An excitatory role for peripheral EP₃ receptors in bladder afferent function


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PGE₂, one of the principal PGs, is synthesized in urothelium and detrusor smooth muscle (19, 24, 25, 27) as well as in neurons and glial cells (18, 23) and is released in response to various physiological (e.g., bladder distension) and pathological (e.g., mediators of inflammation) stimulation. PGE₂ interacts with four EP receptor subtypes (EP₁, EP₂, EP₃, and EP₄) (23).

PGE₂ has been detected at a high level in the urine of overactive bladder patients and may contribute to the clinical pathophysiology of bladder hypersensitivity (12, 13). Intravesical administration of PGE₂ induces bladder overactivity in animals by activation of sensory bladder afferent neurons (9, 18, 29, 31). Furthermore, the reduction of PG formation by cyclooxygenase inhibitors has demonstrated in vivo efficacy in hyperactive bladder models (1, 8, 11, 39). Based on studies in EP₁ receptor-deficient mice, this subtype has been reported to mediate the inflammatory nociceptive response to peritoneal irritation (42).

The role of EP₃ receptors in the regulation of afferent nerve activity from the urinary bladder has not been studied. To evaluate the peripheral role of EP₃ receptors in bladder afferent functions, a peripherally limited EP₃ receptor antagonist, (2E)-3-\{1-[(2,4-dichlorophenyl)methyl]-5-fluoro-3-methyl-1H-indol-7-yl\}-N\{-[4,5-dichloro-2-thienyl]sulfonyl\}-2-propenamide (DG-041; see Ref. 44), was tested on the micturition reflex in a bladder rhythmic contraction model and on bladder pain sensation by measurement of visceromotor reflex (VMR) and cardiovascular (pressor) responses to noxious urinary bladder distension (UBD).

The spontaneously hypertensive rat (SHR) exhibits bladder hypersensitivity (26, 33). One mechanism for this hypersensitivity could be an enhanced afferent limb of the micturition reflex pathway. The mechanism of sensitized afferent nerve activity in the SHR is based on an increased production of nerve growth factor by bladder smooth muscle (2, 33). This induces enlargement of bladder sensory neurons (3) and sensitization of the bladder afferent nerve activity (5). However, an increase in sensitivity of the primary afferent fibers to mechanical stimulation has not been documented in the SHR. Indeed, using the VMR response to noxious UBD as a measure of bladder afferent signal transmission, the SHR did not present a sensitized bladder phenotype (16).

In addition to the physiological examination of the bladder afferent/detrusor function in SHR, pharmacological responses of SHR to tool compounds targeting afferent pathways or detrusor muscle provide an alternative way to study the mechanism of bladder hypersensitivity in the SHR. For example, β₂-adrenoceptor activation results in an enhanced relaxation of bladder smooth muscle in the SHR but fails to change VMR.

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MATERIALS AND METHODS

The experimental protocols were approved by the Institutional Animal Care and Use Committee of GlaxoSmithKline Pharmaceuticals (King of Prussia, PA).

