Loxoprofen inhibits facilitated micturition reflex induced by acetic acid urinary bladder infusion of the rats

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ABSTRACT

Prostaglandins (PGs) are well known as one of the chemical mediators of inflammation. Nonsteroidal anti-inflammatory drugs (NSAIDs), PG synthesis inhibitors, are used for anti-nociception and/or anti-inflammation. We examine the effect of loxoprofen, an NSAID, on micturition in acetic acid-induced bladder inflammation of the rats. In cystometrogram study with saline infusion into the urinary bladder, loxoprofen did not alter the interval of bladder contraction (IC, 107% of the control). IC was shortened by acetic acid infusion (65% of the control) and loxoprofen prolonged the IC (162% of acetic acid infused period). This prolonged IC was approximately same as the control. Loxoprofen did not alter the threshold pressure and the maximal voiding pressure. These data suggest that PGE₂ might not play a part of normal micturition and may play a part of the micturition reflex during acetic acid infusion. That is, loxoprofen might be useful for pathological hyperreflex of the micturition.

Urinary bladder controls two different physiological stages; storage of urine and micturition reflex. Previous studies have mainly investigated the reflex pathway of the micturition (5, 25). In contrast, recent studies were interested in the storage mechanisms because over active bladder was commonly seen in elder people (2, 8, 21). During storage of the urine in the bladder, smooth muscles were stretched and it activated the afferent fibers in the pelvic nerve (18). The activities were mediated through the sacral spinal cord and it stimulated the pontine micturition center (6).

When inflammation of the urinary bladder occurred, painful sensation was noticed and frequency of the micturition were increased (14). As indicated

in many reports, inflammation of the urinary bladder could excite the detrusor muscles with chemical mediators (17) and activate the afferent nerve terminals (1, 7).

Prostaglandins (PGs) have been investigated for one of the chemical mediators of the inflammation. In general, cyclooxygenase (COX) isoenzymes synthesize PGs from arachidonic acid at many organs including bladder smooth muscles and urothelium (4, 15). Indeed, PGE₂ in the urine was increased at the interstitial cystitis patients (11). Recent animal studies also demonstrated that COX-2 and PGE₂ were increased in cystitis rats (9, 24). Thus PGE₂ must be important for changing the micturition reflex of the bladder inflammation.

Nonsteroidal anti-inflammatory drugs (NSAIDs) are widely used for treatment of the inflammation. Under anesthesia, NSAIDs increased the bladder capacity during the inflammation in rats (23). It has been reported that anesthesia have influence to the micturition reflex (26). So unanesthetized animals experiments must be performed. Hence we used

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loxoprofen in cystometrogram study after acetic acid-induced inflammation of the urinary bladder under conscious conditions.

MATERIALS AND METHODS

Male Sprague-Dawley rats weighed from 250 to 400 g ($n = 10$) were used for the experiments. The Animal Care and Use Committee of the Akita University, School of Medicine approved the experimental protocols involving the use of animals. Statistical analysis was performed using a Mann-Whitney test with $p < 0.05$ considered to be significant. All data are presented as mean $\pm$ S.E.M.

The animal was first anesthetized with halothane (1.5–2%) and oxygen (1 L/min). An intravenous catheter (PE50) was inserted into the external jugular vein for the purpose of drug administration. After laparotomy, we conducted cystostomy and inserted the PE50 catheter into the bladder dome. The catheter was connected to a pressure transducer for measuring the bladder pressure, and to an infusion pump. The pressure was recorded continuously using data acquisition software (Chart 4, ADInstruments, Sydney, Australia) on a computer system equipped with an analog-digital converter (PowerLab, ADInstruments, Sydney, Australia). We placed the animal into a restrain cage (Bollman cage, Natsume, Tokyo, Japan) after preparation, and recorded cystometrogram after the animal recovered from the anesthesia. Continuous cystometrogram was performed with constant infusion of saline (rate 2.4 ml/h). Two to three hours after initiation of saline infusion, loxoprofen sodium (loxoprofen, 1 mg/kg, 0.2 ml, i.v., given from Sankyo Co., Ltd., Tokyo, Japan) was administered. The following parameters were measured and compared before and after the drug administration; the interval of bladder contraction, the threshold pressure and the maximal voiding pressure.

In five animals, 0.1% acetic acid (pH 3.3) infusion was performed. We changed bladder infusion

solution from saline to 0.1% acetic acid after a stable cystometrogram was recorded. Loxoprofen (1 mg/kg, 0.2 ml, i.v) was administrated one to two hours after acetic acid infusion.

RESULTS

In control animals, the interval of bladder contraction (IC) was 11.4 ± 2.7 min, the threshold pressure (TP) was 10.4 ± 0.9 cmH₂O and the maximal voiding pressure (MVP) was 39.3 ± 3.3 cmH₂O (Fig. 1, Table 1). Following administration of loxoprofen (1 mg/kg), the values of IC, TP and MVP were 12.2 ± 2.6 min (107% of the control), 10.4 ± 0.8 cmH₂O (100% of the control) and 36.9 ± 3.3 cmH₂O (94% of the control), respectively (Fig. 1B, Table 1). Hence, loxoprofen did not significantly alter the cystometric parameters during saline infusion.

