

## FUNCTIONAL AND MOLECULAR MECHANISMS UNDERLYING BLADDER OVERACTIVITY AND AFFERENT HYPEREXCITABILITY IN RATS WITH CHEMICALLY INDUCED PROSTATIC INFLAMMATION

### Hypothesis / aims of study

Lower urinary tract symptoms (LUTS) are commonly seen in males with benign prostatic hyperplasia (BPH), and their storage LUTS such as urinary frequency and urgency overlap with the symptoms of overactive bladder (OAB) syndrome. Although BPH-induced bladder outlet obstruction (BOO) has been studied as a mechanism inducing storage LUTS, there is also increasing clinical evidence showing that asymptomatic prostatic inflammation is involved in not only the development of histological BPH, but also the emergence of male LUTS suggesting that prostatic inflammation could be another potential mechanism inducing storage LUTS in BPH patients. Therefore, this study utilized a rat model of non-bacterial prostatic inflammation [1] to investigate the changes in bladder activity, and functional and molecular characteristics of bladder and prostatic afferent pathways following prostatic inflammation.

### Study design, materials and methods

Male SD rats were used, and prostatic inflammation was induced by formalin (5%; 50  $\mu$ l per lobe) injection into bilateral ventral lobes of the prostate [1]. (1) **Bladder function**: Before and at 1 week after formalin injection, voiding behaviour was evaluated in metabolic cages. Thereafter, continuous cystometrograms (CMG) in a conscious condition were also recorded 1 week after formalin treatment to measure intercontraction intervals (ICI) of the micturition reflex. (2) **Tissue inflammation**: Ventral lobes of the prostate and the bladder were removed at 1 week post-prostatic inflammation, and myeloperoxidase (MPO) activity was also measured to evaluate neutrophil infiltration using ELISA. (3) **Afferent neuron characterization**: L6-S1 dorsal root ganglia (DRG) are removed at 1 week post-prostatic inflammation, whole-cell patch clamp recordings were performed on dissociated bladder and/or prostatic afferent neurons labelled by fluorescent dyes (Fast Blue [FB] and Dil) injected into the prostate and bladder wall, respectively, to examine the electrical properties of neurons. Also, dye-labelled bladder and/or prostatic afferent neurons were collected from DRG sections using laser-capture microdissection (LCM) methods, and mRNA levels of TRP receptors (TRPV1, TRPA1) and an A-type K<sup>+</sup> channel (K<sub>A</sub>) subunit, Kv1.4 were measured by real-time RT-PCR.

### Results

(1) **Bladder function**: Compared to vehicle-injected rats, formalin-treated rats with prostatic inflammation exhibited a significant ( $p < 0.05$ ) decrease in voided volume per micturition (metabolic cage study) and a significant ( $P < 0.05$ ) reduction in ICI (CMG study) (Fig. 1).

(2) **Tissue inflammation**: MPO activity in the formalin-injected prostate was increased 5-fold compared to vehicle injected controls, whereas there was no difference in MPO activity of the bladder between rats with intraprostatic vehicle or formalin injection. (3) **Afferent neuron characterization**: In DRG sections or dissociated DRG neurons, FB-labelled bladder afferent neurons, Dil-labelled prostatic afferent neurons and double-labelled (e.g., Dil and FB-positive) dichotomized afferent neurons innervating both prostate and bladder were identified. In patch clamp recordings, capsaicin-sensitive bladder and prostatic afferent neurons from prostatic inflammation rats had significantly ( $P < 0.05$ ) lower thresholds for spike activation ( $-28.3 \pm 1.4$  mV and  $-28.7 \pm 1.4$  mV) compared to control rats ( $-19.5 \pm 1.1$  mV and  $-21.3 \pm 2.2$  mV, respectively) (Fig. 2). The number of action potentials of bladder or prostatic afferent neurons during an 800 msec depolarizing pulse was significantly greater after prostatic inflammation compared to control rats (Fig. 2). In the voltage clamp condition, the peak densities of K<sub>A</sub> currents during membrane depolarization to 0 mV were significantly ( $P < 0.05$ ) lower in neurons from prostatic inflammation rats than in those from control rats. In RT-PCR, mRNA levels of TRPV1 and TRPA1 were increased, and Kv 1.4 was decreased in FB, Dil and double-labelled neurons compared to non-labelled neurons in prostatic inflammation rats.

