

## THE ROLE OF CAPSAICIN-SENSITIVE C-FIBER AFFERENT PATHWAYS IN THE CONTROL OF MICTURITION IN NORMAL MICE AND LOWER URINARY TRACT DYSFUNCTION IN MICE WITH SPINAL CORD INJURY

### Hypothesis / aims of study

Bladder afferent pathways controlling the micturition reflex consist of C-fiber and A $\delta$ -fiber afferent nerves. While normal micturition is dependent on activation of A $\delta$ -fiber bladder afferents, C-fiber afferent activation has been considered to be involved in lower urinary tract dysfunction (LUTS) such as overactive bladder (OAB). In a rat model of neurogenic LUTS induced by spinal cord injury (SCI), capsaicin pretreatment that desensitizes TRPV1 receptor-expressing C-fiber afferents eliminates detrusor overactivity (DO) shown by nonvoiding contractions (NVCs) without affecting the voiding reflex, indicating that the capsaicin-sensitive bladder afferents have a selective role in initiating DO in SCI rats [1]. However, the role of capsaicin-sensitive bladder afferents in the control of micturition is not fully elucidated in mice that have increasingly been used for the LUTS research. In order to clarify the contribution of capsaicin-sensitive C-fiber afferent to micturition in mice, we therefore examined bladder and urethral activities in mice with or without SCI after capsaicin pretreatment.

### Study design, materials and methods

A total of 60 female C57BL/6N mice (9 weeks age) were divided into 4 groups; (1) spinal intact (SI), (2) SI-Capsaicin pretreatment (Cap), (3) SCI and (4) SCI-Cap groups.

In SCI groups, mice underwent complete transection of the Th8/9 spinal cord, and 4 weeks after surgery, continuous cystometry (CMG) and external urethral sphincter (EUS)-electromyogram (EMG) analyses were conducted under an awake condition. In SCI animals, the bladder was emptied postoperatively twice a day until CMG measurement. In CAP groups, capsaicin (25, 50 and 100 mg/kg) was given subcutaneously 4 days before experiments.

Prior to CMG and EUS-EMG recordings, animals were anaesthetized with isoflurane, and a polyethylene catheter (PE-50) was inserted into the bladder through the bladder dome. After the recovery from anaesthesia, bladder activity was monitored by continuous CMG (infusion rate: 0.01 ml/min). Thereafter, epoxy-coated stainless steel wires (50  $\mu$ m diameter) were placed percutaneously in the EUS under isoflurane anaesthesia for EMG recordings. Simultaneous measurements of continuous CMG and EUS-EMG were then performed after the recovery from anaesthesia. After rhythmic bladder contractions became stable for at least 60 min, the number of NVCs and the duration of reduced EMG activity of EUS-EMG activity during voiding bladder contraction were evaluated. Also, in SCI groups, the number of NVCs was measured before and after intraperitoneal injection of a ganglion blocker (hexamethonium, 30 mg/kg).

### Results

In the SI-Cap group, 100 mg/kg capsaicin pretreatment significantly increased the bladder capacity and significantly reduced the duration of reduced EMG activity compared to SI mice without pretreatment. In the SCI-Cap group, 50 and 100 mg/kg capsaicin pretreatment significantly decreased the number of NVCs and the duration of reduced EMG activity, respectively, compared to the SCI group without capsaicin pretreatment (Figure 1). In addition, desensitization of capsaicin-sensitive afferent pathways after 100 mg/kg capsaicin pretreatment was confirmed by a negative response in the eye-wipe test. In a separate group of SCI mice without capsaicin pretreatment, hexamethonium administration almost completely blocked the NVCs (Figure 2).

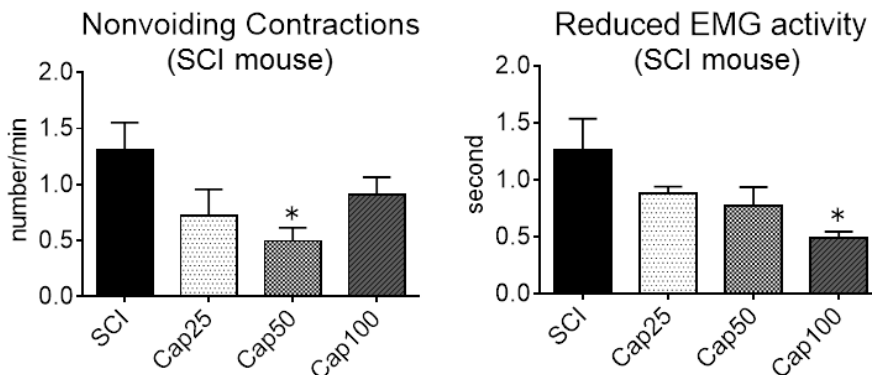


Figure 1. Comparison of number of nonvoiding contractions (NVCs) and duration of reduced electromyogram (EMG) activity with or without capsaicin treatment (CAP; 25, 50 and 100 mg/kg) in mice with spinal cord injury (SCI). n=6-10 per group. \* p<0.05 vs. SCI control group.

