The five primary prostaglandins stimulate contractions and phasic activity of the urinary bladder urothelium, lamina propria and detrusor

Zane Stromberga
Bond University
Russ Chess-Williams
Bond University
Christian Moro (cmoro@bond.edu.au)
Bond University https://orcid.org/0000-0003-2190-8301

Research article

Keywords: inflammation, prostaglandins, urinary bladder, urothelium, detrusor

Posted Date: March 18th, 2020

DOI: https://doi.org/10.21203/rs.3.rs-17651/v1

Version of Record: A version of this preprint was published at BMC Urology on April 29th, 2020. See the published version at https://doi.org/10.1186/s12894-020-00619-0.

Abstract

Background: Inflammation is often associated with several bladder dysfunctions, including overactive bladder (OAB) and interstitial cystitis/bladder pain syndrome (IC/PBS). As such, inflammation of the bladder and the actions of inflammatory mediators may contribute to the development of the urinary symptoms. This study assessed the actions of PGE_2, PGF_2, PGD_2, TXA_2, and PGI_2 on the urinary bladder urothelium with lamina propria (U&LP), as well as the detrusor smooth muscle.

Methods: Studies were carried out using isolated tissue baths, where strips porcine bladder U&LP or detrusor were exposed to varying concentrations of prostaglandin agonists (1 μM and 10 μM).

Results: All assessed prostaglandin agonists contracted both the U&LP and detrusor smooth muscle, with the rank order of contractile response effectiveness as: $PGE_2 > PGF_{2\alpha} > TXA_2 > PGD_2 > PGI_2$. In U&LP, treatment with PGE_2 (10 μM) increased tonic contractions by 1.36 ± 0.09 g ($n = 42$, $p < 0.001$) and phasic contractions by 40.4 ± 9.6% ($n = 42$, $p < 0.001$). In response to $PGF_{2\alpha}$ (10 μM), U&LP tonic contractions increased by 0.79 ± 0.06 g ($n = 14$, $p < 0.001$) and phasic activity by 13.3% ± 5.3% ($n = 15$, $p < 0.05$). In detrusor preparations, PGE_2 (10 μM) increased tonic contractions by 1.32 ± 0.13 g ($n = 38$, $p < 0.001$) and $PGF_{2\alpha}$ (10 μM) by 0.97 ± 0.14 g ($n = 12$, $p < 0.001$). Only 34% ($n = 48$) of all detrusor preparations exhibited spontaneous activity prior to the addition of any agonist at a frequency of 2.03 ± 0.12 cpm. In preparations that did not exhibit initial phasic activity, all of the prostaglandin agonists were capable of commencing phasic activity.

Conclusions: The urinary bladder U&LP and detrusor respond to a variety of prostaglandin agonists, with their activation resulting in direct contractions, as well as increases to spontaneous contractile activity. This study presents the prostaglandin receptor system as a potential therapeutic target for lower urinary tract dysfunction.

Background

Urinary bladder inflammation has been observed in various lower urinary tract dysfunctions, including interstitial cystitis/painful bladder syndrome (IC/BPS) [1] and overactive bladder (OAB) [2, 3]. It is also widely reported that there is an increase in the presence of inflammatory mediators within the bladder wall [4, 5] and urine [6–8] of patients suffering from these conditions. The mediators include histamine, nerve growth factor, proteases and chemokines released from nearby mast cells [9, 10], serotonin [11], and prostaglandins synthesised in the bladder wall [12]. Furthermore, significantly increased expression of histamine receptors had been noted in patients with BPS/IC [13]. The actions of inflammatory mediators not only can cause urinary bladder contractions [14–17] but also are known to sensitise afferent nerve endings resulting in an increased spinal cord neuronal activation [18, 19]. Therefore, inflammation and the actions of these pro-inflammatory mediators may contribute to the development of the urinary frequency and urgency symptoms observed in OAB, and pain in IC/BPS.

The involvement of prostaglandins in bladder physiology was first recognised from their release during or immediately after urinary bladder distension or inflammatory injury of the urothelium [20, 21]. An increase of prostaglandins in the urine of patients suffering from OAB has been well-reported previously [22–25], suggesting the prostaglandin system as a potential future therapeutic target in various bladder dysfunctions. The exact role and mechanisms of endogenous prostaglandins in the urinary bladder are not well understood. However, previous studies utilising exogenous prostaglandins have shown that these chemicals can alter contractility and micturition reflex in human bladders [26].

Prostaglandin production is generally low in healthy tissue but can increase immediately following acute inflammation [27]. They are synthesised in the bladder by cyclooxygenase (COX) and then subsequently converted into five primary prostanoids via their respective synthases: PGE_2, PGD_2, $PGF_{2\alpha}$, prostacyclin (PGI_2) and thromboxane (TXA_2) [28]. Prostaglandins are synthesised in both the bladder urothelium with lamina propria (U&LP) and in detrusor smooth muscle in response to stretch, nerve stimulation, U&LP damage or other inflammatory mediators [12, 29]. The production of prostaglandins is determined by the cells present at sites of inflammation capable of synthesising prostaglandins and the activity of the two cyclooxygenase isoenzymes, namely COX-1 and COX-2. For example, macrophages predominantly generate PGE_2 and TXA_2, whereas mast cells produce PGD_2 [30]. COX-1 is present in most cells, whereas the expression of COX-2 is generally low in cells, but can increase dramatically upon stimulation by immune cells [31]. Prostaglandin I_2 is the main prostaglandin synthesised in the human bladder, followed by PGE_2, $PGF_{2\alpha}$ and PGA_2 [32, 33].

These five prostaglandins exert their function by activating eight different G-protein-coupled receptors. These receptors include EP1, EP2, EP3 and EP4 subtypes of prostaglandin E_2 receptor; FP receptor for $PGF_{2\alpha}$; TP receptor for thromboxane; DP receptor for PGD_2; and IP receptor for PGI_2 [34]. The majority of studies conducted on the effects of prostaglandins in the urinary bladder have explicitly focused on PGE_2-mediated contractions. Generally, the stimulation EP1 and EP3 receptors are thought to cause bladder contractions, whereas EP2 and EP4 induce bladder relaxation [35]. Indeed, the EP1 receptor is involved in initiating micturition in both humans and animals and has been shown to be responsible for bladder overactivity in an animal model of bladder obstruction [36]. In a guinea pig model, application of PGE_2 has shown increases in amplitude of urinary bladder phasic contractions without affecting the frequency [37]. Furthermore, the stretch-induced release of PGE_2 from the urothelium has been suggested to exert a direct effect of detrusor smooth muscle cells to evoke contraction or to enhance the release of local ATP via stimulation of EP1 receptor resulting in an increased afferent activation [38]. The role of other prostaglandins in the urinary bladder has also been explored, albeit to a lesser extent. PGD_2, the major prostaglandin released from mast cells at sites of inflammation, has been shown to cause inhibitory effects on detrusor smooth muscle cells [39, 40]. The use of PGI_2 antagonists have been shown to decrease neurogenic detrusor overactivity [41], and the frequency of bladder contractions in citric-acid induced detrusor overactivity [42] of rat models, but the actions of the agonist on the layers of the bladder are unclear. Thromboxane and $PGF_{2\alpha}$ have been shown to induce direct contractions of the isolated

human detrusor [43]; however, it is unclear how these mediators affect the urothelium with lamina propria.

Although past studies have explored the effects of the different prostaglandins on the urinary bladder with a large focus on the actions of PGE_2, a complete understanding of the contractile effects of the other four prostaglandins on the urinary bladder remain unclear. Specifically, of interest is to determine how the actions of the prostaglandins affect urothelium with lamina propria that is separated from the detrusor smooth muscle. Therefore, this study aimed to assess the influence of PGE_2, $PGF_{2\alpha}$, PGD_2, TXA_2 PGI_2 on the urinary bladder urothelium with lamina propria and detrusor smooth muscle contractions and spontaneous activity.

