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ENHANCED BLADDER PAIN IN CYCLOPHOSPHAMIDE-INDUCED CYSTITIS IN RATS-INVOLVEMENT OF PELVIC NERVE AND HYPOGASTRIC NERVE SENSITIZATION

Hypothesis / aims of study

Bladder pain syndrome/interstitial cystitis (BPS/IC) is a serious disease whose main symptoms include bladder pain and frequent urination. The etiology is not well known and there is no appropriate animal model of the disease. On the other hands, cyclophosphamide (CYP)-induced cystitis is recognized as chemical cystitis that has an inflammatory status without bacterial infection. Although CYP cystitis is different from BPS/IC, it similarly shows frequent urination and bladder pain. Thus we investigated whether chronic CYP cystitis enhances bladder pain sensation induced by C-fiber activation in rats. Furthermore, we evaluate the contribution of pudendal nerves, pelvic nerves, and hypogastric nerves to pain behavior in chronic CYP cystitis.

Study design, materials and methods

Female Sprague-Dawley rats weighing 240-260 gram were used.

(1) Effects of chronic CYP cystitis: 75 mg/kg of CYP was injected intraperitoneally once in 3 days for 4 times to induce chronic cystitis. Saline was similarly injected 4 times in the control group. At the day 10, a behavioral study was performed in a metabolic cage under an awake condition as previously described [1]. Licking and freezing behaviors induced by C-fiber stimulation using intravesical administration of 3μM RTx were evaluated every 5 seconds for 15 minutes, and voided volume was recorded simultaneously (n=8 each).

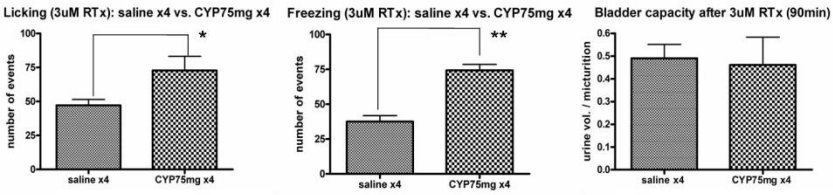
(2) Effects of nerve transection: At the first day of CYP injection, rats underwent either sham operation, pudendal nerve transection, pelvic nerve transection, or hypogastric nerve transection. After 4 times of CYP injections, at the day 10, a behavioral study was performed (n=5-6). A separate group of rats without CYP injection was used as controls of nerve transection surgeries, and divided into 4 groups which were sham operation, pudendal transection, pelvic nerve transection, and hypogastric nerve transection. Seven days after surgery, a behavioral study was performed (n=4-6).

Results

(1) Both licking and freezing behaviors induced by intravesical RTx application were significantly increased in the chronic CYP cystitis group compared to the control group (Fig.1a, b). However, changes in bladder capacity after RTx stimulation were not different in two groups (Fig.1c).

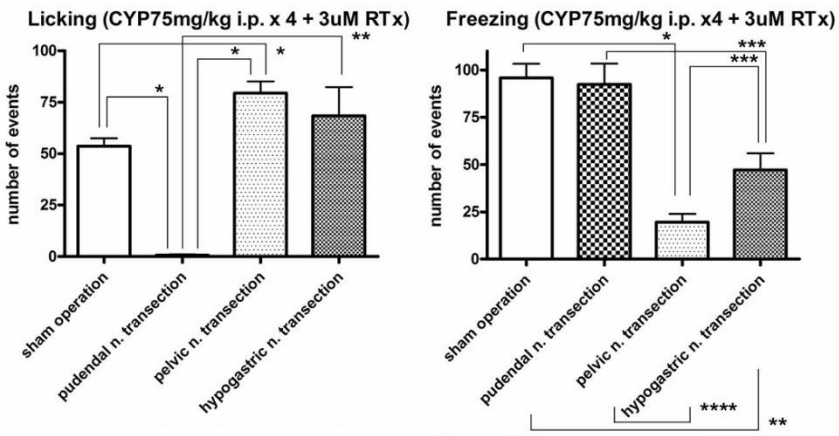
(2) In chronic CYP cystitis rats, licking was significantly decreased after pudendal nerve transection (Fig.2a). Freezing was decreased in both pelvic nerve and hypogastric nerve transected groups, with pelvic nerve transection showing the greater reduction in freezing behavior than hypogastric nerve transection (Fig.2b). In control rats without CYP treatment, pudendal nerve transected rats also showed a reduction of licking (Fig. 3a), while pelvic nerve, but not hypogastric nerve, transection decreased freezing behavior (Fig.3b).

