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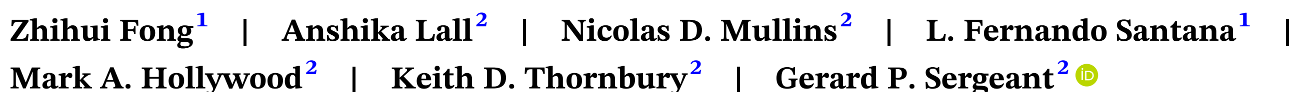
Blockade of Kv7 channels reverses the inhibitory effects of exchange protein directly activated by cAMP activation on purinergic contractions of the murine detrusor



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Abstract

Purinergic contractions of the detrusor are reduced by cAMP, but the underlying mechanisms are unclear. We examined the effects of BK and Kv7 channel modulators on purinergic contractions of the detrusor and tested if the inhibitory effects of activators of the cAMP effectors, PKA and EPAC, were reduced by blockade of BK or Kv7 channels. Purinergic contractions of the murine detrusor were induced by electric field stimulation (EFS) or application of the P2X receptor agonist α,β-MeATP. EFS responses were inhibited by the L-type Ca^{2+} channel blocker nifedipine, but not by the SERCA inhibitor CPA or the SOCE blocker GSK7975A. The Kv7 channel opener retigabine and BK channel activator compound X inhibited purinergic responses, while blockade of Kv7 or BK channels with XE991 or iberiotoxin, respectively, augmented these responses. Application of the EPAC activator 007-AM or PKA activator 6-MB-cAMP inhibited EFS responses. These effects were unaffected by iberiotoxin; however, XE991 reduced the effects of 007-AM, but not 6-MB-cAMP. Kv7.5 was the only Kv7 transcript detected in isolated detrusor myocytes. These data suggest that purinergic contractions of the detrusor are regulated by BK and Kv7 channels and the latter may also play a role in EPAC-dependent inhibition of this activity.

KEYWORDS
bladder and urethra pharmacology, ion channels as drug targets, potassium channels, urinary incontinence

1 | INTRODUCTION

Electrical stimulation of parasympathetic nerves in the urinary bladder leads to contractions induced by co-release of acetylcholine (ACh) and ATP.[1] ATP-induced contractions (referred to as purinergic contractions) are brought about by activation of P2X1 receptors on detrusor myocytes,[2] while the effects of ACh are primarily due to stimulation of postjunctional M3 muscarinic receptors.[3,4] The relative contribution of each neurotransmitter to nerve-mediated contractions of the bladder varies according to species.[5] For example, the purinergic component accounts for ∼50% of the overall contractile response in rodents, whereas in humans, it only accounts for ∼2%.[6] However, in tissues

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taken from patients with bladder disorders such as interstitial cystitis,[7] outflow obstruction,[8] idiopathic detrusor instability,[9] and some types of neurogenic bladder,[10] the purinergic component of nerve-evoked contractions of the human bladder can be increased by up to 40%. Spontaneous release of ATP from nerve terminals has been shown to enhance spontaneous contractions of the detrusor, which are associated with these bladder storage disorders[11]; consequently, inhibiting nerve-mediated ATP-evoked contractions has been advanced as a novel therapeutic approach to target the atropine-resistant contractions that occur in these disorders.[12] Therefore, it is of interest to develop a better understanding of the mechanisms that generate and regulate these responses using a murine model where purinergic contractions are developed in their normal bladders.

Stimulation of P2X1 receptors in detrusor myocytes leads to excitatory junction potentials which trigger action potentials and contractions that are sensitive to the L-type Ca^{2+} channel (LTCC) blocker, nifedipine.[13] BK channels act as key regulators of detrusor excitability, due to their ability to hyperpolarize membrane potential and limit activation of voltage-gated Ca^{2+} channels.[14–17] In addition, purinergic contractions of the detrusor are enhanced by pharmacological blockade or genetic ablation of BK channels.[18,19] Kv7 channels have also been shown to regulate detrusor excitability[20–22]; however, little is known about their role in modulation of purinergic contractions. Fong et al. showed that purinergic contractions of the mouse bladder were inhibited by selective activators of PKA (6-MB-cAMP) and EPAC (007-AM),[23] but the mechanisms underlying these effects have not been fully determined. 007-AM reduced the amplitude of ATP-evoked currents in isolated detrusor myocytes[23] and in HEK293 cells expressing P2X1 receptors,[24] highlighting an important modulatory pathway for regulation of purinergic responses in the detrusor by EPAC. However, as the contractile responses of the bladder to ATP involve Ca^{2+} influx via LTCC, it is possible that EPAC could modulate purinergic contractions of the detrusor via additional mechanisms.

The purpose of the present study was to examine the effects of BK and Kv7 channel modulators on purinergic contractions of the detrusor and investigate if the inhibitory effects of the PKA and EPAC activators, 6-MB-cAMP and 007-AM, on purinergic contractions of the detrusor were affected by blockade of Kv7 or BK channels.

2 | MATERIALS AND METHODS

2.1 | Ethical approval

All procedures were carried out in accordance with EU Directive 2010/63/EU and with approval from Dundalk Institute of Technology Animal Care & Use Committee. The study was conducted in accordance with the Basic & Clinical Pharmacology & Toxicology policy for experimental and clinical studies.[25]

2.2 | Tissue preparation and cell isolation

Male and female C57BL/6 mice (ENVIGO, UK) aged between 9 and 14 weeks were killed by cervical dislocation. Bladders were removed, opened longitudinally, and pinned to a Sylgard Petri dish with the luminal side facing upwards. The mucosa was removed by sharp dissection, exposing the detrusor. Two longitudinal strips of detrusor from each bladder were used for tension recording, cell dispersal, or molecular biology. Strips of detrusor were chopped into pieces (~1 mm^3) using sharp dissecting scissors and stored in Ca^{2+}-free Hanks solution for 30 min prior to cell dispersal. Tissue pieces were incubated in dispersal medium containing (per 5 ml) of Ca^{2+}-free Hanks solution:15-mg collagenase (Sigma type 1A), 1-mg proteinase (Sigma type XXIV), 10-mg bovine serum albumin (Sigma), and 10-mg trypsin inhibitor (Sigma) for 10–15 min at 37°C. Tissue was transferred to Ca^{2+}-free Hanks solution and stirred for 10 min to release single SMC.

2.3 | Tension recordings

Detrusor strips (~8 × 2 mm) were mounted in water-jacketed organ baths (volume 14 ml), maintained at 37°C, and bathed with Krebs' solution bubbled with 95% O_2–5% CO_2. Strips were adjusted to a tension of ~5 mN and allowed to equilibrate for 50 min. Contractions were recorded using a multichannel Myobath system and data were acquired using DataTrax2 software (WPI, Europe). Electric field stimulation (EFS) was applied via two platinum electrode wires (5-mm length, 2 mm apart) by a MultiStim system-D330 stimulator (Digitimer Ltd, England), which delivered 0.3-ms pulses of 20 V (nominal) at a frequency of 4 Hz for 10 s at 100-s intervals. Drugs were delivered by adding them to the organ bath, where they were diluted in Krebs' solution to their final concentration.

2.4 | Immunocytochemistry

Freshly isolated single murine detrusor SMCs were immunolabelled using an anti-Kv7.5 antibody (PA5-101768; ThermoFisher Scientific) for fluorescence

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imaging. Dissociated cells were fixed with paraformaldehyde (4%) in phosphate-buffered saline solution (PBS) for 10 min at room temperature, followed by washes with PBS (3 × 10 min). Cells were then incubated with glycine solution (50 mM in PBS) for 10 min, washed again with PBS (3 × 10 min), and permeabilized with a blocking solution composed of BSA (3% w/v) and Triton X-100 (0.25% v/v) in PBS for 1 h at room temperature. Next, cells were incubated in rabbit anti-Kv7.5 polyclonal antibody (1:200 dilution; ThermoFisher Scientific) overnight at 4°C. The primary antibody solution was removed by PBS washes (3 × 10 min), followed by secondary antibody incubation with goat anti-rabbit Alexa Fluor 568 (1:1000 dilution) for 1 h at room temperature. Secondary antibodies were washed with PBS (3 × 10 min). Immunofluorescence of isolated detrusor SMCs was detected using an Olympus FV3000 confocal microscope equipped with 60× oil immersion lens (NA = 1.40). Images were collected in optimized optical planes with a *z*-axis of 0.41 μm/step.

2.5 | Solutions

Solutions used were of the following composition (mM): *Krebs' solution*: NaCl (120), KCl (5.9), $NaHCO_3$ (25), $NaH_2PO_4.2H_2O$ (1.2), glucose (5.5), $MgCl_2$ (1.2), and $CaCl_2$ (2.5). pH was maintained at 7.4 by continuous bubbling with 95%O_2−5% CO_2. PBS (10X; Fisher Scientific). PBS 1X (11.9 PO_4^{3-}, 137 NaCl, 2.7 KCl; pH 7.4).

