# The role and signaling of isoprostanes in the regulation of detrusor muscle tone in murine and human urinary bladder

**PhD Thesis**


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# 1. Introduction

Regulation of urinary bladder function under physiological and pathophysiological conditions has not been understood completely to date despite numerous animal studies and human investigations. Disorders of the urinary bladder affect a large number of patients and are often associated with systemic inflammation. Release of inflammatory mediators may have a substantial impact on the impairment of micturition and therefore the elucidation of these pathways may help to understand the pathogenesis of frequent urinary bladder disorders like overactive bladder syndrome (OAB). The prostaglandin-like isoprostanes are released in large amounts during systemic inflammation and previous studies indicated their potential role in the pathogenesis of urinary bladder dysfunctions.

Isoprostanes are members of the isoeicosanoid family, they are PG-like compounds, however there are marked differences between the two lipid groups. Unlike PGs that are produced from arachidonic acid via COX enzymes, isoprostanes are formed primarily non-enzymatically through lipid peroxidation, as a result of reactive oxygen species (ROS) interaction with polyunsaturated fatty acids (e.g. arachidonic acid). Isoprostane families designated by the letters D, E, F, A and J are distinguished by the type of the cyclopentane ring in their structure. This nomenclature is similar to that of PGs', for instance, F2-isoprostanes are structural isomers of $PGF_{2\alpha}$.

Systemic isoprostane formation is remarkably increased under pathological conditions associated with oxidative stress and systemic chronic inflammation, such as diabetes, atherosclerosis, Alzheimer's disease, asthma and OAB. Isoprostane levels are elevated in these disorders, and they are suitable compounds for serving as biomarkers of diseases associated with oxidative stress, as they are formed *in vivo* in the human body as a result of elevated ROS concentration. Moreover, they are chemically stable compounds and are present in detectable amounts in both human urine and plasma. In addition, isoprostanes have been identified recently as biological mediators in the progression of the above-mentioned conditions linked to oxidative stress, including their possible role in inducing smooth muscle contraction in the airways and the vasculature. Interestingly, isoprostanes have also been implicated in human neurogenic bladder dysfunction, as their urinary concentration is elevated in patients with hyperreflexic bladder whereas decreased in patients affected by areflexic bladder.

The general concept of smooth muscle regulation through $Ca^{2+}$-dependent and $Ca^{2+}$-sensitizing pathways apply to the detrusor muscle as well. $Ca^{2+}$-dependent

contraction requires elevated intracellular $Ca^{2+}$ concentration and formation of $Ca^{2+}$-calmodulin complex which activates the myosin light chain kinase (MLCK) leading to the phosphorylation of myosin light chain and consequently to contraction. The cross-bridge cycle is also regulated by myosin light chain phosphatase (MLCP), which cleaves the phosphate from MLC reducing its activity. The Rho – Rho-kinase (ROCK) pathway inhibits MLCP resulting in a sustained smooth muscle contraction. Many contractile stimuli, acting through G protein–coupled receptors, induce MLC phosphorylation via activation of the $G_q$ and $G_{11}$ proteins and subsequent stimulation of phospholipase C-β, resulting in inositol 1,4,5-trisphosphate (IP3)-mediated $Ca^{2+}$ release from the sarcoplasmic reticulum and the $Ca^{2+}$ and calmodulin–dependent activation of MLCK. The receptors of many contractile mediators also couple to the $G_{12}$ and $G_{13}$ proteins to activate the Rho/ROCK pathway, resulting in the inhibition of MLCP. TP receptors have been reported to mediate vascular, bronchial, and prostate smooth muscle contractions. The downstream signaling pathways mediating TP receptor activation have been examined extensively in platelets and smooth muscle as well. It has been reported that both $G\alpha_{q/11}$ and $G\alpha_{12/13}$ proteins can couple to TP receptors and both the PLC-ß – $IP_3$ – CaM-$(Ca^{2+})_4$ – MLCK and the RhoGEF – RhoA – ROCK – MLCP pathways can be involved in the signal transduction of TP-mediated responses (e.g. platelet aggregation, vasoconstriction, bronchoconstriction).

# 2. Objectives

The working hypothesis of our study is that isoprostanes that are present in the urine are not only useful biomarkes of systemic oxidative stress, but may be possible mediators of detrusor contractions leading to detrusor overactivity under pathological conditions.

The aim of this study was to investigate the potential effects of isoprostanes and identify the receptor(s) mediating these effects in the murine detrusor muscle. Furthermore, we aimed to examine the intracellular signaling of isoprostane-evoked contractions with the help of transgenic mouse models and pharmacological tools in order to provide novel, more specific therapeutic targets for the treatment of the bladder overactivity. To gain further clinical significance, we have performed experiments investigating the effects of isoprostanes and the signaling of the responses in human urinary bladders, as well.

