Mechanism of Action by Which Aspirin Alleviates Detrusor Hyperactivity in Rats

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Abstract. We examined the effect of aspirin on urodynamic parameters in normal and cyclophosphamide-induced cystitic rats and compared them in rats with or without sensory denervation. Cystometry was performed under urethane anesthesia; and volume threshold for micturition (VT), micturition frequency (MF), micturition pressure (MP), and micturition volume (MV) were determined. Cystitis was induced by pretreatment with cyclophosphamide and sensory denervation was performed by pretreating animals with a large dose of capsaicin. PGE2 and 6-keto-PGF1α contents in the bladder were determined by ELISA. Sensory intact, cystitic rats showed decrement of VT and increment of MF. Aspirin increased VT and decreased MF in the cystitic condition. Both PGE2 and 6-keto-PGF1α contents in the bladder were significantly increased in cystitic rats, but such increases were completely inhibited by aspirin. In sensory denervated rats, aspirin showed a marginal tendency of increment of VT. Cystitic rats showed overflow micturition in the sensory denervated condition, but VT was the same as that of normal rats. Furthermore, following capsaicin pretreatment, aspirin had no effect on the cystometrogram in cystitic rats. From these findings, it is concluded that suppression of sensory C-fiber via inhibition of PGs synthesis in the bladder is involved in the pharmacological action of aspirin in the detrusor hyperactivity.

Keywords: aspirin, cystometry, cystitis, detrusor hyperactivity, sensory denervation
PGs production may lead to an increase in neural activity, resulting in bladder hyperactivity. Several clinical trials have demonstrated the therapeutic potential of non-steroidal anti-inflammatory drugs (NSAIDs) such as indomethacin (14, 15), flurbiprofen (16, 17), and diclofenac (18) in patients with detrusor hyperactivity and nocturnal enuresis. Since NSAIDs inhibit PGs synthesis, it is strongly suggested that NSAIDs could be useful for urinary-incontinence-related diseases.

Aspirin is an NSAID widely used as an analgesic and anti-thrombotic drug in the clinical setting. The mode of action of aspirin has been well investigated and is known to inhibit the activity of the COX enzyme. Velasco et al. (19) reported that i.v. administration of aspirin increased bladder capacity in cyclophosphamide-induced cystitic rats and acetic acid-induced detrusor hyperactivity in a conscious cystometry. Furthermore, Schroder et al. (20) reported that aspirin prevented the dysfunction of bladder smooth muscle after partial urethral outlet obstruction. While these aspects clearly demonstrate the usefulness of aspirin for lower urinary dysfunction, the mechanism by which aspirin prevents detrusor hyperactivity has yet to be investigated.

The aim of this study is to clarify the mechanism of action by which aspirin alleviates detrusor hyperactivity. In order to achieve this purpose, we compared the effect of aspirin on the cystometrogram in rats with or without sensory C-fiber denervation in the cystitis model.

Materials and Methods

Animals

Female Sprague-Dawley rats (8-week-old; Charles River Japan, Yokohama) were used in all experiments. They were housed with free access to food and water ad libitum and maintained on a light-dark cycle at 22 – 24°C except during the experiment. During the experiment under the anesthetized condition, the animals were kept under a heating lamp to prevent decrement of body temperature. The animals were handled according to the principles accepted by The Animal Welfare Committee in Research Center Kyoto of Bayer Yakuhin, Ltd.

In some experiments, the denervation of capsaicin-sensitive afferent neurons (sensory denervation) was performed as described in a previous paper (21). Briefly, the animals were subcutaneously injected with capsaicin (Wako, Osaka) once daily for three consecutive days (20, 30, and 50 mg/kg), 1 week before the experiments. All capsaicin injections were performed under ether anesthesia, and the animals were pretreated with terbutaline (0.1 mg/kg, i.m.) and aminophylline (10 μg/kg, i.m.) to prevent respiratory impairment associated with capsaicin injection. The effectiveness of treatment was tested by examining the protective wiping movements of the eye.

