

## PROSTATIC INFLAMMATION INDUCES BLADDER OVERACTIVITY THROUGH PROSTATE-TO-BLADDER AFFERENT CROSS-SENSITIZATION IN RATS

### 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 proposed as a mechanism inducing enhanced bladder afferent activity and storage LUTS, there is also increasing clinical evidence of a positive relationship between the severity of LUTS and the degree of prostatic inflammation in BPH specimens. Therefore, it is possible that afferent cross-sensitization between the prostate and bladder due to prostatic inflammation could be another potential mechanism inducing storage LUTS in BPH patients. Thus, we investigated the effects of prostatic inflammation on bladder activity and afferent nerve function using rats with chemically induced prostatic inflammation.

### Study design, materials and methods

Prostatic inflammation was induced by formalin (5%; 50 µl per lobe) injection into bilateral ventral lobes of the prostate of male SD rats. (1) Rats were placed in metabolic cages overnight for 12 hours before and 1 week after formalin injection to evaluate the changes in voiding behavior. Continuous cystometrograms in an awake condition were also obtained 1 week after formalin treatment to measure intercontraction intervals (ICI) of the voiding reflex. (2) Ventral lobes of the prostate and bladder were harvested at 1 week prostatitis, and hematoxylin/eosin staining was performed. Myeloperoxidase (MPO) activity was also measured to evaluate neutrophil infiltration using ELISA. (3) Fluorescent dyes (Dil and Fast Blue [FB]) were injected into the prostate and bladder wall, respectively, to label organ-specific afferent neurons. The afferent neurons in S1 dorsal root ganglia (DRG) that innervate prostate and/or bladder were dissected and collected using laser-capture microdissection (LCM) methods and mRNA levels of TRP receptors (TRPV1, TRPA1) and ATP receptors (P2X2 and P2X3) were measured by real-time RT-PCR.

### Results

(1) In metabolic cage study, the average micturition volume is decreased significantly in formalin injected rats ( $0.46 \pm 0.08$  to  $0.33 \pm 0.07$  mL,  $p = 0.005$ ) without changes in vehicle-treated control rats ( $0.42 \pm 0.03$  to  $0.41 \pm 0.03$  mL) (Fig. 1). In cystometry, the average ICI was significantly shorter in rats with prostatic inflammation than in control ( $791 \pm 139$  vs  $1175 \pm 146$  sec,  $p = 0.026$ ) (Fig. 2). (2) Inflammatory changes were observed focally in the formalin-treated prostate. In the severe inflammation area, a large number of leukocytes were present in a slightly edematous stromal region and the epithelium shrank whereas other regions had almost normal appearance. The bladder showed no sign of inflammation. MPO activity in the formalin-injected prostate was increased 5-fold compared to control, whereas there was no difference in MPO activity of the bladder between prostatitis and control rats. (3) In addition to Dil-labelled prostate afferent neurons and FB-labelled bladder afferent neurons, double-labelled (e.g., Dil and FB-positive) afferent neurons innervating both prostate and bladder were found in  $20.3 \pm 3.4\%$  of Dil or FB labelled neurons. TRPV1, TRPA1 and P2X2 mRNA were increased in Dil, FB and double-labelled neurons compared to non-labelled neurons (Fig. 3). In contrast, there was no difference in P2X3 mRNA between groups.

