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Increased Expression of TREK-1 K⁺ Channel in the Dorsal Root Ganglion of Rats with Detrusor Overactivity After Partial Bladder Outlet Obstruction

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Statistical Analysis C
Data Interpretation D
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Background: Changes in expression and activity of ion channels are important pathophysiological mechanisms underlying detrusor overactivity (DO) in partial bladder outlet obstruction (PBOO). The objective of this study was to examine the expression of TREK-1 channel in the bladder and central nervous system of DO rats.

Material/Methods: Thirty Sprague-Dawley rats were subjected to PBOO operations and those displaying non-voiding contractions (NVCs) in cystometry were classified as DO. Sham-operated rats without NVCs in cystometry served as controls. The expression and distribution of TREK-1 in the bladder, spinal cord, and dorsal root ganglion (DRG) were detected by real time-PCR, western blot, and immunohistochemistry.

Results: TREK-1 channel expression in the DRG was significantly increased at the mRNA level (11.20 ± 3.762 vs. 3.209 ± 1.505 , $P < 0.01$) and protein level (2.195 ± 0.058 vs. 1.713 ± 0.066 , $P < 0.01$) in DO rats as compared to control rats. However, the expression of TREK-1 mRNA in the bladder (1.380 ± 0.810 vs. 4.206 ± 3.827 , $P > 0.05$) and spinal cord (0.764 ± 0.357 vs. 0.696 ± 0.188 , $P > 0.05$) was comparable between the 2 groups. Immunohistochemistry showed enhanced immunoreactive signals of TREK-1 channel in the DRG, but not in the spinal cord and bladder.

Conclusions: TREK-1 channel was upregulated in the DRG of DO rats after chronic PBOO, which might suppress neuronal excitability and play a protective role in bladder overactivity in PBOO.

MeSH Keywords: **Potassium Channels, Tandem Pore Domain • Urinary Bladder Neck Obstruction • Urinary Bladder, Overactive**

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Background

Detrusor overactivity (DO), characterized by involuntary detrusor contractions during the filling phase, is a common bladder dysfunction associating with storage symptoms in bladder outlet obstruction patients [1]. The prevalence of DO was reported to be as high as 60% in men with benign prostatic hyperplasia [2]. The cause of DO is proposed to be neurogenic or myogenic [3,4], but the underlying mechanisms remains the subject of debate. More investigations of the pathophysiological conditions linked to DO is important for better understanding of the disease, as well as laying a foundation for further investigations on novel pharmacological agents.

Changes in the expression and/or activity of ion channels, such as calcium-activated K^+ channels or Ca^{2+} channels, were implicated as key factors in regulating detrusor myocyte cell excitability and causing bladder overactivity in partial bladder outlet obstruction (PBOO) cases [5–7]. Prior studies focused more on the changes of ion channels in bladder wall [6,8]. Recently, a few studies found that upregulation or suppression of ion channels in the spinal cord was associated with neuronal excitability and might be involved in the afferent transduction in obstruction condition [9,10]. For example, Igawa et al. reported that T-type Ca^{2+} channel was upregulated in the dorsal horn of spinal cord in obstructed rats, and blocking it increased bladder capacity [9]. Another study demonstrated that TRAAK, a type of K^+ channel, was upregulated in the L6–S1 spinal cord of bladder outlet obstruction rats, which might suppress neuronal excitability in the spinal cord and further exert a protective effect on bladder overactivity [10]. These reports suggested that DO was not only associated with the abnormality of ion channels in bladder wall, but also the ion channel changes in the central nervous system distal to the bladder.

TREK-1 belongs to the TWIK-related K^+ channel (TREK) family that is known to play an essential role in maintaining the resting membrane potential of cells [11–13]. TREK-1 channel was reported to be downregulated in detrusor myocytes of PBOO mice, and these changes might be associated with bladder overactivity secondary to PBOO [5]. TREK-1 can also regulate neuronal excitability in the process of micturition reflex. However, the expression patterns of TREK-1 channel in the central nervous system has not been tested in PBOO models, which might be involved in the neurogenic mechanism of DO. Hence, the objective of this study was to examine the expression of TREK-1 channel in the bladder, spinal cord, and dorsal root ganglion (DRG) of DO rats.

