Enhanced bladder capacity and reduced prostaglandin E2-mediated bladder hyperactivity in EP3 receptor knockout mice

Gerald P. McCafferty, Blake A. Misajet, Nicholas J. Laping, Richard M. Edwards, and Kevin S. Thorneloe

Urogenital Biology, Cardiovascular and Urogenital Center for Excellence in Drug Discovery, GlaxoSmithKline Pharmaceuticals, King of Prussia, Pennsylvania

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McCafferty GP, Misajet BA, Laping NJ, Edwards RM, Thorneloe KS. Enhanced bladder capacity and reduced prostaglandin E2–mediated bladder hyperactivity in EP3 receptor knockout mice. Am J Physiol Renal Physiol 295: F507–F514, 2008. First published May 28, 2008; doi:10.1152/ajprenal.00054.2008.—Nonsteroidal anti-inflammatory cyclo-oxygenase inhibitors that function to reduce prostaglandin E2 (PGE2) production have been widely reported as effective agents in models of urinary bladder overactivity. We therefore investigated a potential role for the PGE2 receptor, EP3, in urinary bladder function by performing conscious, freely moving cystometry on EP3 receptor knockout (KO) mice. EP3 KO mice demonstrated an enhanced bladder capacity compared with wild-type (WT) mice (~185% of WT) under control conditions, based on larger voided and infused bladder volumes. Infusion of the EP3 receptor agonist GR63799X into the bladder of WT mice reduced the bladder capacity. This was ineffective in EP3 KO mice that demonstrated a time-dependent increase in bladder capacity with GR63799X, an effect similar to that observed with vehicle in both genotypes. In addition, infusion of PGE2 into WT mice induced bladder overactivity, an effect that was significantly blunted in the EP3 KO mice. The data reported here provide the first evidence supporting a functional role for EP3 receptors in normal urinary bladder function and implicate EP3 as a contributor to bladder overactivity during pathological conditions of enhanced PGE2 production, as reported previously in overactive bladder patients.

GR63799X; conscious cystometry

CYCLOOXYGENASE (COX) inhibitors, nonsteroidal anti-inflammatory drugs, are widely utilized to treat pain, targeting both constitutively expressed COX-1 and/or inducible COX-2 isoforms (6). This is based on a large body of evidence that COX-2 expression is increased in response to cytokines, injury, and inflammation (3). COX-2 expression is also enhanced in preclinical models of urinary bladder dysfunction, including bladder outlet obstruction and interstitial cystitis (11, 24, 25). Consistent with this finding, COX inhibitors have demonstrated in vivo efficacy in bladder overactivity models performed in multiple species (1, 11, 13, 32, 36). COX inhibitors have also been evaluated in small clinical voiding studies yielding positive outcomes (2, 4, 27).

COX enzymes function to metabolize arachidonic acid to prostaglandin H2 that is rapidly converted to prostaglandin E2 (PGE2), as well as to other prostaglandins (PGI2, PDG2, PGF2α, and TXA2) via their respective synthase enzymes. PGE2 is produced by bladder smooth muscle in response to stretch (24, 25) and electrical field stimulation (7). The bladder urothelium also releases PGE2 in a stretch-dependent manner (19, 26), an effect potentiated in spinal cord-injured rats that present with bladder overactivity and increased PGE2 levels in their urine (19). PGE2 contracts isolated bladder strips in vitro (23, 26) and evokes bladder overactivity in vivo when infused intravesicularly into bladders of rats and mice via a mechanism involving capsaicin-sensitive sensory bladder afferents (12, 17, 18, 29). Likewise, intravesicular PGE2 and sulprostone, an EP1/EP3 agonist, induce bladder instability and reduce bladder capacity in humans (30). As PGE2 is enhanced in the urine of male and female overactive bladder patients (14, 15) and in preclinical models (11, 24, 25), PGE2 likely contributes to the clinical pathophysiology of bladder overactivity. Taken together, the literature suggests a role for PGE2 in multiple aspects of urinary bladder physiology/pathophysiology in numerous species.

PGE2 mediates its effects by activating the EP family of G protein-coupled receptors that consist of four known isoforms, EP1-EP4 (9). Of these, it has been previously suggested that EP1 receptors are involved in PGE2-induced bladder overactivity and bladder outlet obstruction (16, 18, 29). Here, we investigated a potential role for the EP3 receptor isoform in urinary bladder function by characterizing the urodynamics and pharmacological responses of EP3 knockout (KO) mice.