2.6 | Molecular biology

Total RNA from tissue strips was prepared using the Trizol method (Invitrogen). RNA samples were DNase (DNase 1, Invitrogen) treated to remove any contaminating genomic DNA. First-strand cDNA was prepared from tissue RNA preparations using Superscript II Reverse Transcriptase (RT) (Invitrogen). Two hundred micrograms per microlitre random hexamers were used to reverse transcribe RNA. Real-time quantitative PCR (qPCR) was performed on a QUANTICA real-time PCR system (TECHNE) using the SYBR Green PCR Master Mix (Applied Biosystems). The cycling conditions were as follows: An initial 5-min denaturation at 95°C was followed by 40 cycles of denaturation at 95°C for 30 s, annealing at 56°C for 1 min, and extension at 72°C for 45 s. Tissue samples were analysed in triplicates with cDNA obtained from six different mice, in total. In addition, nontemplate controls (NTC) were included for all primer sets. After real-time qPCR acquisition, a dissociation curve (70–90°C) was obtained. Subsequent analysis of the individual melting curves allowed us to verify the specificity of primer sets. β-Actin was used as a reference gene for quantification of Kv7.1–Kv7.5 in murine detrusor. Standard curves were generated, for the reference gene (β-actin) and the genes of interest, from regression analysis of the mean CT values of triplicate samples for the $\log_{10}$ diluted cDNA. Mouse brain cDNA was used as a template in the construction of standard curves. Unknown quantities relative to the standard curves for Kv7.1–Kv7.5 were calculated, yielding transcriptional quantification of each isoform cDNA, relative to the endogenous standard (β-actin). The following PCR primers were used. In each case, the number in parentheses represents the NCBI reference sequence: β-actin (NM_007393.3): sense nucleotide (nt) 192–208, CTAGGCACCAGGGTGTG and antisense nt 396–381. GTGAGCAGCACAGGGT. Amplicon size: 205 base pairs (bp). Kv7.1 (NM_008434.2): sense nt 1525–1544, AGCACACCCCATTTCTTGAG and antisense nt 1798–1779, ACTGATCCAGCCTTCTCTGT. Amplicon size: 274 bp. Kv7.2 (NM_010611.3): sense nt 1707–1727, CGGCAGAATTCAGAAGAAGCA and antisense nt 1917–1898, CCGAGTACTGTTCGATGACG. Amplicon size: 211 bp. Kv7.3 (NM_152923.2): sense nt 1788–1807, CCAGCAGTCTCCAAGGAATG and antisense nt 1991–1972, GCCCCCTTAGTTGGGTAGTA. Amplicon size: 204 bp. Kv7.4 (NM_001081142.2): sense nt 836–855, CGATCACACTGACGACCATT and antisense nt 1051–1032, ACGCAGCCTGGATGAGATTA. Amplicon size: 216 bp. Kv7.5 (NM_001160139.1): sense nt 2107–2126, TCTTGGCTCA GGTTTTGCAT and antisense nt 2299–2280, CTTCTGATTGGTAGGGCTGC. Amplicon size: 193 bp. Each gene specific primer set was designed to span an exon–exon boundary. The identity of amplified PCR products was confirmed by DNA sequencing (Source Bioscience Ltd, Ireland).

2.7 | Single cell PCR

Freshly dispersed murine detrusor SMCs were plated on a Nunclon dish (35 mm), mounted on the stage of an inverted microscope of a standard patch clamp setup, and left to settle at room temperature for 30 min, following continuous perfusion with Ca^{2+}-free Hanks solution. Borosilicate micropipettes with a tip diameter of approximately 100 μm, pre-filled with Ca^{2+}-free Hanks solution, were used for cell collection. Detrusor SMCs were identified based on their morphology (spindle-shaped and slightly elongated). Once an SMC was identified, a micropipette was navigated adjacent to the target SMC using a micromanipulator, and negative pressure was applied to extract the SMC into the micropipette. Each SMC was

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then expelled and collected in an Eppendorf tube (1.5 ml) containing cell lysis buffer (350 μl) supplemented with β-mercaptoethanol (3.5 μl). Approximately 30 individual cells were collected per sample and were immediately used for RNA extraction. RNA extraction from these samples was performed using an RNeasy Micro Kit (Qiagen). Ethanol was added to the cell lysate and the suspension was transferred to an RNeasy MinElute spin column according to the manufacturer's instructions. RNA was eluted from the spin column into RNase-free water and then stored at −80°C. cDNA synthesized from isolated smooth muscle cells in this study were always checked for contamination from other cell types by performing PCR with primers against cell-specific markers as follows: smooth muscle cells (smooth muscle myosin, heavy chain 11), neurons (ubiquitin carboxy-terminal hydrolase L1), mast cells (carboxypeptidase A3), fibroblasts (proline 4-hydroxylase), and PDGFRα+ cells (platelet derived growth factor receptor, α polypeptide). Only samples that were positive to smooth muscle myosin, heavy chain 11 and negative for all other markers were used in this study.

2.8 | Drugs

α,β-Methylene ATP (α,β-MeATP) (Tocris), atropine (Sigma Aldrich), 8-pCPT-2′-O-Me-cAMP-AM, referred to as 007-AM (8-(4-Chlorophenylthio)-2′-O-methyladenosine-3′,5′-cyclic monophosphate, acetoxymethyl ester) (Biolog), 6-MB-cAMP (N^6-Monobutyryladenosine-3′,5′-cyclic monophosphate, sodium salt) (Biolog), XE991 (Tocris), retigabine (Tocris), iberiotoxin (Smartox), FPL 64176 (Tocris), GSK7975A (Tocris), and cyclopiazonic acid (CPA, Tocris). Compound X, a racemic mixture of two enantiomers, Y and Z, which are among the most potent novel BK channel openers described to date, was prepared following the procedure of Ponte et al.[26] Briefly, a solution of triphenyl phosphine (230 mg, 0.875 mmol) and perchloric acid (70%, 76 μl, 0.875 mmol) in acetonitrile (2 ml) was added to a stirring solution of 4-aminobenzoic acid (300 mg, 2.19 mmol), 1-naphthaldehyde (360 mg, 2.19 mmol), freshly cracked cyclopentadiene (289 mg, 4.38 mmol), and anhydrous sodium sulphate (311 mg, 2.19 mmol) in acetonitrile (9 ml). The reaction mixture was stirred at room temperature for 4 h, and the precipitate that formed was isolated by filtration and purified by flash chromatography (methanol–dichloromethane) to afford the title compound as a white solid (316 mg, 42%). Spectral data were in agreement with that already reported. All drugs were dissolved in the manufacturer recommended solvent and then diluted into Krebs solution for use in tension recording experiments. Drugs were removed by emptying the bath solution through a drain port at the base of the bath and replacing with Krebs solution without the drug.

2.9 | Statistics

In each data set, '*n*' reflects the number of cells or tissue strips, and '*N*' refers to the number of animals from which these were obtained. Statistical analysis was performed using one-way ANOVA (with a Bonferonni post-hoc test) or a Student's paired *t*-test where appropriate. Normalized data were compared using a Wilcoxin signed-rank test.

3 | RESULTS

Fong et al. showed that atropine-resistant contractions of the murine bladder evoked by EFS were inhibited by tetrodotoxin, NF449, and α,β-MeATP confirming that they were neural in origin and due to activation of P2X1 receptors.[23] Acevedo and Conteras previously found that atropine-resistant contractions of the murine bladder, induced by EFS, were reduced by application of LTCC blockers,[27] indicating that purinergic contractions of the detrusor were reliant on Ca^{2+} influx by LTCC. To confirm that EFS-evoked purinergic contractions of murine detrusor strips in the present study were reliant on Ca^{2+} influx via LTCC, we examined the effects of an LTCC blocker (nifedipine, 1 μM) and an LTCC activator (FPL 64176, 300 nM). Figure 1A shows a representative trace demonstrating that nifedipine greatly reduced the amplitude of purinergic nerve-evoked contractions of the murine bladder. Summary data in Figure 1B show that nifedipine reduced the mean amplitude of EFS-evoked contractions from 2.4 ± 0.4 to 0.16 ± 0.3 mN ($p < 0.0001$, $n = 15$, $N = 8$). In contrast, FPL 64176 augmented the mean amplitude of the EFS responses from 2.6 ± 0.5 to 8.9 ± 1.5 mN ($p < 0.001$, $n = 16$, $N = 8$; Figure 1C,D). These data confirm that purinergic contractions of the detrusor involve Ca^{2+} influx via LTCC. Experiments were also conducted to assess if the purinergic EFS responses involved Ca^{2+} release from intracellular stores by examining the effects of CPA (10 μM), a sarcoplasmic/endoplasmic reticulum Ca^{2+} ATPase (SERCA) inhibitor and GSK7975A (10 μM), which inhibits store-operated Ca^{2+} entry.[28] Data in Figure 2A–D show that neither agent significantly reduced the amplitude of EFS-evoked contractions ($p > 0.05$), but that subsequent addition of nifedipine inhibited the responses. CPA notably increased the amplitude of the responses and raised basal tension, as noted previously by Ziganshin et al. in guinea pig bladder.[29]

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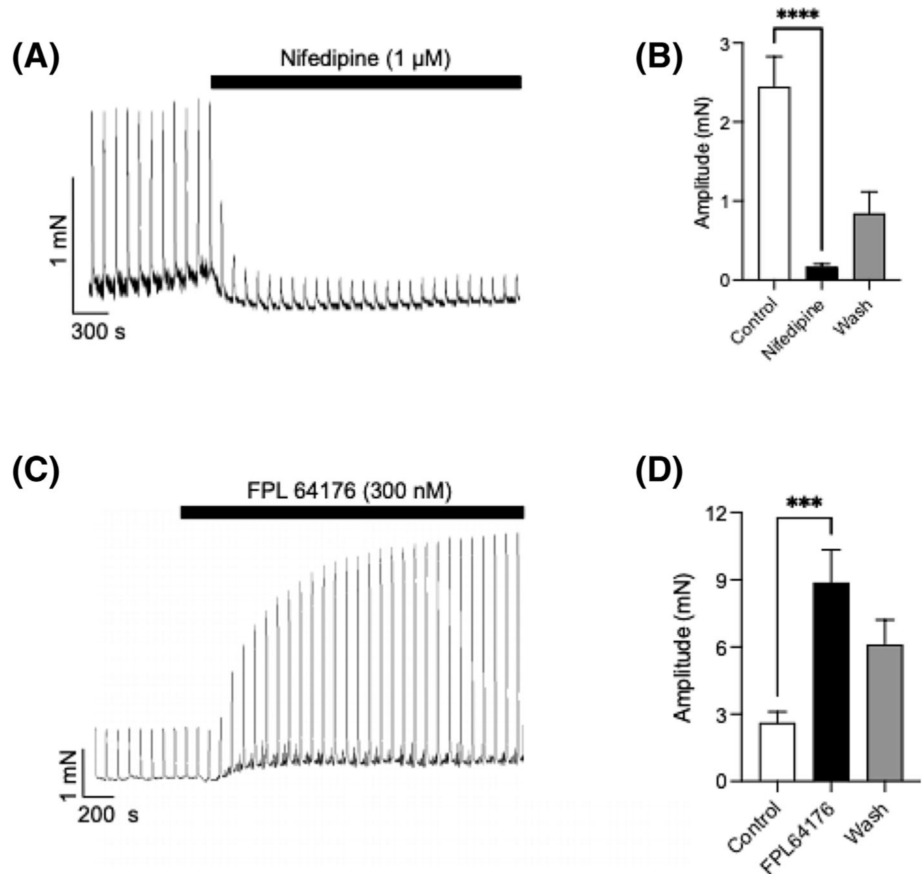

FIGURE 1 Purinergic nerve-evoked contractions of the detrusor rely on Ca^{2+} influx via L-type Ca^{2+} channels. (A) Representative trace showing effect of nifedipine (1 μM) on EFS-evoked contractions of the detrusor in the presence of atropine (1 μM). (B) Summary bar chart showing mean amplitude of EFS responses before, during, and following wash out of nifedipine. (C) Representative trace showing effect of FPL 64176 (300 nM) on EFS-evoked contractions of the detrusor in the presence of atropine (1 μM). (D) Summary bar chart showing mean amplitude of EFS responses before, during, and following wash out of FPL 64176 (300 nM). Error bars represent SEM. ***$p < 0.001$; ****$p < 0.0001$.