# 3. Materials and methods

## Animals

All procedures were carried out according to the guidelines of the Hungarian Law of Animal Protection (28/1998) and were approved by the Government Office of Pest County (Permission number: PEI/001/2709-13/2014).
Urinary bladders were obtained from adult male (90-120 day-old, 30-35 g) wild-type mice (C57BL/6 strain from Charles River Laboratories, Isaszeg, Hungary, referred to as WT) and from animals deficient for the TP receptor (TP-KO), or from mice in which either the $G\alpha_{q/11}$- or the $G\alpha_{12/13}$-protein encoding genes were conditionally inactivated in a smooth muscle specific manner ($G\alpha_{q/11}$-KO and $G\alpha_{12/13}$-KO). The mouse lines with smooth muscle specific inducible deletion of the $G_{q/11}$ or $G_{12/13}$ signaling pathway were generated on $G\alpha_{11}$-deficient ($G\alpha_{11}^{-/-}$) or $G\alpha_{12}$-deficient ($G\alpha_{12}^{-/-}$) background with floxed alleles of the genes coding Gαq ($Gnaq^{flox/flox}$) or Gα13 ($Gn\alpha13^{flox/flox}$), and expressing a fusion protein of the Cre recombinase with a modified estrogen receptor binding domain (Cre-ERT2) under the control of the smooth muscle myosin heavy chain (SMMHC) promoter. Deletion of Gnaq or Gna13 was induced by intraperitoneal tamoxifen treatment (1 mg/day for five consecutive days) in SMMHC-$CreERT2^{+/-}$;$Gnaq^{flox/flox}$;$Gna11^{-/-}$ and SMMHC-$CreERT2^{+/-}$;$Gna12^{-/-}$;$Gna13^{flox/flox}$ mice, respectively as described. Tamoxifen-treated SMMHC-$CreERT2^{-/-}$;$Gnaq^{flox/flox}$;$Gna11^{+/+}$ and SMMHC-$CreERT2^{-/-}$;$Gna12^{+/+}$;$Gna13^{flox/flox}$ mice served as controls and are referred to as $G\alpha_{q/11}$-CTRL and $G\alpha_{12/13}$-CTRL.

## Human tissues

All procedures involving human urinary bladder tissues have been approved by the Scientific and Research Committee of the Medical Research Council of Hungary (License No.: 21545-2/2019/EKU). Urinary bladder tissue samples were obtained from 19 patients (15 males, 4 females; age 65.5±9.3 years, range between 44-78 years) undergoing open radical cystectomy due to muscle-invasive bladder cancer after having obtained informed patient consent. None of the patients has had any symptom of overactive bladder syndrome or any voiding dysfunction before surgery, nor was taking drugs for OAB. The urinary bladders were placed into physiological saline solution immediately after the surgical removal and examined by an expert uro-pathologist who has cut out 3x2 cm tumor-free parts of the urinary bladders containing full width of bladder wall. Samples were collected preferentially from either the dome or the cranial part of the lateral bladder wall. These specimens were placed into Hank's Balanced Salt Solution (HBSS) containing $10^{-6}$ M of indomethacin and were

transported to the place of the experiments. The addition of indomethacin was necessary in order to avoid any potential prostanoid receptor desensitization, since there is evidence for *in vitro* prostanoid synthesis in the urinary bladder, which can be increased by mechanical stretch. However, in order to avoid any potential side effects of COX inhibition, indomethacin was not present in the Krebs solution during the experiments.

**Preparation of UBSM strips**

After the mice were sacrificed with cervical dislocation the urinary bladders were removed from a lower midline incision and placed into Krebs solution (119 mM NaCl, 4.7 mM KCl, 1.2 mM $KH_2PO_4$, 2.5 mM $CaCl_2 \cdot H_2O$, 1.2 mM $MgSO_4 \cdot 7H_2O$, 20 mM $NaHCO_3$, 0.03 mM EDTA, and 10 mM glucose, pH 7.4) at 37 °C. Under dissection microscope (M3Z; Wild Heerbrugg AG, Gais, Switzerland), adipose and connective tissues were removed from the serosal surface. The whole mucosal layer (urothelium + submucosa) was also gently and completely removed in order to prevent the potential release of paracrine factors from the mucosal epithelium or submucosal tissue and to avoid potential tension changes induced by myofibroblasts. The cleaned detrusor muscle was cut into four strips of equal length for myography. In some specific experiments, the mucosal layer was not removed to evaluate whether its presence alters the contractile responses.
Human urinary bladder specimens were also placed into Krebs solution (same as described above, T = 37 °C) containing $10^{-6}$ M of indomethacin during the preparation. Under dissection microscope, the serosal tissue and the mucosal layer were removed. The isolated, denuded detrusor specimens were cut into equal, approximately 10x3x3 mm strips for myography.

