Prostaglandin facilitates afferent nerve activity via EP₁ receptors during urinary bladder inflammation in rats

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ABSTRACT

We examined the effects of loxoprofen, a cyclooxygenase inhibitor, and ONO-8711, an EP₁-receptor antagonist, on afferent nerve activity during acetic acid (AA, 0.1% v/v)-induced inflammation of the rat urinary bladder. Distension stimulation was performed (vesical pressure of 30 cm H₂O) for 2 min. The neuronal discharge was recorded from L6 dorsal root filaments. The discharge was observed just after the beginning of distension and increased gradually thereafter. When the vesical pressure returned to control value, the discharge diminished abruptly. AA infusion increased the total number of spikes to $198 \pm 38.8\%$ of control values. AA infusion also produced asynchronous discharge in 39% of the animals. Loxoprofen administration (1 mg/kg, i.v.) reduced the number of spikes to $40.3 \pm 14.8\%$ of control values. ONO-8711 administration (1 and 3 mg/kg, i.v.) reduced the number of spikes to $81.6 \pm 1.6\%$ and $32.2 \pm 7.4\%$ of control values, respectively. These data indicate that loxoprofen or EP₁-receptor antagonist inhibit the inflammation-related neuronal activity. EP₁ receptors in the peripheral afferent nerve terminal and/or urothelium may facilitate the primary afferent nerve activity, which elicits the inflammation-induced micturition reflex.

Prostaglandins (PGs) are synthesized in various tissues where they carry out a number of functions. In the urinary bladder, production of PGs in the smooth muscle and urothelium (6) is induced by physiological stimuli such as distension of bladder (25), or by pathological conditions, such as injury of the vesical mucosa (1, 12). PGE₂ levels in urine have been shown to increase in patients with chronic cystitis, indicating that PGE₂ is likely released during bladder inflammation (32).

Intravesical administration of PGE₂ facilitated micturition, and increased basal intravesical pressure (IVP) in anesthetized rats (15). In humans, PGE₂ administration into the urinary bladder caused a strong

sensation of urgency (27). It was speculated that PGE₂ reduced bladder capacity and increased bladder instability (27). Other studies have indicated that PGE₂ affects bladder smooth muscle directly, and activates capsaicin-sensitive bladder afferent (18, 19).

Many published reports have proposed models of bladder inflammation (3, 8, 17, 21, 29, 32). Acetic acid (AA) infusion into the urinary bladder of rats shortened inter-contraction interval (ICI) in cystometrograms (20, 28). In this animal model, the number of C-fos positive neurons in the lumbo-sacral spinal cord increased following inflammation (3). Cyclooxygenase synthesizes PGs from arachidonic acid, whereas non-steroidal anti-inflammatory drugs (NSAIDs) are known to inhibit cyclooxygenase. Because NSAIDs have been shown to prolong ICI, it is possible that they would inhibit afferent nerve activity during AA-induced inflammation (28, 29).

PGE₂ receptors are classified into four subtypes

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(24). EP₁ and EP₃ receptors cause contraction of smooth muscle, whereas EP₂ and EP₄ receptors cause relaxation. Each receptor uses a different G-protein-coupled signal transduction system. EP₁ receptors have been demonstrated to be expressed in the rat urinary bladder (9, 30). EP₁ receptor mRNA was also expressed in dorsal root ganglion neurons, suggesting that EP₁ receptors may function in the primary afferent nerve terminals (24). A recent study revealed that EP₁ receptors were not essential for normal micturition (26), leading us to an idea that EP₁ receptors could rather be used for transmission of inflammatory signals.

When an EP₁-receptor antagonist was used, the level of PGE₂ stayed within a normal range, although its specific effects were abolished. Here, we report of the effects of loxoprofen, an NSAID, and ONO-8711, an EP₁-receptor antagonist, on bladder afferent nerve activity during AA infusion.