Gene expression by TaqMan study. Rats were anesthetized initially with 3% isoflurane and killed by exanguination. The dorsal root ganglia (DRGs) at level L6/S1 and T13/L1 and bladders were removed immediately and kept at −80°C. Tissues were homogenized in Trizol Reagent (Invitrogen, Carlsbad, CA) and after phase separation with chloroform. Total RNA was extracted using the RNeasy Mini Kit (Qiagen) following the manufacturer’s instructions. Any genomic DNA contamination was removed using DNase I (Ambion, Austin, TX). RNA samples were judged to be free of genomic DNA by no amplification in a standard TaqMan assay using 10 ng of RNA and β-actin (ACTB) primer/probe oligonucleotides. The RNA was quantified using Ribogreen RNA quantitation reagent (Invitrogen) and converted to cDNA by reverse transcription utilizing the High Capacity cDNA Archive Kit (Applied Biosystems, Foster City, CA). The equivalent of 10 ng mRNA/well was arrayed into 384-well plates using a Biomek FX robot (Beckman Coulter), and quantitative RT-PCR was carried out immediately and kept at −80°C. Tissues were homogenized in Trizol Reagent (Invitrogen, Carlsbad, CA) and after phase separation with chloroform. Total RNA was extracted using the RNeasy Mini Kit (Qiagen) following the manufacturer’s instructions. Any genomic DNA contamination was removed using DNase I (Ambion, Austin, TX). RNA samples were judged to be free of genomic DNA by no amplification in a standard TaqMan assay using 10 ng of RNA and β-actin (ACTB) primer/probe oligonucleotides. The RNA was quantified using Ribogreen RNA quantitation reagent (Invitrogen) and converted to cDNA by reverse transcription utilizing the High Capacity cDNA Archive Kit (Applied Biosystems, Foster City, CA). The equivalent of 10 ng mRNA/well was arrayed into 384-well plates using a Biomek FX robot (Beckman Coulter), and quantitative RT-PCR was carried out using a 7900HT Sequence Detector System (Applied Biosystems) in a 5-μ1 reaction volume. TaqMan Universal PCR Master Mix (2×; Applied Biosystems) and universal PCR conditions recommended by the manufacturer were followed. The mRNA expression of EP3 receptors and three splice variants (EP3A, D14869; EP3B, NM102704; and EP3C, D16443) were studied. All primers and probes (6-carboxyfluorescein, tetramethyl-6-carboxyrhodamine) are listed in Table 1. To normalize the data, samples were scaled relative to each other by the geometric mean of the set of valid housekeeping genes for that sample. Each data point was then expressed as the ratio of housekeeping gene abundance in the sample to the average of that in all samples and marked invalid if it had statistically inconsistent behavior to the other housekeeping genes in those samples with similar tissue types. The housekeeping genes used were ACTB, glyceraldehyde-3-phosphate dehydrogenase (GAPDH), and cyclophilin (peptidylpropyl isomerase A), and the data was floored at a relative abundance of 0.5.

Pharmacokinetic studies. At least 3 days before the start of the study, three rats received surgically implanted femoral vein and arterial catheters for infusion of DG-041 (1 mg/kg with a volume of 4 ml/kg in 16% Caviron and 3.2% dimethyl sulfoxide, over a 30-min infusion) and blood sampling. Blood (0.25 ml) was drawn at predetermined time points, up to 2 h, postdosing in lithium heparin-containing tubes. Plasma was separated by centrifugation and stored at −20°C before mass spectral analysis.

Table 1. Primers of Taqman study

<table>
<thead>
<tr>
<th>Gene</th>
<th>Forward Primer</th>
<th>Reverse Primer</th>
<th>Probe</th>
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<tbody>
<tr>
<td>EP3</td>
<td>CAACCTGGGCAGGAATCAAGG</td>
<td>AAGCTGGATAGCCGCTCTCCG</td>
<td>FAM-CTGGCACTGGAGCCCGCCAGTG</td>
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<tr>
<td>EP3B</td>
<td>CTGCTTCCGTTGGTATGATT</td>
<td>GGCCTCCGCTCTTGGATATGAG</td>
<td>CTGGATCCCTGGTTTATCTTGCTGCT</td>
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<tr>
<td>EP3A</td>
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<td>GGTCTTTCATGGGAGACACTT</td>
<td>CTGGATCCCTGGTTTATCTTGCTGCT</td>
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<tr>
<td>EP3C</td>
<td>TGGCGACGAGCTGCTGCTGCT</td>
<td>GTGTCTTTCCTTCTTCAGGCCC</td>
<td>CCAACCCAGAGGGGAGCCGATCTCCTT</td>
</tr>
<tr>
<td>ACTB</td>
<td>GAGCTATGATGCTGCTGAC</td>
<td>AGGTCTGATGAGCTGAGAGG</td>
<td>CATGCTAGATGCGCAATGGCAGT</td>
</tr>
<tr>
<td>GAPDH</td>
<td>GGAGCTAGATTAGCTGCCTT</td>
<td>GGCCAGTTCCAGAGTTT</td>
<td>ACCAGAAGAAAAAGGAGCCCAGATCTT</td>
</tr>
<tr>
<td>PPIA</td>
<td>TTAATGACCATCTGGTGAGTAGA</td>
<td>CCACAAGCAGTGCCTGTGCTTTC</td>
<td>CCAAAAGACACATGTCCTGGAGCA</td>
</tr>
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</table>

GAPDH, glyceraldehyde-3-phosphate dehydrogenase.

