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SPINAL CORD PHOSPHORYLATION OF EXTRACELLULAR SIGNAL-REGULATED KINASES (ERKS) IS INDUCED BY SENSORY INPUT CONVEYED IN CAPSAICIN-RESISTANT BLADDER AFFERENTS AND CONTRIBUTES TO PAIN AND BLADDER REFLEX OVERACTIVITY IN A CHRONIC CYSTITIS RAT MODEL

Hypothesis / aims of study

The mitogen activated protein kinase (MAPK) is a family of serine/threonine protein kinases that transduce extracellular stimuli into post-translational and transcriptional responses [1]. This family is composed of several members such as extracellular signal-regulated kinases 1 and 2 (ERKs1/2), p38, c-Jun N-terminal kinase/stress-activated protein kinase (JNK/SAPK) and ERK5 [1]. In the spinal cord noxious stimuli induce rapid ERKs 1/2 phosphorylation in second order sensory neurones. In rats, inhibition of ERKs phosphorylation results in reduction of acute pain behaviour [1].

The occurrence of ERKs phosphorylation in the spinal cord after chronic bladder inflammation, a well-known cause of pain and increased bladder reflex activity [2,3], is undetermined. In this study, we investigated (i) spinal ERKs phosphorylation after innocuous and noxious distension of intact and inflamed bladders, (ii) which bladder afferents convey nociceptive input responsible for ERKs phosphorylation and (iii) the role of ERKs activation in bladder reflex activity.

Study design, materials and methods

Bladders of intact and cyclophosphamide (CYP)-inflamed rats (75mg/kg i.p. once every 3 days during 10 days) were distended at 15 (innocuous) or 60 cm H_2O (noxious) for 2 minutes. After immediate perfusion, spinal cord segment L6 was removed, post-fixed and 40 μm transverse sections immunoreacted with a specific antibody raised against phosphorylated ERKs. Immunoreactive (IR-) cells were counted in 10 sections/animal and averaged.

To identify bladder afferents conveying sensory input responsible for ERKs phosphorylation, intact and inflamed animals were treated intravesically with either resiniferatoxin 100nM (RTX) or its vehicle. On the following day rats were submitted to bladder distension for 2 minutes at 60 and 15 cm H_2O, respectively. As above, animals were immediately perfused and L6 segment analysed.

To evaluate the role of spinal ERKs phosphorylation in bladder reflex activity, intrathecal catheters were implanted at L6 spinal level. Three days later, animals were i.p. injected with either saline or CYP (200 mg/Kg). Four hours later, animals were anaesthetised with urethane and bladders exposed. After bladder stabilization, cystometrograms were obtained while saline was infused (6ml/h) through the dome. The urethra remained open and body temperature maintained at 36-37ºC. The frequency of bladder contractions was determined while saline, 1 and 5 μg of PD98059 (a specific ERKs phosphorylation inhibitor) were injected intrathecally.

Results

Scarce IR-cells were observed in sections from non-stimulated intact (1.5±0.58) or inflamed animals (1.75±0.96). Bladder stimulation at 15 cmH_2O induced low ERKs phosphorylation in intact animals (8.75±1.71) and strong activation in inflamed rats (21.13 ± 6.61; $p<0.001$, against intact animals). Bladder distension at 60 cm H_2O significantly increased ERKs phosphorylation in both intact (30.95±3.46; $p<0.001$) and inflamed rats (41.13 ± 2.63; $p<0.001$). Inflamed animals had, however, significantly more IR-cells ($p<0.01$) than intact animals. In all experiments, IR-cells were bilaterally distributed in laminae I-II, lamina X and intermediolateral grey matter.

Desensitization of capsaicin-sensitive bladder afferents by intravesical RTX did not affect ERKs phosphorylation induced by bladder distension in both intact and inflamed rats.

Intact animals had 0.50±0.07 bladder contractions/minute which was not changed by intrathecal 1 or 5 μg of PD98059. In inflamed animals bladder contractions/minute were 0.94±0.33 /minute ($p<0.05$ against intact animals). Intrathecal 1 μg reduced bladder reflex

activity to 0.66±0.25/minute ($p<0.05$). Although 5 µg further reduced bladder frequency to 0.52±0.20/minute, this decrease was not significantly different from that obtained after 1 µg PD98059.

Interpretation of results

This study shows that ERKs activation participates in bladder pain perception in both intact and inflamed animals and may contribute to allodynia in inflammatory bladder conditions. As ERKs phosphorylation is not affected by C-fibre desensitization by RTX, this study suggests that bladder sensory input leading to ERKs phosphorylation in the spinal cord is conveyed by capsaicin-resistant, presumably Aδ fibres. Furthermore, ERKs activation has a specific role in regulating bladder reflex hyperactivity associated to inflammatory states.

Concluding message

Since ERKs inhibition by intrathecal PD98059 decreased bladder reflex activity in inflamed but not in intact animals, this study forwards that ERKs inhibitors may be relevant to treat allodynia and increased micturition frequency associated to chronic bladder inflammation.

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