K_V7 CHANNELS IN RAT URINARY BLADDER SMOOTH MUSCLE: EXPRESSION AND ROLE IN EXCITABILITY AND CONTRACTILITY

Sarah E. Maxwell[1], Wenkuan Xin[1], Rana Ajeeb[1], Frank Park[1], John Malysz[1], Georgi V. Petkov[1]

[1]Department of Pharmaceutical Sciences, College of Pharmacy, University of Tennessee Health Science Center, Memphis, TN

Abstract

INTRODUCTION AND OBJECTIVE: Voltage-gated K+ (K_V) type 7 channels (K_V7.1–K_V7.5/KCNQ1-5) regulate various body functions and display organ or cell type specific expression profile. Our group has initiated studies aimed at elucidating the roles of K_V7 channels in urinary bladder smooth muscle (UBSM) focusing primarily on the guinea pig animal model where all K_V7 channels are expressed and contribute to function. Given potential interspecies differences and translational value of the rat model in pre-clinical drug discovery, we are now investigating the role of K_V7 channels in this species. We hypothesize that K_V7 channels are expressed in rat UBSM, and their activation attenuates UBSM excitability and contractility.

METHODS: UBSM tissue strips without mucosa were obtained from adult male Sprague Dawley rats. UBSM homogenates were used in Western blots to detect K_V7 channel subtypes. UBSM strip contractility recordings were conducted under isometric tension conditions. Single UBSM cells were freshly isolated using enzymatic treatment for immunocytochemistry and amphotericin-B perforated current-clamp (I=0) electrophysiology.

RESULTS: Retigabine (K_V7.2-K_V7.5 activator), ICA-069673 (selective K_V7.2/K_V7.3 activator, also active on K_V7.4), and ML213 (preferential K_V7.2 and K_V7.4 activator) all reduced spontaneous phasic, 20 mM K^+-induced, 1 μM carbachol-induced, and electrical field stimulated UBSM contractions in a concentration-dependent manner (EC_{50} values and max inhibition for amplitude, force, and frequency: 1.1 to 111 μM, 40 to 100%, n=4-13). The three activators (each tested at 10 μM) also induced membrane potential hyperpolarization in single DSM cells (Δ -7.6 to -13.8 mV, each n=5 and $p<0.05$), whereas the K_V7.1-K_V7.5 blocker XE991 (10 μM) caused depolarization (Δ +8.7 mV, n=12, $p<0.01$). In the presence of XE991, retigabine, ICA-069673, and ML213 failed to alter the membrane potential. Western blot experiments detected K_V7.4 and K_V7.5 channel proteins in UBSM and control brain tissue. Immunocytochemistry revealed that freshly isolated single UBSM cells expressed both alpha smooth muscle actin and K_V7.4 channel proteins.

CONCLUSIONS: Rat UBSM expresses K_V7 channels, particularly the K_V7.4 subtype. Pharmacological activation of UBSM K_V7 channels reduces single UBSM cell excitability via membrane potential hyperpolarization that in turn decreases UBSM contractility. In rats – just as previously reported for guinea pigs – K_V7 channels regulate UBSM function. Future studies are needed to address the role of individual K_V7 channel subtypes in rat and, more importantly, human UBSM.

Introduction

K_V7 channels (K_V7.1-K_V7.5/KCNQ1-5), a family of voltage-gated K^+ (K_V) channels, have been shown to display cell type specific expression profiles, playing roles in multiple tissues regulating function of various organs. [1]. More recently, they have emerged as key regulators of excitability and contractility in urinary bladder smooth muscle [2,3,4,5]. While the guinea pig animal model has previously been the focus of these studies, due to potential interspecies differences we have shifted to the rat model for K_V7 channel studies, increasing their translational value as well.

Objective

Our overall goal is to determine the role of UBSM KV7 channel subtypes in regulation of urinary bladder function.

Methods

Animals: Male adult Sprague Dawley rats (300 - 400 g).

Immunocytochemistry: Single UBSM cells were freshly isolated using enzymatic treatment and subsequently plated and labelled for alpha smooth muscle actin and K_V7.4 with Alexa Fluor 488 and 594 conjugated antibodies respectively.