MATERIALS AND METHODS

Mice. The generation of the EP3 receptor KO mouse was described previously (8). The EP3 KO allele was bred into 129P3/J and B6 backgrounds and maintained as intercrossed homozygous colonies at Jackson Labs. Wild-type (WT) mice were also obtained from Jackson Labs 129P3/J and C57Bl/6J colonies. All experimental procedures were approved by GlaxoSmithKline IACUC.

Bladder catheter surgery. Bladder catheter implantation was performed essentially as described (10, 33). Mice were anesthetized using isoflurane (Abbott Laboratories, N. Chicago, IL) and the urinary bladder was exposed by an abdominal midline incision. A purse string suture was placed in the dome of the bladder into which a sterile, saline-filled bladder catheter (PE-10 flanged catheter tip fused to PE-50 tubing) was inserted and secured. The catheter was routed subcutaneously to the back of the neck and stored in a skin pouch. The abdominal wall and skin were sutured to close.

Cystometry. Mice were allowed to recover from surgery for 6–10 days before cystometry. On the day of cystometry, under light isoflurane anesthesia, the end of the bladder catheter was retrieved from the skin pouch. Saline-filled polyethylene (PE-50) tubing was used to extend the externalized catheter through a mouse infusion harness (Covance, NJ).

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The catheter was then connected to a pressure transducer that was inline with the syringe pump of a Small Animal Cystometry Lab Station (MED Associates, St. Albans, VT). The mice were placed in a wire bottom cage located over the balance and allowed to recover from the light anesthesia (~15–30 min) before initiating the continuous intravesicular infusion of saline at 25 μl/min for the duration of the study. Mice were euthanized on completion of cystometric profiling by carbon dioxide inhalation with diaphragm disruption as a secondary euthanasia method.

**Drug administration.** PGE2 and glacial acetic acid were purchased from Sigma. 4-[(Phenylcarbonyl)amino]phenyl (4Z)-7-[(1R,2R,3R)-

![Graphs showing control urodynamics in EP3 wild-type (WT) and EP3 knockout (KO) mice. A and B: representative cystometric recording from EP3 WT and EP3 KO mice, respectively. Intravesicular bladder pressure (top) and voided (black) and infused (gray) volumes (bottom). C and D: average infused and voided volumes, respectively, from EP3 WT and EP3 KO mice.](image)

**Fig. 1.** Control urodynamics in EP3 wild-type (WT) and EP3 knockout (KO) mice. A and B: representative cystometric recording from EP3 WT and EP3 KO mice, respectively. Intravesicular bladder pressure (top) and voided (black) and infused (gray) volumes (bottom). C and D: average infused and voided volumes, respectively, from EP3 WT and EP3 KO mice.

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**Table 1. Comparison of control urodynamics and the response to AA (0.25%) in EP3 WT and KO mice, bladder pressures (mmHg), and volumes (μl)**

<table>
<thead>
<tr>
<th></th>
<th>Infused Volume</th>
<th></th>
<th>Void Volume</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>AA</td>
<td>%Control</td>
<td>Control</td>
</tr>
<tr>
<td>EP3 WT (n = 8)</td>
<td>205 ± 32</td>
<td>27 ± 2*</td>
<td>15 ± 2</td>
<td>168 ± 31</td>
</tr>
<tr>
<td>EP3 KO (n = 8)</td>
<td>371 ± 34†</td>
<td>88 ± 28*</td>
<td>24 ± 6</td>
<td>321 ± 30†</td>
</tr>
<tr>
<td></td>
<td>Threshold Pressure</td>
<td></td>
<td>Peak Pressure</td>
<td></td>
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<tr>
<td>EP3 WT (n = 8)</td>
<td>14 ± 2</td>
<td>23 ± 3*</td>
<td>176 ± 30</td>
<td>35 ± 2</td>
</tr>
<tr>
<td>EP3 KO (n = 8)</td>
<td>12 ± 1</td>
<td>17 ± 2*</td>
<td>147 ± 14</td>
<td>41 ± 2†</td>
</tr>
<tr>
<td></td>
<td>Average Pressure</td>
<td></td>
<td>%Control</td>
<td></td>
</tr>
<tr>
<td>EP3 WT (n = 8)</td>
<td>11 ± 2</td>
<td>20 ± 3*</td>
<td>208 ± 34</td>
<td></td>
</tr>
<tr>
<td>EP3 KO (n = 8)</td>
<td>9 ± 1</td>
<td>15 ± 1*</td>
<td>169 ± 16</td>
<td></td>
</tr>
</tbody>
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Values are means ± SE. AA, acetic acid; WT, wild-type; KO, knockout. *P < 0.05 vs. same genotype control, paired t-test. †P < 0.05 vs. other genotype control, unpaired t-test.
3-hydroxy-2-[(2R)-2-hydroxy-3-(phenyloxy)propyl]oxy-5-oxocyclopentyl]-4-heptenoate (GR63799X) was synthesized in house at GlaxoSmithKline Pharmaceuticals. PGE2 and GR63799X were dissolved in ethanol and DMSO, respectively, and diluted 1:1,000 in saline just before bladder infusion. In all studies, infusion of saline was initiated with a 30-min equilibration period that was followed by 30 min of control urodynamics. Mice were then challenged intravesically with various agents; GR63799X (10 μM) was infused into male 129P3/J mice for 120 min, PGE2 (120 μM) was infused into female B6 mice for 120 min, and acetic acid (0.25% vol/vol) was infused into male 129P3/J mice for 60 min.