Methods

Tissue preparation

Urinary bladders were obtained from Large White-Landrace pigs (approximately six months old, weighing between 80 and 100 kg) from the local abattoir after slaughter for the routine commercial provision of food. All methods were carried out in accordance with relevant Australian guidelines and regulations, and all experimental protocols were in accordance the Australian Code of Practice for the Care and Use of Animals for Scientific Purpose [44]. As no animals were bred, harmed, culled, interfered, or interacted with as part of this research project, Animal Ethics Approval was not required for offal use [45]. Urothelium with lamina propria was dissected from the underlying detrusor layer, consistent with methods carried out in past studies [14, 46], and cut in strips. Adjacent strips of U&LP and detrusor (10 mm x 5 mm) were tied vertically between an isometric force transducer (MCT050/D, ADInstruments, Castle Hill, Australia) and a fixed hook in 10 mL organ baths (Labglass, Brisbane, Australia), and superfused with Krebs-bicarbonate solution (NaCl 118.4 mM, $NaHCO_3$ 24.9 mM, $CaCl_2$ 1.9 mM, $MgSO_4$ 2.41 mM, KCl 4.6 mM, KH_2PO_4 1.18 mM and D-glucose 11.7 mM) and carbogen gas (95% oxygen and 5% carbon dioxide) at 37°C. After tissue mounting, strips of U&LP and detrusor were washed three times, tension adjusted to 1.5 – 2.0 g and tissues left to equilibrate for 30 min. After the equilibration period, a single dose of a prostaglandin receptor agonist was added to the tissue strip.

Pharmaceutical agents

The following compounds were used in this study: prostaglandin E_2, prostaglandin $F_{2\alpha}$, prostaglandin D_2, prostaglandin I_2 and thromboxane A_2 (U-46619, Cayman Chemicals, Michigan, USA). Prostaglandin E_2, prostaglandin $F_{2\alpha}$, prostaglandin D_2, and prostaglandin I_2 were dissolved in 100% ethanol and diluted with distilled H_2O. U-46619 was supplied as a solution in methyl acetate, which was diluted with distilled H_2O. Two concentrations of each prostaglandin receptor agonists were selected, 1 μM and 10 μM.

Data analysis

Data were graphed and analysed using GraphPad Prism version 8.3 for Windows (GraphPad Software, La Jolla, California, USA). Statistical analysis was conducted using a paired Student's *t*-test, where $p < 0.05$ was considered as significant. All values were reported as mean change ± SEM. *n* equates to the number of individual bladders used in this study.

Results

Prostaglandin agonists for increasing U&LP spontaneous phasic activity

Strips of U&LP exhibited spontaneous phasic contractions in the absence of any stimulation at a mean frequency of 3.26 ± 0.07 cycles per minute (cpm, n = 146). Treatment with PGE_2 caused the most prominent increases to U&LP spontaneous contractile activity. When PGE_2 (1 μM) was added to isolated tissues, spontaneous activity increased by 39.2% ± 6.7% (n = 38, $p < 0.001$, Figure 1). A greater concentration of PGE_2 (10 μM) showed similar increases of 40.4% ± 9.6% to the U&LP spontaneous activity (n = 42, $p < 0.001$). Treatment with $PGF_{2\alpha}$ showed smaller increases of 10.5% ± 4.6% to spontaneous activity when treated with 1 μM (n = 10, $p < 0.05$) and 13.3% ± 5.3% when treated with 10 μM (n = 14, $p < 0.05$). The addition of PGI_2 (10 μM) increased spontaneous activity by 6.2% ± 1.6% (n = 8, $p < 0.01$) but had no effect at a lower concentration (1 μM, n = 8). The frequency was not significantly affected by PGD_2 (1 - 10 μM, n = 12) or TXA_2 (1 - 10 μM, n = 16).

The average amplitude of these spontaneous phasic contractions exhibited in U&LP strips in the absence of any stimulation was 0.57 ± 0.02 g (n = 146). In response to treatment with 1 μM PGE_2, amplitude decrease of 0.14 ± 0.04 g (n = 38, $p < 0.001$, Table 1) were observed. Similar decreases of 0.16 ± 0.03 g were also observed in response to a higher PGE_2 concentration (10 μM, n = 42, $p < 0.01$). Treatment with TXA_2 (1 μM) showed a significant decrease in the amplitude by 0.28 ± 0.06 g (n = 8, $p < 0.01$), which was not observed at a higher concentration (10 μM, n = 6). The addition of PGI_2 (10 μM) decreased amplitude of spontaneous activity by 0.14 ± 0.05 (n = 8, $p < 0.05$) but had no effect at a lower concentration (1 μM, n = 8). The amplitude of spontaneous contractions was not altered by the addition of either $PGF_{2\alpha}$ (1-10 μM, n = 24) or PGD_2 (1-10 μM, n = 12, Table 1). None of the decreases in the amplitude of spontaneous phasic contractions of the U&LP were significantly affected by the two different prostaglandin receptor agonist concentrations (1 μM and 10 μM).

Table 1: U&LP changes in the amplitude of phasic contractions in response to the five primary prostaglandin agonists (mean ± SEM).

	1 µM of agonist			10 µM of agonist		
Agonist	Absence (g)	Presence (g)	n	Absence (g)	Presence (g)	n
PGE_2	0.53 ± 0.05	0.40 ± 0.03***	38	0.53 ± 0.04	0.37 ± 0.03**	42
$PGF_{2\alpha}$	0.30 ± 0.03	0.29 ± 0.01	10	0.51 ± 0.06	0.46 ± 0.08	14
TXA_2	0.90 ± 0.16	0.62 ± 0.14**	8	0.75 ± 0.16	0.71 ± 0.25	6
PGD_2	0.59 ± 0.10	0.46 ± 0.04	4	0.55 ± 0.08	0.43 ± 0.06	8
PGI_2	0.64 ± 0.07	0.56 ± 0.07	8	0.57 ± 0.09	0.43 ± 0.06*	8

*p < 0.05, **p < 0.01, ***p < 0.001. Paired Student's *t*-test.

Prostaglandin agonists in stimulating phasic contractions in detrusor

Total of 34% (n = 48) of the detrusor preparations that were set up in the organ baths exhibited spontaneous activity prior to the addition of any agonists. These contractions occurred at an average frequency of 2.03 ± 0.12 cpm (n = 48) with an average amplitude of 0.26 ± 0.02 g (n = 48). However, the majority of the detrusor preparations, that were otherwise quiescent developed spontaneous phasic contractions after the addition of the agonist.

Of those detrusor preparations that did not exhibit initial phasic activity during baseline: PGE_2 (1 µM) sparked contractions in 68% of preparations (n = 19) and PGE_2 (10 µM) in 69% (n = 22); $PGF_{2\alpha}$ (1 µM) initiated contractions in in 56% (n = 5) and $PGF_{2\alpha}$ (10 µM) in 88% (n = 7); TXA_2 (1 µM) initiated contractions in 63% (n = 5) and TXA_2 (10 µM) in 80% (n = 4); PGD_2 (1 µM) initiated phasic activity in 50% (n = 2) and PGD_2 (10 µM) in 75% (n = 6); and lastly PGI_2 (10 µM) initiated contractions in 40% (n = 2) of preparations. This demonstrates the ability of prostaglandin agonists to induce spontaneous activity in otherwise quiescent detrusor tissue strips.