Next, we examined the effects of the BK channel opener compound X[26] and the BK channel blocker iberiotoxin (IbTx) on EFS-evoked contractions of the detrusor. Compound X (3 μM) reduced contraction amplitude by 45%, from 2.9 ± 0.4 to 1.6 ± 0.2 mN ($n = 12$; $N = 6$; $p < 0.001$; Figure 3A,B), while IbTx produced a fivefold increase in mean contraction amplitude from 3.6 ± 0.3 to 17.8 ± 1.2 mN ($n = 37$; $N = 35$; $p < 0.0001$; Figure 3C,D). DSM strips in this study generated spontaneous phasic contractions (SPCs), and it was notable that IbTx also produced an increase in SPC amplitude. Mean SPC amplitude increased from 0.18 ± 0.02 mN under control conditions to 0.39 ± 0.06 mN in IbTx ($n = 37$, $p < 0.001$). Data shown in Figure 3E–H demonstrate that compound X and IbTx exerted similar effects on contractions elicited by exogenous application of α,β-MeATP (1 μM). Compound X reduced the amplitude of α,β-MeATP responses by ∼54%, from 3.9 ± 0.8 to 1.8 ± 0.3 mN ($n = 12$; $N = 12$; $p < 0.01$; Figure 3E,F), while IbTx increased mean contraction amplitude, from 3.3 ± 0.3 to 8.7 ± 1.5 mN ($n = 6$; $N = 6$; $p < 0.05$; Figure 3G,H). These data indicate that contractions of the detrusor induced by stimulation of postjunctional P2X1 receptors are affected by modulation of BK channels.

P2X1 receptor-dependent contractions of the detrusor were also affected by modulators of Kv7 channels (Figure 4). Figure 4A,B,E,F demonstrate that retigabine (10 μM), an opener of Kv7.2–7.5 channels,[30–32] reduced the amplitude of EFS and α,β-MeATP-induced contractions of the detrusor. EFS responses were reduced by 53% from 3.3 ± 0.6 to 1.6 ± 0.3 mN ($n = 7$; $N = 7$; $p < 0.01$; Figure 4A,B) and α,β-MeATP responses reduced by 54% from 5.5 ± 0.7 to 2.5 ± 0.3 mN ($n = 6$; $N = 6$; $p < 0.01$; Figure 4E,F). In contrast, the Kv7 channel blocker, XE991,[29] increased the amplitude of EFS responses from 2.8 ± 0.2 to 7.0 ± 0.6 mN EFS ($n = 26$; $N = 20$; $p < 0.0001$; Figure 4C,D) and α,β-MeATP responses from 4.3 ± 0.4 to 6.5 ± 0.4 mN ($n = 26$; $N = 17$; $p < 0.0001$; Figure 4G,H). XE991 also caused a robust increase in SPC amplitude, increasing mean amplitude from 0.13 ± 0.02 to 1.48 ± 0.22 mN ($n = 26$, $p < 0.0001$).

The data presented thus far indicate that purinergic contractions of the detrusor rely on Ca^{2+} influx via LTCC and can be modulated by drugs which affect BK and Kv7 channels. Since there are five Kv7 subtypes (Kv7.1–7.5),[33] we examined the transcriptional expression of each Kv7 subtype in murine detrusor strips using RT-PCR, to help determine which subtypes may underlie the effects elicited by retigabine and XE991. The electrophoresis gel shown in Figure 5A shows expression of Kv7.1–7.5 in brain and intact detrusor tissue, while expression of Kv7.1–7.5 in isolated detrusor myocytesis shown in

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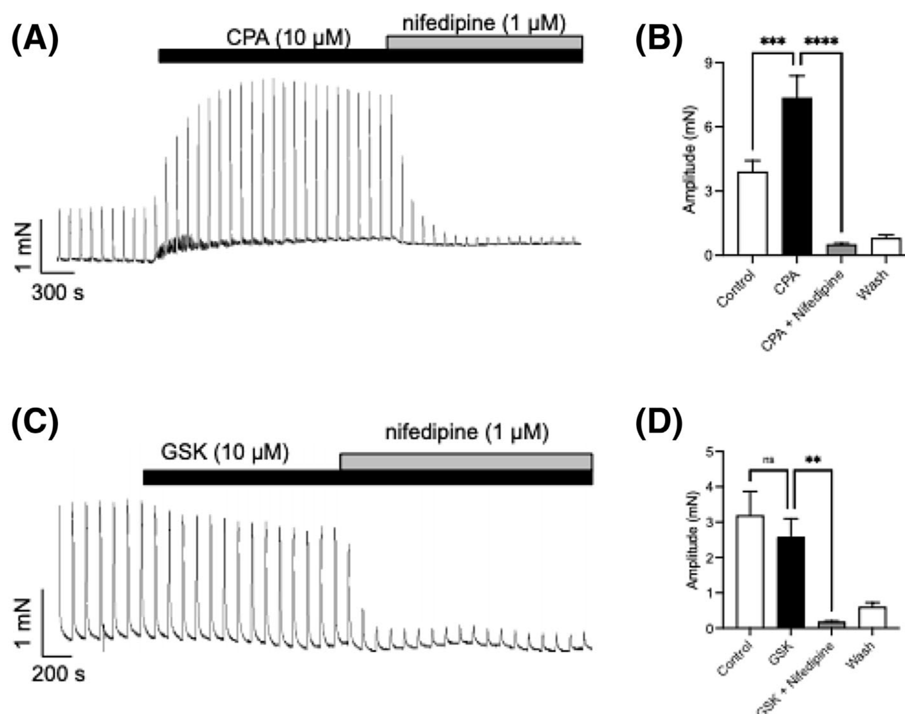

FIGURE 2 Purinergic nerve-evoked contractions of the detrusor are not mediated by Ca^{2+} release from intracellular stores. (A) Representative trace showing effect of CPA (10 μM), followed by nifedipine (1 μM), on EFS-evoked contractions of the detrusor in the presence of atropine (1 μM). (B) Summary bar chart comparing mean amplitude of EFS responses in control, CPA (10 μM), CPA (10 μM) + nifedipine (1 μM), and wash out. (C) Representative trace showing effect of GSK7975A (10 μM), followed by nifedipine (1 μM), on EFS-evoked contractions of the detrusor in the presence of atropine (1 μM). (D) Summary bar chart comparing mean amplitude of EFS responses in control, GSK7975A (10 μM), GSK7975A (10 μM) + nifedipine (1 μM), and wash out. Error bars represent SEM. $^{**}p < 0.01$, $^{***}p < 0.001$, $^{****}p < 0.0001$.

Figure 5B. Bands for each subtype were detected in brain, while Kv7.1 and Kv7.3–Kv7.5 were found in intact bladder strips. However, only Kv7.5 was detected in isolated detrusor myocytes. PCR was also performed on reaction mixtures lacking cDNA (no-template controls) for each primer to test for contamination and non-specific amplification. No amplification product was detected from these reactions (data not shown). Analysis of Kv7.1–7.5 expression in intact detrusor strips using real-time quantitative PCR (Figure 5C) revealed higher expression of Kv7.5 compared with the other subtypes. Finally, to further examine Kv7.5 expression in isolated detrusor myocytes, immunoreactivity to an anti-Kv7.5 antibody was examined. Detrusor myocytes displayed Kv7.5 immunoreactivity with predominant staining concentrated to the periphery of the cells (Figure 5D). Negative controls (secondary antibody, no primary antibody incubation) were prepared simultaneously and imaged using the same parameters to ensure no positive immunoreactivity occurred (see example in Figure 5D,vi).