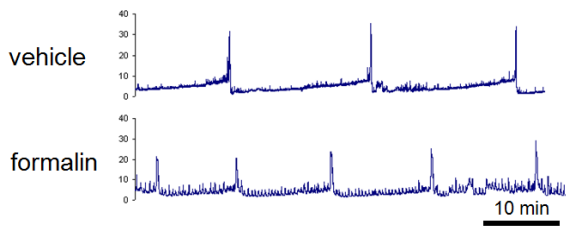
### Interpretation of results

These results indicate that: (1) formalin-induced inflammation after prostatic injection is confined in the prostate, without inducing inflammatory changes in the bladder, (2) prostatic inflammation induces bladder overactivity as evidenced by reduced voided volume per micturition and ICI, (3) prostatic inflammation induces hyperexcitability of capsaicin-sensitive C-fiber prostatic afferent neurons and also bladder afferent neurons, which is associated with reductions in K<sub>A</sub> current density and Kv1.4  $\alpha$ -subunit expression, and (4) prostatic inflammation induces upregulation of TRPV1 and TRPA1, which are expressed predominantly in C-fiber afferent neurons, not only in prostate afferent neurons, but also in dichotomized afferent neurons and bladder afferent neurons. Thus, it is assumed that prostatic inflammation that induces sensitization of C-fiber prostatic afferent pathways can also elicit functional and molecular changes in C-fiber bladder afferent pathways, possibly via afferent cross-sensitization from the prostate to the bladder through dichotomized afferents innervating both organs, thereby resulting in bladder overactivity.

### Concluding message

Chemically-induced inflammation localized in the prostate induces bladder overactivity evident as frequent micturition and enhances bladder afferent function shown by TRPV channel overexpression and increased excitability with reduced K<sub>A</sub> channel activity. Because there is clinical association between prostatic inflammation and BPH-induced LUTS, prostate-to-bladder afferent cross-sensitization through dichotomized afferents could contribute to storage LUTS in BPH patients with prostatic inflammation.

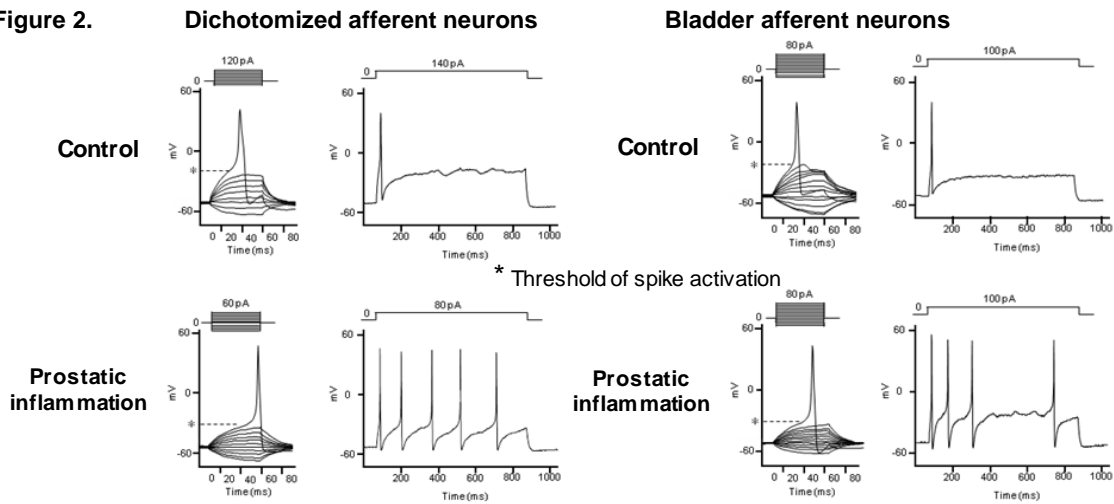
**Figure 1.**



	vehicle	formalin
Baseline pressure (cm H <sub>2</sub> O)	3.6 ± 1.5	5.1 ± 1.3
Threshold pressure (cm H <sub>2</sub> O)	8.4 ± 1.5	9.0 ± 1.2
Peak pressure (cm H <sub>2</sub> O)	39.0 ± 2.6	34.5 ± 2.1
Intercontraction interval (sec)	1309 ± 146	790 ± 139*

vehicle; N = 6, formalin; N = 7  
 \*; p < 0.05 by U-test

**Figure 2.**



References

1. Prostate, 74: 337-345, 2014.

Disclosures

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