

A PROPOSAL OF A NEW ANIMAL MODEL FOR THE EVALUATION OF BLADDER PAIN IN RATS

Hypothesis / aims of study

Bladder pain is one of the primary symptoms in patients with painful bladder syndrome and interstitial cystitis. Several bladder pain models have been proposed in recent years, but currently there is no globally authorized animal model for the evaluation of bladder pain. The present study thus aims to propose a suitable model. Pain-related responses, which include both pain-related behavior and bladder distension-induced amplification of the abdominal muscle electromyogram (EMG) were investigated in rats treated with cyclophosphamide. Moreover, the effects of some analgesic agents on these responses were also investigated.

Study design, materials and methods

Female Sprague-Dawley rats were used. An electrode was attached surgically 3 or 4 days before measurement. Rats were anesthetized with sodium pentobarbital, after which a ventral incision was made in the skin, and an electrode was anchored to the abdominal external oblique muscle with silk ligatures. The electrode was fed through a subcutaneous tunnel and anchored to the skin on the back of the neck. Each rat was housed in individual cages until measurement. Two days before measurement, cyclophosphamide (150 mg/kg) or saline was administered intraperitoneally to rats that had been denied access to water for 6 hours. The rats were again allowed access to water between 18 hours after administration until measurement.

On the test day, the rats were anesthetized with diethyl ether. A catheter was inserted into the bladder through the urethra and anchored by ligation around the urethra through a small incision in the lower abdomen. The catheter leading out from the external oblique muscle was fed through a flexible metal coil to guard against biting. The rats were then placed in individual Ballman cages until recovered from anesthesia (at least 1.5 hours). After recovery, rats were placed in individual metal cages for measurement. The catheter and electrode were connected to a syringe filled with physiological saline and an amplifier, respectively. The EMG was taken and intravesical pressure was measured via the electrode and catheter, respectively, connected to a pressure transducer. Physiological saline was infused into the bladder through the catheter with a syringe pump at a rate of 45 mL/h. All measurements were taken from freely moving rats. The time point at which pain-related behavior and amplification of the EMG were observed simultaneously was considered to be the bladder-pain threshold. The volume of saline infused into the bladder was calculated from the time at which the threshold occurred.

Results

Treatment with cyclophosphamide significantly decreased the bladder pain threshold when compared with that in saline-treated rats. This decreased bladder pain threshold increased significantly after treatment with morphine (1 mg/kg iv or more) and amitriptyline (1 mg/kg iv or more) as well as after denervating the capsaicin-sensitive C-fiber by pretreating with resiniferatoxin. No sedative effect was observed at the doses used in this experiment.

Interpretation of results

Several bladder pain models have been proposed in recent years. In a model reported previously [1], visceromotor responses reflecting abdominal contractile responses related to urinary bladder distension were used as an indicator of urinary bladder nociception. A behavioral approach using freely moving conscious rats treated with cyclophosphamide was also proposed by another group [2]. In addition, the amplification of abdominal EMG induced by distension of the colon was commonly used as a model of abdominal pain associated with irritable bowel syndrome. In the present study, we proposed a novel *in vivo* experimental model for evaluating bladder pain based on these previous studies with some modifications.

Bladder distension was chosen as the pain inducer because it is a clear source. Abdominal EMG was chosen as the pain indicator because it has been used previously [1]. In previous report, experiments were done under light anesthesia to prevent the micturition reflex from influencing the EMG [1]. In addition, it is reported that micturition reflex induced the EMG amplification [3]. However, it is distinguishable from bladder pain because the EMG amplification caused by micturition reflex is transient and concurrent with a transient elevation in intravesical pressure. In addition, the present experiment used conscious, freely moving rats so that pain-related behavior could be observed. The behavior caused by micturition reflex is different from that caused by pain; the micturition reflex causes rats to lift their gluteal region, while visceral pain causes them to writhe or stretch their abdominal region.

Evaluation of pain in animals always raises the question of whether these responses really reflect the pain. As mentioned above, we first performed the experiment using conscious and freely moving rats to observe pain-related behavior. The rats adopted a writhing and stretching posture when they felt visceral pain (such as acetic acid-induced writhing). Second, we examined the effects of the typical analgesic drugs morphine and amitriptyline. These drugs increased the bladder pain threshold significantly. Third, we evaluated the contribution of C-fiber, which mediates sensory signals, especially the pain signal. Pretreatment with resiniferatoxin caused denervation of the C-fiber, which resulted in an elevated bladder pain threshold. This result suggests that the C-fiber contributes to pain in this model. Additionally, it is well known that cyclophosphamide induces bladder pain. In the present study, pretreatment of cyclophosphamide significantly decreased the bladder pain threshold compared with that in saline-treated rats. Taken together, these results clearly suggest that our method is accurately evaluating the bladder pain.

Pain in the lower urinary tract is one of the main complaints in patients with painful bladder syndrome and interstitial cystitis. Painful bladder syndrome is defined as "the complaint of suprapubic pain related to bladder filling, accompanied by other symptoms such as increased daytime and night-time frequency, in the absence of proven urinary infection or other obvious pathology." The pain evaluated in this study is visceral pain "related to bladder filling." In addition, cyclophosphamide-induced bladder inflammation model rats are commonly used as a model of interstitial cystitis. Therefore, the rat model evaluated in this study seems to be useful for the evaluation of drugs developed for these indications. Amitriptyline was shown to be effective in this study as well as in a clinical study in patients with interstitial cystitis. These results also support the usefulness of this model for evaluating bladder pain.

Concluding message

Observation of EMG amplification and behavior were combined in this *in vivo* experimental model. These responses were inhibited by analgesic agents. Moreover, contribution by the C-fiber was also shown in this model. These results suggest that this

experimental model could be useful for evaluating bladder pain and provide a better therapeutic approach to painful bladder syndrome and interstitial cystitis.

References

[1] Neurosci Lett (2001) 306; 97-100.

[2] J Urol (2000) 164; 203-208.

[3] Am J Physiol Regul Integr Comp Physiol (2006) 290; R1436-R1445.

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