Material and Methods

Animals and grouping

The experiments were approved by the Ethics Committee on Animal Experiments of Sun Yat-Sen University. Thirty Sprague-Dawley rats (female, age 6–8 weeks) were randomly selected to undergo PBOO operation or sham-operation. Six weeks of post-operation, PBOO rats that displayed non-voiding contractions (NVCs) in cystometry test were classified into the DO group [14]. Sham-operated rats without NVCs in cystometry were selected as controls. All rats were housed in a SPF unit and the operations were performed using standard sterile techniques.

PBOO operation

PBOO operation was performed as previously reported [15]. Briefly, the rats were anesthetized with urethane (intraperitoneal injection, 1 g/kg). The bladder and urethra were exposed through a lower abdominal incision. A plastic tube (diameter 0.1 cm) was inserted into the urethra. Then, obstruction was created by ligating the urethra using a 2/0 silk. Finally, the catheter was removed before closure of the abdomen. All rats received penicillin (20 000 units) for 3 days after the operation to prevent post-operative infection. Sham-operated rats underwent a similar procedure without ligation of the urethra.

Cystometry

Six weeks after the obstruction, cystometry was performed on all PBOO and sham-operated rats as previously described [10]. The same anesthetic protocol as in the PBOO operation was used. The dome of the bladder was exposed through a lower abdominal incision and punctured with a 22-G angiocatheter. Through a 3-way stopcock, a syringe pump and a BL-420E+ pressure transducer (TME Technology, Sichuan, China) were connected to the angiocatheter. Warm saline (about 37°C) was infused into the bladder at a speed of 0.2 mL/min. Bladder capacity, NVCs, intravesicular pressure, and residual urine volume were recorded.

Quantitative RT-PCR

For real-time polymerase chain reaction (RT-PCR), rats were fully anesthetized with urethane (intraperitoneal injection, 4 g/kg) and transcardially perfused with heparinized saline. Tissues of bladder, L6–S1 spinal cord, and DRG were rapidly collected. Since DRG is very small, bilateral DRGs were combined to provide adequate tissue for total RNA extraction. To ensure rapid purification of high-quality RNA, tissue lapping instrument (TissueLyser II, QIAGEN, Germany) with TRIzol (Life Technologies, Germany) was used for homogenization, and

RNeasy mini kit was used for total RNA extraction. The quality and concentration of RNA were determined by ultraviolet spectrophotometry. Using a qPCR RT kit (TOYOBO, Japan), total RNA was reverse transcribed into cDNA. The cDNA was then amplified using the RT-PCR kit (Takara, Japan) with a Real Time system (Eppendorf, Germany). The special primers used for TREK-1 were as follows: CACCATTGGATTTGGCGATT (sense) and CAACGAGGATCCAGAACCACA (reverse). Cycle amplifications included 95°C for 30 s (1 cycle), 95°C for 5 s and 60°C for 30 s (40 cycles). β -actin expression was used as an internal control.

Western blot

The bladder, spinal cord, and DRG tissues were dissected and homogenized using RIPA buffer. Bradford assay was used to determine the protein concentrations with bovine γ -globulin as the reference standard. Then, the proteins were separated by 12% SDS-PAGE and transferred to polyvinylidene fluoride membranes. The membranes were incubated with TREK-1 primary antibody (Alomone Labs, Israel), diluted at 1: 1000. Then, the membranes were washed 3 times for 10 min each time, incubated with secondary antibody (dilution 1: 5000), and finally visualized using the enhanced chemiluminescence system (Millipore, USA). β -actin was used to confirm equal protein loading.

Immunohistochemistry

The rats were fully anesthetized and transcardially perfused with 4% paraformaldehyde. Then, the bladder, spinal cord, and DRG tissues were collected. After fixing in 4% paraformaldehyde for 3 days, the tissues were embedded in paraffin and sliced into thin sections (4–5 μ m). The sections were incubated with 0.3% H₂O₂ at room temperature for 1 h, and blocked with bovine serum albumin for 30 min. Then, the sections were incubated with TREK-1 primary antibody (diluted 1: 100, Alomone Labs, Israel) at 4°C overnight, washed 3 times for 10 min each time and further incubated with secondary antibody at room temperature (37°C) for 30 min. Negative controls were incubated with 0.01 mol/L PBS instead of TREK-1 primary antibody. The number of positive cells and distribution of TREK-1 channel were blindly evaluated in the tissue sections of DO and control rats.

