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Piezo1 expression increases in rat bladder after partial bladder outlet obstruction

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ABSTRACT

Aims: For patients with benign prostatic hyperplasia (BPH), storage symptoms due to bladder dysfunction are bothersome, and that mechanism elucidation is needed. Piezo1, a mechanically activated ion channel, is believed to play a role in sensing bladder distension. To investigate the involvement of Piezo1 in bladder dysfunction, we examined the expression and distribution of Piezo1 and neurofilament (NF-L) to understand pathological alterations in rat bladders with partial bladder outlet obstruction (pBOO), an animal model of BPH.

Main methods: Female Sprague–Dawley rats were subjected to sham or pBOO operations. On days 3, 7, and 14 after pBOO, *Piezo1* mRNA levels in the bladder were examined by quantitative real-time PCR. The expression of light NF-L was also examined by western blotting. On day 7, the distributions of Piezo1 were examined by in situ hybridization.

Key findings: The expression levels of *Piezo1* mRNA in whole bladder were significantly increased from days 3 to 14 after pBOO. On day 7 in pBOO rats, significant increases in *Piezo1* mRNA were observed in the detrusor layer as well as the suburothelial layer, while the predominant distribution was observed in the urothelium of sham rats. Coinciding with the increase in Piezo1, the decreases in NF-L expression were observed in the bladder from pBOO rats.

Significance: The increase in Piezo1 in pBOO rat bladders might be involved in the compensatory mechanism associated with bladder denervation including the decrease in NF-L. Inhibition of Piezo-1 may be a new therapeutic approach to ameliorate the storage dysfunction shown in pBOO.

1. Introduction

Benign prostatic hyperplasia (BPH) is a condition of nonmalignant enlargement of the prostate gland. Patients with BPH commonly show voiding symptoms (e.g. weak urine stream and urinary retention) related to the partial bladder outlet obstruction (pBOO) [1,2]. Approximately half of the patients also exhibit storage symptoms (urinary frequency, urgency, and nocturia) due to bladder dysfunction secondary to pBOO [2,3]. The storage symptoms are bothersome and lower the patient's quality of life; however, the pathophysiology of such bladder dysfunction remains to be elucidated. To understand it better, pBOO animal model, especially obstruction is created by a surgical placement of a ligature, has been developed [4,5]. In pBOO rats on days 3, 7, 14 after surgery, bladder hypertrophy shown by wall thickness and significant increase in bladder weight is observed similarly to patients with pBOO [6,7]. In addition, non-voiding contractions (NVCs) are also observed in that model [8,9], which indicate bladder dysfunction including detrusor instability showed in patients with BPH. As the mechanism of bladder dysfunction, previous studies have reported that animals with pBOO showed myogenic and neurogenic changes in the bladder. For example, up-regulation of connexin 43 enhances intracellular communication [10], and partial denervation induces hypersensitivity to acetylcholine in the bladder [11]. More recently, the contribution of certain molecules including channels and neurotransmitters in the urothelium to the pathogenesis has been highlighted [12]. Interestingly, it has been reported that the expression of stretch-activated channels (SACs) such as transient receptor potential (TRP) channels, and the release of neurotransmitters were significantly increased in the urothelium of bladders with pBOO [13–17]. These increases possibly enhance the activity of afferent nerves (c-fibers) in the bladder, which can affect the micturition reflex in the storage urine phase. As mentioned above, the pathophysiology has been elucidated step by step; however, more exact mechanism and critical targets are needed to develop a drug for storage symptoms.

Piezo1 and Piezo2 have been identified recently as mechanically activated ion channels [18]. Piezo1 is expressed in several tissues including bladder, kidney, lung, skin and endothelial cells. Piezo1 commonly plays important roles in sensing stretch. For example, it acts as a sensor of shear stresses and determines vascular structure to detect blood flow, and it detects intraluminal pressure change and urine flow sensing in renal tubular [19,20]. Piezo2 is expressed in

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sensory trigeminal ganglia, dorsal root ganglia, Merkel cells, lung, and bladder, and participates in the recognition of light touch and noxious stimuli [18,19]. In bladder, little has been reported on the role of Piezo2, but Piezo1 is localized in the urothelium of normal mice and humans. Piezo1 in bladder has been suggested to contribute to sensing bladder distention similar to SACs [21]. Therefore, Piezo1 could be involvement of bladder dysfunction as well as SACs in bladder with pBOO. However, little is known about the role of Piezo1 in pathological bladder conditions in any animal model or patients with bladder dysfunction. In order to investigate the involvement of Piezo1 in bladder dysfunction secondary to pBOO, we examined the distribution and expression changes of Piezo1 in the bladder of the disease models for the first time.

2. Material and methods

Eighty-six female Sprague–Dawley rats were used in this study (aged 9–11 weeks). Forty-eight rats were used for real-time quantitative PCR and histology, 24 rats were used for western blot (6 groups, operation and control). Fourteen rats were used for cystometry (2 groups, operation and control). Rats were obtained from Charles River Laboratories Japan Inc. (Tokyo, Japan), and housed in an air-conditioned room and fed a standard laboratory diet (CRF-1; Oriental Yeast, Tokyo, Japan). All animal experiments were conducted in accordance with Basic policies for the conduct of animal experimentation in the Ministry of Health, Labour and Walfare, and were approved by the Committee on Ethics in Animal Experiments of Asahi Kasei Pharma Corporation.