Fong et al. demonstrated that the amplitude of purinergic nerve-evoked contractions of the detrusor were reduced by activation of PKA and EPAC.[23] We next investigated if the inhibitory effects of the PKA and EPAC activators, 6-MB-cAMP, and 007-AM on purinergic EFS-evoked contractions were affected by blockade of Kv7 channels with XE991. Figure 6A is a representative trace showing the effect of 6-MB-cAMP (100 μM) on EFS-evoked contractions of the detrusor, before and in the presence of XE991 (10 μM). Figure 6B,C are summary bar charts showing the effects of 6-MB-cAMP on mean contraction amplitude (normalized to peak contraction amplitude under control conditions and in the presence of XE991, respectively). 6-MB-cAMP reduced mean amplitude by 58 ± 2.9% under control conditions and 63 ± 1.3% in XE991 ($n = 7$; $N = 6$; $p < 0.05$; Wilcoxon test). Therefore, the inhibitory effects of 6-MB-cAMP were not reduced in the presence of XE991. In contrast, the representative trace in Figure 6D shows that the effects of 007-AM were greatly reduced in the presence of XE991. Summary data in Figure 6E,F indicate that 007-AM reduced mean contraction amplitude by 52 ± 4% under control conditions, compared with only 14 ± 3% in the presence of XE991 ($n = 7$; $N = 5$; $p < 0.05$; Wilcoxon test). Time controls for these experiments demonstrate that the effects of 007-AM were reproducible in the absence of XE991. Application of 007-AM reversibly

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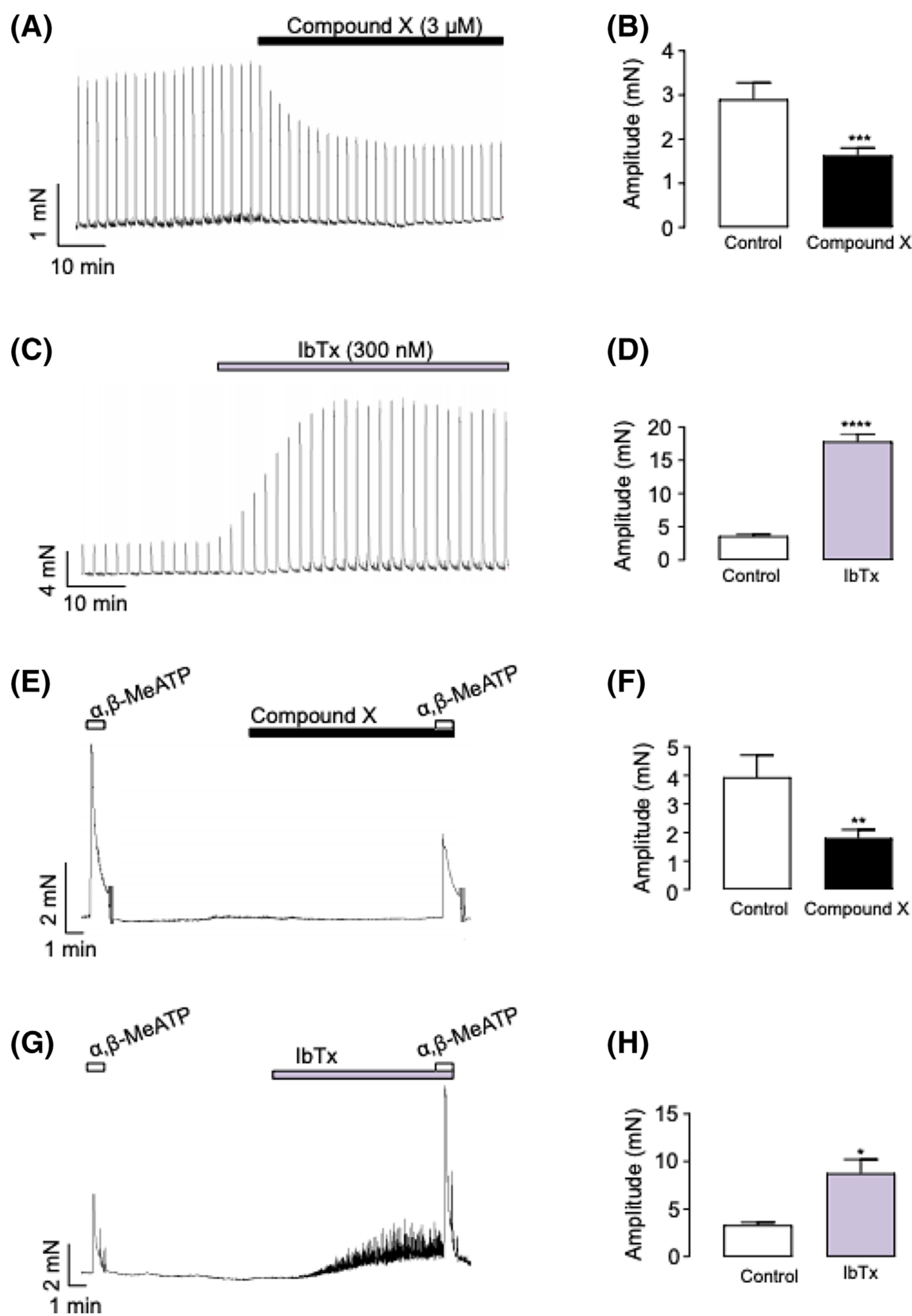

FIGURE 3 Purinergic nerve-evoked contractions of the detrusor are affected by BK channel modulators. (A, B) Representative trace and corresponding summary bar chart showing effect of the BK channel opener, compound X (3 μM) on EFS-evoked contractions of the detrusor in the presence of atropine (1 μM). (C, D) Representative trace and corresponding summary bar chart showing effect of iberiotoxin (300 nM) on purinergic nerve-evoked contractions of the detrusor. (E, F) Representative trace and corresponding summary bar chart showing effect of compound X (3 μM) on contractions of the detrusor induced by α,β-methylene (1 μM). (G, H) Representative trace and corresponding summary bar chart showing effect of iberiotoxin (300 nM) on contractions of the detrusor induced by α,β-methylene (1 μM). Error bars represent SEM. $^{**}p < 0.01$; $^{***}p < 0.001$; $^{****}p < 0.0001$.

reduced the mean amplitude of EFS-induced contractions from 2.14 ± 0.51 to 1.2 ± 0.32 mN, and when added for a second time in the absence of XE991, responses were reduced from 2.2 ± 0.59 to 1.2 ± 0.25 mN ($p < 0.05$, $n = 5$). These data indicate that blockade of Kv7 channels reduced the inhibitory actions of EPAC, but not PKA, on purinergic contractions of the detrusor.

The representative traces in Figure 7A,D indicate that blockade of BK channels with IbTx only slightly reduced the inhibitory effects of 6-MB-cAMP and 007-AM on purinergic contractions of the detrusor. Summary data in Figure 7B,C indicate that 6-MB-cAMP reduced mean contraction amplitude by 64 ± 2% under control conditions, compared with 55 ± 2% in IbTx ($n = 6$; $N = 6$; $p < 0.05$; Wilcoxon test). 007-AM reduced mean contraction amplitude by 50 ± 3% under control conditions (Figure 7E) compared with 41 ± 2% in IbTx (Figure 7F; $n = 6$; $N = 6$; $p < 0.05$; Wilcoxon test).

The data in Figures 6 and 7 show that the inhibitory effects of 6-MB-cAMP on purinergic contractions of the detrusor were not prevented by addition of either XE991 or IbTx alone. However, co-application of both drugs greatly reduced the effects of 6-MB-cAMP (Figure 8A–C). In control conditions, 6-MB-cAMP reduced mean contraction amplitude by 63 ± 3% ($n = 7$; $N = 5$; Figure 8B), whereas, in the presence of XE991 and IbTx, only an 11%

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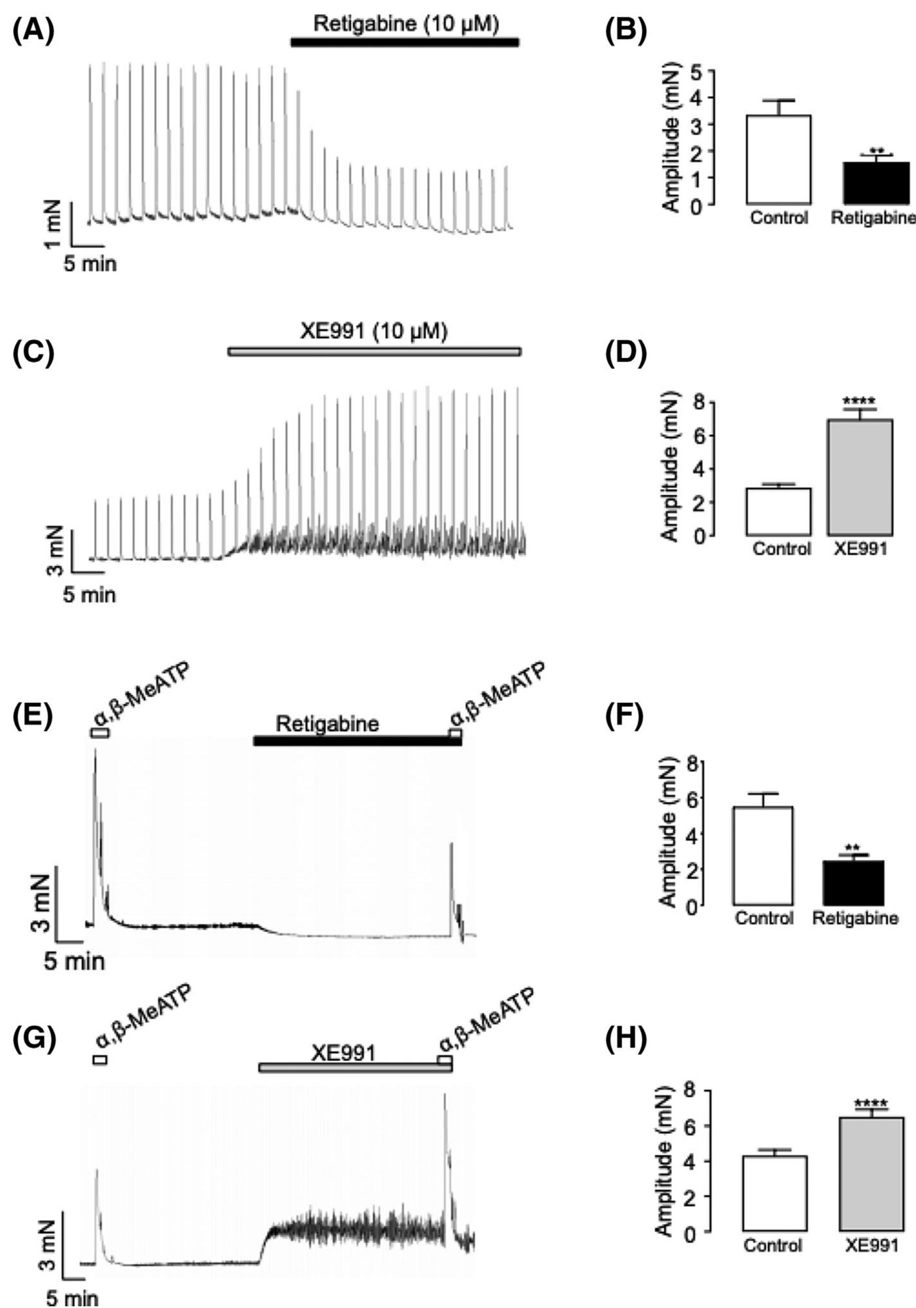