In saline infused period of experimental animals for acetic acid infusion, IC, TP and MVP were 21.0 ± 1.5 min, 13.6 ± 0.9 mmH₂O and 36.3 ± 2.3 mmH₂O, respectively. In 0.1% acetic acid infused period of the experimental animals, IC, TP and MVP were 13.9 ± 0.5 min, 10.3 ± 0.9 mmH₂O and 36.9 ± 3.2 mmH₂O, respectively. Infusion of 0.1% acetic acid in the bladder significantly decreased IC (65% of the control, $p < 0.01$, Fig. 2, Table 2). But it did not change TP and MVP (76% of the control, $p = 0.06$ and 102% of the control, $p = 0.84$, respectively). Loxoprofen (1 mg/kg) prolonged IC during acetic acid infusion significantly (Fig. 2B, Table 2). After loxoprofen administration, IC was prolonged to 22.5 ± 1.7 min (162% of acetic acid infused peri-

Table 1 Effect of loxoprofen on parameters of cystometrograms in control animals.

	Control	Loxoprofen
No. of animals	5	5
Interval of bladder contraction (min)	11.4 ± 2.7	12.2 ± 2.6
Threshold pressure (cmH ₂ O)	10.4 ± 0.9	10.4 ± 0.8
Maximal voiding pressure (cmH ₂ O)	39.3 ± 3.3	36.9 ± 3.3

Table 2 Effect of loxoprofen on parameters of cystometrograms in acetic acid infusion experimental animals.

	Control	Acetic acid	Loxoprofen
No. of animals	5	5	5
Interval of bladder contraction (min)	21.0 ± 1.5	$13.9 \pm 0.5^*$	$22.5 \pm 1.7^{**}$
Threshold pressure (cmH ₂ O)	13.6 ± 0.9	10.3 ± 0.9	13.7 ± 1.7
Maximal voiding pressure (cmH ₂ O)	36.3 ± 2.3	36.9 ± 3.2	36.6 ± 2.2

* $p < 0.01$ compared with control. ** $p < 0.01$ compared with acetic acid infused period.

Fig. 1 A: Effects of loxoprofen on continuous cystometrogram in a control animal. Arrows indicate the administration of saline or loxoprofen. Loxoprofen did not change the interval of bladder contraction. B: Bargraph represents loxoprofen effect on averaged interval of bladder contraction (IC).

Fig. 2 A: Effects of loxoprofen on continuous cystometrogram in an acetic acid infusion experimental animal. Arrows indicate the administration of saline or loxoprofen. Loxoprofen prolonged the interval of bladder contraction (IC). B: Bargraph represents 0.1% acetic acid and loxoprofen effects on averaged interval of bladder contraction.

od, $p < 0.01$). This was not different from saline infused period (107% of control, $p = 0.15$). TP and MVP were not altered by the drug administration (13.7 ± 1.7 mmH₂O, 133% of acetic acid infused period, $p = 0.1$ and 36.6 ± 2.2 mmH₂O, 99% of acetic acid infused period, $p = 1$, respectively). Two hours after the drug administration, IC was 10.4 ± 0.4 min (75% of acetic acid infused period).

DISCUSSION

Loxoprofen did not alter IC during the saline infusion into the urinary bladder (107% of the control). In contrast, loxoprofen increased IC during the acetic acid infusion (162% of acetic acid infused period). Acetic acid infusion could induce the inflammation in the urinary bladder and could increase PGE₂ contents in the bladder (3, 9, 10, 24). Indeed PGE₂ in the urine was increased cystitis patients (13). These data indicated that PGE₂ did not play a part of normal micturition and may play a part of the micturition reflex during acetic acid infusion.

PGE₂ infusion into the urinary bladder facilitated the micturition reflex (11) and made contraction of the detrusor smooth muscles (19). PGE₂ could be released from the immune cells during the inflammation and from fibroblast cells in the submucosal connective tissue (13, 16). Hence PGE₂ receptors were present in the urinary bladder (19), we speculate that systematic administration of loxoprofen might act in the urinary bladder for reduction of PGE₂ during the acetic acid infusion.

Aspirin, indomethacin or ketoprofen prolonged IC in the bladder inflammation (22). The present study demonstrated that loxoprofen prolonged IC during the acetic acid infusion. However it did not alter TP and MVP. Loxoprofen or EP1 receptor antagonist inhibits the neural activity in the primary afferent fiber from the urinary bladder during the acetic acid infusion (12). Thus locally released PGE₂ during the inflammation in the urinary bladder might act on the primary afferent terminals and activate the micturition reflex.

Micturition reflex in the EP1 knockout mice mimicked the control mice. However detrusor over activity was not developed after partial obstruction of bladder outlet in knockout mice (20). This suggests that PGE₂ is involved in the developing the pathological hyperreflex of the micturition. Recent study reported that loxoprofen reduced frequency of the micturition during the night in nocturia patients (2). These suggest that loxoprofen could prevent developing hyperreflex of the micturition.

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