Western Blot: Homogenates of mucosa-free UBSM tissues were used to detect K_V7 channel subtypes

Isometric UBSM tension recordings: Mucosa-free isolated UBSM strips were mounted for isometric tension recordings as previously described [3,4,5]. The effects of the K_V7 channel modulators retigabine, ICA-069673, and ML213 were evaluated on UBSM spontaneous phasic, 20 mM K^+-induced, 1 μM carbachol-induced, or electrical field stimulated (EFS) contractions.

Patch-clamping: Single UBSM cells prepared using enzymatic dissociation were used, and cell membrane potential was recorded in current-clamp mode (I=0) at room temperature using the amphotericin-B perforated patch clamp method as previously described [3,4].

Statistics: ANOVA or Student t test analyses were performed on n (number of UBSM strips or cells; N is the number of rats) to determined significance ($p<0.05$).

Results

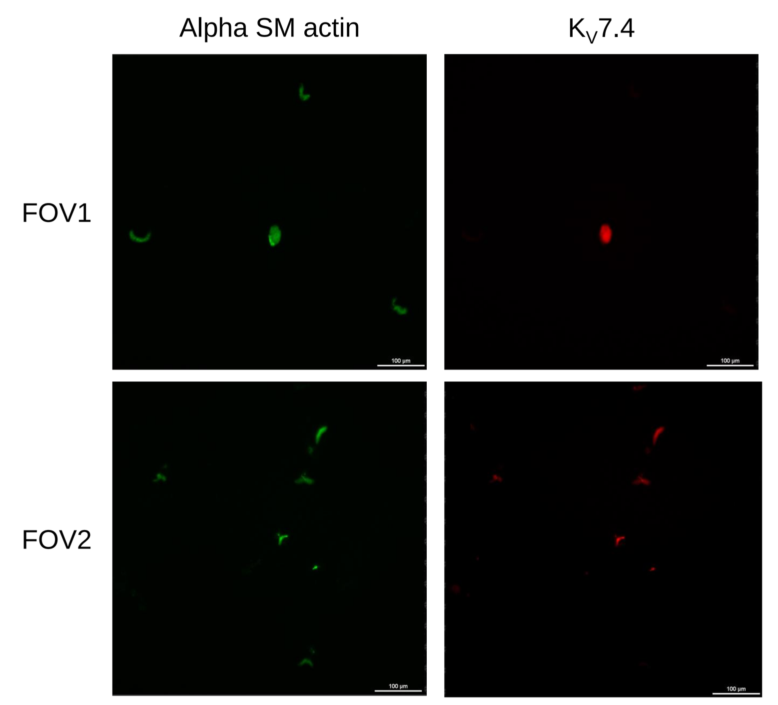


Figure 1. K_V7.4 channel proteins are detected in UBSM single cells. Immunocytochemical experiments were used to detect K_V7.4 channel protein in freshly isolated UBSM single cells using a channel specific antibody. Red staining indicates the presence of K_V7.4 and alpha smooth muscle (SM) actin shown in green. Two separate fields of view (FOV) are displayed.