Prostaglandin agonists in stimulating tonic contractions in U&LP

All assessed prostaglandin agonists contracted the U&LP with the rank order of contractile response effectiveness as: PGE_2 > $PGF_{2\alpha}$ > TXA_2 > PGD_2 > PGI_2. The addition of PGE_2 (1 µM) to isolated U&LP induced tissue contractions, with increases of 1.01 ± 0.08 g (n = 38, p < 0.001) to the tonic contractions. When a greater concentration of PGE_2 (10 µM) was selected, increases of 1.36 ± 0.09 g (n = 42, p < 0.001, Figure 2) were observed. Treatment with 1 µM $PGF_{2\alpha}$ showed a small increase to tonic contractions of 0.15 ± 0.04 g (n = 10, p < 0.01) when compared to a higher concentration of 10 µM, which exhibited increases of 0.79 ± 0.06 g (n = 14, p < 0.001). The addition of two concentrations of TXA_2 induced similar

contractions, where tonic contraction increased by 0.70 ± 0.07 g when treated with 1 μM ($n = 8$, $p < 0.001$), and by 0.65 ± 0.12 g after treatment with 10 μM ($n = 6$, $p < 0.001$).

When PGD_2 (1 μM) was added to the U&LP tissue preparations, tonic contractions increased by 0.19 ± 0.04 g ($n = 4$, $p < 0.05$, Figure 3). Treatment with a higher concentration of PGD_2 (10 μM) exhibited increases of 0.63 ± 0.09 g ($n = 8$, $p < 0.001$). The addition of PGI_2 showed small increases in tonic contractions of 0.11 ± 0.02 g in response to 1 μM PGI_2 ($n = 8$, $p < 0.001$), and 0.22 ± 0.03 g in response to 10 μM PGI_2 ($n = 8$, $p < 0.001$, Figure 3).

Prostaglandin agonists in stimulating tonic contractions in detrusor

All assessed prostaglandin agonists contracted the detrusor smooth muscle preparations with the rank order of contractile response effectiveness as: $PGE_2 > PGF_{2\alpha} > TXA_2 > PGD_2 > PGI_2$. In detrusor preparations, PGE_2 (1 μM) increased the tonic contractions by 0.73 ± 0.09 g ($n = 34$, $p < 0.001$), whereas PGE_2 (10 μM) nearly doubled the response, producing an average increase of 1.32 ± 0.13 g ($n = 38$, $p < 0.001$, Figure 4). Treatment with 1 μM $PGF_{2\alpha}$ showed a small increase of 0.20 ± 0.05 g ($n = 10$, $p < 0.01$), whereas 10 μM of $PGF_{2\alpha}$ increased the tonic contractions by 0.97 ± 0.14 g ($n = 12$, $p < 0.001$). When TXA_2 was added, tonic contractions increased by 0.47 ± 0.12 g when treated with 1 μM ($n = 8$, $p < 0.001$), and by 1.03 ± 0.14 g ($n = 6$, $p < 0.001$, Figure 4) when treated with 1 μM TXA_2.

PGD_2 showed a small increase in the tonic contractions of 0.12 ± 0.04 g when 1 μM was added ($n = 4$, $p < 0.05$), and an increase of 0.36 ± 0.06 g when 10 μM PGD_2 was added ($n = 6$, $p < 0.01$, Figure 5). PGI_2 showed small increases in tonic contractions at both concentrations, showing an increase of 0.16 ± 0.02 g when treated with 1 μM ($n = 8$, $p < 0.001$), and 0.13 ± 0.03 g when treated with 10 μM PGI_2 ($n = 8$, $p < 0.001$, Figure 5). The effects of prostaglandin agonists on tonic contractions of the detrusor smooth muscle were significantly different between the two concentrations (1 μM and 10 μM) for PGE_2 ($p < 0.001$), $PGF_{2\alpha}$ ($p < 0.001$) and PGD_2 ($p < 0.05$).

Discussion

Urinary bladder inflammation is observed in various lower urinary tract disorders, including IC/BPS [1] and OAB [2]. The inflammation can be mediated by immune cells, such as mast cells capable of releasing a variety of pro-inflammatory mediators, including histamine and prostaglandins [10]. This study determined the influence of the five major prostaglandins on urinary bladder U&LP and detrusor smooth muscle contractility and spontaneous activity and demonstrated the relative differences in the agonist-evoked contractions. U&LP strips are known to exhibit spontaneous phasic contractions in the absence of any stimulation [47]. These spontaneous contractions that are thought to be propagated by the muscularis mucosae present within the U&LP [48–50] and which can be mediated by prostaglandin agonists observed in this study may have a modulatory role in the bladder function. Immunohistochemical analysis has demonstrated that this muscularis mucosae layer is distinct from its

adjacent detrusor smooth muscle layers and has been observed in pig, human and guinea pig bladders [48]. This is also further reinforced with consistent findings where U&LP preparations still developed large spontaneous contractions when the apical urothelial layer and larger blood vessels were removed [51, 52]. Interestingly, both rat and mouse bladders lack muscularis mucosae and this may be the underlying reason as to why the spontaneous contractions developed remain very small [48]. Nonetheless, these spontaneous contractions occurring in rat U&LP arise from noradrenaline stimulation of the vasculature [53], which remains similar to that observed in pig tissue [54].

Previous research has shown that stimulation of the M3 muscarinic receptor in U&LP causes immediate contractions, as well as increases in the frequency of spontaneous phasic contractions, and reduction in their amplitude [47]. This may be one of the actions of muscarinic receptors in disease, and one of the mechanisms underlying antimuscarinic therapy as the first-line pharmaceutical treatment in people suffering from overactive bladder [55]. In our study, the prostaglandin agonists have shown similar contractile responses to both tonic contractions and spontaneous activity, thereby associating the actions of prostaglandins with many of the bladder contractile dysfunctions, such as OAB and IC/BPS.

This spontaneous contractile activity has been suggested to occur as a means to prevent the stretching of the microvasculature upon bladder distension [51]. Muscularis mucosae, specifically, appears to be the main contractile element present in the U&LP capable of generating ten times more contractile strength when normalised to a cross-sectional area [51]. The effects of prostaglandins on U&LP contractility and their ability to increase spontaneous phasic contractions are of particular interest, as there is growing evidence that this system can modulate the underlaying detrusor smooth muscle contractions [56]. The five prostaglandin agonists had varying effects on the frequency of spontaneous phasic contractions, with PGE_2 causing the most significant contractile response, as well as the most substantial increases in the frequency of spontaneous contractions. $PGF_{2\alpha}$ also induced spontaneous activity, while stimulating the tissues to contract, although at a smaller response compared to the same concentration of PGE_2. After addition of PGE_2, the amplitude of the spontaneous contractions was significantly smaller when compared to baseline activity; however, this reduction was not reproduced in response to $PGF_{2\alpha}$. Our result is not replicated in all species, with Rahnama'i, van Koeveringe [37] reporting that treatment with PGE_2 reduced the amplitudes of phasic contractions in intact guinea pig bladders. Finally, D_2, TXA_2 and I_2 had no effect on the spontaneous activity exhibited by U&LP.

The ability to contract the tissue was varied between the different prostaglandin agonists. The rank order of agonist response in stimulating contractions in U&LP and detrusor was: $PGE_2 > PGF_{2\alpha} > TXA_2 > PGD_2 > PGI_2$. These findings contrast Palea, Artibani [57], where agonist potency in contracting detrusor muscle was: $PGF_{2\alpha} > TXA_2 > PGE_2$. In this study, prostaglandin E_2 had the most substantial effect on increasing the tonic contractions when compared to the other agonists in both U&LP and detrusor. This finding is consistent with previous research that reported the involvement of PGE_2 in the initiation of micturition in both humans and animals [58], suggesting a contribution to bladder overactivity. Treatment with $PGF_{2\alpha}$ showed minimal increases at a concentration of 1 μM, yet responses were significantly enhanced in both

U&LP and detrusor when increased to 10 μM. At the smaller concentration of 1 μM, treatment with TXA_2 reached maximal contractile responses, and as such, was not enhanced at the higher agonist concentration of 10 μM. This was not the case with detrusor preparations, wherein the higher concentration of TXA_2 (10 μM) resulted in significantly enhanced contractions. The responses observed in porcine tissue in response to $PGF_{2\alpha}$, and TXA_2 are consistent with the Palea [43] findings. In addition, our study has established that U&LP isolated tissue is also capable of responding and producing definite increases in tonic contractions in response to these prostaglandin agonists.