Cystometry

The animals were anesthetized with i.p. injection of urethane (1.2 g/kg) (Wako). After laparotomy, the bladder was exposed and both ureters were ligated and cut. A polyethylene cannula (PE50; Becton Dickinson, Tokyo) was implanted into the bladder dome, and the abdomen was closed. The bladder was emptied by gently pressing on the hypogastrum. The bladder catheter was connected to a pressure transducer for the measurement of intrabladder pressure (MLT0698; ADInstruments, Nagoya) and an infusion pump (model 864, ADInstruments) for continuous infusion of saline solution via a t-shaped tube. The pressure transducer was further connected to an amplifier (ML119, ADInstruments) and a recorder (PowerLab, ADInstruments). Cystometry under the anesthetized condition was performed after a 1 h equilibration period subsequent to the surgical procedure. Saline was infused into the bladder at a flow rate of 3.6 ml/h. The effect of treatments was calculated on the volume threshold (VT) and micturition volume (highest amplitude during micturition: MV) for the first micturition, on the micturition frequency (MF), and on the mean amplitude of bladder contractions (micturition pressure: MP) at 60 min after the beginning of cystometry. VT was calculated as follows: time required for first micturition (min) × 3.6 (ml) / 60 (min). The first voided urine was collected by means of a microtube and the volume of urine was measured by microsyringe. For drug treatment, the left femoral vein was cannulated with a polyethylene catheter (PE50). The injection volume was 0.5 ml/kg. Cystometry started 5 min after the injection of vehicle or aspirin.

Cystometry in cystitic rats was performed according to the method described by Lecci et al. (22) with slight modification. Briefly, the animals were given cyclophosphamide (Wako) at the dose of 150 mg/kg, i.p. 18 h before cystometry.

Determination of bladder PGs levels

PGE\textsubscript{2} and 6-keto-PGF\textsubscript{1α} levels in the bladder were determined at 18 h after the administration of cyclophosphamide (150 mg/kg, i.p.) or saline. Since 6-keto-PGF\textsubscript{1α} is a metabolite of PGI\textsubscript{2}, 6-keto-PGF\textsubscript{1α} level is correlated to PGI\textsubscript{2} level in the bladder. Vehicle or aspirin was administered via the jugular vein 5 min before sacrifice. Under urethane anesthesia, the bladder was isolated, weighed, frozen with liquid nitrogen, and stored at −80°C until assay. The frozen bladder sample was crushed in a small amount of liquid nitrogen and
placed into a tube containing 100% ethanol with 5 mM indomethacin (Wako). Indomethacin was used to prevent further PGs synthesis during homogenization. The crushed sample was homogenized and centrifuged at 12,000 rpm for 10 min at 4°C. The supernatant was evaporated under a stream of nitrogen gas in a water bath at 37°C and the dried sample was then dissolved in an assay buffer and centrifuged at 12,000 rpm for 10 min at 4°C. PGE₂ and 6-keto-PGF₁α levels in the supernatant were determined by EIA (Amersham Bioscience, Piscataway, NJ, USA), while protein content was determined by the Pierce BCA Protein Reagent. Data are expressed as pg/mg protein.

Drugs
Aspirin (acetylsalicylic acid; Nacalai, Osaka) was dissolved in 1% Na₂CO₃, which was also chosen as the vehicle. Under these conditions, Na₂CO₃ has no effect on cystometry variables either in cyclophosphamide-treated rats or in controls when compared to saline-treated animals. Capsaicin was dissolved in a mixture containing ethanol, Tween 80, and saline (EtOH : Tween 80 : saline = 1:1:8) and administered s.c. at the volume of 2 ml/kg. Aminopyline (Wako) and terbutaline (Wako) were dissolved in saline and administered i.m. at the volume of 1 ml/kg.

Statistical analyses
All data are presented as the mean ± S.E.M. from 5 – 8 animals. Statistical analyses were performed by one-way analysis of variance (ANOVA) with Dunnett’s test in the dose-response study in normal rats and ANOVA with Tukey’s multiple comparison test in the other studies. For all statistical tests P<0.05 was considered significant.

Results

Cystometry in normal rats
In the vehicle-treated control group, VT, MF, MP, and MV were 0.37 ± 0.03 ml, 12.4 ± 2.3 times/h, 25.7 ± 1.6 cmH₂O, and 0.14 ± 0.04 ml, respectively (Table 1). Intravenous administration of aspirin significantly increased VT for micturition and MV at 30 mg/kg and showed a tendency to decrease MF (Fig. 1). No effect on micturition amplitude was observed.