Statistics and analysis. Statistical evaluations were made by Student’s paired \( t \)-test within a group, or unpaired \( t \)-test between groups of mice (GraphPad Prism). In all cases, urodynamics were assessed by analysis of the last 30 min of recording in the presence of drug, using the MED Associates analysis module in conjunction with Origin 7.0 (OriginLab, Northampton, MA). Parameters measured were infused maximum (volume infused to induce voiding), void volume (urine voided), pressure threshold (pressure before micturition), average filling pressure, and peak pressure (micturition peak). Since EP3 KO mice demonstrated larger infused and voided volumes compared with WT mice under control conditions, the response to intravesicular drug challenge was expressed as a percentage of control; the percent control values were compared between genotypes. In some cases, the effect on bladder volumes was assessed as a change from control. Nonvoiding contractions were assessed as increases in bladder pressure of greater than 5 mmHg during the filling phase and were analyzed using Mini Analysis software (Synamtosoft, Decatur, GA).

RESULTS

Comparison of EP3 WT and KO mice. Consistent with data reported on another EP3 KO mouse (28), the male 129/P3J EP3 KO mice (8) weighed significantly more than 129/P3J WT
controls (KO 30.0 ± 0.5 g, n = 19; WT 26.2 ± 0.7 g, n = 12; P < 0.0001) of equivalent age (~21 wk; KO 151 ± 1 days, n = 19; WT 152 ± 2 days, n = 12; P = 0.5) when maintained on a regular diet. A cystometric comparison of male EP3 WT and EP3 KO mice suggested that EP3 KO mice have an enhanced bladder capacity, based on an observation of higher voided urine volumes (191% of EP3 WT), and higher infused volumes required to stimulate micturition (181% of EP3 WT) in the EP3 KO mice (Fig. 1). Threshold and average filling pressures were not significantly different between genotypes; however, the peak micturition pressure was significantly higher in KO mice (Table 1). Nonvoiding bladder contractions (pressure oscillations) as assessed during the filling phase of the cystometrigrams were not different in terms of average amplitude or frequency of these events between the two genotypes (n = 8 per genotype; amplitude: WT 10.3 ± 0.5 mmHg, KO 10.6 ± 0.9 mmHg, P = 0.77; frequency: WT 0.016 ± 0.002 Hz, KO 0.021 ± 0.002 Hz, P = 0.13). The average age of the WT and KO mice utilized for this comparison was not significantly different; however, the EP3 KO mice weighed significantly more than the WT mice (KO 30.0 ± 1 g, n = 8; WT 26 ± 1 g, n = 8; P = 0.03), as shown in general for these mice above. To compensate for the differential size of EP3 WT and KO mice, we normalized the infused and voided bladder volumes by body weight. When compensating for this weight difference, the infused and voided bladder volume/body weight ratios (µl/g) were also significantly different (infused: 8 ± 1 WT, 12 ± 1 KO, P = 0.03; void: 6 ± 1 WT, 11 ± 1 KO, P = 0.02), indicating that the enhanced bladder capacity of the EP3 KO mice was not solely due to an increased body size.

Acetic acid-induced bladder overactivity. The same mice utilized for the basal urodynamic comparison were challenged with intravesicular infusion of acetic acid (AA; 0.25% vol/vol). AA evoked a significant decrease in infused and voided volumes and a significant increase in threshold and average filling pressures in both WT and KO mice (Table 1). Peak micturition pressure was also increased in both the WT and KO mice. When AA urodynamic parameters were normalized to their respective controls, no significant difference in the AA response between WT and KO mice was observed (Table 1).