Of the five prostaglandins, PGD_2 and PGI_2 had the smallest effect on both tonic contractions and spontaneous activity. This lack of increases to the tonic contractions or spontaneous contractile frequency may be explained by PGD_2 having potential inhibitory actions via the stimulation of DP receptor [40]. These authors also noted that the excitatory effect was stimulated via the TP receptor system when PGD_2 concentrations were increased. While there is no previous literature investigating the effects of PGI_2 agonists on urinary bladder contractility, studies involving PGI_2 antagonists have shown decreases in the frequency of bladder contractions and increased micturition threshold in rat models [41, 42] suggesting their potential in treating detrusor overactivity. An explanation for the small contractile effects observed in our study in response to PGI_2, the main prostaglandin synthesised in the human bladder [32, 59], is that the aqueous solutions of PGI_2 are extremely chemically unstable with a relatively short half-life, depending on the buffer concentration [60, 61]. As such, future studies utilising more chemically stable PGI_2 agonist analogous might provide further insights into the actions of this inflammatory mediator on the urinary bladder.

The actions of each prostaglandin agonist were varied, with different responses exhibited depending on the concentrations used. At this stage, it is unclear which receptor subtype is activated for the observed changes in tonic contractions and spontaneous activity to occur. There is the potential for agonists to activate alternate receptors, or even for the prostaglandin agonists to convert into other metabolites upon contact with the tissue [62, 63]. Therefore, additional studies that can utilise selective antagonism of each prostaglandin receptor subtype in response to prostaglandin agonists, as well as explore potential receptor systems capable of modulating the effects of prostaglandins, would be beneficial. In addition, future studies utilising immunohistochemical or radioligand binding assessments to determine the location, density, and prevalence of the prostaglandin receptors would provide further insights into this response. Overall, this is the first study to show actions of all five prostaglandin agonists on the two separate layers on the urinary bladder, U&LP and detrusor, presenting a potential therapeutic target for the management of bladder contractile disorders.

Conclusions

The urinary bladder is capable, to some extent, of responding to all five major prostaglandins produced in the urinary bladder. However, the exact underlying cellular mechanisms and receptor subtypes involved in the observed responses are unknown. Out of the five prostaglandins, PGE_2 and $PGF_{2\alpha}$ had the most

significant impact on both contraction and increases to the spontaneous contractile frequency in the U&LP. All five prostaglandin receptor agonists were also capable of inducing spontaneous phasic contractions in otherwise quiescent detrusor tissue strips. Although PGI_2 is thought to be the main prostaglandin synthesised in the human bladder, its effects on inducing contractions or spontaneous phasic activity were minimal. Based on the responses observed in both U&LP and detrusor, the specific involvement of EP1 to EP4, FP and TP receptors in urinary bladder function should be further explored. In addition, the mechanism of action for these prostaglandin responses may represent an additional therapeutic target in the treatment of bladder overactivity or interstitial cystitis/bladder pain syndrome.

Abbreviations

PG
prostaglandin
IC/BPS
interstitial cystitis/bladder pain syndrome
OAB
overactive bladder
U&LP
urothelium and lamina propria
TX
thromboxane

Declarations

Ethics approval

All methods were carried out in accordance with relevant Australian guidelines and regulations, and all experimental protocols were in accordance the Australian Code of Practice for the Care and Use of Animals for Scientific Purpose [44]. As no animals were bred, harmed, culled, interfered, or interacted with as part of this research project, Animal Ethics Approval was not required for offal use [45].

Consent for publication

Not applicable.

Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

Funding

This research was supported by the Australian Bladder Foundation managed by the Continence Foundation of Australia. ZS was supported by an Australian Government Research Training Program Scholarship. The funding bodies had no role in the design of the study and collection, analysis, and interpretation of data and in writing the manuscript.

Authors' contributions

Data was collected by ZS. ZS, RCW and CM were all equally responsible for the study design, data analysis, and preparation of manuscript.

References

1. Grover S, Srivastava A, Lee R, Tewari AK, Te AE. Role of inflammation in bladder function and interstitial cystitis. Therapeutic advances in urology. 2011;3(1):19-33.
2. Comperat E, Reitz A, Delcourt A, Capron F, Denys P, Chartier-Kastler E. Histologic features in the urinary bladder wall affected from neurogenic overactivity--a comparison of inflammation, oedema and fibrosis with and without injection of botulinum toxin type A. Eur Urol. 2006;50(5):1058-64.
3. Loran OB, Pisarev SA, Kleimenova NV, Sukhorukov VS. Allergic inflammation as one of the factors of pathogenesis of overactive urinary bladder. Urologiia (Moscow, Russia : 1999). 2007(2):37-41.
4. Kastrup J, Hald T, Larsen S, Nielsen VG. Histamine content and mast cell count of detrusor muscle in patients with interstitial cystitis and other types of chronic cystitis. Br J Urol. 1983;55(5):495-500.
5. Liu HT, Tyagi P, Chancellor MB, Kuo HC. Urinary nerve growth factor but not prostaglandin E2 increases in patients with interstitial cystitis/bladder pain syndrome and detrusor overactivity. BJU Int. 2010;106:1681-5.
6. El-Mansoury M, Boucher W, Sant GR, Theoharides TC. Increased Urine Histamine and Methylhistamine in Interstitial Cystitis. Journal of Urology. 1994;152(2 Part 1):350-3.
7. Jacobs BL, Smaldone MC, Tyagi V, Philips BJ, Jackman SV, Leng WW, et al. Increased nerve growth factor in neurogenic overactive bladder and interstitial cystitis patients. Can J Urol. 2010;17(1):4989-94.
8. Furuta A, Yamamoto T, Suzuki Y, Gotoh M, Egawa S, Yoshimura N. Comparison of inflammatory urine markers in patients with interstitial cystitis and overactive bladder. International urogynecology journal. 2018;29(7):961-6.
9. Amin K. The role of mast cells in allergic inflammation. Respiratory Medicine. 2012;106(1):9-14.
10. Theoharides TC, Kempuraj D, Tagen M, Conti P, Kalogeromitros D. Differential release of mast cell mediators and the pathogenesis of inflammation. Immunological reviews. 2007;217:65-78.
11. Matsumoto-Miyai K, Yamada E, Shinzawa E, Koyama Y, Shimada S, Yoshizumi M, et al. Serotonergic regulation of distention-induced ATP release from the urothelium. American journal of physiology

Renal physiology. 2016;310(7):F646-f55.