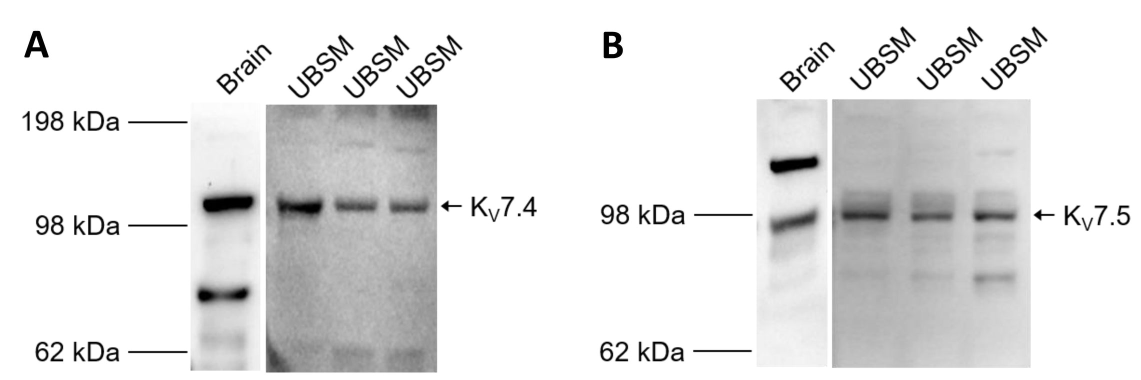


Figure 2. K_V7.4 and K_V7.5 channel proteins are present in UBSM. **A)** Western blots of UBSM showed the presence of K_V7.4 channel protein in UBSM (n=3, N=3). **B)** Western blots of UBSM showed the presence of K_V7.4 channel protein in UBSM (n=3, N=3).