EP3 receptor activation with GR63799X. Infusion of the EP3 receptor agonist GR63799X (10 µM) into WT mice caused a reduction in both the voided and infused bladder volumes, with no significant effect on average, threshold, and peak micturition pressures (Fig. 2A, Table 2). In contrast, there was a time-dependent increase in the voided and infused volumes in vehicle-infused WT mice (n = 7). Although the GR63799X response on voided and infused volumes did not reach statistical significance compared with control values in the same mice before GR63799X infusion (Table 2), the GR63799X effect was significant compared with the vehicle-treated controls. On average, GR63799X caused a −39 ± 23 µl change in void volume and a −40 ± 25 µl change in infused volume, whereas vehicle treatment caused a +92 ± 39 µl change in void volume and +99 ± 38 µl change in infused volume (P = 0.006, for both void and infused).

The reduction in voided and infused bladder volumes in response to GR63799X observed in WT mice was absent in EP3 KO mice. KO mice demonstrated an increase in voided and infused bladder volumes in the presence of GR63799X (Fig. 2, Table 2). GR63799X treatment in KO mice resulted in +86 ± 42 and +83 ± 35 µl changes in infused and voided volumes, respectively (n = 9). Similar increases were observed with vehicle treatment in the KO mice (n = 4). These responses in the EP3 KO were similar to the effect of vehicle treatment in WT mice, and in both genotypes likely reflect a time-dependent increase in bladder capacity resulting from the continuous infusion of saline. Overall, normalizing the GR63799X response to control demonstrated that the effect on voided and infused bladder volumes in WT mice was significantly different from that observed in KO mice (Fig. 2, Table 2). No significant differences were observed between genotypes on peak micturition and average bladder pressures (Table 2). A significant increase in threshold pressure was observed with GR63799X in KO mice. This is consistent with observations in vehicle-treated KO mice and is likely a result of the time-dependent increase in bladder capacity.

PGE2-induced bladder overactivity. We infused EP3 WT and EP3 KO mice with the physiological ligand for the EP3 receptor, PGE2. WT mice infused with 10 µM PGE2 yielded no reduction in bladder volumes (n = 8). However, PGE2 at 120 µM evoked a reduction in infused and voided volumes, with no significant effect on bladder pressures (Fig. 3, Table 3). This concentration is considerably higher than the low nanomolar levels measured in urine from overactive bladder pa-

| Table 2. Effect of intravesicular GR63799 (10 µM) on EP3 WT and EP3 KO bladder pressures (mmHg) and volumes (µl) |
|-----------------|-----------------|-----------------|-----------------|
|                 | Infused Volume  |                 | Void Volume     |
|                 |                 | %Control        |                 |                   |
| Control         | GR63799         |                  | Control         | GR63799          |
|                 |                 | %Control        | %Control        | %Control          |
| EP3 WT (n = 12) | 164 ± 20        | 124 ± 22        | 83 ± 17         |
|                 |                 | 137 ± 17        | 107 ± 20        | 83 ± 18          |
|                 |                 | 271 ± 43†       | 363 ± 60*       | 134 ± 16‡        |
| EP3 KO (n = 9)  | 319 ± 54†       | 418 ± 65*       | 134 ± 16‡       |
|                 |                 | 39 ± 2          | 44 ± 2          | 113 ± 5          |
|                 |                 | 46 ± 2          | 53 ± 3*         | 115 ± 6          |
| Control         | Threshold Pressure | GR63799 | %Control | Peak Pressure | GR63799 | %Control |
|                 | 15 ± 1          | 17 ± 1          | 120 ± 10        | 39 ± 2          | 44 ± 2  | 113 ± 5  |
|                 | 15 ± 3          | 24 ± 2*         | 166 ± 18‡       | 46 ± 2          | 53 ± 3* | 115 ± 6  |
| EP3 WT (n = 12) |                 |                 | 128 ± 11        |
|                 |                 | 123 ± 13        |
| EP3 KO (n = 9)  | 13 ± 2          | 15 ± 2          | 128 ± 11        |
|                 |                 | 123 ± 13        |

Values are means ± SE. *P < 0.05 vs. same genotype control, paired t-test. †P < 0.05 vs. other genotype control, unpaired t-test. ‡P ≤ 0.05 vs. other genotype % control, unpaired t-test.