12. Rahnama'i MS, Van Koeveringe GA, Van Kerrebroeck PE. Overactive bladder syndrome and the potential role of prostaglandins and phosphodiesterases: an introduction. Nephrourol Mon. 2013;5(4):934-45.
13. Shan H, Zhang E-W, Zhang P, Zhang X-D, Zhang N, Du P, et al. Differential expression of histamine receptors in the bladder wall tissues of patients with bladder pain syndrome/interstitial cystitis – significance in the responsiveness to antihistamine treatment and disease symptoms. BMC urology. 2019;19(1):115.
14. Stromberga Z, Chess-Williams R, Moro C. Histamine modulation of urinary bladder urothelium, lamina propria and detrusor contractile activity via H1 and H2 receptors. Scientific Reports. 2019;9(1):3899.
15. Moro C, Edwards L, Chess-Williams R. 5-HT2A receptor enhancement of contractile activity of the porcine urothelium and lamina propria. International Journal of Urology. 2016;23(11):946-51.
16. Saban R, Simpson C, Vadigepalli R, Memet S, Dozmorov I, Saban MR. Bladder inflammatory transcriptome in response to tachykinins: Neurokinin 1 receptor-dependent genes and transcription regulatory elements. BMC urology. 2007;7(1):7.
17. Stromberga Z, Chess-Williams R, Moro C. Alterations in histamine responses between juvenile and adult urinary bladder urothelium, lamina propria and detrusor tissues. Scientific Reports. in press.
18. Grundy L, Caldwell A, Caraballo SG, Erickson A, Schober G, Castro J, et al. Histamine induces peripheral and central hypersensitivity to bladder distension via the histamine H1 receptor and TRPV1. American Journal of Physiology-Renal Physiology. 2019;0(0):null.
19. Davidson S, Copits BA, Zhang J, Page G, Ghetti A, Gereau RW. Human sensory neurons: Membrane properties and sensitization by inflammatory mediators. Pain. 2014;155(9):1861-70.
20. Gilmore NJ, Vane JR. Hormones released into the circulation when the urinary bladder of the anaesthetized dog is distended. Clin Sci. 1971;41:69-83.
21. Rahnama'i MS, van Kerrebroeck PE, de Wachter SG, van Koeveringe GA. The role of prostanoids in urinary bladder physiology. Nat Rev Urol. 2012;9(5):283-90.
22. Tanaka I, Nagase K, Tanase K, Aoki Y, Akino H, Yokoyama O. Improvement in neurogenic detrusor overactivity by peripheral C fiber's suppression with cyclooxygenase inhibitors. The Journal of urology. 2010;183(2):786-92.
23. Reyes AA, Klahr S. Bladder contributes to eicosanoids excreted in urine. Am J Physiol. 1990;259(5 Pt 2):F859-F61.
24. Kim JC. Changes of urinary nerve growth factor and prostaglandins in male patients with overactive bladder symptom. Int J Urol. 2005;12:875-80.
25. Kim JC, Park EY, Seo SI, Park YH, Hwang TK. Nerve growth factor and prostaglandins in the urine of female patients with overactive bladder. J Urol. 2006;175:1773-6.

26. Andersson KE, Ek A, Persson CG. Effects of prostaglandins on the isolated human bladder and urethra. Acta physiologica Scandinavica. 1977;100(2):165-71.
27. Ricciotti E, FitzGerald GA. Prostaglandins and inflammation. Arterioscler Thromb Vasc Biol. 2011;31(5):986-1000.
28. Khan MA, Thompson CS, Mumtaz FH, Jeremy JY, Morgan RJ, Mikhailidis DP. Role of prostaglandins in the urinary bladder: an update. Prostaglandins, leukotrienes, and essential fatty acids. 1998;59(6):415-22.
29. Andersson KE. Overactive bladder - pharmacological aspects. Scand J Urol Nephrol Suppl. 2002;210:72-81.
30. Tilley SL, Coffman TM, Koller BH. Mixed messages: modulation of inflammation and immune responses by prostaglandins and thromboxanes. The Journal of clinical investigation. 2001;108(1):15-23.
31. Smith WL, Dewitt DL. Prostaglandin endoperoxide H synthases-1 and -2. Adv Immunol. 1996;62:167-215.
32. Masunaga K. Prostaglandin E2 release from isolated bladder strips in rats with spinal cord injury. Int J Urol. 2006;13:271-6.
33. Jeremy JY. Eicosanoid synthesis by human urinary bladder mucosa: pathological implications. Br J Urol. 1987;59:36-9.
34. Woodward DF, Jones RL, Narumiya S. International Union of Basic and Clinical Pharmacology. LXXXIII: classification of prostanoid receptors, updating 15 years of progress. Pharmacological reviews. 2011;63(3):471-538.
35. Coleman RA, Smith WL, Narumiya S. International Union of Pharmacology classification of prostanoid receptors: properties, distribution, and structure of the receptors and their subtypes. Pharmacol Rev. 1994;46:205-29.
36. Lee T, Hedlund P, Newgreen D, Andersson KE. Urodynamic effects of a novel EP1 receptor antagonist in normal rats and rats with bladder outlet obstruction. J Urol. 2007;177:1562-7.
37. Rahnama'i MS, van Koeveringe GA, van Kerrebroeck PEV, de Wachter SGG. The effect of indomethacin on the muscarinic induced contractions in the isolated normal guinea pig urinary bladder. BMC urology. 2013;13(1):8.
38. Wang X, Momota Y, Yanase H, Narumiya S, Maruyama T, Kawatani M. Urothelium EP1 receptor facilitates the micturition reflex in mice. Biomedical research (Tokyo, Japan). 2008;29(2):105-11.
39. Guan NN, Nilsson KF, Wiklund PN, Gustafsson LE. Release and inhibitory effects of prostaglandin D2 in guinea pig urinary bladder and the role of urothelium. Biochimica et biophysica acta. 2014;1840(12):3443-51.
40. Guan NN, Svennersten K, de Verdier PJ, Wiklund NP, Gustafsson LE. Receptors involved in the modulation of guinea pig urinary bladder motility by prostaglandin D2. British journal of pharmacology. 2015;172(16):4024-37.

41. Khera M, Boone TB, Salas N, Jett MF, Somogyi GT. The role of the prostacyclin receptor antagonist RO3244019 in treating neurogenic detrusor overactivity after spinal cord injury in rats. BJU Int. 2007;99:442-6.
42. Cefalu JS, Zhu QM, Eggers AC, Kaan TK, Ho MJ, Jett MF, et al. Effects of the selective prostacyclin receptor antagonist RO3244019 on the micturition reflex in rats. The Journal of urology. 2007;178(6):2683-8.
43. Palea S. Pharmacological characterization of thromboxane and prostanoid receptors in human isolated urinary bladder. Br J Pharmacol. 1998;124:865-72.
44. Australia Government. Australian code for the care and use of animals for scientific purposes 2013 [Available from: https://www.nhmrc.gov.au/about-us/publications/australian-code-care-and-use-animals-scientific-purposes.
45. Queensland Government. Using Animals in Science 2016 [Available from: https://www.business.qld.gov.au/industries/farms-fishing-forestry/agriculture/livestock/animal-welfare/animals-science/activities/dead-animals.
46. Moro C, Leeds C, Chess-Williams R. Contractile activity of the bladder urothelium/lamina propria and its regulation by nitric oxide. Eur J Pharmacol. 2012;674(2-3):445-9.
47. Moro C, Uchiyama J, Chess-Williams R. Urothelial/lamina propria spontaneous activity and the role of M3 muscarinic receptors in mediating rate responses to stretch and carbachol. Urology. 2011;78(6):1442.e9-15.
48. Mitsui R, Lee K, Uchiyama A, Hayakawa S, Kinoshita F, Kajioka S, et al. Contractile elements and their sympathetic regulations in the pig urinary bladder: a species and regional comparative study. Cell and tissue research. 2019.
49. Fry CH, Vahabi B. The Role of the Mucosa in Normal and Abnormal Bladder Function. Basic & clinical pharmacology & toxicology. 2016;119 Suppl 3(Suppl 3):57-62.
50. Drake MJ, Fry CH, Hashitani H, Kirschner-Hermanns R, Rahnama'i MS, Speich JE, et al. What are the origins and relevance of spontaneous bladder contractions? ICI-RS 2017. Neurourology and urodynamics. 2018;37(S4):S13-s9.
51. Lee K, Mitsui R, Kajioka S, Naito S, Hashitani H. Role of PTHrP and Sensory Nerve Peptides in Regulating Contractility of Muscularis Mucosae and Detrusor Smooth Muscle in the Guinea Pig Bladder. The Journal of urology. 2016;196(4):1287-94.
52. Heppner TJ, Bonev AD, Nelson MT. Ca(2+)-activated K+ channels regulate action potential repolarization in urinary bladder smooth muscle. The American journal of physiology. 1997;273(1 Pt 1):C110-7.
53. Shimizu Y, Mochizuki S, Mitsui R, Hashitani H. Neurohumoral regulation of spontaneous constrictions in suburothelial venules of the rat urinary bladder. Vascular pharmacology. 2014;60(2):84-94.
54. Moro C, Tajouri L, Chess-Williams R. Adrenoceptor function and expression in bladder urothelium and lamina propria. Urology. 2013;81(1):211.e1-7.