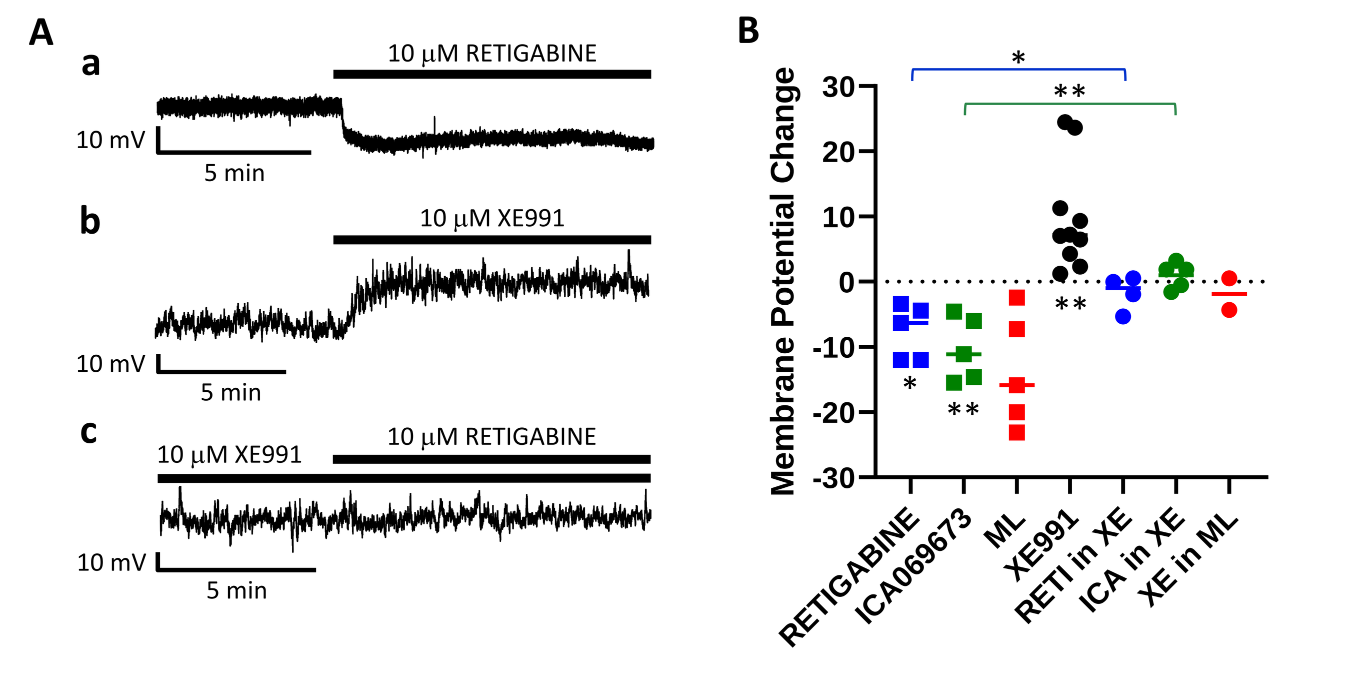
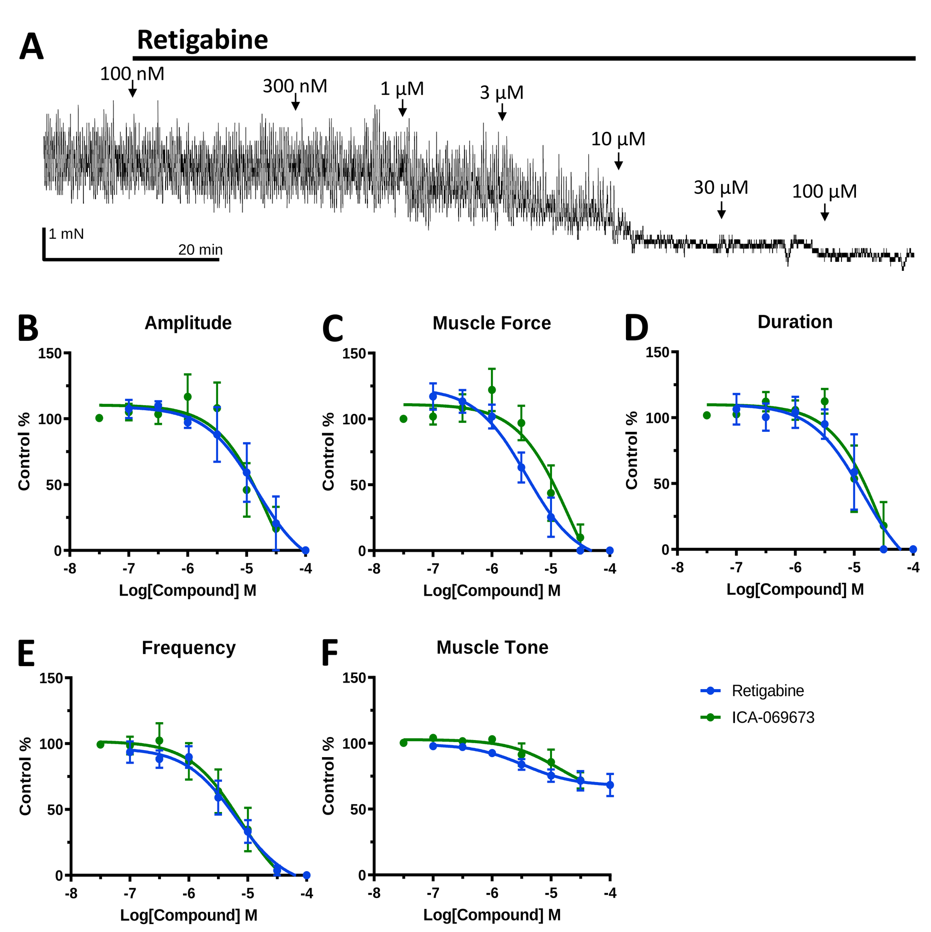