**Myography**

Both murine and human detrusor muscle strips were mounted perpendicularly on two parallel tissue-holding needles of a myograph evenly spaced from the end of the strips (200 µm needles, 6 ml chambers, 610 M Multi Wire Myograph System, Danish Myo Technology A/S, Aarhus, Denmark). Chambers were filled with 6 ml of 37 °C Krebs solution aerated with carbogen (mixture of 5% $CO_2$ and 95% $O_2$). Detrusor muscle contractions were registered under isometric conditions. The direction of emerging contraction force was measured along the longitudinal axis of the samples parallel with the axis of the force transducer.
Every experiment started with a 60-minute resting period while the UBSMs were stretched to and stabilized at a resting tension of 5 mN (murine) or 3 mN (human). After the resting period, UBSMs were challenged twice with 124 mM

$K^+$-containing Krebs solution to examine the viability of the tissues. After several washes with normal Krebs solution, the contractile effect of U-46619 [selective TP agonist ($10^{-5}$M)], $PGE_2$ ($10^{-5}$ M), $PGF_{2\alpha}$ ($10^{-5}$ M), 8-iso-$PGE_2$ ($10^{-5}$ M), 8-iso$PGF_{2\alpha}$ ($10^{-5}$ M), carbamoylcholine chloride [carbachol ($10^{-6}$ M)] or α,β-methyleneadenosine 5'-triphosphate [α,β-methylene ATP, ATP-analogue ($10^{-5}$ M)] was measured. In some of the strips one of the following inhibitors was applied without washing out: SQ-29548 (selective TP antagonist, $10^{-5}$ M, 20 min), L-798106 ($EP_3$-receptor antagonist, $10^{-5}$ M, 20 min), atropine (muscarinic-ACh-receptor antagonist, $10^{-6}$ M, 20 min), pyridoxalphosphate-6-azophenyl-2',4'-disulfonate (PPADS; P2 purinergic receptor antagonist, $10^{-5}$ M, 20 min), Y-27632 (ROCK inhibitor, $10^{-5}$ M, 20 min), tetrodotoxin (TTX, voltage-gated sodium channel blocker to inhibit potential neurotransmitter release from nerve endings, $10^{-6}$ and $10^{-5}$ M, 30 min). When DMSO was the solvent of the inhibitor, it was applied in matched concentration as vehicle control. Furthermore, in some experiments, non-cumulative (single-dose) dose-response relationship assessments of U-46619, 8-iso-$PGE_2$ and 8-iso$PGF_{2\alpha}$ were performed at the following concentrations: $10^{-8}$ M, $10^{-7}$ M, $10^{-6}$ M, $3x10^{-6}$ M, $10^{-5}$ M and $3x10^{-5}$ M. Finally, bladder strips were exposed to 124 mM $K^+$-containing Krebs solution to retest the viability of the detrusor strips. Agonist-induced tension changes were normalized to the reference contraction induced by 124 mM $K^+$-containing Krebs solution (second administration).
MP100 system and AcqKnowledge 3.72 software from Biopac System (Goleta, CA) were used for the acquisition and analysis of myographic measurements.

**Reagents**

U-46619, $PGE_2$, $PGF_{2\alpha}$, 8-iso-$PGE_2$, 8-iso-$PGF_{2\alpha}$, SQ-29548 and L-798106 were purchased from Cayman Chemical (Ann Arbor, MI), and were dissolved in DMSO to stock solutions of $10^{-2}$ M. Carbachol (carbamoylcholine chloride) was purchased from Sigma-Aldrich (St. Louis, MO) and was dissolved in saline to a stock solution of $10^{-3}$ M. Atropine was purchased from Egis Pharmaceutical PLC (Budapest, Hungary) and was diluted in water to a stock solution of $10^{-3}$ M. α,β-methylene ATP, PPADS and Y-27632 were purchased from Cayman Chemical (Ann Arbor, MI) and were dissolved in saline (α,β-methylene ATP: $10^{-2}$ M, PPADS: $10^{-2}$ M and Y-27632: $10^{-3}$ M). Indomethacin was purchased from Sigma-Aldrich (St. Louis, MO) and dissolved in DMSO ($10^{-2}$ M stock concentration), as its aqueous solutions are unstable. Tetrodotoxin citrate (TTX)

was purchased from Tocris Bioscience (Bristol, UK) and was dissolved in DMSO to stock solutions of $10^{-2}$ M.

**Data analysis**

Maximum contraction was defined as the peak value of tension developed after the addition of agonists. Average curves of individual contraction responses were also determined and presented on the left side of the figures, where they were plotted as mean ± SD. All data are presented as mean ± SD except dose-response curves, where mean ± SEM is shown. For statistical analysis, all data sets were subjected first to the D'Agostino-Pearson normality test. If the normal distribution of the data was verified, the *p* values were determined by Student's unpaired *t* test or one-way ANOVA, depending on the number of experimental groups, whereas Mann-Whitney test or Kruskal-Wallis test was used if the normality test failed or if the case numbers were too small for the normality test. Statistical analysis and graph plotting were performed with GraphPad Prism software (v.6.07; GraphPad Software Inc., La Jolla, CA, USA), and $p < 0.05$ was considered as statistically significant difference.