Statistical analysis

All statistical analyses were conducted by SPSS for windows (version 20.0, IBM, USA). Data were presented as means \pm standard deviation. Student's *t*-test was used for comparisons between 2 groups. $P < 0.05$ was considered as statistically significant.

Results

Bladder weight and cystometry

Two PBOO rats died of severe obstruction within 1 week after the operation, 1 sham-operated rat and 1 PBOO rat died due to infections. In the cystometry test, PBOO rats showed significantly increased bladder capacity (13.2 \pm 4.8 vs. 0.8 \pm 0.3 ml, $P < 0.001$) (Figure 1A, 1B), maximum bladder pressure (34.7 \pm 7.0 vs. 23.1 \pm 6.1 cm H₂O, $P < 0.001$), and residual urine volume (10.0 \pm 2.9 vs. 0.2 \pm 0.2 mL, $P < 0.001$) than the sham-operated rats. During bladder filling, 10 out of 16 PBOO rats showed obvious NVCs before micturition, which indicated DO in these rats (Figure 1E). None of the rats in the sham-operation group showed NVCs before micturition (Figure 1F). Thus, the 10 PBOO rats with DO during cystometry were classified as the DO group, and the 10 sham-operated rats without DO as the control group.

Six weeks after obstruction, the ratio of empty bladder weight to body weight in the PBOO rats was significantly higher than the controls (2.84 \pm 0.71 mg/g vs. 0.68 \pm 0.41 mg/g, $P < 0.001$). Hematoxylin and eosin (H&E) staining showed hyperplasia of detrusor myocytes and connective tissue in the bladder wall of PBOO rats as compared to the sham-operated rats (Figure 1C, 1D). These results indicated that bladder hypertrophy was induced by the obstruction.

Expression of TREK-1 mRNA in the bladder, spinal cord and DRG

Quantitative RT-PCR showed that the expressions of TREK-1 mRNA in both bladder (1.380 \pm 0.810 vs. 4.206 \pm 3.827, $P > 0.05$) and L6–S1 spinal cord (0.764 \pm 0.357 vs. 0.696 \pm 0.188, $P > 0.05$) were comparable between the DO rats and controls. However, TREK-1 mRNA expression in the DRG of DO rats was significantly higher than the control rats (11.20 \pm 3.762 vs. 3.209 \pm 1.505, $P < 0.01$) (Figure 2).

Expression and distribution of TREK-1 protein

As shown in Figure 3, TREK-1 protein level in the DRG of DO rats was significantly higher as compared to the control rats (2.195 \pm 0.058 vs. 1.713 \pm 0.066, $P < 0.01$). However, there was no significant difference in TREK-1 protein in the bladder between the 2 groups (0.458 \pm 0.309 vs. 0.338 \pm 0.204, $P > 0.05$). Immunohistochemistry showed the increased number of positive cells and enhanced immunoreactive signals of TREK-1 in DRG neurons of DO rats (Figure 4). The immunohistochemistry showed that TREK-1 protein mainly located in the ventral and dorsal horn of L6–S1 spinal cord, in the mucosa and detrusor of the bladder. However, TREK-1 protein expressions in the bladder and spinal cord were comparable between the