FIGURE 4 Purinergic nerve-evoked contractions of the detrusor are affected by Kv7 channel modulators. (A, B) Representative trace and corresponding summary bar chart showing effect of the Kv7 channel opener, retigabine (10 μM) on EFS-evoked contractions of the detrusor in the presence of atropine (1 μM). (C, D) Representative trace and corresponding summary bar chart showing effect of XE991 (10 μM) on purinergic nerve-evoked contractions of the detrusor. (E, F) Representative trace and corresponding summary bar chart showing effect of retigabine (10 μM) on contractions of the detrusor induced by α,β-methylene (1 μM). (G, H) Representative trace and corresponding summary bar chart showing effect of XE991 (10 μM) on contractions of the detrusor induced by α,β-methylene (1 μM). Error bars represent SEM. $^{**}p < 0.01$; $^{****}p < 0.0001$.

reduction in amplitude was observed with 6-MB-cAMP (Figure 8C). 007-AM reduced mean contraction amplitude by 50 ± 3% under control conditions (Figure 8E) compared with 18 ± 1% in IbTx + XE991 (Figure 8F; $n = 6$; $N = 4$). Therefore, co-addition of IbTx with XE991 did not result in a greater reduction in the effects of 007-AM, compared with the effect of XE991 alone.

4 | DISCUSSION

The present study demonstrated that purinergic contractions of the detrusor were inhibited by Kv7 and BK channel activators and augmented by Kv7 and BK channel blockers. Activators of both PKA and EPAC inhibited the purinergic responses; however, the effects of EPAC activation were greatly attenuated by blockade of Kv7 channels, whereas the PKA effects were not. In contrast, blockade of BK channels had little effect on the inhibitory actions elicited by activation of EPAC or PKA. These findings complement those of Fong et al. which reported that EPAC activation inhibited P2X1 currents in detrusor myocytes,[23] suggesting that EPAC can inhibit purinergic contractions of the bladder via multiple pathways.

Some studies have reported that purinergic contractions of smooth muscle involve Ca^{2+} release from

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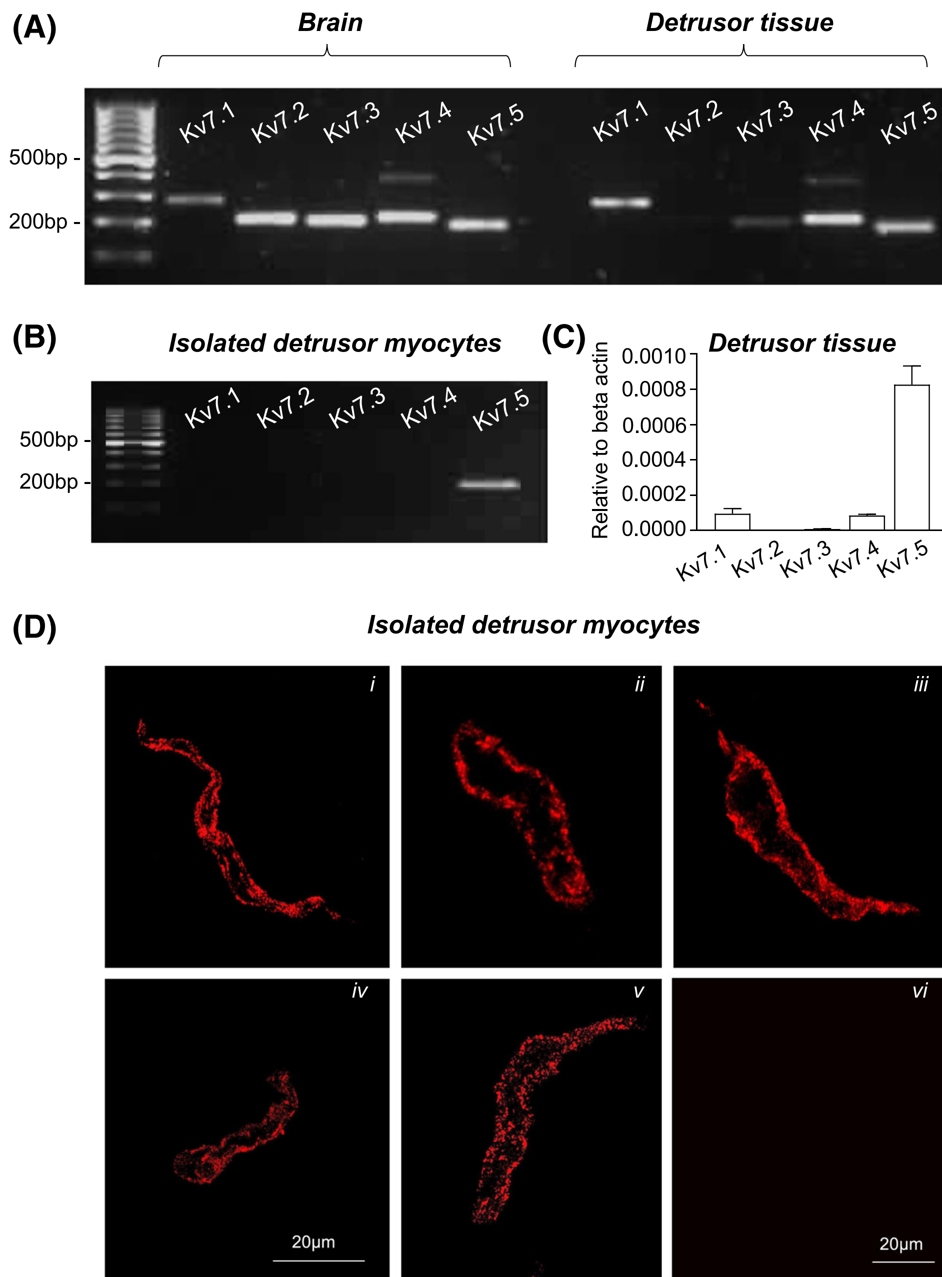

FIGURE 5 Transcriptional expression of Kv7.1–7.5 in the murine detrusor. Representative electrophoresis gels displaying amplification products from RNA derived from mouse brain and detrusor tissue (A), and isolated detrusor myocytes (B) using gene specific primers for Kv7.1–Kv7.5. (C) Summary bar chart plotting relative expression levels of Kv7.1–Kv7.5 (rel. β-actin) in murine detrusor strips using real-time quantitative PCR. (D, i–v) Immunocytochemical staining of isolated detrusor myocytes with anti-Kv7.5 antibody ($n = 5$, $N = 3$). No immunoreactivity was observed in cells treated with secondary antibody only and imaged using the same parameters (representative example in D, vi).

intracellular stores. For example, Hashitani et al. reported that co-application of caffeine and ryanodine inhibited nifedipine-resistant purinergic responses in rat detrusor,[13] and Brain et al. reported that Ca^{2+} influx via P2X1 channels stimulated Ca^{2+}-induced Ca^{2+} release (CICR) via ryanodine receptors in murine vas deferens myocytes.[34] However, the findings of our study showed that purinergic responses were not inhibited by the ORAI blocker, GSK7975A, or the SERCA inhibitor, CPA. Instead, we found that the purinergic responses were greatly attenuated by nifedipine, in agreement with previous studies.[13,27,35–39] Therefore, it appears that the main influence of P2X1 receptor activation in murine detrusor myocytes is to induce depolarization of membrane potential and activate voltage-gated Ca^{2+} channels rather than stimulate Ca^{2+} release from intracellular stores.

There is a significant body of evidence which indicates that bladder excitability is reduced by activation of Kv7 channels; therefore, Kv7 channel activation has been proposed as a therapeutic approach to alleviate the symptoms associated with overactive bladder.[40] Kv7 transcripts have been detected in bladder tissues from several species including rat,[41] guinea pig,[20,42] pig,[43] and humans.[44–48] Cystometry studies in rats showed that the Kv7 channel activator retigabine increased micturition volume and voiding intervals,[41,48] and Brickel et al. reported that urinary retention-related adverse events were experienced by some patients receiving retigabine for treatment of