# 4. Results

## 4.1. Contractile effects of isoprostanes in murine urinary bladder

### 4.1.1. Isoprostanes evoke detrusor muscle contraction independent of the mucosal layer

First, we wanted to examine if the isoprostane analogues of $PGE_2$ and $PGF_{2\alpha}$ do have a contractile effect in murine UBSM *ex vivo*. Similarly, to PGs, 8-iso-$PGE_2$ and 8-iso-$PGF_{2\alpha}$ evoked smooth muscle contractions in isolated murine detrusor muscle strips (Fig. 1/ A-B). Next, we aimed to evaluate the potential contribution of cells of the mucosal layer to the mediation of the contractile responses. Therefore, the effects of isoprostanes were compared in the presence [M(+)] and absence [M(-)] of the mucosal layer. There was no significant difference in the responses evoked by the isoprostanes, indicating that mucosa-derived mediators do not contribute to the contractile effect (Fig. 1/A-B).

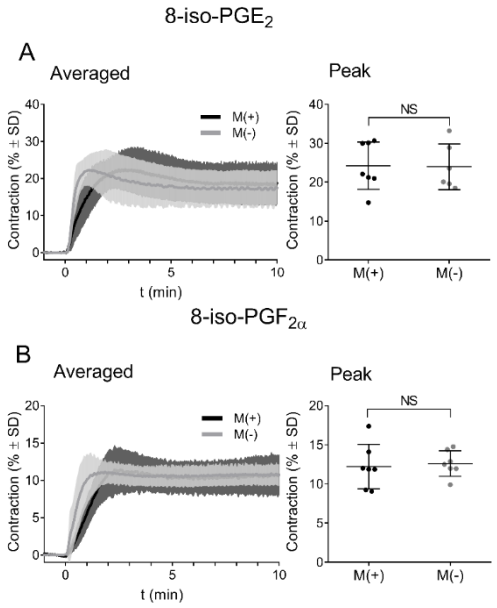


**Figure 1. Isoprostane 8-iso-$PGE_2$ and 8-iso-$PGF_{2\alpha}$ *(A, B)* evoked smooth muscle contractions in the wild-type (WT) murine detrusor muscle.**

### 4.1.2. Isoprostanes act directly on the detrusor muscle independently of neuronal transmitter release

Similarly, we wanted to examine whether isoprostanes' contractile actions are mediated by neurotransmitter release from nerve terminals innervating the bladder. To examine this, UBSM strips were pretreated with either the muscarinic-ACh-receptor antagonist atropine ($10^{-6}$ M, 20 min incubation) or the purinergic P2-receptor antagonist PPADS ($10^{-5}$ M, 20 min incubation) or with the combination of the two antagonists. Neither atropine, nor PPADS, nor even the combination of the two decreased the contractile responses evoked by 8-iso-$PGE_2$ and 8-iso-$PGF_{2\alpha}$ ($10^{-5}$ M) (*Fig. 2/A-B*). As tachykinins acting on the NK2 receptor are also thought to be involved in the neuronal control of micturition, we also tested whether UBSM contractions induced by the NK2 agonist [β-$Ala^8$]-NKA(4–10) are mediated by ACh or ATP release. However, similarly to isoprostanes, [β-$Ala^8$]-NKA(4–10)-induced contractions also remained unaltered in the presence of atropine or PPADS (*Fig. 2/C*). The effectiveness of the inhibitory effect of atropine and that of PPADS was verified by the loss of UBSM contraction induced by the ACh analogue carbachol ($10^{-6}$ M) and the ATP analogue α,β-methylene ATP ($10^{-5}$ M), respectively (*Fig. 2/D-E*). Therefore, we concluded that the detrusor muscle contractions induced by isoprostanes and tachykinins acting on NK2 receptors are not mediated by ACh or ATP release from nerve terminals.

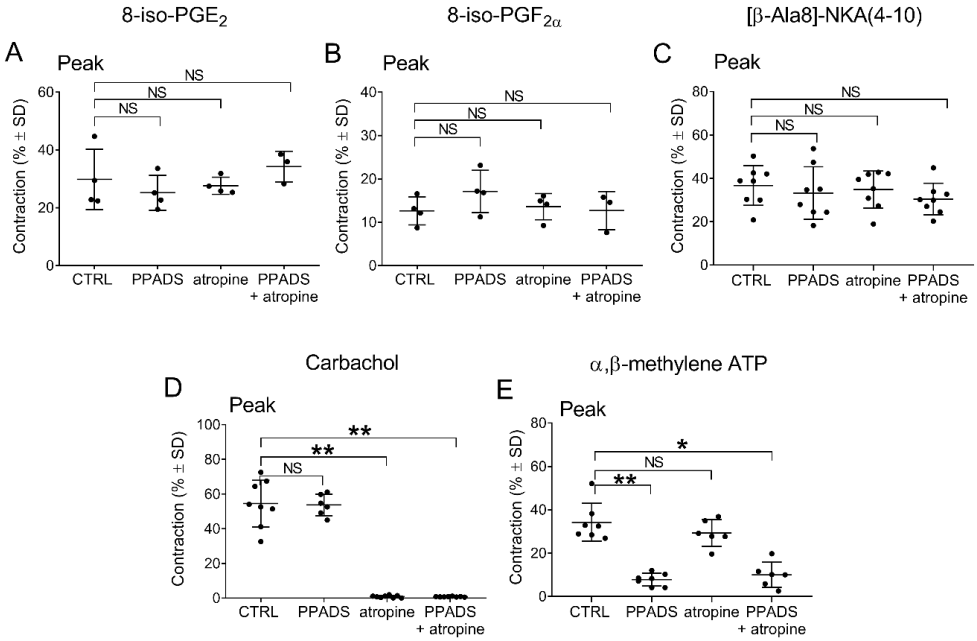


**Figure 2. Isoprostane- and [β-Ala$^8$]-NKA(4–10)-induced detrusor contractions remain unaltered after incubation with atropine or PPADS in murine urinary bladder smooth muscle (UBSM).**