The rat brain-to-blood ratio of DG-041 was also studied. Rats were anesthetized with isoflurane (3%) and dosed with DG-041 (10 mg/kg iv). Arterial blood (0.4 ml) was drawn every 15 min postdose in lithium heparin-containing tubes. Rats then were killed by exanguination. The rat brain was removed and placed in a preweighed container for homogenization, followed by the addition of 2 ml/g acetone to induce a threefold final dilution. The brain tissues were then homogenized with a Polytron homogenizer and centrifuged. The supernatants were transferred and stored at −20°C before analysis.

Quantification of DG-041 was performed using liquid chromatography/tandem mass spectrometric (LC/MS/MS) detection. Samples were thawed, plasma proteins were precipitated with 200 μl of 95:5 acetonitrile-0.1% formic acid in water, containing an appropriate mass spectrometric internal standard, and the resulting mixture was vortex mixed for 2 min followed by centrifugation for 30 min at >2,000 g. With the use of a sensitive and selective LC/MS/MS method on an HTS PAL autosampler (CTC Analytics, Zwingen, Switzerland) coupled to a Sciex API5000 triple-quadrupole mass spectrometer (Applied Biosystems), samples were analyzed for quantitative concentrations of DG-04. Analytical standards (1–2,500 ng/ml) were prepared in rat plasma or brain homogenate to ensure accurate calibration of the mass spectrometer for each biological matrix.

In vivo bladder rhythmic contraction. Female Sprague-Dawley (SD) rats weighing 200–300 g (n = 17) were anesthetized with 3% isoflurane during surgery. Anesthetized rats were maintained with a hot water-circulating heating pad underneath the studies and were killed upon completion of experimental procedures by an intravenous overdose of pentobarbital sodium (120 mg/kg; Vorteq Pharmaceuticals, Dearborn, MI). One jugular vein was cannulated with polyethylene tubing for intravenous administration of urethane and DG-041. A cannula (PE-50) was placed in the bladder via the urethra, and the urethra was ligated to ensure an isovolumetric bladder. Upon completion of surgical procedures, slow intravenous infusion of urethane (1.2 g/kg, ethyl carbonate; Sigma, St. Louis, MO) was given over 15 min. Saline bladder infusion procedures were begun 30 min after the final dose of urethane.

The urethral cannula was connected with a T connector and linked with a low-volume transducer (model MLT0380D; ADInstruments, Colorado Springs, CO). The signal was amplified through a direct current (DC) amplifier (ADInstruments, ML119). The other end of the T connector was linked to a 20-ml syringe with a perfusion pump. For the pharmacological study on bladder rhythmic contraction, the saline infusion in bladder was at a rate of 50 μl/min to induce micturition reflex (here defined as bladder contraction with intensity >10 mmHg). The infusion rate was then lowered to 10 μl/min until three to five rhythmic bladder contractions were established; the infusion was then terminated. The vehicle or test compound was administered after a 15-min control period. Following administration, bladder rhythmic contractions were recorded for 20–30 min. Two parameters of the bladder rhythmic contraction were evaluated (frequency/interval and amplitude). The mean controls were calculated by the average of readouts during the last 5-min interval of the control period. The
effects of compounds were calculated by the mean response in every 5-min period after injection (16).