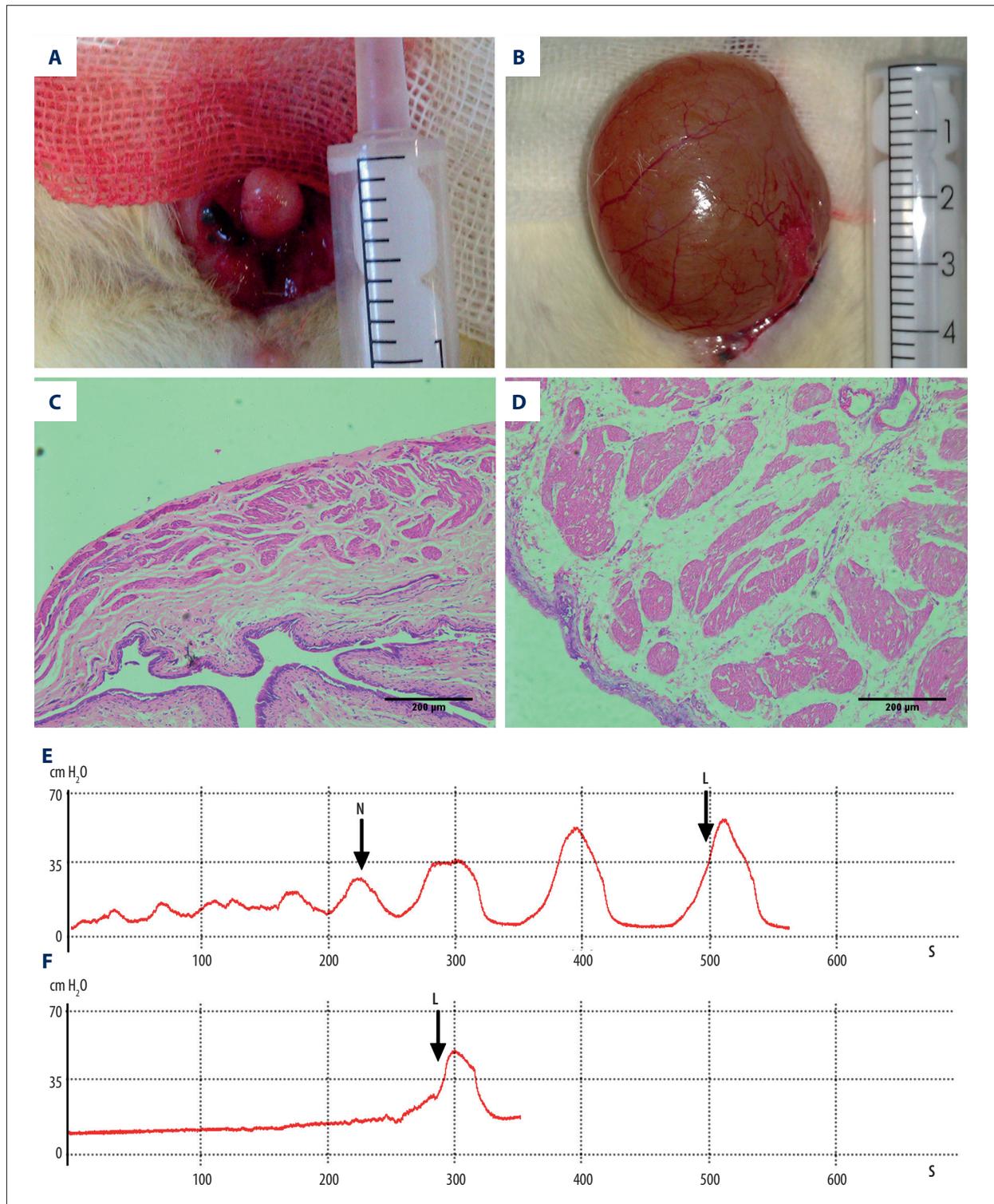


Figure 1. Bladder changes and cystometry results of sham-operated and PBOO rats. (A, B) Increased bladder capacity during filling phase in cystometry in PBOO rats (B) vs. sham-operated rats (A). (C, D) H&E staining showed hypertrophy of bladder wall in PBOO rats (D) vs. sham-operated rats (C). (E) Examples of cystometrograms of DO rats showing non-voiding contractions during bladder filling. (F) Examples of cystometrograms of sham-operated rats during bladder filling. N – non-voiding contractions; L – leakage of urine from the urethra orifice; PBOO – partial bladder outlet obstruction; H&E – hematoxylin and eosin; DO – detrusor overactivity.