55. Chapple CR, Khullar V, Gabriel Z, Muston D, Bitoun CE, Weinstein D. The effects of antimuscarinic treatments in overactive bladder: an update of a systematic review and meta-analysis. Eur Urol. 2008;54(3):543-62.
56. Chakrabarty B, Bijos DA, Vahabi B, Clavica F, Kanai AJ, Pickering AE, et al. Modulation of Bladder Wall Micromotions Alters Intravesical Pressure Activity in the Isolated Bladder. Frontiers in Physiology. 2019;9(1937).
57. Palea S, Artibani W, Ostardo E, Trist DG, Pietra C. Evidence for Purinergic Neurotransmission in Human Urinary Bladder Affected by Interstitial Cystitis. The Journal of urology. 1993;150(6):2007-12.
58. Lee T, Hedlund P, Newgreen D, Andersson KE. Urodynamic effects of a novel EP(1) receptor antagonist in normal rats and rats with bladder outlet obstruction. The Journal of urology. 2007;177(4):1562-7.
59. Jeremy JY, Tsang V, Mikhailidis DP, Rogers H, Morgan RJ, Dandona P. Eicosanoid synthesis by human urinary bladder mucosa: pathological implications. Br J Urol. 1987;59(1):36-9.
60. Stehle RG. [56] Physical chemistry, stability, and handling of prostaglandins E2, F2α, D2, and I2: A critical summary. Methods in Enzymology. 86: Academic Press; 1982. p. 436-58.
61. Moncada S. Biology and therapeutic potential of prostacyclin. Stroke. 1983;14(2):157-68.
62. Abadir PM, Siragy HM. Angiotensin type 1 receptor mediates renal production and conversion of prostaglandins E2 to F2α in conscious diabetic rats. Journal of the Renin-Angiotensin-Aldosterone System. 2015;16(4):774-9.
63. Canete Soler R, Lopez Bernal A, Turnbull AC. Conversion of prostaglandin E2 to prostaglandin F2 alpha by human myometrium. Hormone and metabolic research = Hormon- und Stoffwechselforschung = Hormones et metabolisme. 1987;19(10):515-6.

Figures

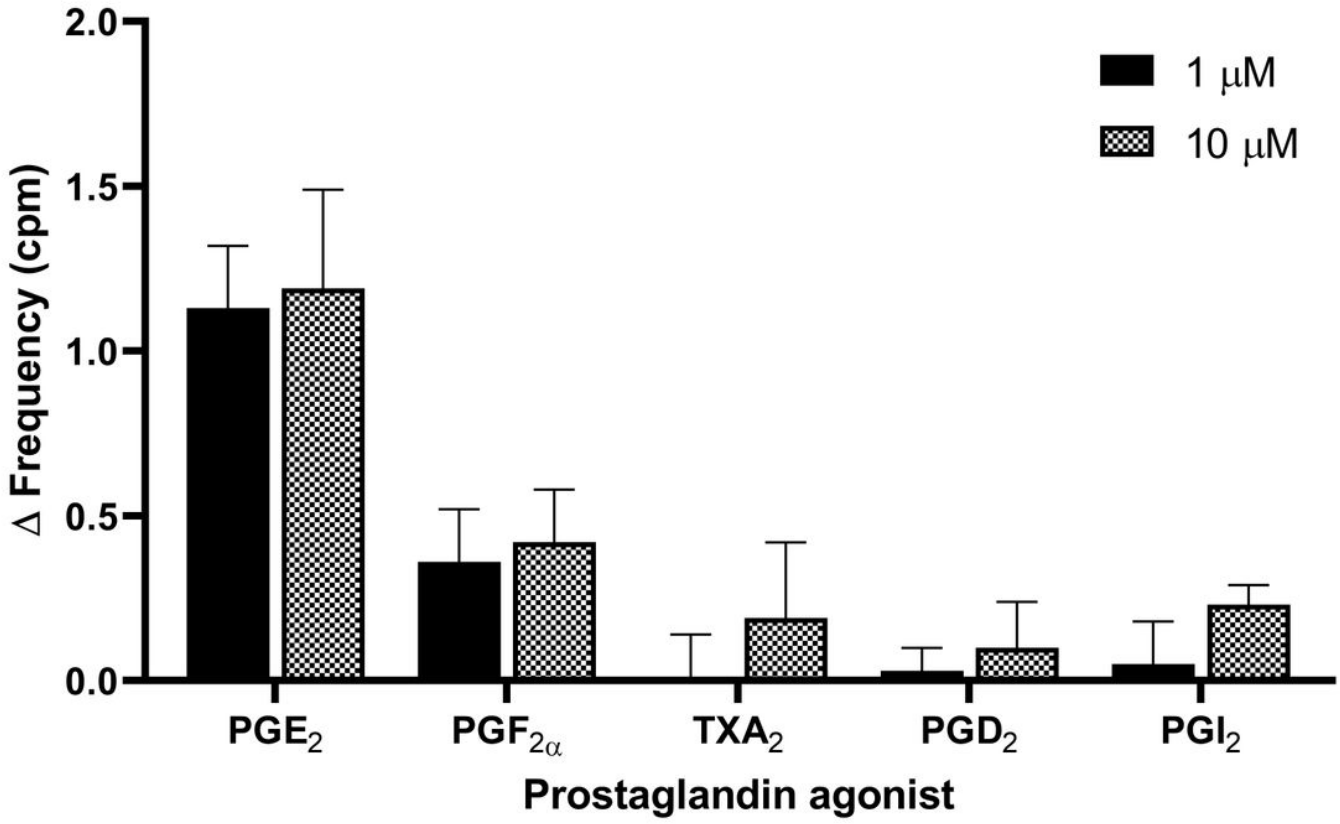

Figure 1

U&LP changes in the frequency of spontaneous phasic contractions after the treatment with 1 μM and 10 μM of each specific prostaglandin agonists E2, F2α, TXA2, D2, and I2¬. There were no statistically significant differences in frequency changes between the 1 μM and 10 μM concentrations for any of the agonists (unpaired Student's 2-tailed t-test).

