Prostaglandin facilitates afferent nerve activity via EP\textsubscript{1} 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 EP\textsubscript{1}-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 H\textsubscript{2}O) 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 ± 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 ± 14.8% of control values. ONO-8711 administration (1 and 3 mg/kg, i.v.) reduced the number of spikes to 81.6 ± 1.6% and 32.2 ± 7.4% of control values, respectively. These data indicate that loxoprofen or EP\textsubscript{1}-receptor antagonist inhibit the inflammation-related neuronal activity. EP\textsubscript{1} 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). PGE\textsubscript{2} levels in urine have been shown to increase in patients with chronic cystitis, indicating that PGE\textsubscript{2} is likely released during bladder inflammation (32).

Intravesical administration of PGE\textsubscript{2} facilitated micturition, and increased basal intravesical pressure (IVP) in anesthetized rats (15). In humans, PGE\textsubscript{2} administration into the urinary bladder caused a strong sensation of urgency (27). It was speculated that PGE\textsubscript{2} reduced bladder capacity and increased bladder instability (27). Other studies have indicated that PGE\textsubscript{2} 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 cystometograms (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).

PGE\textsubscript{2} receptors are classified into four subtypes...
(24). EP$_1$ and EP$_3$ receptors cause contraction of smooth muscle, whereas EP$_2$ and EP$_4$ receptors cause relaxation. Each receptor uses a different G-protein-coupled signal transduction system. EP$_1$ receptors have been demonstrated to be expressed in the rat urinary bladder (9, 30). EP$_1$ receptor mRNA was also expressed in dorsal root ganglion neurons, suggesting that EP$_1$ receptors may function in the primary afferent nerve terminals (24). A recent study revealed that EP$_1$ receptors were not essential for normal micturition (26), leading us to an idea that EP$_2$ receptors could rather be used for transmission of inflammatory signals.

When an EP$_1$-receptor antagonist was used, the level of PGE$_2$ 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 EP$_1$-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 isotonically (30 cm H$_2$O) for 2 min by raising the height of the reservoir tank. This stimulation was repeated every 10 min. Data were shown as means ± 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 ± 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 ± 3.44% of control values, 24). EP$_1$ and EP$_3$ receptors cause contraction of smooth muscle, whereas EP$_2$ and EP$_4$ receptors cause relaxation. Each receptor uses a different G-protein-coupled signal transduction system. EP$_1$ receptors have been demonstrated to be expressed in the rat urinary bladder (9, 30). EP$_1$ receptor mRNA was also expressed in dorsal root ganglion neurons, suggesting that EP$_1$ receptors may function in the primary afferent nerve terminals (24). A recent study revealed that EP$_1$ receptors were not essential for normal micturition (26), leading us to an idea that EP$_2$ receptors could rather be used for transmission of inflammatory signals. When an EP$_1$-receptor antagonist was used, the level of PGE$_2$ 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 EP$_1$-receptor antagonist, on bladder afferent nerve activity during AA infusion.

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Prostaglandin facilitates bladder afferent nerve activity

**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 ± 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 ± 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 ± 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 PGE$_2$ 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 PGE$_2$ 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 PGE$_2$, 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. EP$_1$ receptor mRNA was found in dorsal root ganglion (DRG) neurons (24), and activation of EP$_1$ receptors sensitizes C-fiber DRG neurons (23). Thus, EP$_1$ 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 EP$_1$ receptors was also found in the urothelium (30). When the urothelium is distended, ATP was released to activate P2X$_3$ receptors in the primary afferent terminals (7, 13). Since PGE$_2$ levels increased after inflammation, EP$_1$ 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 PGE$_2$ 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 EP$_1$ receptors at the afferent terminals or may have received indirect signals from EP$_1$ receptors in urothelium. Thus EP$_1$-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.
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 trangental 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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