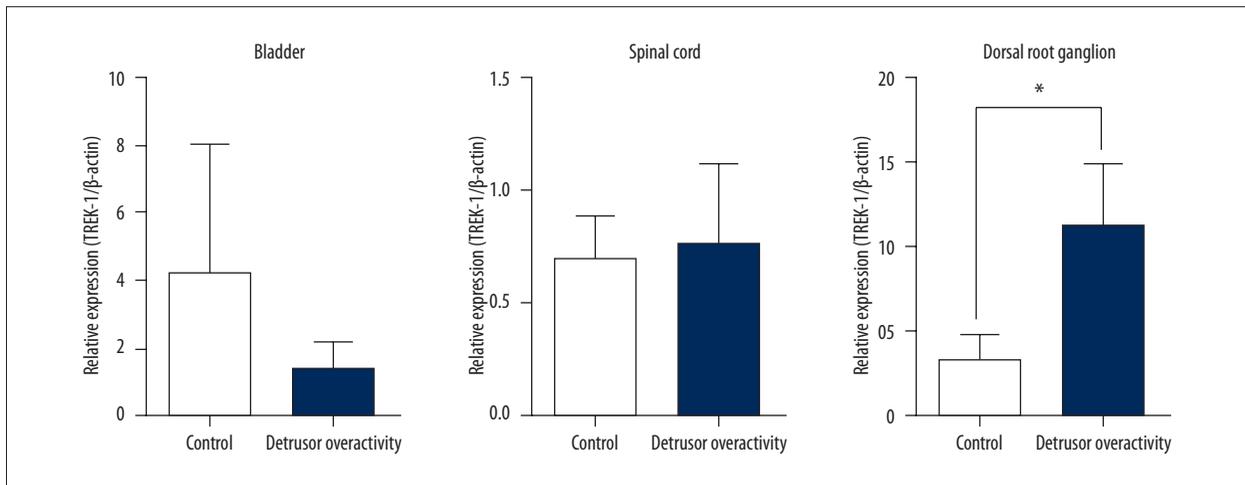


Figure 2. TREK-1 mRNA expression in bladder, spinal cord and dorsal root ganglion of control and DO rats. The mRNA expression is normalized to β -actin and presented as means (SD) of TREK-1/ β -actin. Asterisk indicates $P < 0.01$.

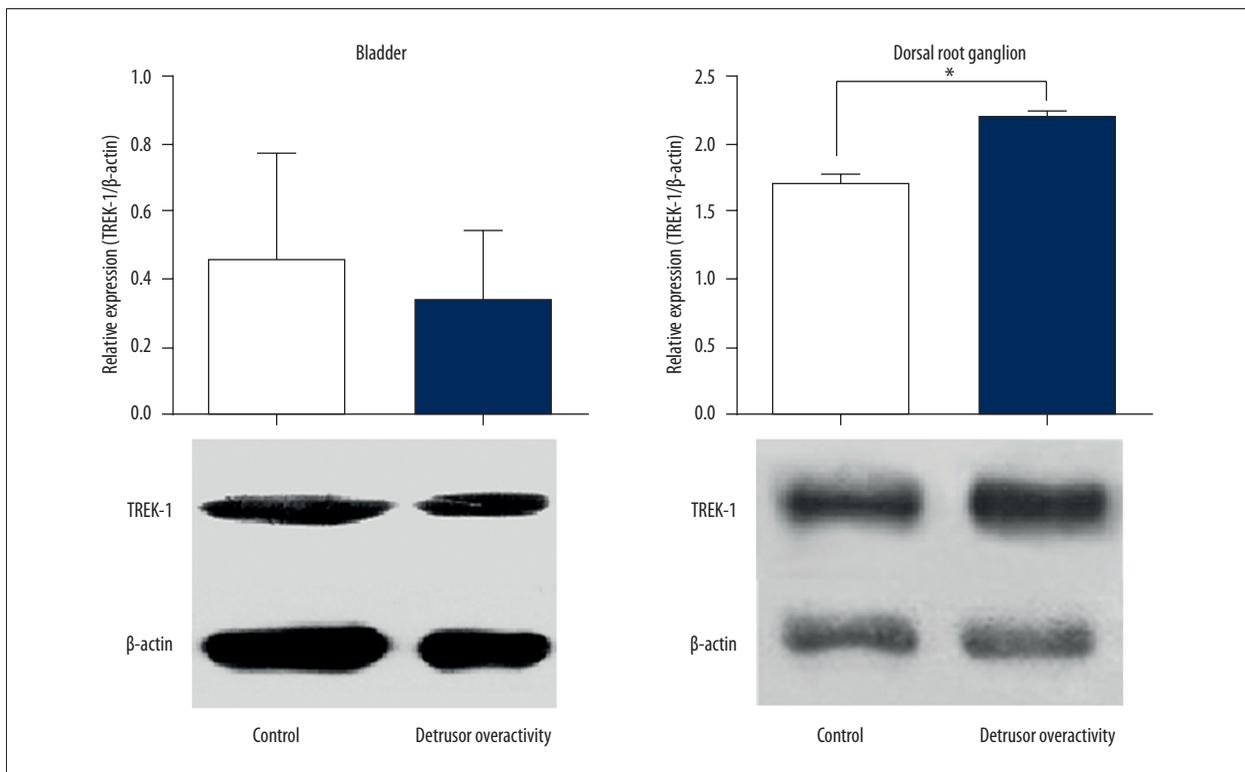


Figure 3. TREK-1 protein expression in the bladder and dorsal root ganglion of control and detrusor overactivity rats. Data are presented as means (SD) of TREK-1/ β -actin. Asterisk indicates $P < 0.01$.