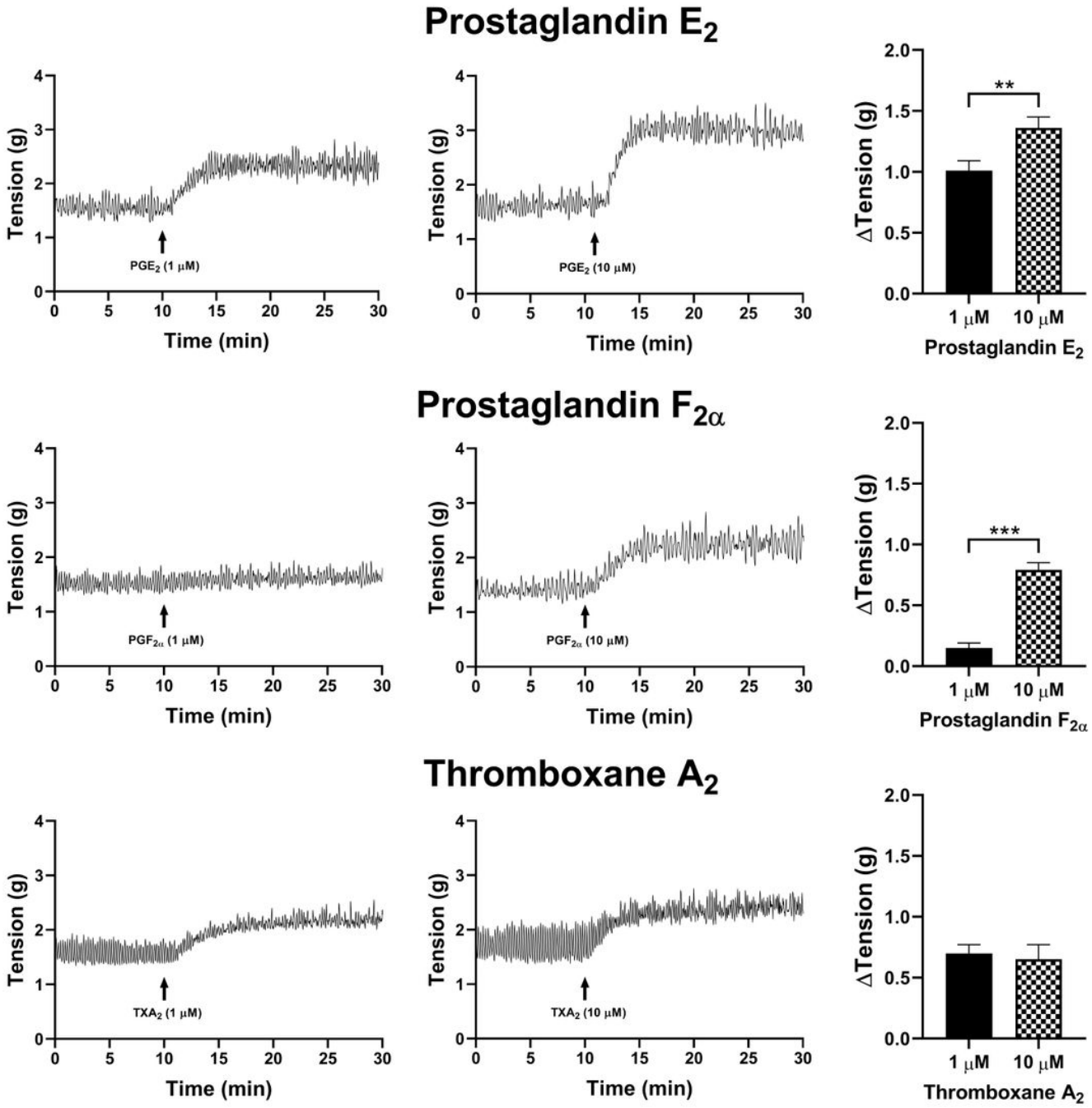

Figure 2

U&LP changes in tonic contractions in response to the treatment with 1 μM and 10 μM of prostaglandin E2 (top row), F2α (middle row) and TXA2 (bottom row). Sample traces of the responses observed to two concentrations of prostaglandin agonist (left & middle columns). Increases in tonic contractions after treatment with each agonist are represented as mean change ± SEM (right column). Significant changes in the tonic contractions between 1 μM and 10 μM were evaluated using an unpaired Student's two-tailed t-test, where *p < 0.05, **p < 0.01, ***p < 0.001.

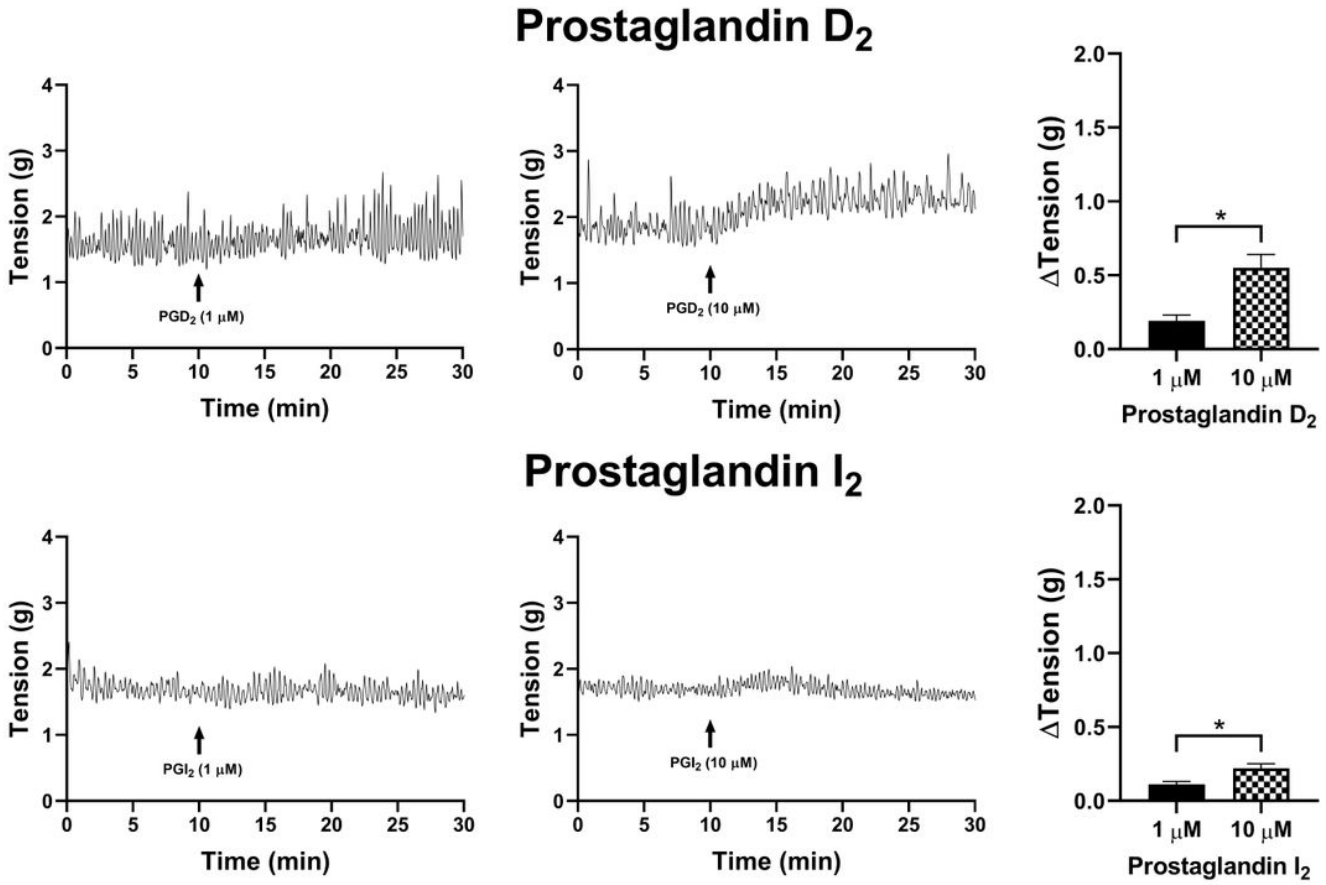

Figure 3

U&LP changes in tonic contractions in response to the treatment with 1 μM and 10 μM of prostaglandin agonists D2 (top row) and I2¬ (bottom row). Sample traces of the responses observed to two concentrations of prostaglandin agonist (left & middle columns). Increases in tonic contractions after treatment with each agonist are represented as mean change ± SEM (right column). Significant changes in the tonic contractions between 1 μM and 10 μM were evaluated using an unpaired Student's two-tailed t-test, where *p < 0.05, **p < 0.01, ***p < 0.001.

