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Full paper

High salt loading induces urinary storage dysfunction via upregulation of epithelial sodium channel alpha in the bladder epithelium in Dahl salt-sensitive rats

Seiji Yamamoto [a], Yuji Hotta [a], Kotomi Maeda [a], Tomoya Kataoka [b], Yasuhiro Maeda [a], Takashi Hamakawa [c], Yasuhiro Shibata [d], Shoichi Sasaki [c], Shinya Ugawa [d], Takahiro Yasui [c], Kazunori Kimura [a, b, *]

[a] Department of Hospital Pharmacy, Graduate School of Pharmaceutical Sciences, Nagoya City University, 3-1 Tanabe do-ri, Mizuho-ku, Nagoya 467-8603, Japan
[b] Department of Clinical Pharmaceutics, Graduate School of Medical Sciences, Nagoya City University, 1-Kawasumi, Mizuho-cho, Mizuho-ku, Nagoya 467-8601, Japan
[c] Department of Nephro-urology, Graduate School of Medical Sciences, Nagoya City University, 1-Kawasumi, Mizuho-cho, Mizuho-ku, Nagoya 467-8601, Japan
[d] Department of Anatomy and Neuroscience, Graduate School of Medical Sciences, Nagoya City University, 1-Kawasumi, Mizuho-cho, Mizuho-ku, Nagoya 467-8601, Japan

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ABSTRACT

We aimed to investigate whether high salt intake affects bladder function via epithelial sodium channel (ENaC) by using Dahl salt-resistant (DR) and salt-sensitive (DS) rats. Bladder weight of DR + high-salt diet (HS, 8% NaCl) and DS + HS groups were significantly higher than those of DR + normal-salt diet (NS, 0.3% NaCl) and DS + NS groups after one week treatment. We thereafter used only DR + HS and DS + HS group. Systolic and diastolic blood pressures were significantly higher in DS + HS group than in DR + HS group after the treatment period. Cystometrogram showed the intercontraction intervals (ICI) were significantly shorter in DS + HS group than in DR + HS group during infusion of saline. Subsequent infusion of amiloride significantly prolonged ICI in DS + HS group, while no intra-group difference in ICI was observed in DR + HS group. No intra- or inter-group differences in maximum intravesical pressure were observed. Protein expression levels of ENaCα in the bladder were significantly higher in DS + HS group than in DR + HS group. ENaCα protein was localized at bladder epithelium in both groups. In conclusion, high salt intake is considered to cause urinary storage dysfunction via upregulation of ENaC in the bladder epithelium with salt-sensitive hypertension, suggesting that ENaC might be a candidate for therapeutic target for urinary storage dysfunction.

1. Introduction

Lower urinary tract symptoms (LUTS) are common among aging individuals, being bothersome and lowering quality of life.[1] LUTS are generally classified into storage, voiding and post micturition symptoms. Among these, storage symptoms are associated with dysfunction in urine storage, leading to increased daytime frequency, nocturia, urgency, and urinary incontinence.[2]

Clinically, researchers have identified hypertension as one of the positive risk factors for nocturia and/or urinary frequency.[3,4] The International Prostate Symptom Score (IPSS) for urinary frequency and nocturia in patients with benign prostatic hyperplasia and hypertension was significantly higher than those of normotensive patients with benign prostatic hyperplasia.[5] As well, urinary storage dysfunction has been identified in animal models of hypertension, such as spontaneously hypertensive rats (SHRs), two kidney-1 clip rats and Dahl salt-sensitive rats.[6–8]

* Corresponding author. 1-Kawasumi, Mizuho-cho, Mizuho-ku, Nagoya 467-8601, Japan. Fax: +81 52 858 7402.
E-mail address: kkimura@med.nagoya-cu.ac.jp (K. Kimura).
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A high salt intake is one of the major risk factors for developing hypertension. In humans, patients with essential hypertension can be subclassified based on the presence of a sensitivity or insensitivity of blood pressure to salt intake.[9] Dahl salt-sensitive (DS) rats have been widely used to study salt-induced hypertension. However, inbreeding of Dahl rats is used to obtain two congenic strains, namely, DS rats and Dahl salt-resistant (DR) rats. DS rats exhibit severe hypertension on a high salt diet, whereas the blood pressure of DR rats does not respond to a high salt diet.[10] Many investigators have reported that DS rats exhibit an enhanced expression and activity of epithelial sodium channels (ENaC) in the kidneys after a high salt intake, compared to a control group, with the expression of ENaC thought to underlie the pathophysiology of salt-sensitive hypertension.[11]

Recently, evidence has been provided that bladder epithelium does not solely provide a permeable barrier to substances in urine but also acts as a sensor to thermal, chemical and mechanical stimulation.[12,13] When stimulated, bladder epithelial cells release several types of physiologically active substances (e.g., adenosine triphosphate, acetylcholine, nitric oxide, nerve growth factor, etc.), which contribute to bladder function. Impairment in the sensing function of bladder epithelial cells is, therefore, considered to be one of pathways underlying the pathogenesis of bladder dysfunction.[12–14]

A number of ion channels (e.g., several transient receptor potential channels, Piezo1 and ENaC) are expressed in bladder epithelium and serve as mechanosensors to translate intravesical pressure into afferent input for micturition.[13–16] Specifically, ENaCs play a role in stretch-evoked release of ATP from bladder epithelium, which is a physiologically active substance for evoking contraction of the detrusor muscle.[17] Moreover, in our previous study, we reported that upregulation of ENaC in the bladder caused frequent urination in rats treated with a mineralocorticoid receptor agonist.[18] Though it has already been reported that DS rats exhibit urinary storage dysfunction after a high salt intake,[7] it is unknown if a high-salt diet induces alterations in bladder epithelium in these animals. Therefore, the aim of our study was to investigate whether a high-salt diet altered expression levels of ENaC in urinary bladder epithelium in DS rats and to evaluate the effects of any alteration in ENaC expression on bladder function.