Cystometry in cystitic rats
When cyclophosphamide was administered at a dose of 150 mg/kg, i.p. 18 h before the experiment, cystometric parameters changed dramatically (Table 2): both VT and MV were decreased (0.17 ± 0.21 vs 0.37 ± 0.02 ml, 0.07 ± 0.01 vs 0.30 ± 0.04 ml in normal rats), while both MF and MP were increased (52.7 ± 7.8 vs 13.7 ± 2.2 times/h, 46.6 ± 4.6 vs 27.2 ± 1.7 cmH₂O) (Fig. 2).

Aspirin increased VT and decreased MF in cystitic rats with significant effect observed at 30 or 10 mg/kg. At the end of the experiment, the bladder wet weight was determined in order to check the degree of inflammation. In cystitic rats, the bladder wet weight was markedly increased as compared with normal rats, the value being 111.4 ± 6.4 vs 65.8 ± 3.9 mg in normal rats. However, aspirin had little effect on the bladder wet weight (data not shown).

Table 1. Effect of aspirin on cystometric parameters in normal rats

<table>
<thead>
<tr>
<th>Dose (mg/kg)</th>
<th>n</th>
<th>VT (ml)</th>
<th>MF (times/h)</th>
<th>MP (cmH₂O)</th>
<th>MV (ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle</td>
<td>7</td>
<td>0.37 ± 0.03</td>
<td>12.4 ± 2.3</td>
<td>25.7 ± 1.6</td>
<td>0.14 ± 0.04</td>
</tr>
<tr>
<td>Aspirin</td>
<td>3</td>
<td>0.33 ± 0.03</td>
<td>11.1 ± 1.2</td>
<td>28.9 ± 3.3</td>
<td>0.29 ± 0.08</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>0.40 ± 0.04</td>
<td>15.7 ± 3.9</td>
<td>30.9 ± 3.1</td>
<td>0.32 ± 0.07</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>0.71 ± 0.08*</td>
<td>7.1 ± 1.3</td>
<td>30.6 ± 4.5</td>
<td>0.53 ± 0.04*</td>
</tr>
</tbody>
</table>

Data are presented as the mean ± S.E.M. *Significantly different from the vehicle-treated control group, at P<0.05. VT: threshold volume, MF: micturition frequency, MP: micturition pressure, MV: micturition volume.

Fig. 1. Typical chart of cystometrogram in normal rats. Cystometry was performed under urethane anesthesia. Vehicle (1% Na₂CO₃) or aspirin (ASA: 30 mg/kg) was administered i.v. 5 min before starting cystometry. * shows the timing of voiding. Note that aspirin prolonged the time required for first micturition and micturition interval without any effect on micturition pressure.
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PGE2 and F1α levels in the bladder

Under normal conditions, both PGE2 and 6-keto-PGF1α levels were quite low in the bladder (Table 3). Aspirin had little effect on basal PGE2 levels, while it decreased 6-keto-PGF1α levels below the detection limit. On the other hand, 18 h after the cyclophosphamide administration, both PGE2 and 6-keto-PGF1α levels were markedly increased up to 30- and 4-fold, respectively. PGs levels were dramatically decreased by aspirin administration in cystitic rats.

Cystometry in sensory denervated rats

The effect of sensory denervation on the action of aspirin was examined (Fig. 3). Although sensory denervation slightly increased VT and MV and decreased MF, these effects were statistically insignificant (Table 4). Aspirin at 30 mg/kg showed a tendency of VT increase, but had no effect on other parameters. On the other hand, VT and MV were slightly decreased by cystitis induction, but this effect was weaker than those observed in sensory intact rats. Furthermore, all rats with cystitis showed overflow micturition by sensory dener-

Table 2. Effect of aspirin on cystometric parameters in cystitis rats

<table>
<thead>
<tr>
<th>Dose (mg/kg)</th>
<th>n</th>
<th>VT (ml)</th>
<th>MF (times/h)</th>
<th>MP (cmH2O)</th>
<th>MV (ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>7</td>
<td>0.37 ± 0.02</td>
<td>13.7 ± 2.2</td>
<td>27.2 ± 1.7</td>
<td>0.30 ± 0.04</td>
</tr>
<tr>
<td>Cystitis</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vehicle</td>
<td>8</td>
<td>0.17 ± 0.02*</td>
<td>52.7 ± 7.8*</td>
<td>46.6 ± 4.6*</td>
<td>0.07 ± 0.01*</td>
</tr>
<tr>
<td>Aspirin</td>
<td>10</td>
<td>0.23 ± 0.02*</td>
<td>26.9 ± 2.5*</td>
<td>35.0 ± 3.4</td>
<td>0.13 ± 0.02*</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>0.29 ± 0.03*</td>
<td>24.3 ± 4.7*</td>
<td>33.6 ± 3.7</td>
<td>0.13 ± 0.04*</td>
</tr>
</tbody>
</table>

