Autonomic Neuroscience: Basic and Clinical 164 (2011) 27–33

Contents lists available at ScienceDirect

Autonomic Neuroscience: Basic and Clinical

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

# Function and expression pattern of TRPM8 in bladder afferent neurons associated with bladder outlet obstruction in rats

Tokumasa Hayashi [a,b,*], Teruyoshi Kondo [c,d], Masaru Ishimatsu [b], Mitsue Takeya [b], Sachiyo Igata [b], Kei-ichiro Nakamura [c], Kei Matsuoka [a]

[a] Department of Urology, Kurume University School of Medicine, Asahimachi 67, Kurume, Japan
[b] Division of Integrated Autonomic Function, Department of Physiology, Kurume University School of Medicine, Asahimachi 67, Kurume, Japan
[c] Division of Microscopic and Developmental Anatomy, Department of Anatomy, Kurume University School of Medicine, Asahimachi 67, Kurume, Japan
[d] Department of Clinical Engineering, Kyushu University of Health and Welfare School of Health Science, Yoshinomachi 1714–1, Nobeoka, Japan

ARTICLE INFO

*Article history:*
Received 26 November 2010
Received in revised form 3 May 2011
Accepted 21 May 2011

*Keywords:*
TRPM8
Urinary bladder
Rat
TRPV1
Bladder cooling reflex
Bladder outlet obstruction

ABSTRACT

We investigated the function and expression pattern of the transient receptor potential melastatin-8 (TRPM8) in urinary bladder afferent neurons from control and bladder outlet obstruction (BOO) rats. BOO was produced and, after six weeks, the effects of intravesical infusion of menthol, the agonist of TRPM8, were investigated using unanesthetized cystometry. The intravesical infusion of menthol produced an increase in the micturition pressure in both sham surgery and BOO rats. In BOO rats, increased basal and threshold pressure and a decreased micturition interval were observed. Next, the population of TRPM8-positive and the co-expression proportion of TRPM8 with neurochemical markers (NF200 or TRPV1) in the bladder afferent neurons were each compared between the control and BOO rats using retrograde tracing and immunohistochemistry. The population of TRPM8-immunoreactive bladder afferent neurons was larger in BOO rats ($3.28 \pm 0.43\%$) than in the control rats ($1.33 \pm 0.18\%$). However, there were no statistical differences between the control and BOO rats in the co-expression proportion of neither TRPM8-NF200 ($84.1 \pm 4.3\%$ vs $79.7 \pm 2.7\%$, $p = 0.41$) nor TRPM8-TRPV1 ($33.3 \pm 3.6\%$ vs $40.8 \pm 2.6\%$, $p = 0.08$) in the bladder afferent neurons. The present results suggest that the neuronal input through TRPM8-positive bladder afferent neurons are augmented after BOO, however, the neurochemical phenotype of the up-regulated TRPM8-positive bladder afferent neurons is not changed after BOO.

© 2011 Elsevier B.V. All rights reserved.

## 1. Introduction

Over active bladder (OAB), which is characterized by urgency or urge incontinence, is a bothersome symptom in the patients with benign prostatic hyperplasia (BPH) (Chapple and Roehrborn, 2006). BPH causes OAB following bladder outlet obstruction (BOO). The etiologies of detrusor overactivity caused by BOO have been demonstrated using an animal model. After partial urethral obstruction, histological and functional changes are observed in the detrusor (Speakman et al., 1987; Seki et al., 1992) and in the neuronal pathway of the micturition reflex (Steers and De Groat, 1988; Steers et al., 1991).

The evaluation of bladder cooling reflex (BCR), or the contraction of urinary bladder caused by the intravesical infusion of cold water, is an appropriate diagnostic tool for neurogenic detrusor overactivity (Bors and Blinn, 1957; Geirsson et al., 1999). Jiang et al. (2002) proposed that the BCR was carried via the C-fibers innervating the urinary bladder, because an intravesical infusion of small amount of cold water facilitated the action potential of C-fibers in the pelvic nerve in an anesthetized normal cat model. The cold-sensitive thermoreceptors that are linked to the C-fibers have been detected in the bladder wall (Fall et al., 1990; Mazières et al., 1998). Additionally, the responsible afferents for the BCR are sensitized by the intravesical infusion of menthol (Lindström and Mazières, 1991; Geirsson, 1993).