MATERIALS AND METHODS

Adult female Wistar rats (150–200 g, $n = 19$) were used. Protocols for these experiments were approved by the Animal Research Committee at Akita University. The rats were anesthetized with intraperitoneally administrated urethane (0.75–0.9 g/kg). A catheter (PE-50) was inserted into the jugular vein for drug administration. The trachea was also cannulated with a larger catheter (PE-200). A double-lumen catheter was inserted into the urinary bladder through the urethra for infusion and recording of IVP. The lumbo-sacral spinal cord was exposed by laminectomy, and covered with mineral oil. A filament of L6 dorsal root was split into thin bundles and fine filaments were selected. Neuronal discharge was recorded with silver-chloride electrodes using an extracellular amplifier (DPA-100E, Diamedical, Tokyo, Japan). The spikes were selected with a built-in window discriminator and the number of spikes per second was counted with a spike counter (DS-8607, Diamedical). IVP, neuronal discharge, and the number spikes were digitalized with an AD converter and recorded with Power Lab system (ver. 5.0, AD instruments, NSW, Australia). Bladder was distended isotonicly (30 cm H₂O) for 2 min by raising the height of the reservoir tank. This stimulation was repeated every 10 min. Data were shown as means $\pm$ S.E. and statistical comparisons were performed using Student's *t* test.

Drugs and solutions. AA was diluted to 0.1% v/v in saline. Displacement of 0.1% AA was performed

during the interval between distensions. The effect was evaluated at the first distension after displacement. Loxoprofen-Na was supplied by Sankyo (Tokyo, Japan), and was dissolved in saline. ONO-8711 was supplied from Ono Pharmaceutical (Osaka, Japan), and was dissolved in NaOH (1N) before it was diluted to the desired concentration in saline. All drug solutions and control solutions were administered at a volume of 0.5 mL/kg. Loxoprofen-Na or ONO-8711 was administered 5 min after the bladder distension. The effects of these drugs were estimated after an additional 15 min. The effects on discharge were evaluated as the change in the number of spikes elicited by distension. After the experiments, rats were sacrificed by intravenous administration of an overdose pentobarbital.

RESULTS

Multi-unit recording of bladder afferent nerve activity was performed ($n = 19$). Discharge was observed just after the beginning of bladder filling and increased gradually thereafter (Fig. 1A). When the vesical pressure returned to the control value, the discharge diminished abruptly.

AA infusion increased the total number of spikes to $198 \pm 38.8\%$ of control values (Fig. 1B). AA infusion also produced asynchronous discharge after IVP returned to control value (39% of animals). The total number of spikes during a bladder distention was reproducible ($99.1 \pm 3.44\%$ of control values,

Fig. 1 The effect of the displacement of 0.1% acetic acid in the urinary bladder. Note that the bladder distension-evoked neuronal discharge was enhanced (266%) after acetic acid infusion. Top trace: intravesical pressure (IVP); middle trace: neuronal discharge (Spikes); bottom trace: the number of spikes/sec (number of spikes). A: control (saline infusion); B: acetic acid infusion. Calibration bar represents 2 min.

Fig. 2 The effect of loxoprofen (1 mg/kg, i.v.) on bladder afferent neuronal discharge after acetic acid infusion. Note that loxoprofen diminished the neuronal discharge elicited by bladder distension. Loxoprofen also inhibited asynchronous discharge. Top trace: intravesical pressure (IVP); middle trace: neuronal discharge (Spikes); bottom trace: the number of spikes/sec (number of spikes). Calibration bar represents 2 min.

Fig. 3 The effect of ONO-8711 (3 mg/kg, i.v.) on bladder afferent neuronal discharge after acetic acid infusion. Note that ONO-8711 diminished the neuronal discharge elicited by bladder distension. ONO-8711 also inhibited asynchronous discharge. Top trace: intravesical pressure (IVP); middle trace: neuronal discharge (Spikes); bottom trace: the number of spikes/sec (number of spikes). Calibration bar represents 2 min.

Fig. 4 The effects of loxoprofen (1 mg/kg, A) and ONO-8711 (1 and 3 mg/kg, B) on afferent activity during bladder distension. All data are expressed as means $\pm$ S.E. Values were calculated as a percentage of the response before solution administration. * and ** represent $p < 0.05$ and $p < 0.01$ vs. vehicle, respectively.

$n = 6$). The maximum number of spikes (28–74 counts/sec) was also reproducible ($102 \pm 8.4\%$, $n = 6$).