In vivo VMR and pressor responses to urinary bladder distension. Female rats (SD, n = 23; SHR, n = 13; WKY, n = 11) were anesthetized initially with 3% isoflurane. Cannulas for intravenous administration and for bladder distension were implanted as described above. To measure blood pressure, the right carotid artery was catheterized with PE-50 tubing. The arterial catheter was linked with a low-volume transducer (ADInstrument; MLT0380D), and signal was amplified through a DC amplifier (ADInstrument; ML119). The bladder catheter was also linked to a bladder distension control device. The bladder was distended with saline by regulating air inflow into a Mariott bottle from a valve interface distension control device (University of Iowa, Bioengineering, B482C-1; see Ref. 37). Two needle electrodes were sutured in the oblique abdominal musculature just above the inguinal ligament. Abdominal contractions were quantified by action potentials of electromyographic activity. Action potentials were initially amplified through a low-noise alternating current (AC) differential amplifier (ADInstrument; EC16/300), processed using the AD data acquisition program (PowerLab 16/30, ML880). Raw action potentials of myoelectric activities, bladder pressure, and blood pressure were displayed on-line continuously. All data were analyzed off-line using the ADInstrument power lab program (Chart 8).

Following completion of the surgical preparation, isoflurane anesthesia was reduced until flexion reflex response could be evoked by pinch of the foot without spontaneous escape behaviors (~1% isoflurane).

For UBD, all rats received a series of at least six phasic bladder distensions at 60 mmHg for 30 s at 3-min intervals to evaluate response stability to repeated bladder distension. The compound or vehicle was administered only after four consistent responses were elicited and given 2 min before the onset of distension.

The electromyographic activity was integrated and calculated as the area under the curve. The VMR response to the stimulus was defined as the increase in electromyographic activity during UBD from the baseline activity before each response. Pressor response was quantified as the peak change in mean arterial pressure during UBD from the baseline activity before each response. Pressor response was normalized to the percentile of mean control response: the average of four UBD responses before compound treatment.

Afferent nerve recording. Male rats weighing 400–500 g (16 SD, 12 SHR, and 10 WKY) were used for this study. Rats were anesthetized initially with pentobarbital sodium (Nembutal) at a dose of 40–45 mg/kg ip and maintained thereafter with supplementary intravenous doses of pentobarbital sodium (5–10 mg·kg⁻¹·h⁻¹). The trachea was cannulated to provide artificial ventilation with room air. For injecting drugs, a catheter was passed to the descending aorta via the fundus. As described above, for UBD, the bladder catheter was connected to a saline bottle from a distension control device. The urethra was ligated close to its entry to the penis, and urine was evacuated constantly via the fundic catheter.

Gene expression by quantitative PCR. The three housekeeping genes (ACTB, GAPDH, and cyclophilin) were expressed in a stable manner to each other across all of the samples, allowing for proper normalization. The localization of mRNA of EP3 receptors in rat bladder and DRG normalized to the mean of housekeeping genes is shown in Fig. 1. The mRNA expression levels of EP3A and EP3v1 were high in bladder, and mRNA of EP3C was high in DRG and brain. A similar expression pattern was observed in SHR and WKY rats (data not shown). Overall, the EP3 receptors were abundant in all tissues, bladder, and neurons.
Pharmacokinetic studies. DG-041 has pharmacokinetics suitable for in vivo studies in rats. Following intravenous infusion of 1 mg/kg DG-041 over 30 min \( (n = 10) \), the maximal concentration was 957 ± 164 ng/ml, the half-time for the elimination phase was 103 ± 26 min, and systemic plasma clearance is 30.4 ± 4.5 ml·min⁻¹·kg⁻¹. DG-041 had a volume of distribution approximately equal to total body water, 0.78 ± 0.29 l/kg. In another group of rats, at 15 min post-10 mg/kg bolus injection \( (n = 4) \), the plasma level was 4.39 ± 1.22 μg/ml. At the same time point, the brain level of DG-041 was much lower than blood levels, yielding a brain-to-blood ratio of 0.027 ± 0.004 \( (n = 4) \).

Effect of intravenous administration of DG-041 on the micturition reflex. Intravenous administrations of vehicle (PEG-400) did not produce significant changes in bladder rhythmic contractions. The administration of DG-041 produced an inhibition of the bladder rhythmic contraction, reducing frequency or eliminating the contraction (“disappearance” of contractions). The disappearing intervals were 9.03 ± 1.28 min (5 out of 6 rats) and 9.72 ± 1.89 min (4 out of 5 rats) at 3 and 10 mg/kg, respectively.