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tients (14, 15) and spinal cord-injured rats (19). However, it is well-known that PGE2 is rapidly metabolized in vivo. In EP3 KO mice challenged with 120 μM PGE2, no significant effect on infused and voided bladder volumes, peak micturition, or threshold pressures was detected (Fig. 3, Table 3). A significant increase in average filling pressure was detected in the EP3 KO with PGE2. However, when the normalized response to PGE2 on average filling pressure was compared between genotypes, no significant difference was observed between genotypes (Table 3). In contrast, normalized data demonstrated that the response to PGE2 on infused and voided volumes was significantly different between genotypes (Fig. 3, Table 3), implying that the response to PGE2 on infused and voided bladder volumes is dependent on the expression of the EP3 receptor gene. Vehicle treatment of EP3 WT and KO mice demonstrated no significant effect (n = 5 each). A comparison between the response of WT to vehicle or PGE2 treatment yielded a significant difference between vehicle and PGE2 on

Fig. 3. Effect of PGE2 intravesicular infusion on EP3 WT and EP3 KO mice. A and B: representative EP3 WT and EP3 KO mouse cystometric profiles, respectively, during infusion of saline (control) and during infusion of PGE2 (120 μM). Intravesicular bladder pressure (top) and voided (black) and infused (gray) volumes (bottom). C and D: average response of WT and KO mice to PGE2 or vehicle (veh) infusion on infused (**P = 0.0004 WT veh vs. WT PGE2, *P = 0.02 WT PGE2 vs. KO PGE2) and voided (**P = 0.006 WT veh vs. WT PGE2, *P = 0.04 WT PGE2 vs. KO PGE2) volumes, respectively.
void and infused volumes (Fig. 3, C and D). In KO mice, no significant difference was observed between vehicle and PGE2. However, the KO mice treated with PGE2 presented a trend toward both a reduction in void and infused volumes with PGE2 relative to vehicle, suggesting a residual response to PGE2 in the EP3 KO.

**DISCUSSION**

To our knowledge, this is the first study implicating a role for EP3 receptors in the regulation of urinary bladder function/dysfunction. We demonstrated that EP3 receptor KO mice present with an enhanced bladder capacity, based on the detection of higher infused and voided bladder volumes compared with WT controls. In addition, WT mice demonstrate bladder overactivity (a reduction in voided and infused volumes) evoked by activation of EP3 receptors with intravesical infusion of the EP3 agonist GR63799X, an effect that is absent in EP3 KO mice. Together, these data provide genetic and pharmacological evidence supporting the ability of EP3 receptors to modulate bladder function. Both preclinical and clinical studies link an enhancement of PGE2 production (the physiological ligand for the EP3 receptor) to urinary bladder overactivity (11, 14, 15, 24, 25). Consistent with this concept, WT mice infused with PGE2 developed bladder overactivity, while EP3 KO mice presented no significant response to PGE2. These data further implicate EP3 not only as playing a role in normal bladder function, but also in the pathophysiology of bladder overactivity.

The enhanced bladder capacity in age-matched EP3 KO mice reported here is not due to the larger size of the EP3 KO mice (28 and confirmed in this study), as normalizing both the infused and voided volumes to mouse weight concluded that EP3 KO mice also have significantly enhanced voided and infused bladder volumes per gram body weight. EP3 is expressed in the kidney, playing a role in the regulation of urine production and urine osmolality (9). However, the enhanced bladder capacity in EP3 KO mice is not due to compensatory changes resulting from alterations in urine production because under basal conditions, urine production and osmolality are unaltered in the EP3 KO (8). Taken together, this suggests that the enhanced bladder capacity observed in EP3 KO mice results from the loss of EP3 receptor activity in mechanisms controlling bladder function. EP3 KO mice also demonstrated a larger peak micturition pressure than the WT controls. The rationale for this difference in peak pressure is unknown, but conceivably could result from the larger bladder capacity in the EP3 KO requiring a heightened micturition pressure to effectively void the larger volume held by the EP3 KO bladders. Alternatively, the EP3 KO may have an enhanced urethral resistance during micturition, due to EP3-dependent changes in the regulation of urethral contractility, thereby increasing the bladder pressure during voiding. This is supported by the reported ability of PGE2 to decrease urethral pressure during micturition (13).