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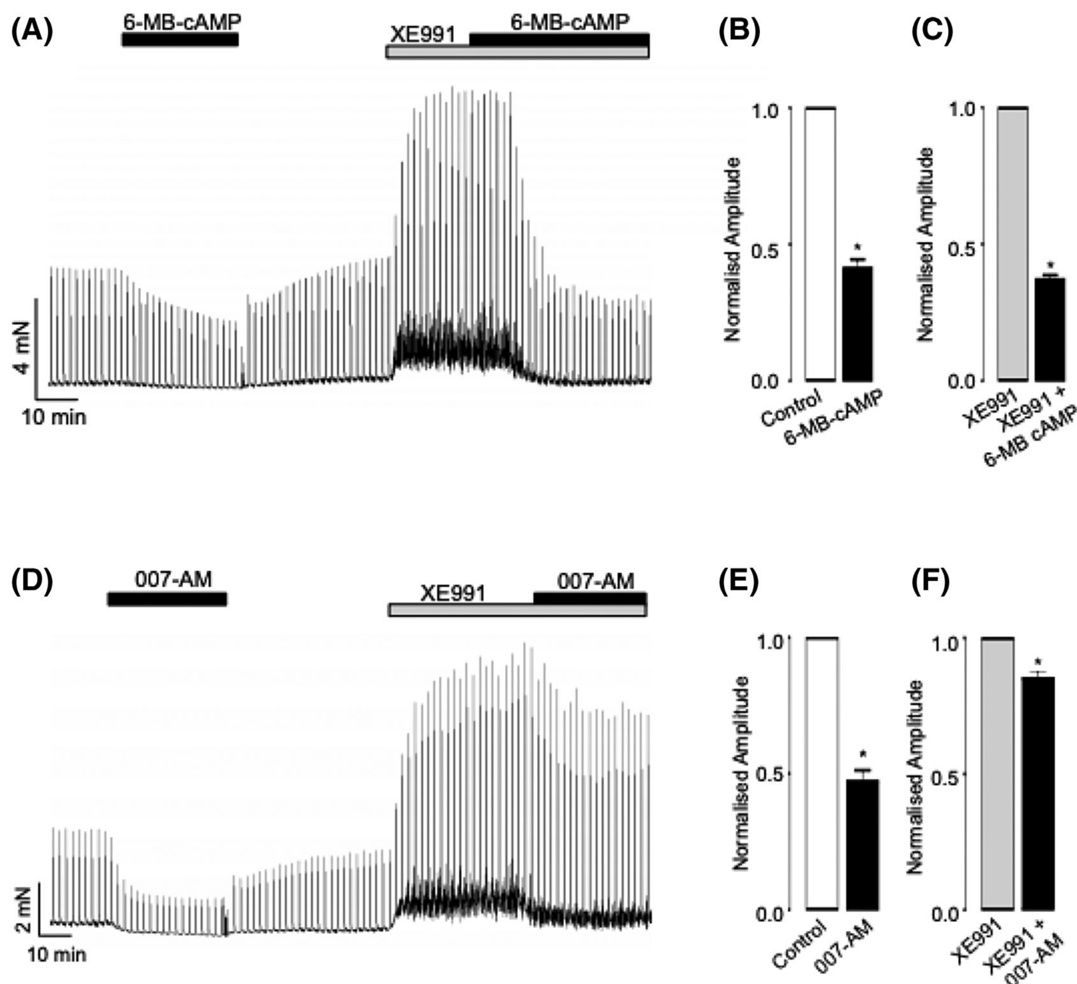

FIGURE 6 Comparison of the effects of 007-AM on purinergic nerve-evoked contractions of the detrusor in the absence and presence of iberiotoxin and XE991. Representative trace (A) and summary bar charts showing effect of 007-AM (10 μM) on EFS-evoked contractions of the detrusor in the absence (B) and presence of iberiotoxin (300 nM) (C). Representative trace (D) and summary bar charts showing effect of 007-AM (10 μM) on EFS-evoked contractions of the detrusor in the absence (E) and presence of XE991 (10 μM) (F). Error bars represent SEM. *$p < 0.05$.

epilepsy in clinical trials.[49] However, while several studies report that Kv7 channel modulators affected the contractility of detrusor strips,[43,46,50,51] there is debate on whether these effects were mediated by effects on Kv7 channels in detrusor myocytes. For example, Tykocki et al. reported that murine urinary bladder smooth muscle cells had no measurable Kv7 channel currents and instead proposed that the effects of retigabine on bladder function arose from inhibition of afferent nerve activity.[52] Tykocki et al. also reported that retigabine reduced the amplitude of voltage-dependent Ca^{2+} currents in isolated murine detrusor myocytes by ∼20% and that this could mediate the inhibitory effects of retigabine on detrusor contractility.[52] However, it is worth noting that a range of Kv7 channel openers have been reported to reduce bladder contractility, including flupertine,[42,47] ML213,[45,53] ML277,[45] meclofenamic acid,[42,54] ICA-069673,[53] and L-364373.[43] Furthermore, the effects of retigabine in some studies were reversed by XE991[41,44] pointing to a Kv7-selective action of retigabine. Nevertheless, it is clear that this issue warrants further investigation, and we cannot discount the possibility that some of the effects of retigabine observed in the present study could involve direct inhibition of LTCC.

The results of the present study indicate that activation of Kv7 channels may play a role in EPAC-dependent inhibition of purinergic contractions of the detrusor, and therefore, it is conceivable that this may contribute to the inhibitory actions of β3-AR agonists on bladder contractility. This idea is consistent with observations in vascular smooth muscle in which β-AR-mediated relaxations of rat mesenteric arteries were found to involve EPAC-dependent activation of Kv7 channels.[55] Their study found that the effects of EPAC were mediated by activation of

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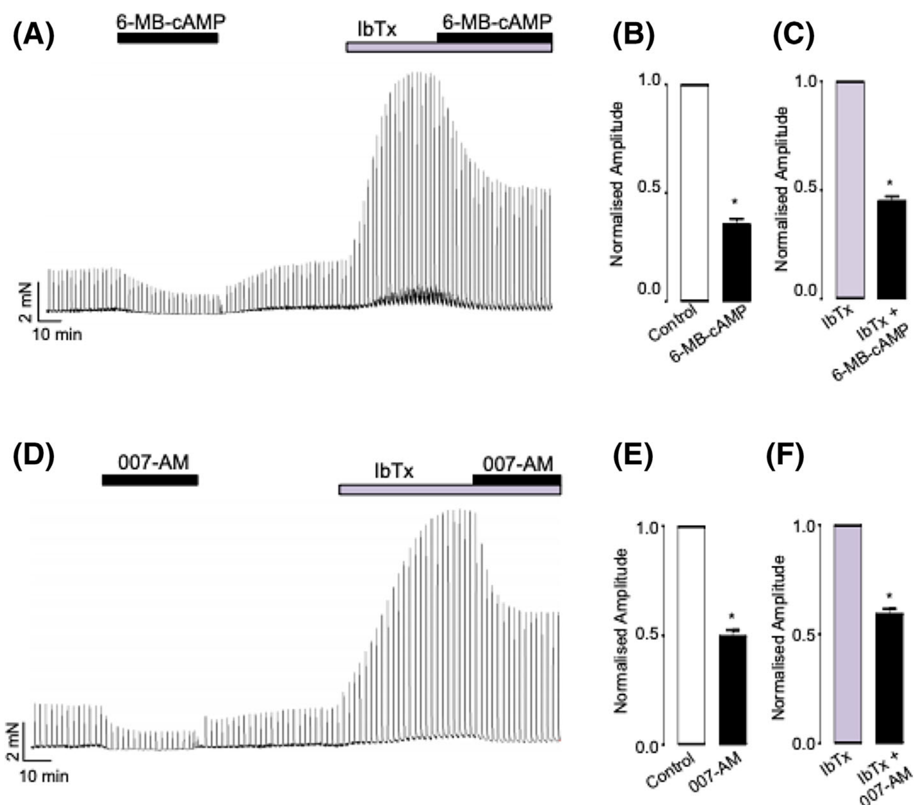

FIGURE 7 Comparison of the effects of 6-MB-cAMP on purinergic nerve-evoked contractions of the detrusor in the absence and presence of iberiotoxin and XE991. Representative trace (A) and summary bar charts showing effect of 6-MB-cAMP (100 μM) on EFS-evoked contractions of the detrusor in the absence (B) and presence of iberiotoxin (300 nM) (C). Representative trace (D) and summary bar charts showing effect of 6-MB-cAMP (100 μM) on EFS-evoked contractions of the detrusor in the absence (E) and presence of XE991 (10 μM) (F). Error bars represent SEM. *$p < 0.05$.

Kv7.4 channels, whereas we found that Kv7.5 was predominantly expressed in intact detrusor strips and was the only Kv7 subtype detected in isolated detrusor myocytes. XE991 diminished the inhibitory effects of 007-AM, but not those of 6-MB-cAMP, on purinergic contractions of the detrusor, indicating that activation of Kv7 channels may be involved in the inhibitory effects of EPAC, but not PKA. Alternatively, since XE991 can induce depolarization of the detrusor,[21] it is possible that the diminished effects 007-AM in the presence of XE991 may reflect an inability of 007-AM to inhibit purinergic responses when the tissue is depolarized. Clearly, more work is required to establish if EPAC activators activate Kv7 channels in detrusor myocytes and, if so, to determine the precise cellular mechanism by which this occurs.

The results of the present study are consistent with others which find that BK channels are important regulators of purinergic contractions of the detrusor.[18,19] However, despite several studies which report that the inhibitory actions of PKA on detrusor contractility involve BK channel activation,[56,57] we found that the inhibitory effects of 6-MB-cAMP in the present study were unaffected by blockade of BK channels. Similarly, the effects of 6-MB-cAMP were not reduced by XE991, suggesting that activation of Kv7 channels was not a factor in PKA-dependent inhibition of purinergic contractions of the detrusor. Interestingly, however, co-addition of XE991 and IbTx nearly abolished 6-MB-cAMP responses. Therefore, it is tempting to speculate that PKA could activate both Kv7 and BK channels and that the reason for the lack of effect of either blocker, when applied alone, is that activation of either population of channels could compensate for blockade of the other. It is unclear whether both types of channels would be activated under control conditions or whether each channel is only activated by PKA when the other channel is blocked. There are examples in the literature of the action of some K^+ channels only being revealed when other types of K^+ channels are blocked. For example, the influence of Kv2 channels on repolarization of action potentials in rabbit urethral myocytes was only observed when BK channels were blocked with penitrem A.[58] Similarly, the contribution of BK channels to vasodilation of murine mesenteric and saphenous arteries induced by GoSlo 5–6 was only revealed when Kv7 channels were blocked with XE991.[59] Therefore, it is apparent that PKA and EPAC are capable of inhibiting purinergic contractions of the detrusor by different mechanisms and that more work is required to elucidate the precise mechanisms underlying the inhibitory of effects of PKA on purinergic contractions of the detrusor.

In summary, our data suggest that activation of EPAC inhibits purinergic contractions of the detrusor via a mechanism involving activation of Kv7 channels which opposes the depolarizing influence of P2X1 receptor activation on membrane potential.