Data are presented as the mean ± S.E.M. *Significantly different from the normal rat and vehicle-treated cystitis group, respectively, at P<0.05. VT: threshold volume, MF: micturition frequency, MP: micturition pressure, MV: micturition volume.

Table 3. PGE2 and 6-keto PGF1α levels in the rat bladder with or without cystitis

<table>
<thead>
<tr>
<th>Dose (mg/kg)</th>
<th>n</th>
<th>PGE2 (pg/mg protein)</th>
<th>PGF1α (pg/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vehicle</td>
<td>5</td>
<td>2.5 ± 0.3</td>
<td>8.9 ± 2.2</td>
</tr>
<tr>
<td>Aspirin</td>
<td>30</td>
<td>3.4 ± 0.4</td>
<td>U.D.</td>
</tr>
<tr>
<td>Cystitis</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vehicle</td>
<td>5</td>
<td>73.8 ± 10.6*</td>
<td>37.3 ± 7.2*</td>
</tr>
<tr>
<td>Aspirin</td>
<td>30</td>
<td>2.4 ± 0.5*</td>
<td>2.6 ± 0.7*</td>
</tr>
</tbody>
</table>

The animals were treated with cyclophosphamide (150 mg/kg, i.p.) 18 h before the experiment. The bladder was taken 5 min after vehicle or aspirin administration. The PGE2 and 6-keto PGF1α contents in the bladder were determined by ELISA. Data are presented as the mean ± S.E.M. *Significantly different from the vehicle-treated group in normal rats and vehicle-treated rats in cystitic rats, respectively, at P<0.05. U.D.: undetectable.
Aspirin and Detrusor Hyperactivity

Discussion

The effect of aspirin on cystometrogram in normal rats was examined and confirmed to increase VT for micturition and decrease MF with no effect on MP.

In general, it is considered that drugs that affect the afferent pathway of reflex micturition increase bladder capacity without affecting voiding itself such as MP. NK-1 antagonists (22) and 5-HT1a antagonists (23) have been shown to prolong micturition interval without affecting MP. Therefore, it is suggested that aspirin suppresses activation of the afferent pathway. In fact, we observed that aspirin prolonged disappearance time without any effect on contraction amplitude in the

Table 4. Effect of aspirin on cystometric parameters in sensory denervated rats with or without cystitis

<table>
<thead>
<tr>
<th></th>
<th>Dose (mg/kg)</th>
<th>n</th>
<th>VT (ml)</th>
<th>MF (times/h)</th>
<th>MP (cmH2O)</th>
<th>MV (ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td></td>
<td>6</td>
<td>0.57 ± 0.06</td>
<td>13.4 ± 2.1</td>
<td>32.7 ± 3.4</td>
<td>0.30 ± 0.08</td>
</tr>
<tr>
<td>Sensory denervation</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Without Cystitis</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vehicle</td>
<td></td>
<td>7</td>
<td>0.66 ± 0.06</td>
<td>9.9 ± 1.3</td>
<td>29.7 ± 2.3</td>
<td>0.43 ± 0.06</td>
</tr>
<tr>
<td>Aspirin</td>
<td>30</td>
<td>6</td>
<td>0.79 ± 0.06</td>
<td>10.9 ± 1.3</td>
<td>25.0 ± 1.8</td>
<td>0.43 ± 0.14</td>
</tr>
<tr>
<td>With Cystitis</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vehicle</td>
<td></td>
<td>6</td>
<td>0.43 ± 0.06</td>
<td>N.C.</td>
<td>61.7 ± 5.8</td>
<td>0.10 ± 0.03</td>
</tr>
<tr>
<td>Aspirin</td>
<td>30</td>
<td>5</td>
<td>0.35 ± 0.06</td>
<td>N.C.</td>
<td>48.1 ± 3.4*</td>
<td>0.05 ± 0.01</td>
</tr>
</tbody>
</table>

Data are presented as the mean ± S.E.M. *Significantly different from normal rats, at P<0.05. VT: threshold volume, MF: micturition frequency, MP: micturition pressure, MV: micturition volume, N.C.: Not calculated.
isovolumetric rhythmic bladder contraction model (data not shown).