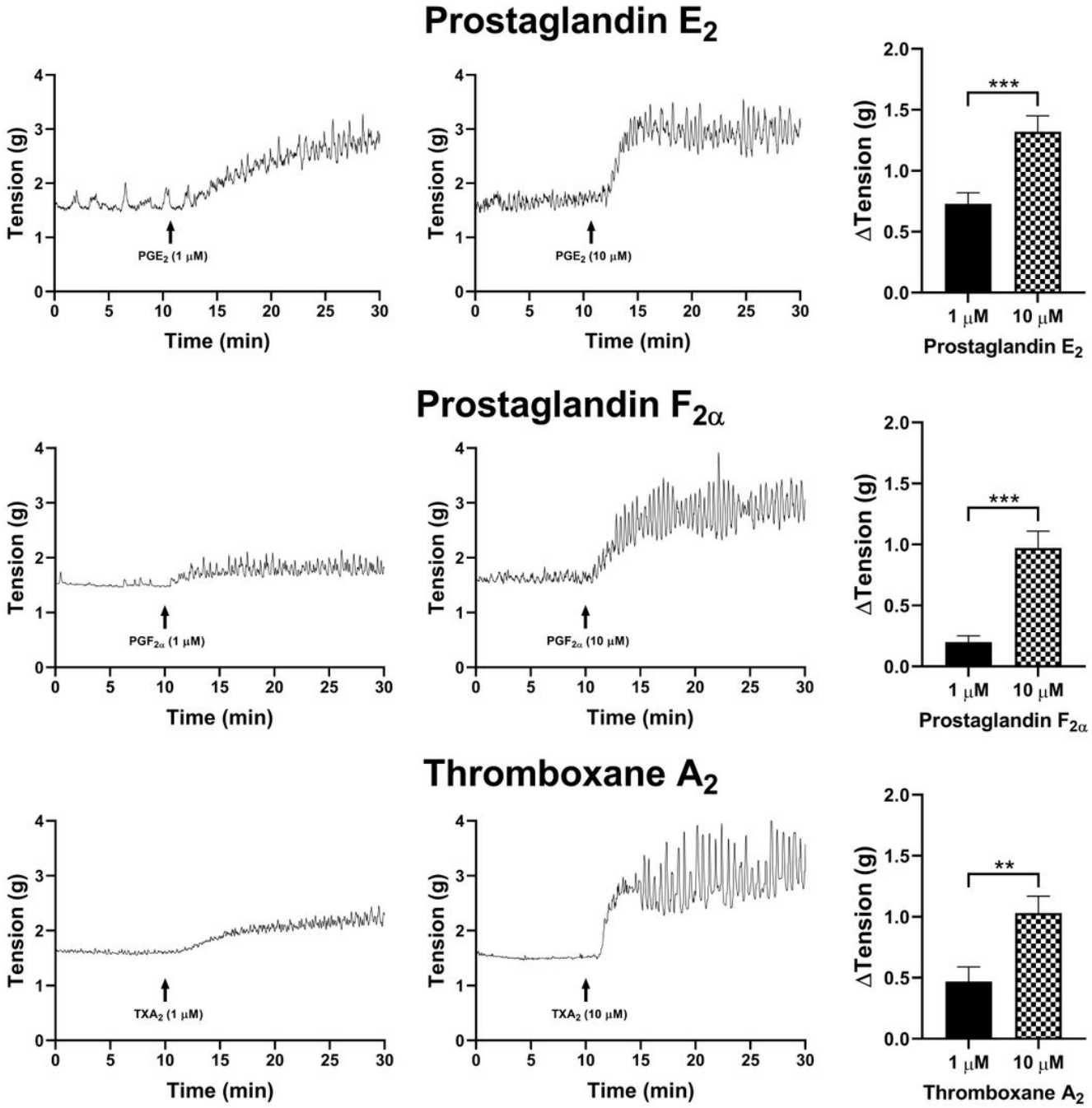

Figure 4

Detrusor changes in tonic contractions in response to the treatment with 1 μM and 10 μM of prostaglandin E2 (top row), F2α (middle row) and TXA2 (bottom row). Sample traces of the responses observed to two concentrations of prostaglandin agonist (left & middle columns). Increases in tonic contractions after treatment with each agonist are represented as mean change ± SEM (right column). Significant changes in the tonic contractions between 1 μM and 10 μM were evaluated using an unpaired Student's two-tailed t-test, where *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

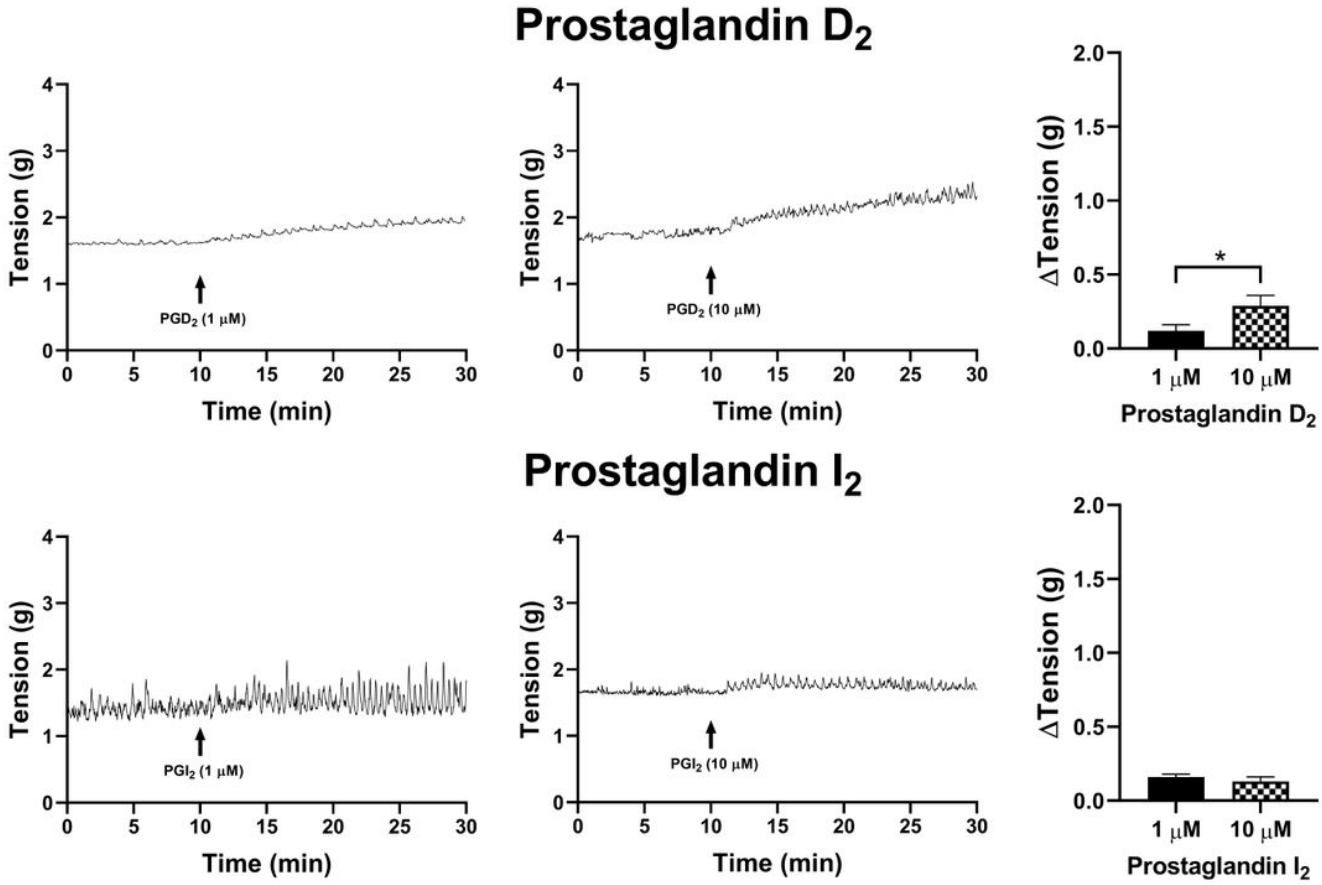

Figure 5

Detrusor changes in tonic contractions in response to the treatment with 1 μM and 10 μM of prostaglandin agonists D2 (top row) and I2¬ (bottom row). Sample traces of responses observed to two concentrations of a prostaglandin agonist (left & middle columns). Increases in the tonic contractions after treatment with an agonist are represented as mean change ± SEM (right column). Significant changes in the tonic contractions between 1 μM and 10 μM were evaluated using an unpaired Student's t-test, where *$p < 0.05$, ***$p < 0.001$.