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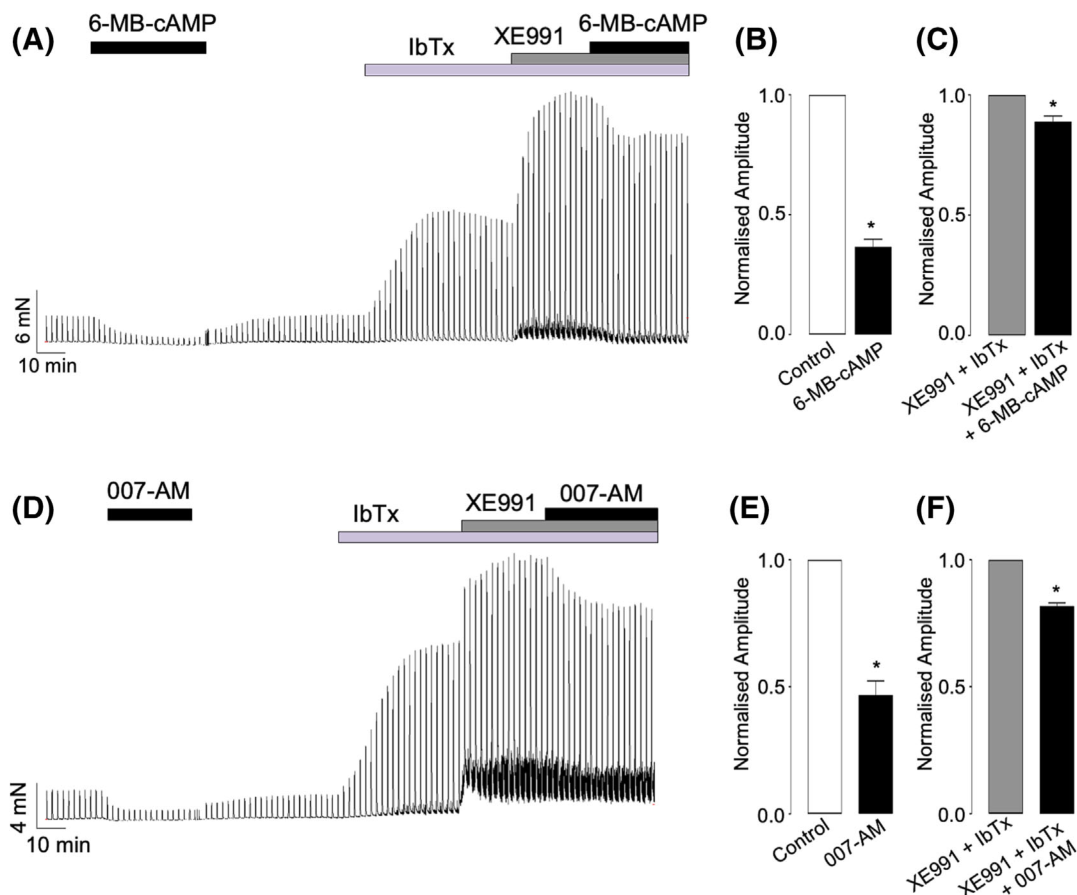

FIGURE 8 Comparison of the effects of 6-MB-cAMP and 007-AM on purinergic nerve-evoked contractions of the detrusor before and during co-application of iberiotoxin and XE991. Representative trace (A) and summary bar charts showing effect of 6-MB-cAMP (100 μM) on EFS-evoked contractions of the detrusor in the absence (B) and presence of iberiotoxin (300 nM) plus XE991 (10 μM) (C). Representative trace (D) and summary bar charts showing effect of 007-AM (10 μM) on EFS-evoked contractions of the detrusor in the absence (E) and presence of iberiotoxin (300 nM) plus XE991 (10 μM) (F). Error bars represent SEM. *$p < 0.05$.

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CONFLICT OF INTEREST STATEMENT

There were no conflicts of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author, GS, upon reasonable request.

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REFERENCES

1. Burnstock G. Purinergic signalling in the urinary tract in health and disease. *Purinergic Signal*. 2014;10(1):103-155. doi:10.1007/s11302-013-9395-y
2. Vial C, Evans RJ. P2X receptor expression in mouse urinary bladder and the requirement of P2X(1) receptors for functional P2X receptor responses in the mouse urinary bladder smooth muscle. *Br J Pharmacol*. 2000;131(7):1489-1495. doi:10.1038/sj.bjp.0703720
3. Matsui M, Motomura D, Karasawa H, et al. Multiple functional defects in peripheral autonomic organs in mice lacking muscarinic acetylcholine receptor gene for the M3 subtype. *Proc Natl Acad Sci U S A*. 2000;97(17):9579-9584. doi:10.1073/pnas.97.17.9579
4. Matsui M, Motomura D, Fujikawa T, et al. Mice lacking M2 and M3 muscarinic acetylcholine receptors are devoid of cholinergic smooth muscle contractions but still viable. *J Neurosci*. 2002;22(24):10627-10632. doi:10.1523/JNEUROSCI.22-24-10627.2002
5. Andersson KE. Purinergic signalling in the urinary bladder. *Auton Neurosci*. 2015;191:78-81. doi:10.1016/j.autneu.2015.04.012

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6. Burnstock G. Purinergic signalling: therapeutic developments. *Front Pharmacol*. 2017;25(8):661. doi:10.3389/fphar.2017.00661
7. Palea S, Artibani W, Ostardo E, Trist DG, Pietra C. Evidence for purinergic neurotransmission in human urinary bladder affected by interstitial cystitis. *J Urol*. 1993;150(6):2007-2012. doi:10.1016/S0022-5347(17)35955-4
8. Smith DJ, Chapple CR. In vitro response of human bladder smooth muscle in unstable obstructed male bladders: a study of pathophysiological causes. *NeurourolUrodyn*. 1994;13:414-415.
9. O'Reilly BA, Kosaka AH, Knight GE, et al. P2X receptors and their role in female idiopathic detrusor instability. *J Urol*. 2002;167(1):157-164. doi:10.1016/S0022-5347(05)65403-1
10. Andersson KE, Hedlund P. Pharmacologic perspective on the physiology of the lower urinary tract. *Urology*. 2002;60(5):13-20. doi:10.1016/S0090-4295(02)01786-7
11. McCarthy CJ, Marangos C, Fry CH, Ikeda Y. ATP transients accompany spontaneous contractions in isolated guinea-pig detrusor smooth muscle. *Exp Physiol*. 2019;104(11):1717-1725. doi:10.1113/EP087960
12. McCarthy CJ, Ikeda Y, Skennerton D, et al. Characterisation of nerve-mediated ATP release from bladder detrusor muscle and its pathological implications. *Br J Pharmacol*. 2019; 176(24):4720-4730. doi:10.1111/bph.14840
13. Hashitani H, Bramich NJ, Hirst GD. Mechanisms of excitatory neuromuscular transmission in the guinea-pig urinary bladder. *J Physiol*. 2000;524(Pt 2):565-579. doi:10.1111/j.1469-7793.2000.t01-2-00565.x
14. Heppner TJ, Bonev AD, Nelson MT. Ca(2+)-activated K+ channels regulate action potential repolarization in urinary bladder smooth muscle. *Am J Physiol*. 1997;273(1 Pt 1):C110-C117. doi:10.1152/ajpcell.1997.273.1.C110
15. Hashitani H, Brading AF. Ionic basis for the regulation of spontaneous excitation in detrusor smooth muscle cells of the guinea-pig urinary bladder. *Br J Pharmacol*. 2003;140(1):159-169. doi:10.1038/sj.bjp.0705320
16. Herrera GM, Etherton B, Nausch B, Nelson MT. Negative feedback regulation of nerve-mediated contractions by KCa channels in mouse urinary bladder smooth muscle. *Am J Physiol Regul Integr Comp Physiol*. 2005;289(2):R402-R409. doi:10.1152/ajpregu.00488.2004
17. Brading AF, Brain KL. Ion channel modulators and urinary tract function. *Handb Exp Pharmacol*. 2011;(202):375-393. doi:10.1007/978-3-642-16499-6_18
18. Thorneloe KS, Meredith AL, Knorn AM, Aldrich RW, Nelson MT. Urodynamic properties and neurotransmitter dependence of urinary bladder contractility in the BK channel deletion model of overactive bladder. *Am J Physiol Renal Physiol*. 2005;289(3):F604-F610. doi:10.1152/ajprenal.00060.2005
19. Werner ME, Knorn AM, Meredith AL, Aldrich RW, Nelson MT. Frequency encoding of cholinergic- and purinergic-mediated signaling to mouse urinary bladder smooth muscle: modulation by BK channels. *Am J Physiol Regul Integr Comp Physiol*. 2007;292(1):R616-R624. doi:10.1152/ajpregu.00036.2006
20. Anderson UA, Carson C, Johnston L, Joshi S, Gurney AM, McCloskey KD. Functional expression of KCNQ (Kv7) channels in guinea pig bladder smooth muscle and their contribution to spontaneous activity. *Br J Pharmacol*. 2013;169(6):1290-1304. doi:10.1111/bph.12210
21. Takagi H, Hashitani H. Effects of K^+ channel openers on spontaneous action potentials in detrusor smooth muscle of the guinea-pig urinary bladder. *Eur J Pharmacol*. 2016;15(789): 179-186. doi:10.1016/j.ejphar.2016.07.041
22. Malysz J, Petkov GV. Detrusor smooth muscle K_V7 channels: emerging new regulators of urinary bladder function. *Front Physiol*. 2020;16(11):1004. doi:10.3389/fphys.2020.01004
23. Fong Z, Griffin CS, Hollywood MA, Thornbury KD, Sergeant GP. β_3-Adrenoceptor agonists inhibit purinergic receptor-mediated contractions of the murine detrusor. *Am J Physiol Cell Physiol*. 2019;317(1):C131-C142. doi:10.1152/ajpcell.00488.2018
24. Fong Z, Griffin CS, Large RJ, Hollywood MA, Thornbury KD, Sergeant GP. Regulation of P2X1 receptors by modulators of the cAMP effectors PKA and EPAC. *Proc Natl Acad Sci U S A*. 2021;118(37):e2108094118. doi:10.1073/pnas.2108094118
25. Tveden-Nyborg P, Bergmann TK, Jessen N, Simonsen U, Lykkesfeldt J. BCPT policy for experimental and clinical studies. *Basic Clin Pharmacol Toxicol*. 2021;128(1):4-8. doi:10.1111/bcpt.13492
26. Ponte CG, McManus OB, Schmalhofer WA, et al. Selective, direct activation of high-conductance, calcium-activated potassium channels causes smooth muscle relaxation. *Mol Pharmacol*. 2012;81(4):567-577. doi:10.1124/mol.111.075853
27. Acevedo CG, Contreras E. Effect of extracellular calcium and calcium channel antagonists on ATP and field stimulation induced contractions of the mouse urinary bladder. *Gen Pharmacol*. 1989;20(6):811-815. doi:10.1016/0306-3623(89)90334-0
28. Derler I, Schindl R, Fritsch R, et al. The action of selective CRAC channel blockers is affected by the Orai pore geometry. *Cell Calcium*. 2013;53(2):139-151. doi:10.1016/j.ceca.2012.11.005
29. Ziganshin AU, Hoyle CH, Ziganshina LE, Burnstock G. Effects of cyclopiazonic acid on contractility and ecto-ATPase activity in guinea-pig urinary bladder and vas deferens. *Br J Pharmacol*. 1994;113(3):669-674. doi:10.1111/j.1476-5381.1994.tb17044.x
30. Tatulian L, Delmas P, Abogadie FC, Brown DA. Activation of expressed KCNQ potassium currents and native neuronal M-type potassium currents by the anti-convulsant drug retigabine. *J Neurosci*. 2001;21(15):5535-5545. doi:10.1523/JNEUROSCI.21-15-05535.2001
31. Schenzer A, Friedrich T, Pusch M, et al. Molecular determinants of KCNQ (K_v7) K^+ channel sensitivity to the anticonvulsant retigabine. *J Neurosci*. 2005;25(20):5051-5060. doi:10.1523/JNEUROSCI.0128-05.2005
32. Wuttke TV, Seebohm G, Bail S, Maljevic S, Lerche H. The new anticonvulsant retigabine favors voltage-dependent opening of the Kv7.2 (KCNQ2) channel by binding to its activation gate. *Mol Pharmacol*. 2005;67(4):1009-1017. doi:10.1124/mol.104.010793
33. Robbins J. KCNQ potassium channels: physiology, pathophysiology, and pharmacology. *Pharmacol Ther*. 2001;90(1):1-19. doi:10.1016/S0163-7258(01)00116-4
34. Brain KL, Cuprian AM, Williams DJ, Cunnane TC. The sources and sequestration of Ca^{2+} contributing to neuroeffector Ca^{2+} transients in the mouse vas deferens. *J Physiol*. 2003; 553(Pt 2):627-635. doi:10.1113/jphysiol.2003.049734
35. Bhat MB, Mishra SK, Raviprakash V. Sources of calcium for ATP-induced contractions in rat urinary bladder smooth