Figure1a Figure1b Figure1c



(Fig1) a, b. Licking and freezing were significantly increased in the chronic CYP cystitis group (*p<0.05, **p<0.001). c. No significant difference in bladder capacity after RTx stimulation.

Figure 2a Figure 2b



(Fig.2) RTx-induced pain behaviour in chronic CYP cystitis rats. a. Pudendal nerve transection showed a significant decrease in licking. b. Both pelvic and hypogastric nerve transected rats showed significantly less freezing compared to sham and pudendal transected rats (*p<0.001, **p<0.01, ***p<0.05, ****p<0.0001).

Figure 3a

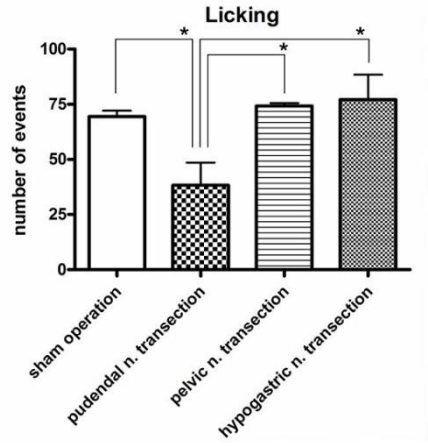
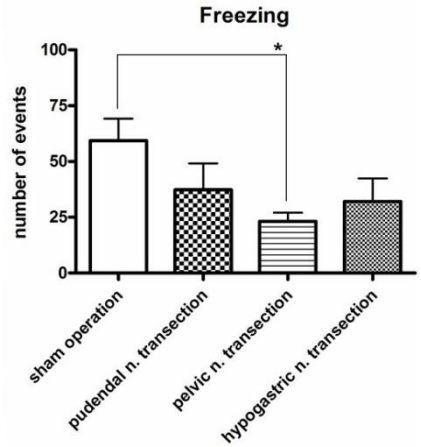


Figure 3b



(Fig.3) RTx-induced pain behaviour in control rats. a. Pudendal nerve transection significantly decreased licking. b. Pelvic nerve transected rats showed significantly less freezing (*p<0.05).

Interpretation of results

Chronic CYP cystitis increases pain behaviors such as licking and freezing induced by RTx-mediated C-fiber stimulation, indicating that chronic cystitis induces bladder hyperalgesia. However, bladder capacity after RTx was not different in control and CYP rats, suggesting that it is not a suitable parameter of bladder hypersensitivity in chronic cystitis. Bladder pain (freezing) in normal rats depends on pelvic nerve activation, but not significantly on hypogastric nerves. In chronic cystitis, both pelvic and hypogastric nerves contribute to enhanced bladder pain sensation, but the contribution of pelvic nerves is still dominant. Urethral pain (licking) depends on pudendal nerve activation in normal and cystitis rats.

Concluding message

Chronic CYP cystitis leads to enhanced bladder pain induced by C-fiber activation due to sensitization of both pelvic and hypogastric nerve afferents while bladder pain in the normal condition predominantly depends on pelvic nerve afferent activation. Thus, the CYP-induced chronic cystitis model could be suitable for the study of mechanisms and new therapies of chronic bladder pain, which is also seen in BPS/IC.

References

1. J Urol. 2008; 179: 359-364

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What were the subjects in the study?	**ANIMAL**
Were guidelines for care and use of laboratory animals followed or ethical committee approval obtained?	**Yes**
Name of ethics committee	**Institutional Animal Care and Use Committee of University of Pittsburgh**