It is known that cyclophosphamide, commonly prescribed for the treatment of lymphoproliferative processes, tumors, and so forth, carries the risk of serious complications such as hemorrhagic cystitis mainly due to the presence of acrolein, a urinary metabolite of cyclophosphamide (24). Since rats with cyclophosphamide-induced cystitis show frequent urination (25, 26), this model is considered to be useful for drug evaluation against detrusor hyperactivity (7, 22). Therefore, we selected this model to investigate the pharmacological action of aspirin to alleviate detrusor hyperactivity in urinary incontinence. In the present study, we confirmed that cystitic rats showed detrusor hyperactivity as previously reported by Lecci et al. (22). The main urodynamic changes observed after cyclophosphamide administration were decrement of VT and MV and increments of MF and MP. In the present study, when aspirin was administered i.v. 5 min before cystometry, the changes induced by cyclophosphamide were significantly improved. Since the acute effect of aspirin was evaluated in this study (aspirin was administered 5 min before cystometry), we considered that the obtained results were not brought on by aspirin’s anti-inflammatory effect. In other words, aspirin is functionally effective in the inflamed bladder. Since it has been reported that COX-2 protein and PGE₁ production are up-regulated in a rat bladder inflammation model (27), it is a distinct possibility that PGs are strongly involved in the detrusor hyperreflexia of cystitic rats. In our study, we confirmed that both PGE₁ and 6-keto-PGF₁α levels are markedly increased in the cystitic bladder. Since 6-keto-PGF₁α is a metabolite of PGL₂, this means that both PGE₁ and PGI₁ levels would be increased in the inflamed bladder. Expectedly, a pharmacological dose of aspirin (30 mg/kg, i.v.) completely inhibited the increase in the syntheses of these PGs synthesis. Therefore, it can be concluded that the inhibition of PGs synthesis in the bladder is involved in the pharmacological action of aspirin in detrusor hyperactivity.

Interestingly, the effective dose of aspirin in cystitic rats is lower than that in normal rats. Inhibitory action of aspirin on PGs synthesis was evident in cystitic rats rather than in normal rats. This observation might be one of the explanations for cystitic rats having higher sensitivity to aspirin. On the other hand, it was reported that sensory C-fiber activation is involved in the development of cyclophosphamide-mediated cystitis (28). From this evidence, we hypothesized that the target site of aspirin might be peripheral C-fibers in the bladder. In order to clarify this hypothesis, we examined the influence of capsaicin-sensitive sensory C-fiber denervation on the pharmacological action of aspirin.

Generally, capsaicin-sensitive sensory C-fiber in human bladder is known to be silent, but it has some contribution in rats even in the normal condition (29). In our study, the efficiency of aspirin was diminished in sensory denervated rats without cystitis as compared with sensory intact rats (20% vs 91% in sensory intact rats). Furthermore, it is well known that PGs decrease the threshold for sensory neurone activation (30). This finding strongly suggests that aspirin affects cystometric parameters via suppression of C-fiber activation.

In sensory denervated rat with cystitis, the changes in cystometric parameters related to detrusor hyperactivity was not observed. Interestingly, these rats showed overflow micturition. This point is quite different from that of rats without cystitis; these rats could void normally even under sensory C-fiber denervated condition. It could be considered that the contribution of Aδ-fiber is predominant in the voiding reflex in non-inflamed bladder, while sensory C-fiber is predominant in it under the inflamed condition. Especially, dribbling micturition in sensory denervated rat with cystitis might be due to the degeneration of Aδ-fiber by cyclophosphamide treatment, as previously suggested by Wakabayashi et al. (31). Under the sensory C-fiber denervated condition, aspirin had no effect on cystometric parameters in cystitic rats. This result also suggests that aspirin alleviates detrusor hyperactivity via suppression of C-fiber activation.

Taken altogether, it is suggested that the suppression of sensory C-fiber via the inhibition of PGs synthesis is involved in the pharmacological action of aspirin in the detrusor hyperactivity.

References

Aspirin and Detrusor Hyperactivity