### 4.1.3. The isoprostane-evoked detrusor contractions are mediated by the thromboxane prostanoid TP receptor

The TP receptor has been suggested to mediate smooth muscle contractions in various organs and species in response to prostanoids and isoprostanes. Therefore, we examined the role of TP receptors in mediating the effect of prostanoids and isoprostanes in the mouse detrusor muscle. The contractile responses evoked by PGs were decreased significantly, and the contractions evoked by the isoprostanes were almost entirely abolished in mice deficient for the TP receptor (TP-KO) (*Fig. 3-4*). Pretreatment with the TP-specific antagonist SQ-29548 ($10^{-5}$ M, 20 min incubation) induced similar changes (data not shown). The remaining responses to PGs in the TP-KO mice decreased further in the presence of the $EP_3$ receptor antagonist L-798106 ($10^{-5}$ M, 20 min incubation, *Fig. 3/A and 4/A*). These results indicate that both PGs and isoprostanes evoke contractile responses in the UBSM, and the responses of the

PGs are mediated partly, whereas those of the isoprostanes mainly by TP receptors.

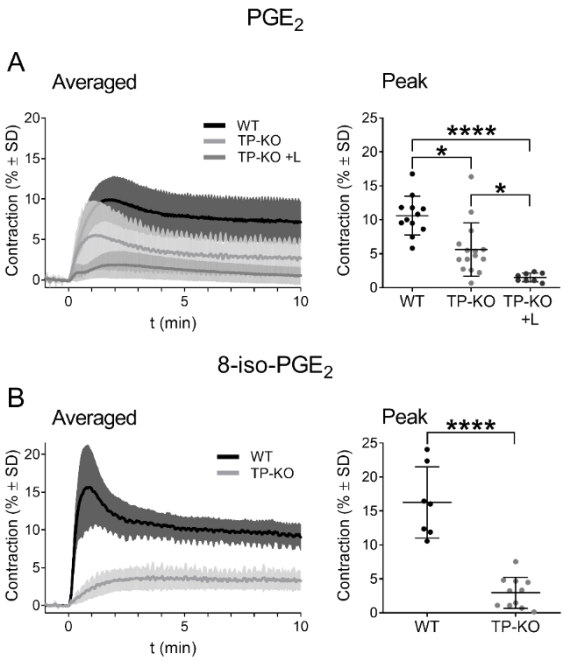


**Figure 3. The role of thromboxane prostanoid (TP) receptors in mediating the $E_2$-prostanoid- and $E_2$-isoprostane-evoked detrusor muscle contraction in murine urinary bladder smooth muscle (UBSM)**.

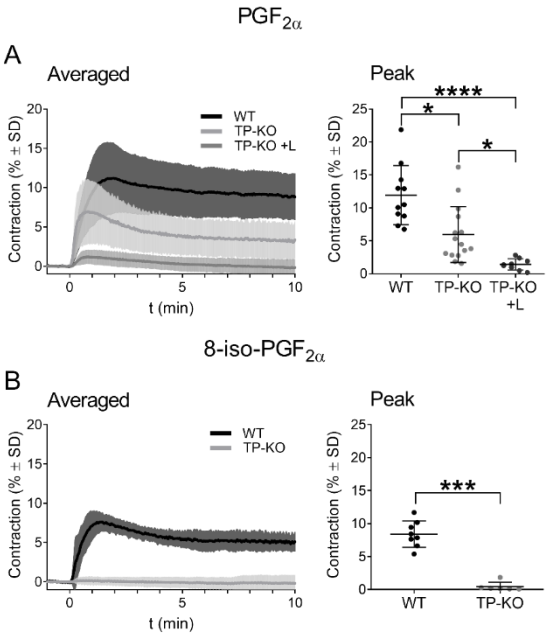


**Figure 4. The role of thromboxane prostanoid (TP) receptors in mediating the $F_{2\alpha}$-prostanoid- and $F_{2\alpha}$-isoprostane-evoked detrusor muscle contraction in murine urinary bladder smooth muscle (UBSM)**.