2. Materials and methods

2.1. Experimental protocols

We used 6-week-old male Dahl salt-resistant (DR) and Dahl salt-sensitive (DS) rats (SLC Inc., Shizuoka, Japan). They were kept in a temperature- and humidity-controlled room, with a 12-h/12-h light/dark cycle. They were fed normal salt diet (NS, 0.3% NaCl; CE-2, CLEA Japan, Inc., Tokyo, Japan) as DR + NS and DS + NS groups or high salt diet (HS, 8% NaCl; CE-2, CLEA Japan, Inc., Tokyo, Japan) as DR + HS and DS + HS groups, with free access to normal water, for one week. Bladder weight was measured in each group.

Next, to evaluate the influences of salt sensitivity on bladder function a cystometrogram was performed in DR + HS and DS + HS groups. For in vitro analyses, rats in DR + HS and DS + HS groups were euthanized, via excessive anesthesia, and their bladders were collected and tissue samples were prepared for fluorescent immunohistochemistry and western blotting analysis.

All animal experiments were performed in accordance with the Guiding Principles for the Care and Use of Laboratory Animals of the Science and International Affairs Bureau of the Japanese Ministry of Education, Culture, Sports, Science and Technology. The study design was reviewed and approved by the Ethics Committee of Nagoya City University.

2.2. Bladder-filling cystometry

Under inhalational anesthesia, with 3% isoflurane for induction and 2% isoflurane for maintenance, the bladders were exposed and polyethylene-50 tubing (with holes in the sides) was inserted into the bladder dome and sealed with 5-0 silk sutures. The tubing was connected to a syringe pump (NE-300, New Era Pump Systems Inc., Farmingdale, NY, USA) that was connected to a pressure transducer (UD5500, Dantec, Denmark) which allowed for saline infusion. Cystometry was then performed while the rats were awake in a restraining cage. In both DR and DS rats, saline infusion (0.08 mL/min at room temperature) was performed, followed by infusion of 1 mM amiloride hydrochloride hydrate (an ENaC inhibitor, 0.08 mL/min at room temperature) for analysis of intercontraction intervals (ICIs, the time between two voiding contractions) and the maximum intravesical pressure (MP, the maximum voiding pressure during a micturition cycle). We then compared the intra- and inter-group changes in ICI and MP during the saline and amiloride infusions. As rats were restrained during cystometry, intravesical pressure was assumed to be equal to detrusor pressure, with no residual urine volume observed.

2.3. Fluorescent immunohistochemistry

Extirpated urinary bladders of the rats of two groups fed high salt diet were initially fixed in 10% formaldehyde, which was then replaced with 70% ethanol, and the fixed samples were subsequently embedded in paraffin. Serial sections (4-μm) were deparaffinized and rehydrated in an ethanol series, and antigens were activated by the microwave method. The sections were blocked with 5% skim milk in PBS for 1 h at room temperature and incubated with a rabbit anti-ENaCα polyclonal antibody (1:200, Bioss Inc., Woburn, MA, USA), which was mixed and diluted in Reagent 1 of IMMUNO SHOT (Cosmo Bio Co., Ltd., Tokyo, Japan), at 4 °C overnight. ENaCα protein was visualized using the Alexa Fluor 488 Goat Anti-Rabbit IgG (H + L) antibody for 30 min (1:1000, Molecular Probes), which was diluted in Reagent 2 of IMMUNO SHOT. The nuclei were then stained for 5 min using a Cellstain DAPI solution (Dojindo Laboratories, Kumamoto, Japan). The kidneys of 10-week-old male Sprague–Dawley rats (SLC Inc., Shizuoka, Japan) were used as positive controls for the primary antibody.

2.4. Protein extraction and western blotting

Protein from the urinary bladder of the rats of two groups fed high salt diet (DR + HS and DS + HS groups) was extracted using the PRO-PREP Protein Extraction Solution (iNtRON Biotechnology Inc., Gyeonggi-do, Korea), with the total protein concentration quantified using the BCA Protein Assay Reagent (Pierce Biotechnology, Rockford, IL, USA). Samples containing 15 μg of total protein were separated, via 7.5% sodium dodecyl sulfate-polyacrylamide gel electrophoresis, and were then transferred onto polyvinylidene difluoride membranes (ImmobilinTM; Millipore Corp., Bedford, MA, USA). The membranes were blocked with 5% skim milk, incubated with the primary rabbit anti-ENaCα polyclonal antibody (1:500; Bioss Inc., Woburn, MA, USA) in Tris-buffered saline and Tween 20 (TBST), and then incubated in TBST with the secondary anti-rabbit immunoglobulin G (IgG), which was conjugated to horseradish peroxidase (1:5000; GE Healthcare, Little Chalfont, UK). The protein bands were visualized using ECL prime (GE Healthcare) and LAS 3000 mini (GE Healthcare). The membranes were then washed with TBST and the bound primary and secondary antibodies were removed using Restore Plus Western Blot Stripping Buffer (Thermo Fisher Scientific, Waltham, MA, USA). Next, the membranes were re-blocked with 5% skim milk,

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