If the contractions were not abolished, their amplitude was not reduced by DG-041. Figure 2, C and D, summarizes the effect of DG-041 on bladder rhythmic contraction following intravenous administration.

Effect of intravenous administration of DG-041 on nociceptive responses to bladder distension. Figure 3, A and B, demonstrates typical examples of intravenous administration of vehicle and DG-041 on both VMR and pressor responses to noxious UBD at 60 mmHg. Intravenous administration of DG-041 produced a significant inhibition of both VMR and pressor responses to UBD. The maximal inhibition occurred at 2 and 5 min postinjection. Figure 3, C and D, summarizes the effects of DG-041 on the VMR response and the pressor response to UBD following intravenous injection.

The inhibition by DG-041 (10 mg/kg iv) on the VMR response to UBD was compared in three strains of rats (SD, SHR, and WKY). DG-041 produced an equal degree of inhibition on the mean VMR response 5 min postinjection (Fig. 4A).

In another group of SHR, responses to three steps of UBD were evaluated in a staircase manner before and after DG-041 (10 mg/kg iv). DG-041 tended to produce stronger inhibition of the response to 60 mmHg UBD than that to 40 mmHg UBD (Fig. 4B).

Effect of DG-041 on response of afferent nerve to bladder distension. A total of 44 mechanosensitive pelvic nerve afferent fibers in the L6 dorsal root were identified by electrical stimulation of the pelvic nerve and UBD (16 from SHR, 12 from WKY, and 16 from SD). The proportions of myelinated...
and unmyelinated afferent fibers in SHR, WKY, and SD rats were similar (Table 2). Most of the afferent nerves exhibited an ongoing discharge as we have previously described (30, 34) with similar mean frequencies in three strains. All fibers were characterized for responses to graded intensities of UBD (5–80 mmHg) and exhibited an increased response to increasing pressures of UBD. The response patterns of afferent fibers from SHR were significantly different from those of WKY rats.

Fig. 2. Effect of DG-041 on volume-induced rhythmic bladder contraction in the anesthetized rat. Typical experimental records showing the effects of intravenous administration (†) of vehicle [polyethylene glycol (PEG) 400, A] and DG-041 (10 mg/kg, B) on bladder rhythmic contraction (mmHg). Time course for the effect of vehicle and DG-041 on the frequency (C) and the amplitude (D) of volume-induced contractions following iv administration. The responses are represented as a percentage of control (%control), where the baseline response before administration is defined as 100%. The significance of differences between the test and control values was determined by ANOVA test.

Fig. 3. Effect of DG-041 on responses to noxious urinary bladder distension (UBD, 60 mmHg, 30 s in 3 min) in the anesthetized rat. Typical experimental recording of the visceromotor reflex (VMR) response (trace on top, mV) and the pressor response (traces on bottom, mmHg) of iv administration (†) of vehicle (PEG-400, A) and DG-041 (100 nmol, B). Time course for the effect of iv administration of vehicle and DG-041 on VMR (C) and pressor (D) responses to UBD. The responses are represented as %control, where the baseline response before administration of drugs is defined as 100%. The significance of differences between the test and control values was determined by ANOVA test.
Table 2. Summary and comparison of mechanosensitive pelvic afferent fibers in SHR, WKY, and SD rats

<table>
<thead>
<tr>
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<th>SHR</th>
<th>WKY</th>
<th>SD</th>
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<tbody>
<tr>
<td>C-fiber CV, m/s</td>
<td>1.60±0.14 (67)</td>
<td>1.77±0.19 (75)</td>
<td>1.61±0.17 (66)</td>
</tr>
<tr>
<td>Aδ-fiber CV, m/s</td>
<td>5.48±1.71 (33)</td>
<td>3.93±0.51 (25)</td>
<td>4.68±0.97 (33)</td>
</tr>
<tr>
<td>Threshold, mmHg</td>
<td>9.39±1.90</td>
<td>4.01±1.21</td>
<td>8.17±1.16</td>
</tr>
<tr>
<td>Resting firing, impulses/s</td>
<td>0.55±0.23 (25)</td>
<td>0.88±0.31 (17)</td>
<td>1.08±0.37 (13)</td>
</tr>
</tbody>
</table>