DO and control rats, which was consistent with the RT-PCR results (Figure 5).

Discussion

The present study focused on examining TREK-1 channel in the bladder, L6–S1 spinal cord, and dorsal root ganglion (DRG)

of rats with detrusor overactivity (DO) secondary to partial bladder outlet obstruction (PBOO). Both mRNA and protein levels of TREK-1 were markedly increased in DRG of the DO rats as compared to the sham rats. However, TREK-1 levels in L6–S1 spinal cord and bladder were comparable between the 2 groups. The upregulation of TREK-1 channel in DRG might suppress the excitability of DRG neurons and play a protective role in DO rats.

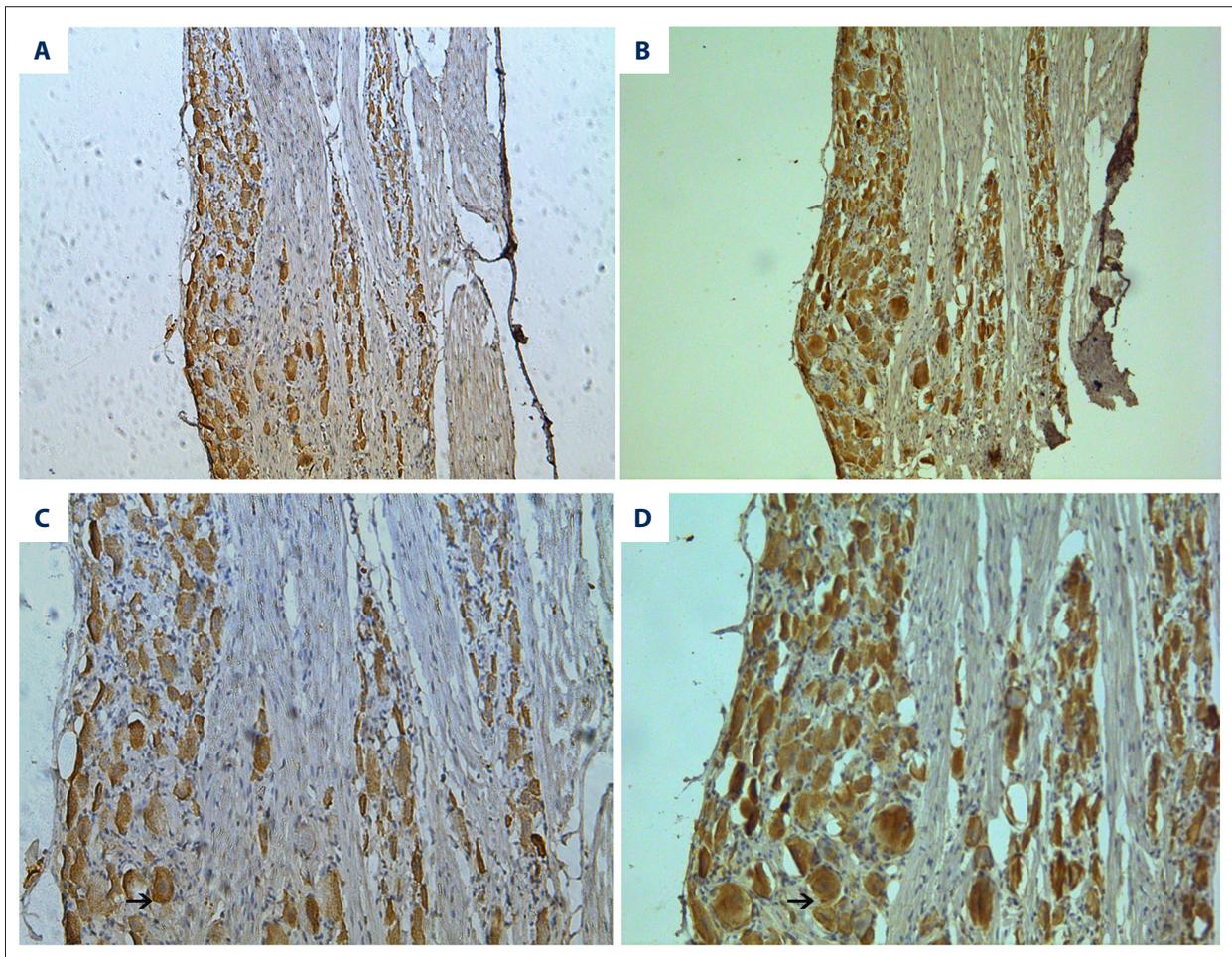


Figure 4. Immunohistochemistry showing the TREK-1 channel distribution in dorsal root ganglion. (A, C) Sections from control rats (magnification: [A] $\times 50$; [C] $\times 200$); (B, D) Sections from detrusor overactivity rats (magnification: [B] $\times 50$; [D] $\times 200$). Black arrows indicate immunopositive neurons.

PBOO was established in female rats for a relatively long period in this study. Six weeks after obstruction, the bladder weight had considerably increased, and morphological examination confirmed hyperplasia of detrusor myocytes in the bladder. The rats that displayed NVCs of the bladder smooth muscle in the cystometry test were enrolled into the DO group. Thus, a successful and reliable DO model induced by PBOO was established in this study.

