed with Flow-View software.

2.6. Statistical analysis

All data are presented as mean ± SE. Statistical significance was evaluated by 1- or 2-way ANOVA, as appropriate, followed by either a Newman–Keuls or Bonferroni post hoc analysis, where appropriate. Differences were considered significant at $P < 0.05$, and individual probability values are noted in the figure legends.

3. Results

3.1. Effect of high salt diet on MAP and HR

In the first experiment, we confirmed the effect of high salt diet on arterial blood pressure and heart rate in DR and DS rats. Mean arterial pressure (MAP) and heart rate (HR) were measured using radiotelemetry before and after switching to high salt diet. Before dietary treatment, neither MAP nor HR was different between Dahl S and Dahl R rats. However, the MAP and HR were significantly increased by 38 ± 4 mmHg and 49 ± 3 bpm, respectively, by 2-week high salt dietary treatment in Dahl S rats (Fig. 1). In Dahl R rats, high dietary salt treatment increased the MAP by only 8 ± 3 mmHg. Together these data demonstrate that the salt-sensitivity of the blood pressure is dramatically enhanced in the Dahl S rats as compared with Dahl R rats.

3.2. Effect of high salt diet on $[Na^+]$ and $[K^+]$ in the CSF and the plasma

We then assessed whether high salt intake alters $[Na^+]$ and $[K^+]$ in the CSF and in the plasma of Dahl S and Dahl R rats. In both Dahl S and Dahl R rats, sodium levels in the CSF are higher than that in the plasma, suggesting that net sodium excretion from choroid plexus is larger than water excretion. High salt intake treatment for 2 weeks significantly enhanced $[Na^+]$ in the CSF of Dahl S rats (Fig. 2A). In contrast, the $[Na^+]$ in the CSF of Dahl R rats was not significantly altered by the high salt intake, suggesting increased sodium excretion or reduced water excretion into CSF in Dahl S rats as compared with Dahl R rats. However, high salt diet treatment did not significantly alter the $[Na^+]$ in plasma of both Dahl S and Dahl R rats. The effects of high salt diet on $[K^+]$ in the CSF and in the plasma