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SOMATIC INJURY INDUCES DE NOVO EXPRESSION OF CHEMOKINES AND THEIR RECEPTORS IN BLADDER PRIMARY AFFERENT NEURONS

Hypothesis / aims of study

The association of painful bladder syndrome with abnormalities in the musculature of the pelvic floor, abdominal wall and hip girdle has been recognized for several decades. Further, many patients can recall a somatic injury that seemed to predate the onset of their painful bladder symptoms, suggesting that relevant somatic injury might alter visceral sensation and/or function. A prime candidate for this type of neuronal plasticity following peripheral injury is the chronic upregulation of the chemokine, monocyte chemoattractant protein-1 (MCP-1). Previous animal studies have demonstrated upregulation of MCP-1 and its cognate receptor, CCR2, in dorsal root ganglion (DRG) neurons directly impacted by a sciatic nerve injury (L4-L6 DRG) as well as adjacent, uninjured neurons (L3 DRG).[1] Importantly, this chemokine/receptor pairing has been directly implicated in the hyperexcitability of sensory neurons and neuropathic pain behavior.[1] Our objective was to determine whether bladder-associated sensory neurons present in the thoracic, lumbar and sacral DRGs might also exhibit upregulation of chemokines/receptors and hyperexcitability following somatic injury.

Study design, materials and methods

Institutional and national guidelines for the care and use of laboratory animals were followed and the study was approved by the animal ethics committee. We used an established model of neuropathic pain (transient focal demyelination of the sciatic nerve) as a model of somatic injury.[2] We utilized a combination of retrograde axon tracing techniques to label primary afferent neurons after bilateral injections into the rat bladder wall (cholera toxin □ subunit conjugated to a fluorescent marker; CTB-555) to identify bladder afferent neurons, and used immunocytochemistry to identify chemokine/receptor-positive neurons in thoracolumbar and sacral DRGs. Control rats underwent retrograde labelling of bladder afferents but did not undergo sciatic nerve injury. In order to study neuronal responsiveness to chemokines, DRGs were acutely dissociated onto laminin-coated coverslips, loaded with calcium-sensitive Fura-2 dye and a baseline signal taken at 340nm. 100mM concentrations of chemokines administered to cells, followed by Capsaicin, High K+ and ATP in turn. Response to ligand challenge by release of sequestered calcium ions was read at 380nm, and the signal quantified by the 340/380 ratio.

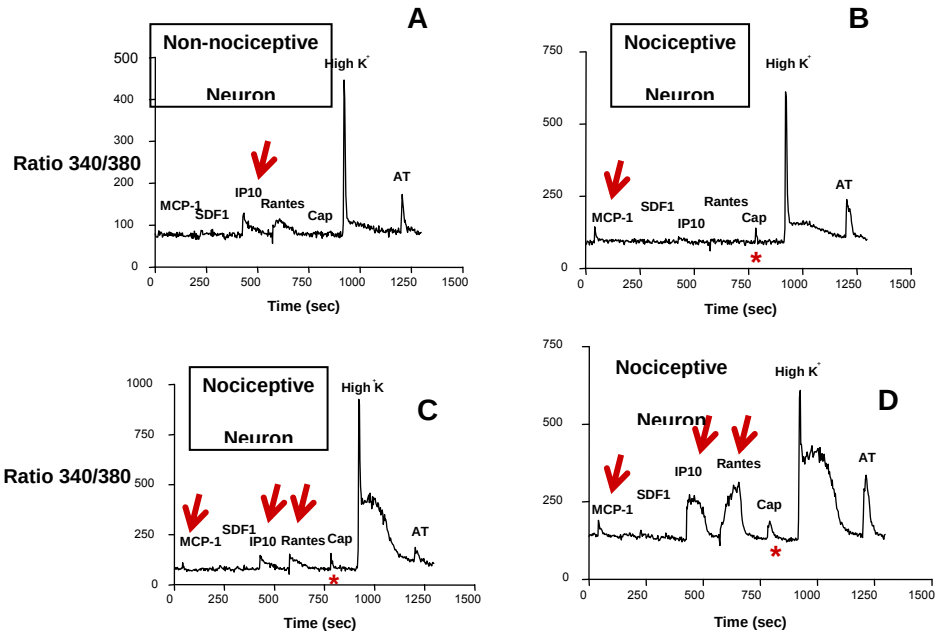
Results

CTB-555 positive bladder afferents were present in T13-L3 and L6-S2 DRGs. Many CTB-555-positive neurons in those ganglia exhibited *de novo* expression of MCP-1 and to a lesser degree, the chemokine receptor CCR2, following sciatic nerve injury. No such expression of chemokines was seen in control rats that underwent retrograde labelling of bladder afferents but did not undergo sciatic nerve injury. Figure 1 shows examples of L1-L3 DRG neuronal response to chemokine administration 14 days after sciatic nerve injury. Under normal conditions, cells rarely respond to chemokines, but do respond to stimuli such as high K^+ or ATP. Increased responsiveness of the cells to chemokines (arrows) occurred after nerve injury in both non-nociceptive neurons (capsaicin-insensitive;**A,C**) and nociceptive (capsaicin-sensitive;**B, D**). Many neurons of both types exhibited responsiveness to MCP-1. For all experiments, MCP-1, IP-10, RANTES, SDF1 were applied at a concentration of 100nM. Capsaicin, high K^+ and ATP were applied at concentrations of 100 nM, 50 mM and 100uM, respectively.

Interpretation of results

Somatic injury in the rodent can produce de novo expression of chemokines and their cognate receptors in primary afferent neurons in the DRGs directly impacted by the somatic injury and also in adjacent, uninjured bladder primary afferents. In particular, MCP-1 signalling is known to influence excitability of sensory neurons and neuropathic pain behaviour. Similar upregulation of this and other ligand/receptor pairings may contribute to the symptomatology of PBS/IC.

FIGURE 1. Nocieptive and Non-nociceptive Sensory Neurons in Adjacent, Uninjured DRG Respond to MCP-1 and Other Chemokines Following Nerve Injury. The figure shows examples of responses of cells acutely isolated from L1-L3 rat DRGs at post-operative day 14 (POD14) after a focal demyelination injury to the sciatic nerve. Under normal conditions, cells rarely respond to any chemokines, but did respond to other stimuli such as high K or ATP. However, increased responsiveness of the cells to chemokines (arrows) occurs after nerve injury in both non-nociceptive neurons (capsaicin-insensitive;**A,C**) and nociceptive (capsaicin-sensitive;**B, D**). Many neurons of both types exhibit responsiveness for MCP-1. For all experiments, MCP-1, IP-10, RANTES, SDF1 were applied at a concentration of 100nM. Capsaicin, high K and ATP were applied at concentrations of 100 nM, 50 mM and 100uM, respectively.



Concluding message

We provide evidence that somatic injury can result in altered responsiveness of bladder afferent neurons. Such somatovisceral effects may play a prominent role in patients with pelvic somatic abnormalities and altered sensitivity to bladder filling.

References

1. Proc Natl Acad Sci U S A 2005;102:14092-7
2. J. Neurosci.2003; 23:3221-3
3. Soc Neurosci Abstract Viewer/Itinerary Planner 2005; 748.9

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ANIMAL SUBJECTS: This study followed the guidelines for care and use of laboratory animals and was approved by SSOM IACUC