Loxoprofen ($n = 6$) and ONO-8711 ($n = 7$) both

reduced the number of spikes during bladder distension (Figs. 2–4). Loxoprofen (1 mg/kg, i.v.) reduced the spike count to $40.3 \pm 14.8\%$ of control values, whereas the vehicle for loxoprofen (saline, $n = 4$)

reduced the count to $87.6 \pm 8.8\%$ of controls (Fig. 4A). The number of spikes after loxoprofen administration was significantly less than that after vehicle administration ($p < 0.05$). ONO-8711 (1 and 3 mg/kg) reduced the spike count to $81.6 \pm 1.6\%$ and $32.2 \pm 7.4\%$ of control values, respectively (Fig. 4B). The number of spikes following 3 mg/kg of ONO-8711 administration was significantly less than the value observed following vehicle administration ($n = 6$; $107 \pm 13.6\%$; $p < 0.01$). Loxoprofen or ONO-8711 inhibited asynchronous discharge at the control IVP value after AA infusion (Figs. 2 and 3). Furthermore, loxoprofen or ONO-8711 had no effect in two cases (Fig. 5). The discharge pattern in these cases was different than what was usually observed; the spike rate increased very rapidly and plateaued during distention of the urinary bladder.

DISCUSSION

In the present study, we have demonstrated that loxoprofen or ONO-8711 inhibited bladder afferent nerve activity, which was elicited by distension stimulation after intravesical infusion of AA. Inflammation of the urinary bladder increased PGE₂ levels in urine (31). NSAIDs were shown to prolong the ICI after AA-induced inflammation of the rat urinary bladder (28). When loxoprofen or ONO-8711 inhibited the afferent nerve activity, the input to the

micturition center of the voiding reflex was reduced. It has been reported that PGE₂ sensitizes C-fiber afferent terminals (14, 18). The inhibitory effects of loxoprofen and ONO-8711 on the neuronal discharge from the urinary bladder may occur at these C-fiber afferent nerve terminals. In other words, acute inflammation induced by AA may result in the production of PGE₂, which then would enhance bladder C-fiber afferent activity.

During multi-unit recordings, many different sensory receptors in the bladder afferent nerve could be activated. Neuronal discharge in the afferent nerve was elicited by activation of various types of receptors, including polymodal receptors. These receptors could be part of mechanosensitive and inflammatory chemical sensitive systems. EP₁ receptor mRNA was found in dorsal root ganglion (DRG) neurons (24), and activation of EP₁ receptors sensitizes C-fiber DRG neurons (23). Thus, EP₁ receptors may localize in the peripheral primary afferent terminals, where they could serve as a key component for the activation of afferent discharge during AA-induced inflammation of the bladder.

Recently expression of EP₁ receptors was also found in the urothelium (30). When the urothelium is distended, ATP was released to activate P2X₃ receptors in the primary afferent terminals (7, 13). Since PGE₂ levels increased after inflammation, EP₁ receptors in the urothelium might elicit the bladder afferent activity after AA infusion (31). However, it is not clear that distention of the urinary bladder causes the release of PGE₂ under normal condition.

Unexpectedly, loxoprofen or ONO-8711 did not alter the neuronal discharge in two cases. It has been reported that urinary bladder have two different sets of afferent fibers (10). Mechanosensitive A-delta afferent fibers were responsible for inducing the normal micturition reflex. On the other hand, C fibers act during inflammation or spinal cord injury (10, 11). We predicted that the recorded units in our non-responding preparations may have been mechanosensitive A-delta fibers. In addition, other recorded units might have contained EP₁ receptors at the afferent terminals or may have received indirect signals from EP₁ receptors in urothelium. Thus EP₁-receptor antagonist should inhibit the inflammation-related neuronal activity without altering the physiological activity. This question could be resolved in the future by measuring nerve conduction velocities. We, however, face some technical difficulties in that our success rate for unit-recording with a stimulation electrode placed at the pelvic nerve is currently very low.

Fig. 5 Loxoprofen (1 mg/kg i.v., A) and ONO-8711 (3 mg/kg i.v., B) had no effect on atypical bladder afferent neuronal discharge after acetic acid infusion. Top traces: intravesical pressure (IVP); bottom traces: the number of spikes/sec (number of spikes). Calibration bar represents 2 min.

