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INTRATHECAL BLOCKADE OF TRK RECEPTOR AND NEUROTROPHINS SEQUESTRATION REDUCES PAIN AND URINARY FREQUENCY IN AN ANIMAL MODEL OF CHRONIC BLADDER INFLAMMATION

Hypothesis / aims of study

Chronic bladder inflammation is accompanied by increased urinary frequency and pain. High levels of Nerve Growth Factor (NGF) play an important role in this process. NGF may be uptaken by bladder sensory afferents, binding to its specific tyrosine kinase receptor (Trk), TrkA. Another important neurotrophin is Brain-Derived Neurotrophic Factor (BDNF) that binds to its specific receptor TrkB, abundantly expressed in bladder sensory afferents. BDNF expression in the cell bodies of sensory afferents is upregulated during peripheral inflammation in an NGF-dependent manner. BDNF is anterogradely transported to the spinal cord where it is released upon afferent stimulation, often contributing to central sensitization and enhancing visceral pain and micturition reflex.
The aim of this work was to study the contribution of NGF and BDNF to pain and enhanced micturition reflex in an animal model of chronic bladder inflammation.

Study design, materials and methods

Female Wistar rats (n=4/group) were anaesthetized for surgical placement of a chronic intrathecal silicone catheter. Four days later, bladder inflammation was induced by a single intraperitoneal injection of cyclophosphamide (CYP, 200 mg/kg). Animals were divided into 4 groups. Each group was injected intrathecally with saline, k252a (general antagonist of Trk receptors), TrkA-Ig2 and TrkB-Ig2 (recombinant proteins that specifically sequester NGF and BDNF, respectively). The mechanical thresholds of the lower abdomen were established using the Von Frey monofilaments before induction of inflammation (baseline values) and at 4h, 24h and 48h post-CYP injection. In all cases, the mechanical threshold was determined 15 minutes after intrathecal injection.
In another set of experiments, animals were also submitted to surgical placement of the intrathecal silicone catheter. Three to 4 days later, animals received an intraperitoneal injection of CYP. Three days later, animals were anaesthetized with urethane, the bladders exposed and a catheter inserted in the dome. Cystometrograms were obtained while saline was infused at a constant rate (6ml/h). Body temperature was kept stable (36-37ºC) and the urethra remained unobstructed throughout the recording period, during which drugs (saline, k252a, TrkA-Ig2 orTrkB-Ig2) were injected in the intrathecal catheter.

Results

Non-inflamed animals had a mechanical threshold of 23.3±3.8 g in the lower abdomen. Bladder inflammation significantly reduced the mechanical threshold at 4 hours post-CYP to 8.5±3.3 g ($p<0.01$ in comparison with baseline observations). In animals receiving intrathecal saline, the mechanical threshold remained similarly low at latter time points. In animals receiving k252a, the lowest dose tested (2 ug) did not produce any effect. However, following injection of the highest dose (6 ug), the mechanical threshold was increased 18.2±9.0 g. Similar values were found at 24h and 48h post-CYP injection in all animals treated with k252a. NGF sequestration with TrkA-Ig2 also improved the abdominal hypersensitivity ($p< 0.05$ versus saline treated rats) at all time points tested. In this case, the mechanical threshold of the abdomen registered (in grams) were 60.0±0.0, 51.5±17, and 34.0±17 at time points 4h, 24 and 48h post-Cyp injection, respectively. Likewise, BDNF sequestration with TrkB-Ig2 also improved the mechanical hypersensitivity of the lower abdomen when compared with intrathecal saline ($p<0.01$). In this case, the mechanical thresholds observed were 80.0±23.0 (4h), 68.8±40.5 (24h) and 23.3±5.5 (48h).
Bladder reflex activity was strongly increased in animals treated with cyclophosphamide, from 0.5±0.1 to 1.0±0.4 ($p<0.01$). Intrathecal saline did not alter this frequency. Intrathecal delivery of k252a dose-dependently reduced bladder frequency to 0.4±0.3 after the highest dose (6 ug). In animals receiving TrkA-Ig2 the frequency of bladder contractions was reduced to 0.7±0.3. Treatment with TrkB-Ig2 significantly reduced bladder frequency to 0.4±0.01 ($p<0.05$).

Interpretation of results

The present study shows that chronic bladder inflammation results in mechanical allodynia in the lower abdomen, evidenced by reduced mechanical sensitivity. Treatment with k252a, TrkA Ig2 or TrkB Ig2 improved mechanical threshold of the abdominal region. Bladder reflex activity caused by cystitis was also reduced by the same treatments. These results support a role for NGF and BDNF for pain and increased urinary frequency arising during bladder inflammation.

Concluding message

Our results indicate that NGF and BDNF are important mediators for development of pain and urinary frequency in animals with chronic bladder inflammation. It is likely that Trk antagonists or neurotrophin sequestering proteins may be useful treatments in the future.

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Were guidelines for care and use of laboratory animals followed or ethical committee approval obtained?	**Yes**
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