### 4.1.4. Intracellular signaling of the isoprostane-evoked contractions in murine detrusor muscle

The general concept of smooth muscle tone regulation through $Ca^{2+}$-dependent and $Ca^{2+}$-sensitizing pathways apply to the detrusor muscle as well. Thus, we intended to investigate the roles of the $Ca^{2+}$-dependent, $G\alpha_{q/11}$- and phospholipaseCβ (PLCβ)-mediated as well as the $Ca^{2+}$-sensitizing, $G\alpha_{12/13}$- and Rho-kinase (ROCK)-mediated signaling in isoprostane-evoked detrusor contractions. Therefore, we first examined whether the $Ca^{2+}$-independent pathway plays a role in the downstream signaling of the TP receptor. The contractile responses evoked by isoprostanes ($10^{-5}$ M) were decreased in mice with smooth-muscle specific $G\alpha_{12/13}$ deficiency compared to WT animals (*Fig 5/A, B*) Similarly, the responses decreased significantly in the presence of the

ROCK inhibitor Y-27632 ($10^{-5}$ M, 20 min incubation) compared to the vehicle-treated control group (*Fig 6/A, B*).

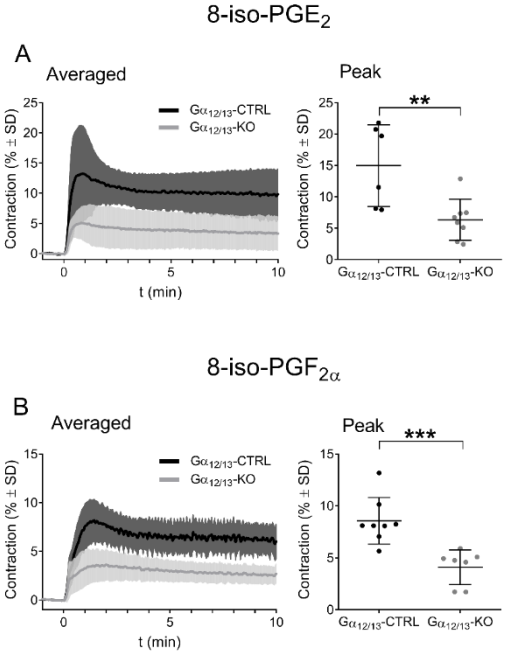


**Figure 5. The Gα$_{12/13}$-coupled signaling pathway plays a significant role in mediating the contractile effect of isoprostanes in murine urinary bladder smooth muscle.**

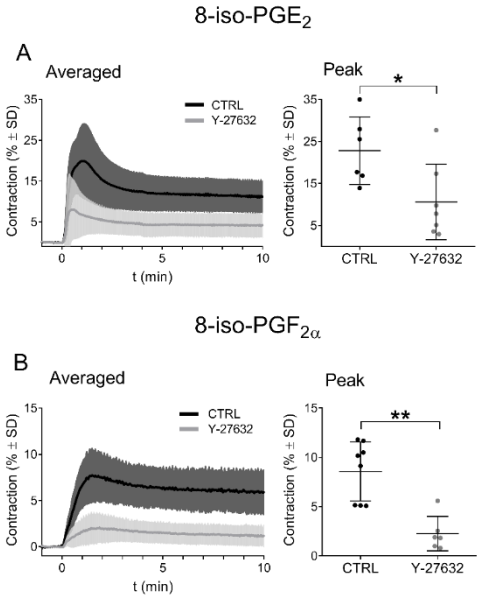


**Figure 6. The Rho-ROCK pathway plays a significant role in mediating the contractile effect of isoprostanes in murine urinary bladder smooth muscle.**

We also examined the role of the $G\alpha_{q/11}$-mediated, classical $Ca^{2+}$-dependent signaling pathway in mediating isoprostane-induced contractions. In UB strips of mice deficient for $G\alpha_{q/11}$-proteins in smooth muscle cells, the contractions evoked by isoprostanes ($10^{-5}$ M) decreased significantly. Furthermore, in $G\alpha_{q/11}$-KO strips the contractile responses to isoprostanes were abolished completely when the ROCK-inhibitor Y-27632 ($10^{-5}$ M, 20 min incubation) was co-administered (*Fig. 7/A-B*).

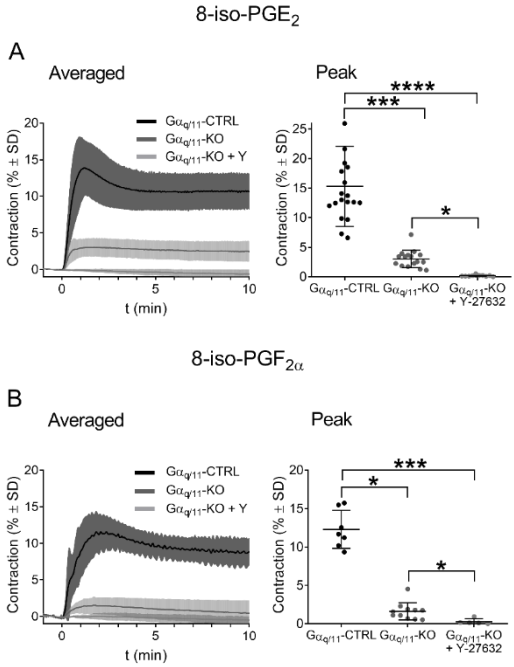


**Figure 7. The $G\alpha_{q/11}$ and the $G_{12/13}$-Rho-ROCK signaling pathways mediate the effects of isoprostanes in murine urinary bladder smooth muscle.**

## 4.2. Isoprostanes evoke contraction in the human detrusor muscle

### 4.2.1. TP receptor activation leads to detrusor contraction in human UBSM strips

In the next part of the study, we first investigated whether functional TP receptors are also present in the human UBSM. We found that the synthetic TP receptor agonist, U-46619 ($10^{-5}$ M) evoked contractions in the human UBSM strips, which were comparable to the effect induced by the muscarinic-ACh-R agonist carbachol ($10^{-6}$ M) (*Fig. 8/A*). The responses to U-46619, but not to carbachol were abolished in the presence of the TP-receptor antagonist SQ-29548 ($10^{-5}$ M, 20 min incubation) (*Fig. 8/B and 8/C*).