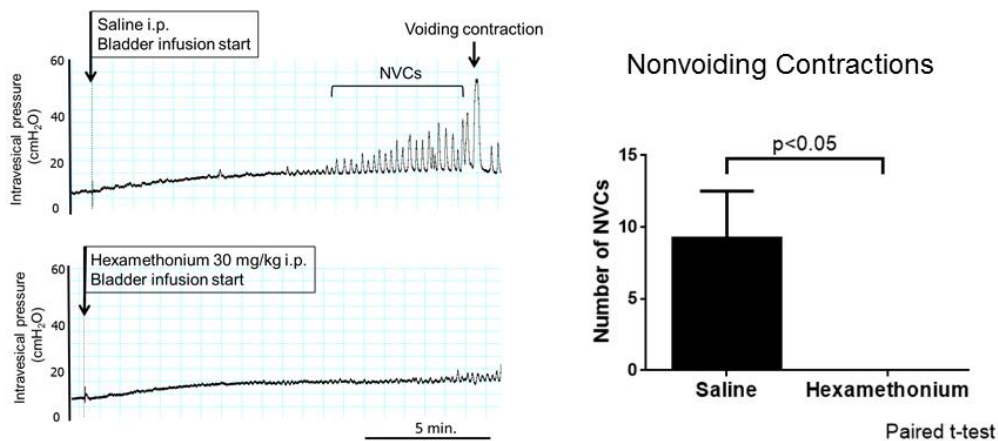


Figure 2. Comparison of nonvoiding contractions (NVCs) before and after intraperitoneum administration of hexamethonium in mice with spinal cord injury (SCI) (n=4).

#### Interpretation of results

In the present study, 100 mg/kg capsaicin pretreatment significantly increased bladder capacity in SI rats, and 50 mg/kg treatment significantly, but not completely, decreased the number of NVCs in SCI mice. These results indicate that capsaicin-sensitive C-fiber bladder afferents facilitate the normal micturition reflex and also contribute to the emergence of DO as shown by NVCs after SCI in mice. The duration of reduced EMG activity during voiding bladder contractions was significantly decreased after 100mg/kg capsaicin pretreatment in both SI and SCI mice. We recently reported that reduced EMG activity during voiding corresponds to synergistic urethral relaxation that helps efficient voiding in mice [2]. Therefore, capsaicin-sensitive C-fiber bladder afferents are likely to enhance the synergistic urethral relaxation during voiding in both SI and SCI mice. In addition, in SCI mice, hexamethonium administration, which blocks ganglionic transmission of the autonomic efferent pathways, almost completely suppressed NVCs, suggesting that NVCs are neurally evoked bladder activity triggered by both capsaicin-sensitive and -insensitive bladder afferents are involved in SCI-induced DO in mice.

#### Concluding message

Capsaicin-sensitive C-fiber bladder afferent pathways are involved in the control of bladder and urethral activity in both SI and SCI mice under the conscious condition. DO as shown by NVCs in SCI is induced by capsaicin-sensitive and -insensitive afferents activities in mice while DO in SCI rats are predominantly dependent on capsaicin-sensitive afferents [1]. Thus, rats and mice have different bladder afferent function, which should be taken into account in the LUTD research.

#### References

1. Cheng CL, de Groat WC. The role of capsaicin-sensitive afferent fibers in the lower urinary tract dysfunction induced by chronic spinal cord injury in rats. *Exp Neurol* 187: 445–454, 2004.
2. Kadekawa K, Yoshimura N et al, Characterization of bladder and external urethral activity in mice with or without spinal cord injury - A comparison study with rats. *Am J Physiol Regul Integr Comp Physiol* 2016 Jan 27. [ajpregu. 00450.2015. doi: 10.1152/ajpregu.00450.](https://doi.org/10.1152/ajpregu.00450.2015)

#### Disclosures

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