2.1. Partial bladder outlet obstruction (pBOO)

pBOO was induced as previously described [4,22]. Briefly, rats were anesthetized by intraperitoneal (i.p.) injection of pentobarbital sodium (30–40 mg/kg). A 22-G Surflo catheter (Terumo Medical Products, Tokyo, Japan) was inserted into the urethra, and the bladder neck and urethra were exposed via a lower abdominal incision. The urethra was ligated with a rod (diameter: 1.1 mm) using a ligature. The rod was removed and the incision closed. Sham-operated rats underwent similar surgery without the procedure of ligation. After the operation, ampicillin (50 mg/kg/day) was given for 3 days to prevent postoperative infection.

2.2. Tissue preparation

At 3, 7, or 14 days after surgery, rats were anesthetized with pentobarbital sodium (50 mg/kg, i.p.) and euthanized by exsanguination. The bladders were rapidly removed, and then, bladder hypertrophy and the ligation around the urethra were confirmed. In terms of bladder hypertrophy, thickened bladder wall was confirmed using bladder sections, as mentioned below, stained with hematoxylin and eosin (Fig. 1). Rats of which bladders weighed <150 mg and >400 mg were excluded because their kidney was abnormally swelled. The bladders on day 7 after pBOO were fixed in 10% formalin neutral buffer solution (Wako Pure Chemical Industries, Osaka, Japan), embedded in paraffin, and finally, serial sections (2 μm thick) were prepared for in situ hybridization analysis and immunohistochemistry. The bladders on days 3, 7, 14 after pBOO were placed in RNAlater (Ambion, Carlsbad, CA, USA) at −80 °C prior to real-time quantitative PCR analysis, or were stored at −80 °C for western blotting.

2.3. Real-time quantitative PCR (qPCR)

The procedure used for qPCR was described previously [6]. Briefly, total RNA was extracted from the isolated bladders using RNeasy Fibrous Tissue kit (Qiagen, Venlo, the Netherlands). Using SuperScript VILO cDNA Synthesis kit (Life Technologies, Carlsbad, CA, USA), cDNA was synthesized. Real-time quantitative PCR, based on the TaqMan technology, was performed with an ABI PRISM 7000 Sequence Detection System (Life Technologies) following the protocol of Platinum Quantitative PCR SuperMix-UDG with ROX (Life Technologies). cDNA was amplified (40 cycles of denaturation for 15 s at 95 °C, and primer annealing and elongation for 30 s at 60 °C). The expression of *RPL19* mRNA was used as the internal control for sample-to-sample variation. Probes and primers were designed with Universal ProbeLibrary Assay Design Center (for *Piezo1*, Roche Diagnostics GmbH, Mannheim, Germany) or Primer Express version 2.0 (for *RPL19*, Life Technologies). The sequences of all primers are shown in Table 1.

2.4. In situ hybridization analysis

The procedure used for this test was described previously [6]. Briefly, the QuantiGene ViewRNA ISH Tissue Assay kit (Affymetrix, Santa Clara, CA, USA) was used according to the manufacturer's protocol. Deparaffinized sections were hybridized with probes for *Piezo1*

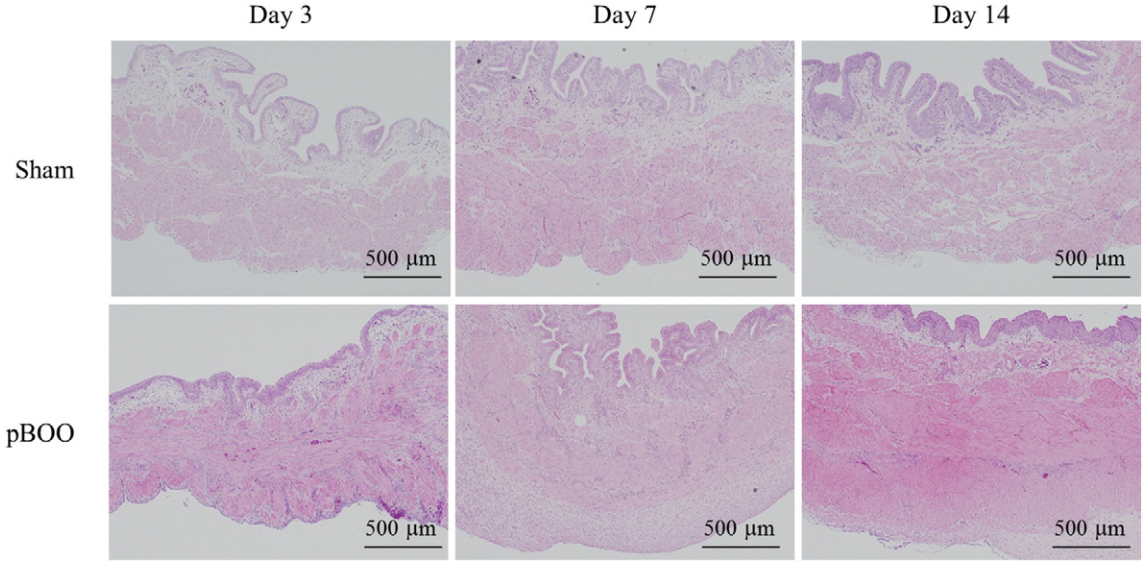

Fig. 1. Histological appearances of bladder sections stained with hematoxylin and eosin in bladders from sham and pBOO rats on days 3, 7, and 14 post-surgery.