As BCR is observed in some patients with BPH (Chai et al., 1998; Gotoh et al., 1999), it is therefore hypothesized that detrusor overactivity with BPH likely results from an increased excitability in C-fibers following BOO (Hirayama et al., 2003). In the rat BOO model, C-fiber dependent sacral micturition reflex enhancement has been demonstrated (Steers and De Groat, 1988) and capsaicin-sensitive C-fiber bladder afferents were found to be involved in functional alterations in bladder afferent pathways following BOO (Tanaka et al., 2003).

Transient receptor potential melastatin-8 (TRPM8) has been shown by two groups (McKemy et al., 2002; Peier et al., 2002) as a cold temperature- and menthol-sensitive cation channel. TRPM8 in the bladder wall was first identified by Stein et al. (2004) using immunohistochemistry and RT-PCR, therefore TRPM8 might be a candidate for

* Corresponding author at: Division of Integrated Autonomic Function, Department of Physiology, Kurume University School of Medicine, Asahimachi 67, Kurume, Fukuoka, Japan. Tel.: +81 942 31 7543; fax: +81 942 31 7728.
*E-mail address:* hayashi_tokumasa@kurume-u.ac.jp (T. Hayashi).

1566-0702/$ – see front matter © 2011 Elsevier B.V. All rights reserved.
doi:10.1016/j.autneu.2011.05.006

inducing the BCR because intravesical infusion of menthol facilitated the micturition reflex in guinea pig (Tsukimi et al., 2005) and rat (Nomoto et al., 2008) models.

Several populations of C-fibers desensitized by capsaicin are considered to be responsible for the BCR in humans (Das et al., 1996) and guinea pigs (Tsukimi et al., 2005). These results suggest that TRPM8 may be expressed in capsaicin-sensitive, i.e. TRPV1-positive, bladder afferent neurons. However, in the rat, it is advocated that intravesically-infused menthol acts on the capsaicin-resistant afferents to facilitate the micturition reflex, because capsaicin pretreatment had no effect on this reflex (Nomoto et al., 2008). We recently described the population and neuronal phenotype of TRPM8-expressing rat bladder afferent neurons. The co-expressed proportion of TRPM8 and TRPV1 in the bladder afferent neurons was 36.1 ± 4.0% under normal conditions (Hayashi et al., 2009).

We hypothesized that the neuronal phenotype of TRPM8-expressed bladder afferents, which appears to induce the BCR, might alter following BOO. These post-BOO alternations might affect the micturition reflex induced by the intravesical infusion of menthol. In other cases of micturition disorders, the increase in TRPM8-immunoreactive nerve fibers in the bladder wall was correlated with the symptom score in the patients with painful bladder syndrome (Mukerji et al., 2006).

In the present study, we compared the micturition reflex induced by intravesical infusion of menthol between control and BOO rats using unanesthetized cystometry. We also compared the population and neuronal phenotype (A-fiber or C-fiber and/or capsaicin-sensitive or -insensitive) of TRPM8-expressing bladder afferent neurons between control and BOO rats using the retrograde tracing technique and immunohistochemistry.

## 2. Materials and methods

### 2.1. Animals

In this study, we used 26 female Wistar rats (250–270 g). The experimental protocols were approved by the Animal Research Committee of the Kurume University School of Medicine.

### 2.2. Procedures of bladder outlet obstruction

Under anesthesia by sodium pentobarbital treatment (50 mg/kg, i.p.), the bladder and proximal urethra were exposed via median laparotomy. The proximal urethra was freed from the vaginal wall by careful dissection to avoid injury to the periurethral blood vessel. Three-zero black silk suture was placed around the proximal urethra and a length of metal rod with an outer diameter of 1.1 mm was placed extralumenally. After tying the suture, the rod was removed. The suture effectively fit the urethra and was freely mobile. In the sham surgery, polyethylene tubing with an outer diameter of 3.0 mm was used to place a ligature around the urethra to stimulate the surgery without creating an obstruction.