PGE₂ was also involved in the central pathways of micturition (22). EP₁ receptors in the spinal cord modulated excitability and excitatory input (22). Intrathecal administration of an EP₁-receptor antagonist inhibited pain sensations and the development of allodynia (16). Since our recordings were isolated from the central nervous system, our observation is the first demonstration that EP₁ receptors facilitate primary afferent nerve terminals at the peripheral end.

Interaction of EP₁ receptors with transient receptor potential vanilloid 1 (TRPV1) channels was also interesting, since TRPV1 was present in the C-fiber afferents and urothelium (2, 4). TRPV1 was activated by capsaicin and capsaicin infusion into the urinary bladder was shown to shorten the ICI (5). EP₁ facilitated the effects of capsaicin on DRG neurons (23). Thus, EP₁ receptors could facilitate bladder afferent nerve activity through TRPV1 channels.

In conclusion, loxoprofen or an ONO-8711 inhibited inflammation-related neuronal activity from the urinary bladder. EP₁ receptors in the peripheral afferent nerve terminal and/or urothelium may facilitate primary afferent nerve activity, which elicits the inflammation-induced micturition reflex.

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REFERENCES

- Andersson KE (1993) Pharmacology of lower urinary tract smooth muscles and penile erectile tissues. *Pharmacol Rev* **45**, 253–308.
- Avelino A, Cruz C, Nagy I and Cruz F (2002) Vanilloid receptor 1 expression in the rat urinary tract. *Neuroscience* **109**, 787–798.
- Birder LA and de Groat WC (1992) Increased c-fos expression in spinal neurons after irritation of the lower urinary tract in the rat. *J Neurosci* **12**, 4878–4889.
- Birder LA, Kanai AJ, de Groat WC, Kiss S, Nealen ML, Burke NE, Dineley KE, Watkins S, Reynolds IJ and Caterina MJ (2001) Vanilloid receptor expression suggests a sensory role for urinary bladder epithelial cells. *Proc Natl Acad Sci USA* **98**, 13396–13401.
- Birder LA, Nakamura Y, Kiss S, Nealen ML, Barrick S, Kanai AJ, Wang E, Ruiz G, de Groat WC, Apodaca G, Watkins S and Caterina MJ (2002) Altered urinary bladder function in mice lacking the vanilloid receptor TRPV1. *Nat Neurosci* **5**, 856–860.
- Brown WW, Zenser TV and Davis BB (1980) Prostaglandin E₂ production by rabbit urinary bladder. *Am J Physiol* **239**, F452–F458.
- Burnstock G (2001) Purine-mediated signaling in pain and visceral perception. *Trends Pharmacol Sci* **22**, 182–188.
- Cervero F (1994) Sensory innervation of the viscera: peripheral basis of visceral pain. *Physiol Rev* **74**, 95–138.
- Coleman RA, Smith WL and Narumiya S (1994) VIII. International union of pharmacology classification of prostanoid receptors: Properties, distribution, and structure of the receptors and their subtypes. *Pharmacol Rev* **46**, 205–229.
- de Groat WC, Booth AM, Yoshimura N (1993) Neurophysiology of micturition and modification in animal model of human disease. In: *The Autonomic Nervous System. Vol 3, Nervous Control of the Urogenital System* (Maggi CA, ed.), pp 227–290, Harwood, London.
- de Groat WC, Kawatani M, Hisamitsu T, Cheng CL, Ma CP, Thor K, Steers W and Roppolo JR (1990) Mechanisms underlying the recovery of urinary bladder function following spinal cord injury. *J Auton Nerv Syst* **30**, Suppl 571–577.
- de Groat WC and Yoshimura N (2001) Pharmacology of the lower urinary tract. *Annu Rev Pharmacol Toxicol* **41**, 691–721.
- Ferguson DR, Kennedy I and Burton TJ (1997) ATP is released from rabbit urinary bladder epithelial cells by hydrostatic pressure changes—a possible sensory mechanism? *J Physiol* **505**, 503–551.
- Gold MS, Beichling DB, Shuster MJ and Levine JD (1996) Hyperalgesic agents increase a tetrodotoxin-resistant Na⁺ current in nociceptors. *Proc Natl Acad Sci USA* **93**, 1108–1112.
- Ishizuka O, Mattiasson A and Andersson KE (1995) Prostaglandin E₂-induced bladder hyperactivity in normal, conscious rats: involvement of tachykinins? *J Urol* **153**, 2034–2038.
- Kawahara H, Sakamoto A, Takeda S, Onodera H, Imaki J and Ogawa R (2001) A prostaglandin E₂ receptor subtype EP₁ receptor antagonist (ONO-8711) reduces hyperalgesia, allodynia, and c-fos gene expression in rats with chronic nerve constriction. *Anesth Analg* **93**, 1012–1017.
- Lanteri-Minet M, Bon K, de Pommery J, Michiels, JF and Menetrey D (1995) Cyclophosphamide cystitis as a model of visceral pain in rats: model elaboration and spinal structures involved as revealed by the expression of c-Fos and Krox-24 proteins. *Exp Brain Res* **105**, 220–232.
- Maggi CA (1992) Prostanoids as local modulators of reflex micturition. *Pharmacol Res* **25**, 13–20.
- Maggi CA, Giuliani S, Conte B, Furio M, Santicioli P, Meli P, Gragnani L and Meli A (1988) Prostanoids modulate reflex micturition by acting through capsaicin-sensitive afferents. *Eur J Pharmacol* **145**, 105–112.
- Matsumoto G, Kawatani M and Takeshige C (1991) Inhibitory effect of 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX) on the micturition reflex in rat. *Neurosci Lett* **133**, 211–224.
- McMahon SB (1988) Neuronal and behavioural consequences of chemical inflammation of rat urinary bladder. *Agents Actions* **25**, 231–233.
- Miura A, Kawatani M, Maruyama T and de Groat WC (2002) Effect of prostaglandins on parasympathetic neurons in the rat lumbosacral spinal cord. *Neuroreport* **13**, 1557–1562.
- Moriyama T, Higashi T, Togashi K, Iida T, Segi E, Sugimoto Y, Tominaga T, Narumiya S and Tominaga M (2005) Sensitization of TRPV1 by EP1 and IP reveals peripheral nociceptive mechanism of prostaglandins. *Mol Pain* **1**, 1–13.
- Narumiya S, Sugimoto Y and Ushikubi F (1999) Prostanoid receptors: structures, properties, and function. *Physiol Rev* **79**, 1193–1226.

