Life Sciences 141 (2015) 8–12

Contents lists available at ScienceDirect

Life Sciences

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

Underlying mechanisms of urine storage dysfunction in rats with salt-loading hypertension

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ARTICLE INFO

Article history:
Received 26 April 2015
Received in revised form 24 August 2015
Accepted 17 September 2015
Available online 21 September 2015

Keywords:
Bladder
Storage dysfunction
Rats
Salt-loading hypertension
Urothelium

ABSTRACT

Aims: Spontaneous hypertensive rats provide a genetic model for exploring the pathogenesis of urine storage dysfunction related to hypertension (HT). In humans, however, HT develops by both genetic and environmental factors including lifestyle factors such as a high-calorie diet, excessive salt intake and stress. We investigated the influence of salt-loading on bladder function and the underlying mechanisms of storage dysfunction related to HT.

Main methods: Six-week-old male Dahl salt-sensitive (DS) and Dahl salt-resistant (DR) rats were fed with a normal or high-salt diet for 12 weeks. Micturition parameters were obtained from a metabolic cage. Whole bladders were excised from 18-week-old rats and distended in an organ bath. The releases of adenosine triphosphoric acid (ATP) and prostaglandin E_2 (PGE_2) from the distended bladder epithelia were measured. Changes in bladder blood flow (BBF) were determined with a laser-speckle-blood-flow imaging system.

Key findings: An increase in mean blood pressure (BP) was noted only in DS rats after salt-loading. During the inactive (sleeping) period, voided volume per micturition gradually increased in DR rats fed a normal or high-salt diet and normal-diet DS rats, while it did not change in the DS rats fed a high-salt diet. Bladder distension significantly increased ATP and PGE_2 release from the urothelium in DS rats fed a high-salt diet. BBF was significantly decreased in high-salt-diet DS rats.

Significance: One mechanism behind the relationship between salt-sensitive HT and urine storage dysfunction may be an increase in ATP and PGE_2 release from the urothelium via suppression of BBF.

1. Introduction

Clinical observations indicate that many non-urological diseases such as hypertension (HT), dyslipidemia and diabetes are associated with lower urinary tract symptoms (LUTS). Lifestyle factors, especially those that result in HT, can influence LUTS [1]. HT is one of the risk factors for worsening lower-urinary-tract symptoms and degrading the improvement of storage symptoms by α1-blocker [2,3]. The spontaneous hypertensive rats (SHRs) provides researchers a genetic model of HT, and is considered a valuable tool for exploring the pathogenesis of urine storage dysfunction related to HT [4]. SHRs exhibit increased urinary frequency and non-voiding detrusor contractions compared with normotensive Wister Kyoto rats. The bladder storage dysfunction in SHRs reportedly depends on multiple factors including overproduction of nerve growth factor in the smooth muscle, autonomic hyperinnervation, altered caveolae-mediated purinergic signaling, enhanced sensitivity of afferent stimulation, and decreased blood supply to the bladder [4–7]. In humans, however, hypertension develops by both genetic and environmental factors including lifestyle factors such as a high-calorie diet, excessive salt intake and stress. Therefore, it is necessary to examine which other factors essentially contribute to the development of storage dysfunction associated with HT. Therefore, the use of other types of HT models is of great importance to clarify the mechanisms leading to urine storage dysfunction.

Excess salt intake is one of the major causes of HT. Inbred Dahl salt-sensitive (DS) and Dahl salt-resistant (DR) rat strains [8], selectively bred from outbred Sprague–Dawley rats under salt-loading conditions, provide contrasting models of high and relatively normal blood pressure (BP), respectively [9]. Supplemental dietary salt increases BP in DS rats, but has little or no influence in DR rats. Although SHRs have been largely investigated for basic understanding of the genetics and the pathophysiology of urine storage dysfunction, no studies have focused on the contribution of salt-sensitive HT to bladder function using this kind of animal model. This study aimed therefore to characterize bladder dysfunction in DS and DR rats and focused on possible mechanisms of urine storage dysfunction.

Abbreviations: HT, hypertension; SHR, spontaneous hypertensive rat; BP, blood pressure; DS rat, Dahl salt-sensitive rat; DR rat, Dahl salt-resistant rat; BBF, bladder blood flow; ATP, adenosine triphosphoric acid; PGE_2, prostaglandin E_2.

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http://dx.doi.org/10.1016/j.lfs.2015.09.010
0024-3205/

2. Materials and methods

This study followed the standard guidelines for the handling of experimental animals and was approved by the animal care committee of Fukui University. Thirty-two 6-week-old male DS rats weighing 190–245 g (mean 209.4 ± 25.4 g, n = 12) and DR rats weighing 225–240 g (mean 229.3 ± 22.4 g, n = 12) were purchased from Sankyo Laboratory (Tokyo, Japan).

2.1. Animals

Sympathetic nerve activity is augmented with salt-loading in DS rats, but not in DR rats. Six-week-old male DS and DR rats were randomly assigned to two different dietary treatments, namely, a normal-salt (0.252%) or high-salt (8%) diet together with 35 ml water/day for 12 weeks (6 rats for each group). The concentration of salt diet was determined by the original articles [8,9]. DS and DR rats were selectively bred from outbred Sprague–Dawley rats [8], therefore, healthy rats would be divided to 2 groups; salt-sensitive and -resistant under high-salt diet. Based on other similar works [10,11], it is not necessary to include a control group under normal- or high-salt diets in this study. The rats were housed at a constant temperature of 23 ± 2 °C (mean ± SEM) and 50% to 60% humidity under a regular 12-hour light–dark schedule (the lights came on automatically at 8:00 am).