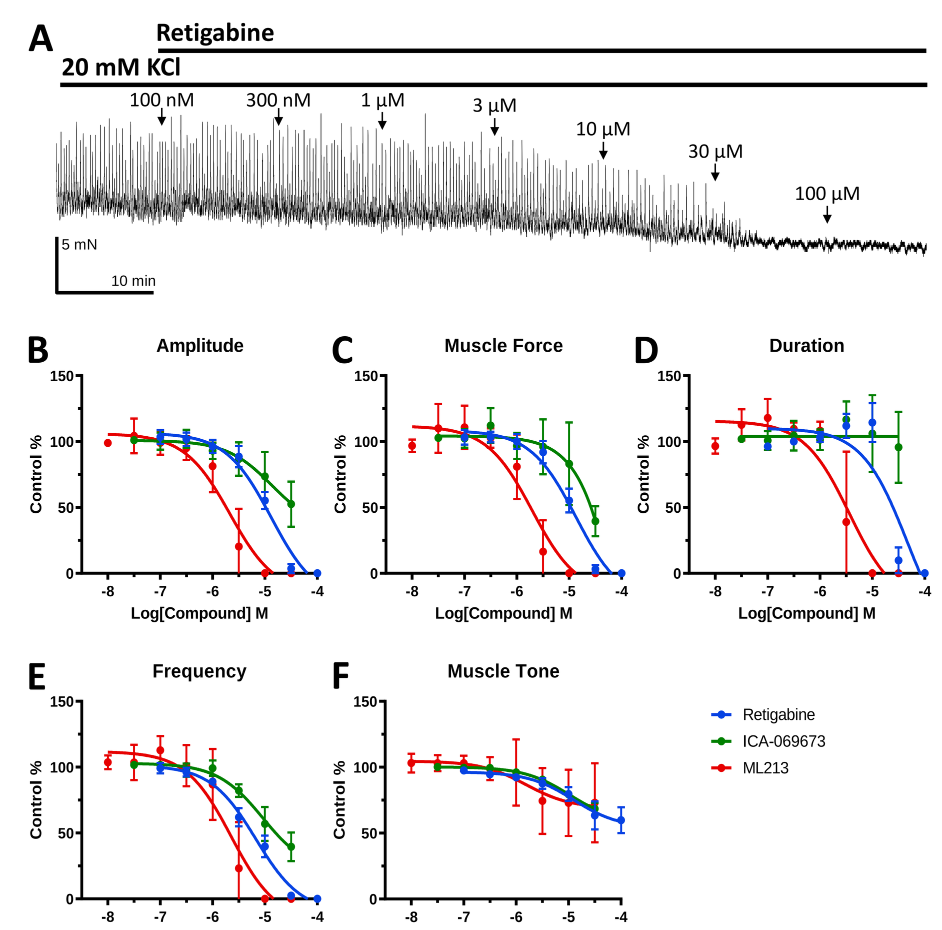