Values are reported as means ± SE. SHR, spontaneously hypertensive rats; WKY, Wistar-Kyoto; SD, Sprague-Dawley; CV, conduction velocity. For C-fiber and Aδ-fiber, values in parentheses are % for resting firing, nos. in parentheses indicate % silent.
The pelvic afferent nerve was recorded from the L6 dorsal root, since 84% of the pelvic afferent fibers from the lower urinary tract enter the spinal cord via the L6 dorsal root and a smaller fraction enters in S1 (43). The present study shows that the mechanosensitive pelvic afferent fibers in the L6 dorsal root from SHR, WKY, and SD rats have the proportion of unmyelinated afferent fibers and exhibit ongoing resting activity, similar to observations in SD rats (30, 34, 43). We defined the response threshold by extrapolation but did not separate to the “high threshold” or “low threshold” afferents due to small sample size and the lack of clear criteria to differentiate the groups. As we reported previously in SD rats (32), response thresholds of the afferent fibers did not correlate with conduction velocities. Unexpectedly, afferents from WKY rats had a lower response threshold and a higher response magnitude. In contrast, the SRFs of mechanosensitive afferent nerves were

Fig. 5. Stimulus-response functions of pelvic nerve afferent fibers to graded intensities of UBD (5–80 mmHg, 30 s in 3 min, A). The response threshold and the conduction velocity of afferent fibers did not correlate (B).

Fig. 6. Examples of effects of vehicle (PEG-400) and DG-041 (10 mg/kg iv) on responses of a pelvic nerve afferent fiber to UBD (60 mmHg, 30 s in 3 min). A: responses of the fiber are illustrated as peristimulus time histograms in 0.01-s bandwidth before and after DG-041 (shadow area). Phasic distending pressure is presented below. B: time course for the effect of iv administration of vehicle and DG-041 on responses of the fiber to UBD.
not enhanced in SHR compared with SD rats. This is consistent with our previous study where a sensitized afferent signal was not detected in SHR as measured by the VMR response to bladder distension (16). In addition, inhibition by DG-041 of the VMR response and afferent response to UBD in SHR was not enhanced. The SHR detrusor showed decreased compliance and was more sensitive to inhibition by β3-adrenoceptor activation (16). If an afferent mechanism is involved in the hypersensitivity in SHR, mechanisms targeting afferent nerves, like an EP3 receptor antagonist, would be expected to be more effective against the responses to UBD, although the pathological mechanism of hypersensitivity is not necessarily linked to an altered pharmacology. However, DG-041 did not produce an enhanced inhibition on bladder nociception in the SHR. Thus both physiological and pharmacological arguments do not support afferent sensitization in the SHR.

DG-041 (10 mg/kg iv) inhibited the bladder rhythmic contraction frequency and often temporarily eliminated the rhythmic voiding contractions. Compounds targeting the bladder afferent pathway often produce this effect (6, 7, 17, 40). “Shutdown” of bladder rhythmic contraction reflects an increased threshold for induction of contractions and an increased bladder capacity in the conscious cystometrographic study (17). Because DG-041 is unlikely to achieve effective concentrations in the CNS following systemic administration, its effects are probably due to the inhibition of EP3 receptors located on peripheral neurons or detrusor muscle. DG-041 failed to change the amplitude of the rhythmic bladder contractions; thus, detrusor functions might not be modulated by an EP3 receptor antagonist. On the other hand, we found mRNA for the EP3A and EP3v1 splice variants to be highly expressed in bladder, either detrusor muscle or urothelium. Unfortunately, DG-041 does not differentiate different EP3

Fig. 7. Inhibition by DG-041 on responses of all afferent fibers from SD, SHR, and WKY rats to UBD (60 mmHg, 30 s in 3 min). A: mean inhibition of responses to UBD (as %control) by DG-041 was significantly different from that by vehicle (P = 0.04, nonpaired Student’s t-test). The inhibition correlated well with response thresholds (B) and conduction velocities (C) of afferent fibers but was not dependent on the control response frequencies (D).