A notable finding was the upregulation of TREK-1 channel in DRG of DO rats. DRG predominantly contains the cell bodies of afferent nerves. TREK-1 is a member of the TREK K^+ channels subfamily that are associated with resting potential and cellular excitability [11–13]. Upregulation of TREK-1 channel in the sensory neurons (afferent) can produce inward K^+ current and increase the resting membrane potential, subsequently decreasing the cellular excitability and suppressing the overactivity of afferent system in DO rats. Thus, it is possible that increased TREK-1 channel may suppress the excitability of

afferent neurons and play a protective role against bladder overactivity in PBOO rats. The afferent innervation has been increasingly recognized as an important therapeutic target [16]. Whether TREK-1 channel can be a potential interventional target in the treatment of DO needs further investigation.

Upregulation of TREK-1 potassium channel in the DRG may be a negative feedback in response to bladder overactivity in DO rats. Micturition reflex is a complex process, involving both the peripheral and central nervous systems [17]. Studies demonstrated that PBOO might contribute to hypersensitivity of the bladder afferent nerve fibers, which include myelinated A δ -fibers and non-myelinated C-fibers [18,19]. Under physiological conditions, C-fibers are commonly “silent” and insensitive to bladder mechanical or chemical stimuli [20]. During chronic bladder outlet obstruction, persistent increase of intravesical pressure might damage the detrusor myocytes and neuronal fibers within the bladder wall, thereby leading to activation of the “silent” C-fibers [21,22]. The activated C-fibers