Our results demonstrating a significant alteration in basal bladder function in EP3 KO mice are in contrast to the lack of difference in basal urodynamics reported for EP1 KO mice (29). This suggests that EP3 receptors play a prominent role in basal urodynamics under normal conditions, while EP1 receptors do not. Although, acute pharmacological manipulation with antagonists targeting the EP1 receptor suggests EP1 receptor activity may be involved in normal rat bladder function (16, 18). Interestingly, the markedly reduced response to PGE2 infusion in EP3 KO mice on bladder volumes reported here is similar to that reported for EP1 KO mice (29). In the current study, EP3 WT mice demonstrated a significantly larger reduction in bladder volumes in response to PGE2 than EP3 KO mice implicating EP3 as a key player in PGE2-induced bladder overactivity. Although there was no significant response to PGE2 on bladder volumes in EP3 KO mice (as compared with control values in the same mice, or vehicle-treated KO mice), the difference between vehicle- and PGE2-treated EP3 KO mice suggests part of the responsiveness to PGE2 may remain in the EP3 KO. This residual response to PGE2 on bladder volumes in the EP3 KO may represent an EP1 component as described in EP1 KO mice (29). Taken together, the current study and Schröder et al. (29) suggest a role for both EP3 and EP1 receptors under conditions of PGE2-induced bladder overactivity, as observed in preclinical models and in overactive bladder patients (11, 14, 15, 24, 25).

The reductions in voided and infused bladder volumes with the EP3 selective agonist GR63799X in WT mice are lost in the EP3 KO, indicating that GR63799X-induced bladder overactivity in WT mice is mediated selectively through the EP3 receptor. EP3 KO mice demonstrated an increase in voided and infused bladder volumes in the presence of GR63799X. This
observation was similar to that encountered with vehicle treatment in both the GR63799X and PGE2 studies, likely representing a time-dependent increase in bladder capacity in response to continuous filling cystometry. Alternatively, a response to vehicle could potentially be involved. These EP3 receptor effects of GR63799X infused directly into the bladder, combined with the reduced response to PGE2 infusion in EP3 KO mice, suggest a role for EP3 receptors at the level of the bladder in regulating bladder function. The cell type(s) responsible for the functional effects described here with EP3 receptor deletion and EP3 receptor activation remain to be elucidated. EP3 receptors are expressed on dorsal root ganglion neurons (22, 35) and therefore, it is conceivable that EP3 receptors are expressed on sensory afferent nerve terminals in the bladder, functioning to regulate afferent sensitivity in response to alterations in PGE2 levels. This mechanism could involve the role of P2X3 receptors in the bladder (5, 34), as P2X3 receptors recently demonstrated a modulation by EP3 receptor activity in isolated dorsal root ganglion neurons (35). Both PGE2 and AA urodynamic responses have been shown to involve capsaicin-sensitive bladder afferents (17, 21). Here, we show that EP3 KO mice have a reduced response to PGE2, but an unaltered response to AA. This indicates that EP3 plays a major role in the response to PGE2 and a minimal role, if any, in the response to AA. Even though in both cases bladder overactivity is mediated via afferent nerves, AA-induced bladder overactivity is likely to involve other mechanisms besides PGE2 production. In contrast, PGE2 is established as a selective agonist of EP receptors. This is supported by the loss in functional response to PGE2 and the lack of a detectable alteration in the response to AA in the EP3 KO. Therefore, the increased bladder capacity in EP3 KO mice, and bladder overactivity induced by EP3 activation, may involve a reduction and an enhancement, respectively, of the sensitivity of bladder afferents. Alternatively, or in addition to an expression of EP3 on afferent nerves, EP3 may be expressed on other cell types within the bladder (i.e., urothelial or smooth muscle cells) where its activity could modulate bladder activity. Further studies to localize the expression of EP3 receptors in the bladder and in bladder-associated nerves will be required to unravel the mechanisms involved in the in vivo responses described here. We can also not eliminate a role for CNS/spinal-based functional expression of EP3 receptors in the phenotype presented by the EP3 KO mice, as reported for EP3-mediated PGE2-induced pain responses (20).

Here, we provide the first description of the role of EP3 receptors in regulating urinary bladder function, with EP3 receptors contributing to normal bladder function, and acting as a pathophysiological mediator of bladder dysfunction under conditions of enhanced PGE2 production as observed in overactive bladder patients. EP3 receptor antagonists are therefore likely to provide efficacy in preclinical models of bladder overactivity and in overactive bladder patients without the gastrointestinal side effects coincident with COX inhibitors.

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DISCLOSURES

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