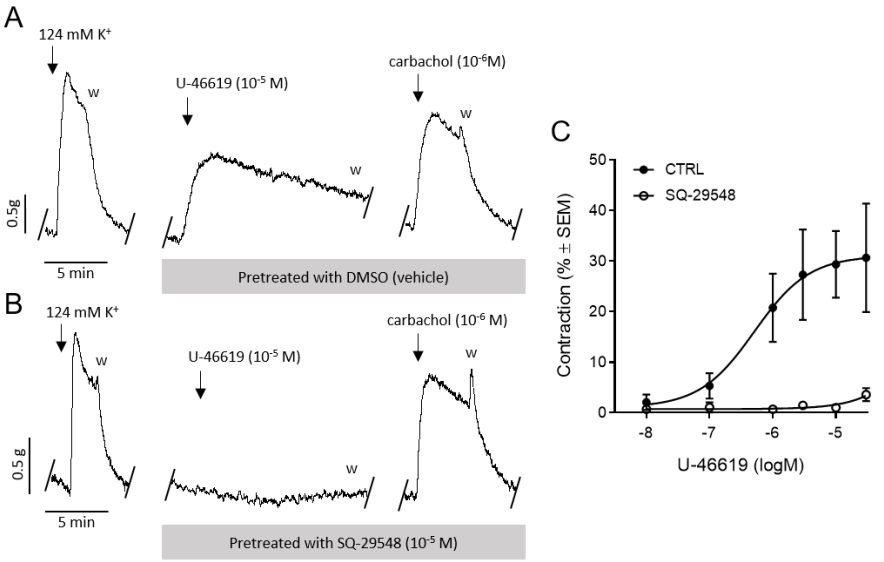


**Figure 8. Functional thromboxane prostanoid (TP) receptors are present in the human urinary bladder smooth muscle and their activation leads to significant contraction.**

After verifying the presence of functional TP receptors in human UBSM, we aimed to examine whether the TP receptors eventually mediate the effect pre-junctionally via activating neurotransmitter release, as we did previously in murine bladders. The responses evoked by U-46619 ($10^{-5}$ M) were unaltered by the muscarinic-ACh-R antagonist atropine ($10^{-6}$ M, 20 min incubation) or the purinergic P2-receptor antagonist PPADS ($10^{-5}$ M, 20 min incubation), but were abolished by SQ-29548 ($10^{-5}$ M, 20 min incubation) (*Fig. 9/A*). The positive control for the inhibitory effect of atropine was the loss of carbachol ($10^{-6}$ M)-induced contractions, whereas the effectiveness of PPADS was verified by inhibition of the contraction induced by the ATP-analogue α,β-methylene ATP ($10^{-5}$ M) *Fig. 9/B-C*. The contractions evoked by either carbachol or α,β-methylene ATP were unaltered by SQ-29548 *(Fig. 9/B-C)*. These data indicate that the contractions evoked by TP activation are independent of either cholinergic or purinergic signaling, and therefore the TP receptors are likely to

be localized directly on the detrusor muscle in the human urinary bladder as well.

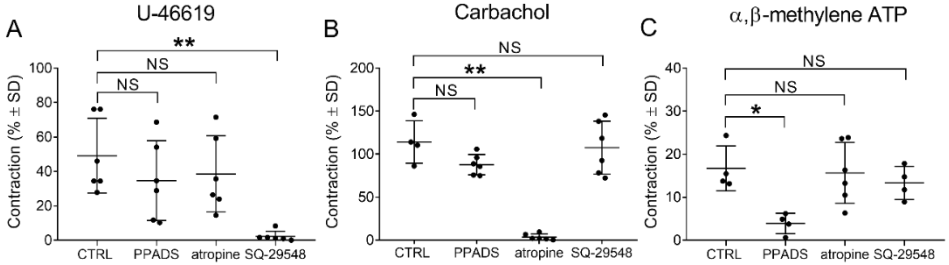


**Figure 9. The thromboxane prostanoid TP receptor-mediated smooth muscle contraction in the human urinary bladder is independent of cholinergic or purinergic signaling.**