### Interpretation of results

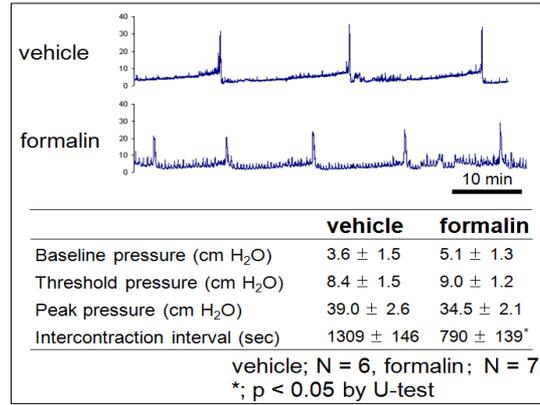
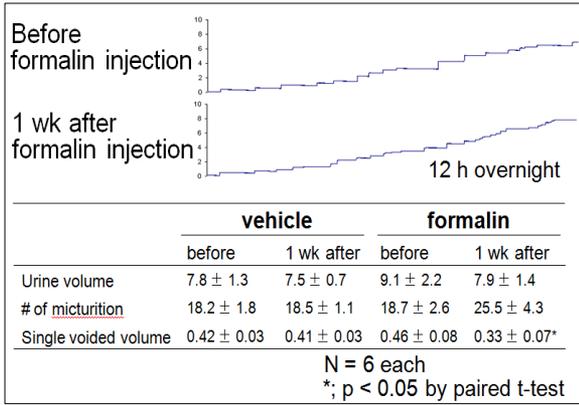
These results indicate that: (a) formalin-induced inflammation after prostatic injection is limited in the prostate, without an extension to the bladder, (b) there are a significant number of DRG neurons with dichotomized afferent nerve fibers that innervate both prostate and bladder, (c) prostatic inflammation induces bladder overactivity as evidenced by reduced voided volume per micturition and ICI and (4) prostatic inflammation induces upregulation of TRPV1, TRPA1 and P2X2 expression not only in prostate afferent neurons, but also in dichotomize afferent neurons innervating both organs as well as bladder afferent neurons that do not innervate the prostate. Thus, it is assumed that bladder overactivity and changes in receptors expression in bladder afferent neurons after prostatic inflammation is indirectly induced by afferent cross-sensitization from the prostate to the bladder through activation of dichotomized afferent pathways that innervate both prostate and bladder. The up-regulation of TRPV1, TRPA1, and P2X2 receptors, which respond to noxious stimuli, could lead to hyperexcitability of bladder afferent pathways, thereby inducing bladder overactivity after prostatic inflammation.

### Concluding message

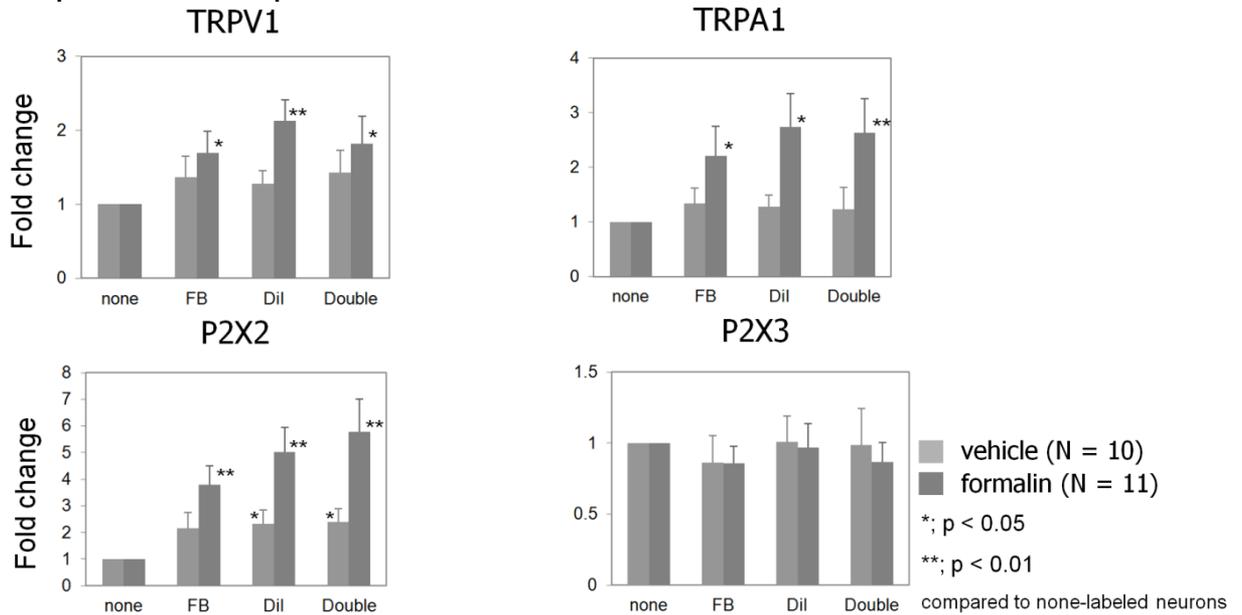
Formalin-induced inflammation localized in the prostate induces bladder overactivity as shown by frequent micturition and enhances bladder afferent function as indicated by TRPV1, TRPA1 and P2X2 upregulation. Since there is clinical association between prostatic inflammation and BPH-induced LUTS, prostate-to-bladder afferent cross-sensitization through dichotomized afferents following prostatic inflammation is a potential mechanism inducing storage LUTS in symptomatic BPH patients.

**Fig. 1 Voiding behaviour**

**Fig. 2 Cystometry**



**Fig.3** Relative expression level compared to none-labelled neurons



Disclosures

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