25. Poggesi L, Nicita G, Castellani S, Selli C, Galanti G, Turini D and Masotti G (1980) The role of prostaglandins in the maintenance of the tone of the rabbit urinary bladder. *Invest Urol* **17**, 454–458.
26. Schroder A, Newgreen D and Andersson KE (2004) Detrusor responses to prostaglandin E₂ and bladder outlet obstruction in wild-type and Ep1 receptor knockout mice. *J Urol* **172**, 1166–1170.
27. Schussler B (1990) Comparison of the mode of action of prostaglandin E₂ (PGE₂) and sulprostone, a PGE₂-derivative, on the lower urinary tract in healthy women. A urodynamic study. *Urol Res* **18**, 349–352.
28. Shinozaki S, Saito M and Kawatani M (2005) Loxoprofen inhibits facilitated micturition reflex induced by acetic acid urinary bladder infusion of the rats. *Biomed Res* **26**, 29–33.
29. Takagi-Matsumoto H, Ng B, Tsukimi Y and Tajimi M (2004) Effects of NSAIDs on bladder function in normal and cystitis rats: a comparison study of aspirin, indomethacin, and ketoprofen. *J Pharmacol Sci* **95**, 458–465.
30. Wang X, Momota Y, Yanase H, Narumiya S, Nakamura Y, Kanbayashi T, Maruyama T, Nimura T and Kawatani M (2005) EP1-receptor in the urothelium might facilitate the micturition reflex in mice. *Society for Neuroscience abstracts*: #48.6, 2005.
31. Wheeler MA, Hausladen DA, Yoon JH and Weiss RM (2002) Prostaglandin E₂ production and cyclooxygenase-2 induction in human urinary tract infections and bladder cancer. *J Urol* **168**, 1568–1573.
32. Wheeler MA, Yoon JH, Olsson LE and Weiss RM (2001) Cyclooxygenase-2 protein and prostaglandin E₂ production are up-regulated in a rat bladder inflammation model. *Eur J Pharmacol* **417**, 239–248.