

## ABLATION OF LAMINA I SPINAL NEURONS EXPRESSING THE NEUROKININ 1 RECEPTOR REDUCES BLADDER HYPERACTIVITY INDUCED BY INTRAVESICAL INSTILLATION OF CAPSAICIN

**Aims of Study:** Substance P and neurokinin 1 (NK1) receptors in the spinal cord reportedly play an important role in the micturition reflex [1] as well as in nociceptive responses [2]. Previous studies also demonstrated that intrathecal injection of substance P-saporin (SSP-SAP), which is a conjugate of substance P and ribosome-inactivating protein, saporin, suppressed responses to nociceptive stimuli [3, 4]. We therefore investigated the effect of ablation of NK 1 receptor-expressing neurons in the spinal cord using SSP-SAP on the micturition reflex in rats.

**Methods:** Using female SD rats (230-260g), an intrathecal catheter was implanted at the level of the L6-S1 spinal cord. Three to four days after implanting catheter, SSP-SAP (1.0  $\mu$ M, 8 $\mu$ l) was injected through an intrathecal catheter to reduce the number of NK1 receptor-expressing neurons in the spinal cord. Control animals underwent intrathecal application of saporin (1.5  $\mu$ M, 8 $\mu$ l). Continuous cystometry under an awake condition was performed 3 weeks after intrathecal injection of saporin or SSP-SAP. Polyethylene tubes were placed into the bladder through the bladder dome under halothane anesthesia, and 0.9% saline solution was infused at a rate of 0.04 ml/min. Saline voided from the urethral meatus was collected and measured to determine voided volume (VV). After constant VVs were obtained, the infusion was stopped and residual volume (RV) was measured. Bladder capacity (BC) was calculated as the sum of VV and RV. Based on these values, voiding efficiency (VE) was estimated. Intercontraction interval (ICI), maximal voiding pressure (MVP), pressure threshold for voiding (PT), intravesical baseline pressure (BP) were also measured and compared between two groups of animals. Thereafter, bladder hyperactivity was induced by intravesical instillation of capsaicin (15  $\mu$ M), and percent reduction in ICI after capsaicin treatment was compared between two groups of animals. After cystometry, animals were perfused by 4% formaldehyde solution under urethane anesthesia and the L6 spinal cord was removed for immunohistochemical study. The relative percentage of areas, which were stained with NK1 receptor antibodies in lamina I at the dorsal horn, was compared between SSP-SAP and saporin-treated rats.

**Results:** During saline infusion into the bladder, ICI was not different in saporin and SSP-SAP-treated rats [ $11.7 \pm 0.8$  (n = 8) vs.  $14.4 \pm 2.1$  (n = 6) min, respectively]. MVP, BP, PT, VE or BC were not altered either after SSP-SAP treatment. When bladder hyperactivity was induced by intravesical instillation of capsaicin, ICI was reduced by  $59.3 \pm 3.1\%$  in control rats treated with saporin (n = 8). However, in SSP-SAP-treated rats (n = 6), the reduction of ICI after capsaicin instillation was significantly (p=0.03) smaller ( $43.0 \pm 6.2\%$  of reduction) than in saporin-treated rats. Immunohistochemical staining with NK1 receptor antibodies revealed that NK1 receptor-expressing neurons were found most prominently in the superficial layer (lamina I) of the dorsal horn in the L6 spinal cord. However, in SSP-SAP treated rats, the area positively stained with NK1 receptor antibodies in the lamina I of the dorsal horn was significantly (p=0.001) reduced by 35 %, compared with saporin-treated rats, indicating that intrathecal injection of SSP-SAP reduced the number of NK1 receptor-expressing neurons in the superficial layer of the spinal cord.

### Conclusions:

These results suggest that NK1 receptor-expressing neurons in the dorsal horn of the spinal cord play an important role in inducing bladder hyperactivity elicited by intravesical capsaicin. Thus ablation of NK1 receptor-expressing neurons in the spinal cord using the saporin-substance P conjugate at this concentration could be effective to treat bladder hyperactivity induced by bladder irritation without affecting normal bladder function.

### Reference

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