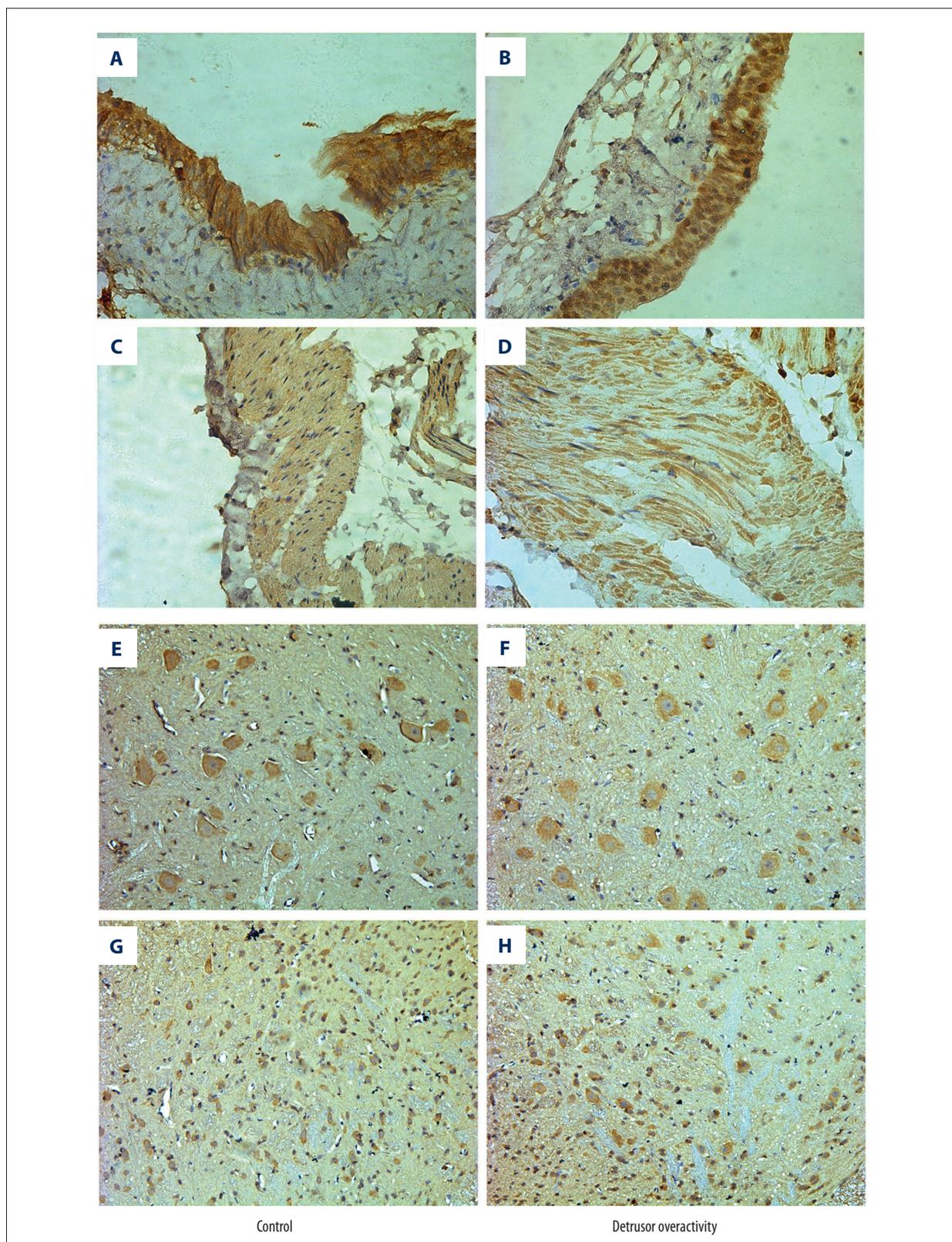


Figure 5. The distribution of TREK-1 channel in the bladder and spinal cord of control and detrusor overactivity rats. (A–D) Sections of bladder showing TREK-1 channel located in the bladder mucosa (A, B) and smooth muscle (C, D). (E–H) Sections of the spinal cord showing TREK-1 located in the ventral horn (E, F) and dorsal horn (G, H).

amplify the bladder sensations and further increase the extent of afferent input. The central nervous system may respond to the increased afferent firing by enhancing the expression of TREK-1 channel to compensate for the over-excitability of afferent system in DO rats.

This study found no difference in TREK-1 level in bladder tissue from DO and control rats. Nevertheless, TREK-1 was previously observed to be downregulated in detrusor myocytes of PBOO mice, which could be responsible for inducing bladder overactivity in the PBOO mouse model [5]. The discrepancy in findings of this study and previous studies might be due to the following reasons: the duration of bladder outlet obstruction differed between our study (6 weeks) and previous reports (2 weeks), which suggest different stages of the disease. The pathophysiology of DO might evolve dynamically in different stages of the disease. Moreover, we detected TREK-1 expression in the whole bladder, rather than in the mucosal and detrusor layers separately, which might also partly explain the negative TREK-1 expression in bladder.

This study had some limitations. First, we did not test the expression of TREK-1 channel in PBOO rats without DO, which might be useful to elucidate whether the TREK-1 change was

caused by PBOO. It is difficult to avoid false negatives when identifying PBOO rats without DO from a period of cystometry records. Also, anesthetization was required in cystometry for the animal model, which may potentially decrease the detection rate of DO by cystometry. Thus, PBOO rats that did not display NVCs during the cystometry test can't suggest that these rats were non-DO rats. Second, how over-expression of TREK-1 channel in the DRG regulates the neuronal excitability remains unknown and requires further investigation. To better elucidate the role of TREK-1 channel in bladder overactivity, the use of TREK-1 knockout animal model or TREK-1 inhibitor are needed in future studies.

Conclusions

This study showed marked upregulation of TREK-1 potassium channel in the DRG of DO rats after chronic PBOO, which might suppress the excitability of DRG neurons, and play a protective role against bladder overactivity.

Competing interests

None.

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