Figure 3. Kv7 channel pharmacological modulation alters UBSM cell membrane potential. **A)** Original recordings of membrane potential demonstrating the effects of (Aa) retigabine (10 μM), (Ab) XE991 (10 μM), (Ac) retigabine (10 μM) in the presence of XE991. Amphotericin-B perforated patch-clamp technique (current-clamp mode, I=0) was used to measure the membrane potential. **B)** Summary graph illustrates the Kv7 channel activator induced hyperpolarization by retigabine, ICA069673 and ML213, and depolarization by the inhibitor XE991 from control (*$P<0.05$, **$P<0.01$, paired t-test, n=5-10, N=4-7). Note that retigabine (10 μM, n=5, N=3) and ICA069673 (10 μM, n=5, N=4) did not alter membrane potential (paired t-test) in the presence of XE991 (10 μM) but differed significantly from retigabine or ICA069673 alone (*$P<0.05$, **$P<0.01$, unpaired t-test). Compared with ML213 (10 μM, n=5, N=4) alone, ML213 (10 μM) in the presence of XE991 (10 μM, n=2, N=2) did not alter membrane potential (p=0.05, paired t-test).



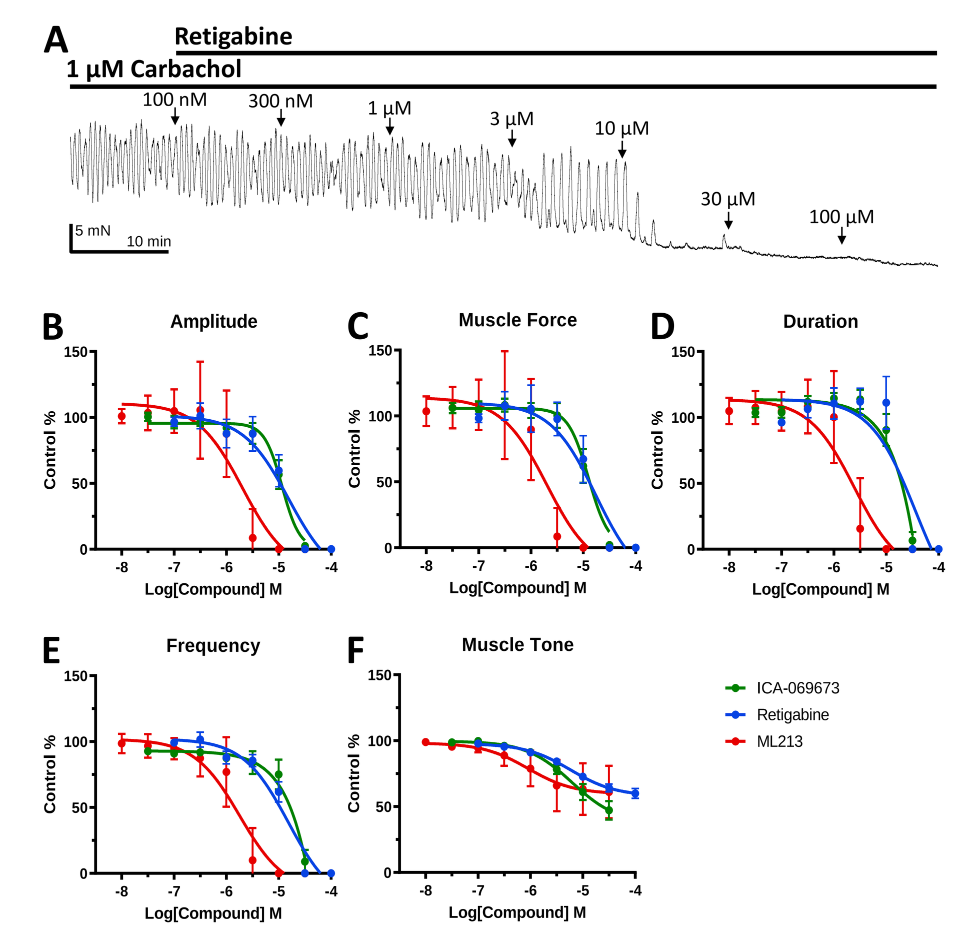
G	IC50, Mean (95% CI) Max Inhibition, Mean ± SEM	
Phasic Contraction Parameter	Retigabine (n=6, N=3)	ICA-069673 (n=5, N=3)
Amplitude	14.6 (4.4 - 55.3) μM 100 ± 0 %	23.8 (5.4 – 30.4) μM 83.5 ± 16.5 %
Muscle Force	3.9 (2.0 - 7.4) μM 100 ± 0 %	20.3 (5.9 – 30.5) μM 90.1 ± 9.9 %
Duration	14.2 (4.5 - 44.4) μM 100 ± 0 %	33.3 (7.0 – 190.3) μM 82.1 ± 17.9 %
Frequency	6.4 (3.1 - 13.1) μM 100 ± 0 %	7.5 (2.3 - 34.2) μM 96.4 ± 3.6 %
Muscle Tone	3.4 (0.8 - 14.5) μM 31.7 ± 8.6 %	13.9 (1.9 – 128.8) μM 28.2 ± 6.3 %

Figure 5. Activation of K_V7 channels attenuates spontaneous phasic UBSM contractions. **A)** Representative isometric tension recording of response to increasing concentrations of retigabine are shown here. **B-F)** Cumulative concentration-response curves for the inhibitory effects of retigabine (100 nM–100 μM) and ICA-069673 (30 nM–30 μM) on UBSM phasic contractions are presented for: amplitude **(B)**, muscle force **(C)**, duration **(D)**, frequency **(E)**, and muscle tone **(F)**. A summary of potency and maximum efficacy values is given **(G)**.