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muscle. *Eur J Pharmacol*. 1989;164(1):163-166. doi:10.1016/0014-2999(89)90244-6
36. Katsuragi T, Usune S, Furukawa T. Antagonism by nifedipine of contraction and Ca2(+)-influx evoked by ATP in guinea-pig urinary bladder. *Br J Pharmacol*. 1990;100(2):370-374. doi:10.1111/j.1476-5381.1990.tb15811.x
37. Bo XN, Burnstock G. The effects of Bay K 8644 and nifedipine on the responses of rat urinary bladder to electrical field stimulation, beta,gamma-methylene ATP and acetylcholine. *Br J Pharmacol*. 1990;101(2):494-498. doi:10.1111/j.1476-5381.1990.tb12736.x
38. Kura H, Obara K, Yabu H. Contractile responses to electrical field stimulation and ATP in guinea-pig urinary bladder. *Comp Biochem Physiol C Comp Pharmacol Toxicol*. 1992;102(1):193-197. doi:10.1016/0742-8413(92)90063-D
39. Hashitani H, Suzuki H. Electrical and mechanical responses produced by nerve stimulation in detrusor smooth muscle of the guinea-pig. *Eur J Pharmacol*. 1995;284(1–2):177-183. doi:10.1016/0014-2999(95)00386-Y
40. Haick JM, Byron KL. Novel treatment strategies for smooth muscle disorders: targeting Kv7 potassium channels. *Pharmacol Ther*. 2016;165:14-25. doi:10.1016/j.pharmthera.2016.05.002
41. Svalø J, Hansen HH, Rønn LC, Sheykhzade M, Munro G, Rode F. Kv7 positive modulators reduce detrusor overactivity and increase bladder capacity in rats. *Basic Clin Pharmacol Toxicol*. 2012;110(2):145-153. doi:10.1111/j.1742-7843.2011.00765.x
42. Afeli SA, Malysz J, Petkov GV. Molecular expression and pharmacological evidence for a functional role of kv7 channel subtypes in guinea pig urinary bladder smooth muscle. *PLoS ONE*. 2013;8(9):e75875. doi:10.1371/journal.pone.0075875
43. Svalø J, Bille M, Parameswaran Theepakaran N, Sheykhzade M, Nordling J, Bouchelouche P. Bladder contractility is modulated by Kv7 channels in pig detrusor. *Eur J Pharmacol*. 2013;715(1–3):312-320. doi:10.1016/j.ejphar.2013.05.005
44. Svalø J, Sheykhzade M, Nordling J, Matras C, Bouchelouche P. Functional and molecular evidence for Kv7 channel subtypes in human detrusor from patients with and without bladder outflow obstruction. *PLoS ONE*. 2015;10(2):e0117350. doi:10.1371/journal.pone.0117350
45. Provence A, Malysz J, Petkov GV. The novel KV7.2/KV7.3 channel opener ICA-069673 reveals subtype-specific functional roles in guinea pig detrusor smooth muscle excitability and contractility. *J Pharmacol Exp Ther*. 2015;354(3):290-301. doi:10.1124/jpet.115.225268
46. Bientinesi R, Mancuso C, Martire M, Bassi PF, Sacco E, Currò D. K_V7 channels in the human detrusor: channel modulator effects and gene and protein expression. *Naunyn Schmiedebergs Arch Pharmacol*. 2017;390(2):127-137. doi:10.1007/s00210-016-1312-9
47. Seefeld MA, Lin H, Holenz J, et al. Novel K_V7 ion channel openers for the treatment of epilepsy and implications for detrusor tissue contraction. *Bioorg Med Chem Lett*. 2018;28(23–24):3793-3797. doi:10.1016/j.bmcl.2018.09.036
48. Streng T, Christoph T, Andersson K-E. Urodynamic effects of the K^+ channel (KCNQ) opener retigabine in freely moving, conscious rats. *J Urol*. 2004;172(5 Pt 1):2054-2058. doi:10.1097/01.ju.0000138155.33749.f4
49. Brickel N, Gandhi P, VanLandingham K, Hammond J, DeRossett S. The urinary safety profile and secondary renal effects of retigabine (ezogabine): a first-in-class antiepileptic drug that targets KCNQ (K_v7) potassium channels. *Epilepsia*. 2012;53(4):606-612. doi:10.1111/j.1528-1167.2012.03441.x
50. Rode F, Svalø J, Sheykhzade M, Rønn LC. Functional effects of the KCNQ modulators retigabine and XE991 in the rat urinary bladder. *Eur J Pharmacol*. 2010;638(1–3):121-127. doi:10.1016/j.ejphar.2010.03.050
51. Wang Y, Tar MT, Shibo F, Melman A, Davies KP. Diabetes attenuates urothelial modulation of detrusor contractility and spontaneous activity. *Int J Urol*. 2014;(10):1059-1064. doi:10.1111/iju.12491
52. Tykocki NR, Heppner TJ, Dalsgaard T, Bonev AD, Nelson MT. The K_V7 channel activator retigabine suppresses mouse urinary bladder afferent nerve activity without affecting detrusor smooth muscle K^+ channel currents. *J Physiol*. 2019;597(3):935-950. doi:10.1113/JP277021
53. Provence A, Angoli D, Petkov GV. K_V7 channel pharmacological activation by the novel activator ML213: role for Heteromeric $K_V7.4/K_V7.5$ channels in guinea pig detrusor smooth muscle function. *J Pharmacol Exp Ther*. 2018;364(1):131-144. doi:10.1124/jpet.117.243162
54. Anderson UA, Carson C, McCloskey KD. KCNQ currents and their contribution to resting membrane potential and the excitability of interstitial cells of Cajal from the guinea pig bladder. *J Urol*. 2009;182(1):330-336. doi:10.1016/j.juro.2009.02.108
55. Stott JB, Barrese V, Greenwood IA. Kv7 channel activation underpins EPAC-dependent relaxations of rat arteries. *Arterioscler Thromb Vasc Biol*. 2016;36(12):2404-2411. doi:10.1161/ATVBAHA.116.308517
56. Petkov GV, Nelson MT. Differential regulation of Ca^{2+}-activated K^+ channels by β-adrenoceptors in guinea pig urinary bladder smooth muscle. *Am J Physiol Cell Physiol*. 2005;288(6):C1255-C1263. doi:10.1152/ajpcell.00381.2004
57. Xin W, Li N, Cheng Q, Fernandes VS, Petkov GV. Constitutive PKA activity is essential for maintaining the excitability and contractility in guinea pig urinary bladder smooth muscle: role of the BK channel. *Am J Physiol Cell Physiol*. 2014;307(12):C1142-C1150. doi:10.1152/ajpcell.00167.2014
58. Kyle B, Bradley E, Ohya S, et al. Contribution of Kv2.1 channels to the delayed rectifier current in freshly dispersed smooth muscle cells from rabbit urethra. *Am J Physiol Cell Physiol*. 2011;301(5):C1186-C1200. doi:10.1152/ajpcell.00455.2010
59. Zavaritskaya O, Dudem S, Ma D, et al. Vasodilation of rat skeletal muscle arteries by the novel BK channel opener GoSlo is mediated by the simultaneous activation of BK and K_v7 channels. *Br J Pharmacol*. 2020;177(5):1164-1186. doi:10.1111/bph.14910

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