Fig. 8. Dose-dependent inhibition by sodium channel blocker mexiletine on responses of pelvic nerve afferent fibers to UBD (60 mmHg, 30 s in 3 min). Mean inhibition of responses to UBD are expressed as %control. There were no apparent differences in effects of DG-041 in SD, SHR, and WKY rats.
splice variants (X. Su, C. W. Wu, D. M. Morrow, and J. P. Jaworski, unpublished observations). Thus inhibition of detrusor muscle contraction cannot be conclusively ruled out. A more selective subtype antagonist could be useful for further clarifying the mechanism of EP3 receptors in bladder function.

The action of an EP3 receptor antagonist on bladder afferent nerves was demonstrated by the inhibitory effect of DG-041 on the VMR response to bladder distension and is consistent with the high expression of EP3 receptor mRNA in primary sensory neurons. The effect of DG-041 on bladder compliance was not measured, but such an effect would not contribute to the antinociceptive activities, since we have demonstrated that an increase in bladder compliance did not change the readout of VMR and pressor responses (16). Thus the antinociceptive effect of DG-041 is predominantly due to inhibition of receptors located on peripheral nerves. It should be noted that the voiding-associated abdominal wall response (4) is triggered by urethra afferent nerve for the physiological voiding (32). It differs from the passive VMR response to noxious bladder distension (60 mmHg), which corresponds to pain sensation. Whether the voiding-associated VMR response contaminates the nociceptive VMR response to 60 mmHg bladder distension should be further investigated.

To further test the interpretations drawn from the VMR experiments, direct effects of DG-041 on the afferent nerve firing were examined. As expected, DG-041 inhibited the response of afferent fibers to bladder distension. This could result from an action at the sensory endings, axon or cell bodies, where the mRNA for EP3 receptors has been detected. The beneficial effects of EP3 antagonism for the inhibition of bladder afferent nerve activity concur with previous studies of hyperalgesia and allodynia associated with the cutaneous pain model (10). It is known that intravesical administration of PGE2 enhances the micturition reflex (9) and that this effect is mediated in part by lowering the threshold of capsaicin-sensitivistic bladder afferent nerves (18). PGE2 has been reported to sensitize peripheral terminals of small-diameter, high-threshold, primary afferent fibers to thermal, chemical, and mechanical stimuli (14, 15, 21, 28). In the present study, we have demonstrated for the first time that an antagonist, DG-041, attenuated the response of afferent nerves to bladder distension, selectively on high-threshold and low-conductivity afferent nerves. In contrast, a nonselective sodium channel blocker inhibited responses of all pelvic afferent fibers to visceral organ distensions to the same degree. The nonselective inhibition of afferent activity by sodium channel blockade was reported in our previous studies (35, 36). Accordingly, the inhibition of afferent nerves innervating the urinary bladder by an EP3 antagonist may be more selective for nociceptors and therefore safer for therapeutic intervention. Whether the sites of action of DG-041 are on myenteric and submucosal plexus neurons or on interstitial cells with their associated visceral sensation requires further investigation. Both studies, the rhythmic contraction and the VMR response, require bladder overdistensions. Further investigation of EP3 antagonists in conscious cystometry will highlight the role of EP3 receptors in basal urodyamics under conditions of physiology or pathology of bladder overactivity.

In summary, we found that mechanosensitive afferent hypersensitivity is not the mechanism of the hypersensitive bladder in SHR. We also have demonstrated for the first time that EP3 receptors are involved in the regulation of the micturition reflex and bladder nociception by an action on afferent nerves and hence added further evidence for the potential usefulness of EP antagonists in the treatment of urgency or pain associated with bladder dysfunction.

ACKNOWLEDGMENTS

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REFERENCES