2.2. Physiological characteristics

The average body weight and mean BP were monitored every two weeks. The mean BP was measured in conscious rats with a computerized tail-cuff system (BA-98A system; Softron Co., Tokyo, Japan) that determines mean BP using a photoelectric sensor. The rats were housed in metabolic cages, and the urine was collected using a plastic box placed on an electronic balance through a urine collection funnel. The cumulative weight of the collected urine was recorded every 3 min by a computer connected to the electronic balance. The rats were kept for about 48 h in the cage; we used only the values recorded in the last 24 h. The micturition frequency for 24 h and the mean voided volume per micturition were calculated according to the recorded data. The inactive (sleeping) period urine volume ratio, which was defined as urine volume during the inactive period divided by the 24-hour urine output, was calculated.

2.3. Adenosine triphosphoric acid (ATP) and prostaglandin E_2 (PGE_2) released from the stretched bladder

The amount of ATP and PGE_2 released from the stretched bladder epithelium at the age of 18 weeks was measured according to the method described by Tanaka et al. [12]. Using halothane anesthesia, the whole bladder with the urethra was excised. For intravesical infusion, an infusing catheter was inserted through the urethra and fixed with a surgical suture at the bladder neck. The bladder was fixed vertically in a 10-ml organ bath with Krebs solution gassed with 5% CO_2 and 95% O_2 at 37 °C. Krebs solution was composed of 118 mM NaCl, 4.7 mM KCl, 2.5 mM $CaCl_2$, 1.2 mM $MgSO_4$, 1.2 mM KH_2PO_4, 25.0 mM $NaHCO_3$ and 11.1 mM D-(+)-glucose. The inside of all bladders was initially washed with 0.3 ml Krebs solution. To set up the distension condition 0.3 ml Krebs solution (baseline level) was infused and 1.5 ml (distension) vehicle was then manually infused at about 0.04 ml per second. Solutions were intravesically infused by a syringe, allowed to stand for 10 min and collected by dropping in free fall after removal of the syringe. Each sample was collected in a tube on ice to avoid metabolism by ecto-adenosine triphosphatase. ATP was measured by ATPlite™ luciferin–luciferase assay using a Fusion luminometer (PerkinElmer, Waltham, Massachusetts). A 100 μl sample was added to 50 μl each of mammalian cell lysis solution and ATPlite™ buffer. The solution was mixed and luminescence was measured. PGE_2 was measured by enzyme-linked immunoassay (PGE_2 Express EIA Kit—Monoclonal Cayman Chemicals, Ann Arbor, Michigan) using a SpectraMax 250 plate reader. A 50 μl sample was incubated and mixed. Absorbance was measured after adding 200 μl Ellman's reagent.

2.4. Measurement of bladder blood flow (BBF)

The BBF values of 18 week-old DS rats fed a normal or high-salt diet were determined with a laser speckle blood-flow-imaging system (Omegazone OZ-2, Omegawave Inc., Tokyo, Japan). Each rat was anesthetized using halothane. The bladder was exposed by an infusion pump and physiological saline. The infusion pump speed was 6 ml/h and infused a volume of 1.0 ml. The BBF during the filling phase was expressed as a percentage of that in the empty bladder, which was expressed as 100%.

2.5. Histological examination

The microvasculature of bladders from 18-week-old DS rats fed a normal- or high-salt diet for 12 weeks was evaluated by hematoxylin–eosin stain and fibrin-specific phosphotungstic acid hematoxylin stain.

2.6. Data analysis

Results are presented as means ± SEMs. All data were analyzed by Student's t-test or Dunnett's multiple comparison test using SPSS, version 16.0J for Windows. Values of $p < 0.05$ were considered statistically significant.

3. Result

3.1. BP, body weight, and micturition parameters

No week-to-week change in mean BP was found in DS rats fed a normal diet or DR rats fed a normal or high-salt diet. The mean blood pressure of DS rats fed a high-salt diet increased from 90.3 ± 3.6 to 124.6 ± 4.1 mm Hg after two weeks of salt-loading, and further increased to 139.5 ± 7.7 mm Hg after four weeks of salt-loading, remaining at that level for the remainder of the experiment (Fig. 1A). Body weight in normal-diet DS and DR rats were 422.0 ± 12.1 and 420.7 ± 54.0 g

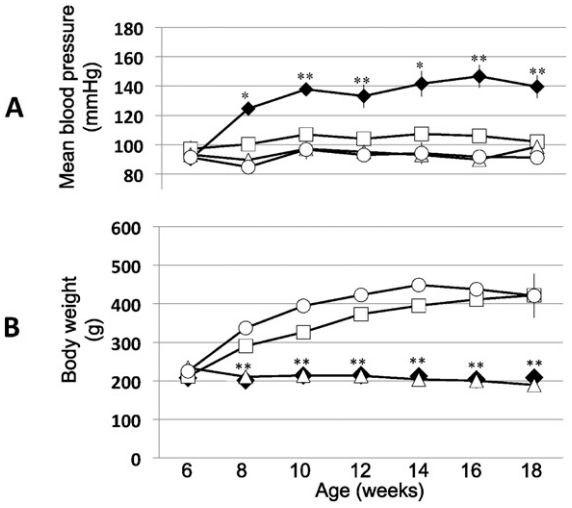

Fig. 1. Changes of physical characteristics in experimental animals. A, mean blood pressure in normal-diet DR rats (○; n = 6), high-salt-diet DR rats (△; n = 6), normal-diet DS rats (□; n = 6), and high-salt-diet DS rats (♦; n = 6). Values are means ± SEMs. *: $p < 0.05$ vs normal-diet DS rats, **: $p < 0.01$ vs normal-diet DS rats. B, body weight in experimental animals. **: $p < 0.01$ vs normal-diet DS and DR rats.