ROLE OF NERVE GROWTH FACTOR IN HYPEREXCITABILITY OF CAPSAICIN SENSITIVE BLADDER AFFERENT NEURONS IN SPINAL CORD INJURED MICE

Hypothesis/aims of study
Spinal cord injury (SCI) rostral to the lumbosacral level eliminates voluntary and supraspinal controls of voiding, leading initially to areflexic bladder, urinary retention and bladder hypertrophy, resulting in increased levels of growth factors including nerve growth factor (NGF) in the bladder wall [1]. NGF is taken up by afferent nerves and transported to the dorsal root ganglion (DRG) cells where NGF changes the phenotypes of C-fiber bladder afferent neurons, thereby inducing bladder afferent hyperexcitability, which contributes to the emergence of neurogenic detrusor overactivity (NDO) following SCI [1]. In this study, we examined the effects of NGF neutralization using anti-NGF antibody on the SCI-induced changes in electrophysiological properties of capsaicin-sensitive bladder afferent neurons in the mouse model, focusing on action potentials (APs) and voltage-gated K⁺ (Kv) currents, which are major determinants of neuronal excitability.

Study design, materials and methods
In female C57BL/6 mice (9-10 weeks old), the spinal cord was transected at the Th8/9 level. In some SCI mice, 2 weeks after SCI, an osmotic pump was placed subcutaneously in order to administer anti-NGF antibody at 10 µg/kg/h for 2 weeks. The dosage of the antibody was determined according to previous studies [1,2] and our preliminary experiments. Bladder afferent neurons were labelled by axonal transport of Fast Blue (FB), a fluorescent retrograde tracer, injected into the bladder wall at 3 weeks after SCI. Four weeks after SCI, freshly dissociated L6-S1 DRG neurons were prepared. Whole cell patch clamp recordings were performed in FB positive neurons (=bladder afferent neurons). After recording APs or Kv currents, capsaicin sensitivity was identified by a transient inward current evoked by capsaicin application.

Two major types of Kv currents expressed in small-sized DRG neurons, namely slow decaying A-type K⁺ (slow KA) and sustained delayed rectifier-type K⁺ (sustained KOr) currents were evaluated [3]. In these neurons, slow KA currents are activated by depolarizing voltage steps from hyperpolarized membrane potentials and inactivated when the membrane potential is maintained at a depolarized level more than -40 mV [3], therefore, slow KA currents are estimated by the difference in these currents activated by depolarizing voltage pulses from a holding potential (HP) of -40 mV and from a HP of -120 mV (see Fig. 2).

Results
In capsaicin sensitive bladder afferent neurons, the resting membrane potentials and the peak and duration of APs did not change by SCI (Table). On the other hand, the threshold for eliciting APs was significantly reduced in SCI compared to spinal intact (SI) mice (Fig. 1 and Table). Also, SCI increased the number of APs during 800 ms membrane depolarization (Table). In addition, the cell diameter and input capacitance of capsaicin sensitive bladder afferent neurons from SCI mice were significantly greater than those of SI mouse neurons (Table). Densities of slow KA and sustained KOr currents evoked by depolarization to 0 mV in capsaicin sensitive bladder afferent neurons from SCI mice were significantly lower than those measured in SI mouse neurons (Fig. 2 and Table). NGF neutralization significantly reversed SCI-induced changes to the threshold, the number of APs and the density of slow KA current, while other SCI-induced changes were not affected by NGF neutralization (Figs. 1 and 2, and Table).

Interpretation of results
Our results indicate that; (1) capsaicin sensitive bladder afferent neurons from SCI mice show hyperexcitability as evidenced by lower spike activation thresholds and tonic firing pattern, and (2) Kv current densities, both slow KA and sustained KOr, are reduced in these neurons from SCI mice. Therefore, functional changes in Kv channels could be responsible for the hyperexcitability of bladder afferent neurons in SCI mice. NGF neutralization in SCI mice ameliorates the hyperexcitability by reversing the KA current reduction. In addition, SCI induces somal hypertrophy of these neurons in mice as evidenced by greater cell diameter and input capacitance, which is proportional to membrane surface area; however, these morphological changes are not affected by NGF neutralization in SCI mice. Taken together, NGF plays an important role in SCI-induced functional, but not morphological, changes in capsaicin sensitive C-fiber bladder afferent neurons, thereby enhancing synaptic transmission in the spinal cord and leading to NDO in SCI mice.

Concluding message
NGF plays an important role in hyperexcitability of capsaicin sensitive C-fiber bladder afferent neurons due to KA current reduction in SCI mice. Thus, NGF-targeting therapies could be effective for the treatment of C-fiber afferent hyperexcitability and NDO in SCI.
References

Disclosures
Funding: NIH Grant (P01DK093424) Clinical Trial: No Subjects: ANIMAL Species: Mice Ethics Committee: The Institutional Animal Care and Use Committee at the University of Pittsburgh