### 4.2.2. Isoprostanes evoke concentration-dependent contractions via TP receptor in human UBSM strips

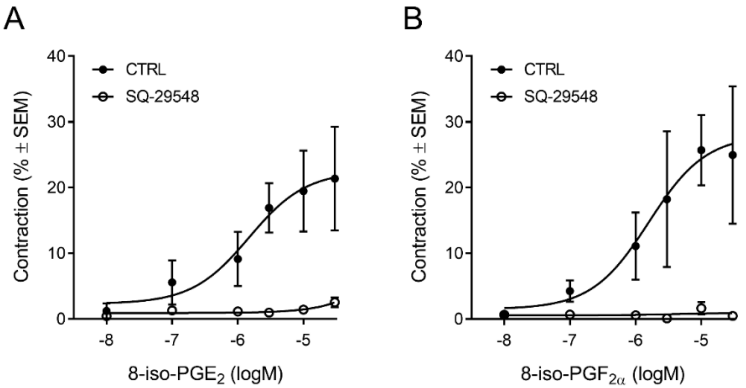


**Figure 10. The isoprostane 8-iso-PGE$_2$ and 8-iso-PGF$_{2\alpha}$ evoke significant, dose-dependent detrusor muscle contraction in human urinary bladder, which is mediated exclusively by the TP receptor.**

The isoprostane 8-iso-PGE$_2$ and 8-iso-PGF$_{2\alpha}$ evoked dose-dependent contractions in the human UBSM strips (*Fig. 10/A-B*) (8-iso-PGE$_2$: $E_{max}$: 22.5%, $EC_{50}$: 1.48 x $10^{-6}$ M; 8-iso-PGF$_{2\alpha}$: $E_{max}$: 28.0%, $EC_{50}$: 1.59 x $10^{-6}$ M). Importantly, the contractile responses evoked by the two examined isoprostanes were abolished in the presence of the TP receptor antagonist SQ-29548 ($10^{-5}$ M, 20 min incubation) *(Fig. 10/A-B)*, indicating that the effects of isoprostanes are

mediated by the TP receptor in the human urinary bladder, similarly to the results obtained in mice.

### 4.2.3. Role of ROCK-enzyme in the signaling of isoprostane-evoked contractions

Finally, we aimed to analyze the intracellular signaling of isoprostanes in the human UBSM. The contractile responses evoked by 8-iso-$PGE_2$ and 8-iso-$PGF_{2\alpha}$ were markedly reduced in the presence of the ROCK inhibitor Y-27632 ($10^{-5}$ M, 20 min incubation) in human urinary bladder strips (*Fig. 11/A-B*).

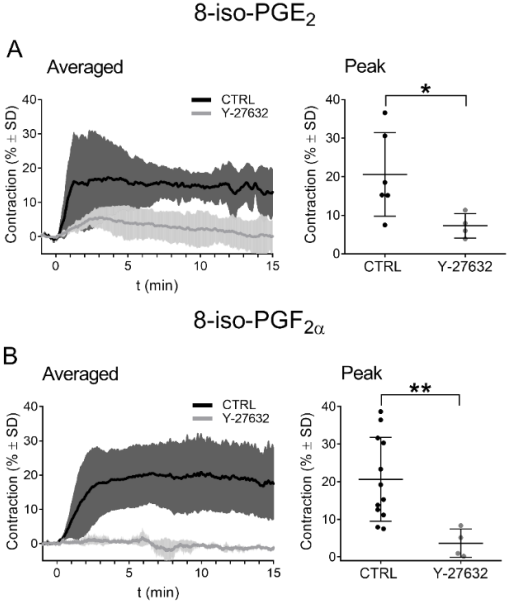


**Figure 11. Rho-ROCK signaling plays a major role in mediating the detrusor contraction evoked by isoprostanes in human urinary bladder.**

# 5. Conclusion

Since both the storage and voiding phase of the urinary bladder is under a complex regulation, even minor alterations can lead to dysfunctions such as involuntary detrusor contraction resulting in detrusor overactivity and the clinical symptoms of overactive bladder syndrome. The aim of this research work was to examine the potential role of isoprostanes in murine and human urinary bladder functions, based on the hypothesis, that isoprostanes are not only present in the urine as biomarkers of systemic oxidative stress, but may have a significant influence on the tone of the urinary bladder smooth muscle. Furthermore, we aimed to examine the receptor(s) and the intracellular signaling of isoprostane-evoked contractions in the detrusor muscle. Our results gained from the *ex vivo* experiments performed on murine and human urinary bladder detrusor strips are the following:

- We demonstrated, that the isoprostane 8-iso-$PGE_2$ and 8-iso$PG_{2\alpha}$ evoke significant smooth muscle contraction in both murine and human urinary bladder smooth muscle.
- The isoprostane-evoked contractile effect is independent of the presence of the mucosal layer or the submucosal tissue.
- The contractile responses induced by both isoprostanes are mediated by the thromboxane prostanoid TP receptor in both murine and human urinary bladders.
- The activation of the TP receptor is independent of neurotransmitter release, acetyl-choline or ATP do not play a role in the contractile effect, the isoprostanes evoke the smooth muscle contraction directly acting on the detrusor muscle.
- The isoprostane-induced TP receptor activation is mediated intracellularly by the $G\alpha_{12/13}$-Rho-ROCK and the $G\alpha_{q/11}$-coupled signaling pathways simultaneously.

# 6. Bibliography of the candidate's publications

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