### 2.3. Cystometry

Six weeks after the surgery, BOO (n = 8) and control (n = 8) rats were anesthetized with sodium pentobarbital, and polyethylene tubing (3 Fr size; Kunii, Japan) with the end flared by heat was inserted into the dome of the bladder and secured in place with a 6–0 nylon purse-string suture. At the same time, the suture for BOO around the proximal urethra was carefully removed. The distal end of the tubing was sealed, subcutaneously tunneled and externalized through a small incision at the back of the neck and animals received Piperacillin (Taisho Toyama Pharmaceutical Co., Ltd, Japan) (50 mg/kg body weight, intramuscularly administered). Three days following the tube implant, animals were placed unrestrained in a cage and the distal end of the tube was connected to polyethylene tubing (5 Fr size; Kunii, Japan) which was in turn connected to a heat generator (UA-10S; EYELA, Japan) to keep physiological saline infused to bladder at 37 °C. The tube was connected via T-tube to a pressure transducer (DX-360; Nihon Kohden, Japan) for bladder pressure monitoring and an injection pump (TE-312; Terumo, Japan) for the infusion of physiological saline at a rate of 20 ml per hour. The signal of the intravesical pressure was amplified using AP-621 (Nihon Kohden, Japan) and digitized by a MiniDigi 1A instrument (Axon Instruments Inc, USA). The pCLAMP 9 software program (Axon Instruments Inc, USA) installed on a computer (DELL Inc, USA) was used for the continuous data acquisition (AxoScope 9.0) and the later analysis (Clampfit 9.0). At the same time, the micturition volume was measured by means of a fluid collector connected to an isometric transducer (TB-611 T; Nihon Kohden, Japan). Because preliminary experiments revealed that the micturition intervals were not steadied at the beginning of saline infusion, the control data were obtained 2 h after the start of infusion. Menthol (Sigma, MO, USA) diluted in saline with 2% ethanol (0.06 mM, 0.6 mM or 6 mM) which was maintained at 37 °C was continuously infused into the bladder after saline infusion. A minimum of three reproducible micturition cycles were recorded in each menthol concentration infusion, which thus allowed us to calculate the mean of cystometrical parameters including basal pressure (BP), threshold pressure (TP; bladder pressure at the start of detrusor contraction for micturition), micturition pressure (MP) and micturition interval (MI). Pre-micturition contractions (PC), defined as the spontaneous detrusor contractions for 2 min prior to micturition, were counted in BOO rats.

### 2.4. Immunohistochemistry in DRG

Retrograde tracing of DRG neurons innervating the bladder and triple-immunostaining using tyramide signal amplification (TSA) was performed as previously described (Hayashi et al., 2009). Briefly, BOO (n = 5) and sham surgery (n = 5) rats were created as described above. After six weeks, they were anesthetized, and the urinary bladder was exposed. At each of approximately 6–10 sites on the dorsal surface of the bladder, 1 μl of 2% wheat-germ agglutinin-horseradish peroxidase (WGA-HRP) (Sigma., MO, USA) was injected. The rats were re-anesthetized 2 days after WGA-HRP injection, and transcardially perfused with heparinized saline followed by 500 ml of 4% paraformaldehyde in 0.1 M phosphate buffer. The bilateral L6-S1 DRGs were extirpated and incubated in 0.1 M phosphate buffer containing 30% sucrose for 18 h. The extirpated DRGs were embedded in OCT compound and frozen. Serial sections (16 μm-thick) were prepared from the DRGs in 2 sets consisting of every fourth section using cryostat sectioning.

The sections were incubated in biotinylated tyramide for 15 min (1:100; PerkinElmer., MA, USA). Sections were then washed and incubated with 3% $H_2O_2$ for 10 min. The sections were rinsed and incubated in blocking buffer (Blocking reagent packed in the TSA kit). The sections were incubated with a rabbit anti-TRPM8 antibody (×8000; Abe et al., 2005) for 18 h at 4 °C in PBSTNB buffer (0.01 M PBS pH 7.5, 0.05% Triton X-100, 0.1%, $NaN_3$, 1% BSA). After several rinses, the sections were incubated with an anti-rabbit IgG-conjugated polymer-HRP, EnVision$^+$ (DAKO., CA, USA) for 60 min, rinsed, and were incubated with Cy3-conjugated tyramide (1:50; PerkinElmer., MA, USA) for no more than 5 min. After washing, the sections were incubated with mouse anti-NF200 antibody (1:100,000; Sigma., MO, USA) or rabbit anti-TRPV1 antibody (1:300; Oncogene., Germany) for 18 h at 4 °C. Subsequently, the sections were rinsed and incubated with Alexa488-conjugated anti-mouse IgG (1:2000; Invitrogen., CA, USA) or Alexa488-conjugated anti-rabbit IgG (1:2000; Invitrogen., CA, USA) for 90 min. The incorporated biotin-tyramide was visualized by adding streptavidin-Alexa350 (1:300; Invitrogen., CA, USA) to the final incubation solution. After washing, the specimens were mounted using PermaFluor aqueous mounting medium (Thermo., PA, USA).

Download English Version:

https://daneshyari.com/en/article/6004720

Download Persian Version:

https://daneshyari.com/article/6004720

Daneshyari.com