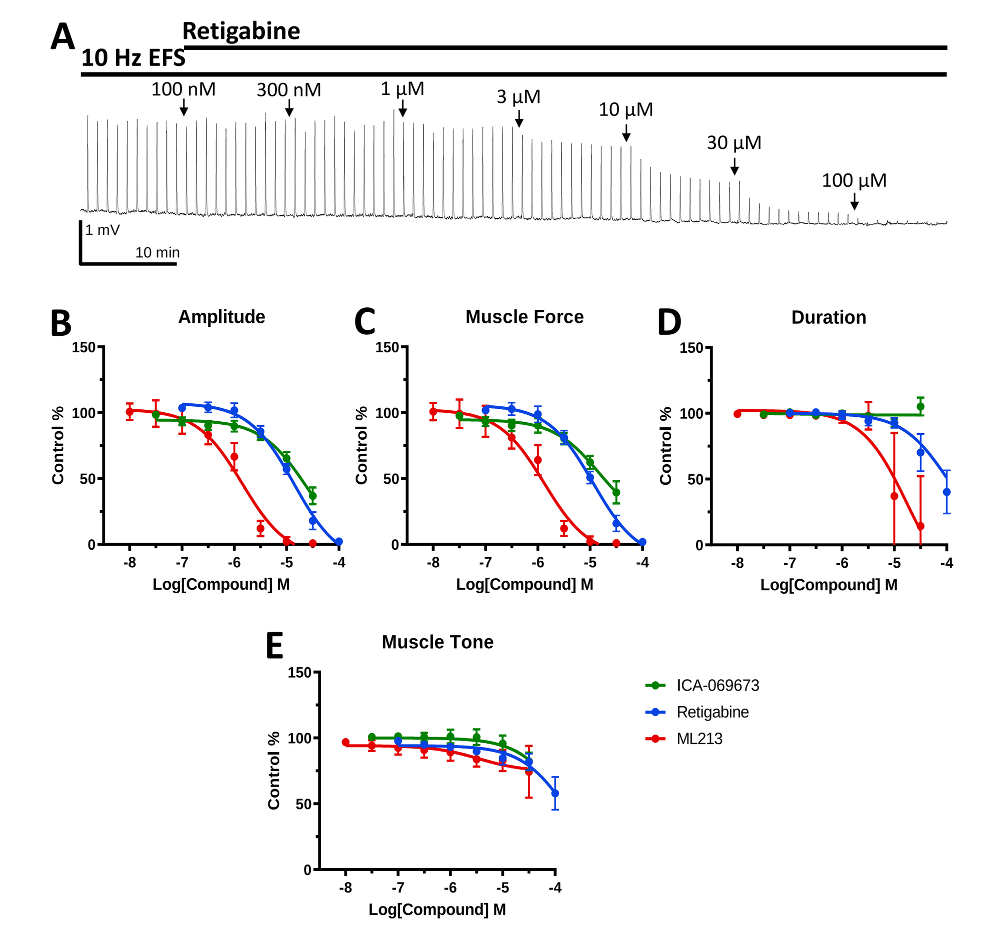
G	IC50, Mean (95% CI) Max Inhibition, Mean ± SEM		
Phasic Contraction Parameter	Retigabine (n=8, N=5)	ICA-069673 (n=6, N=4)	ML213 (n=5, N=4)
Amplitude	13.2 (8.6 - 20.4) μM 100 ± 0 %	13.6 (0.8 – 20.3) μM 47.4 ± 17.2 %	2.1 (1.4 - 3.4) μM 100 ± 0 %
Muscle Force	13.4 (8.0 - 2.3) μM 100 ± 0 %	35.9 (2.6 – 95.6) μM 41.3 ± 21.3 %	2.0 (1.2 - 3.2) μM 100 ± 0 %
Duration	46.8 (15.8 - 139.0) μM 100 ± 0 %	N/A 4.3 ± 26.928	3.6 (2.0 - 6.9) μM 100 ± 0%
Frequency	6.7 (4.4 - 10.2) μM 100 ± 0 %	9.1 (3.1 - 36.4) μM 60.5 ± 10.9	2.3 (1.3 - 3.9) μM 100 ± 0 %
Muscle Tone	12.5 (2.7 - 58.5) μM 40.1 ± 9.9 %	10.3 (5.0 - 2.4) μM 31.6 ± 4.5 %	1.6 (0.2 - 11.3) μM 26.9 ± 13.4 %

Figure 6. Activation of K_V7 channels attenuates 20 mM KCl-induced phasic UBSM contractions. **A)** Representative isometric tension recording of response to increasing concentrations of retigabine are shown here. **B-F)** Cumulative concentration-response curves for the inhibitory effects of retigabine (100 nM–100 μM), ICA-069673 (30 nM–30 μM), and ML213 (10 nM–30 μM) on UBSM phasic contractions are presented for: amplitude **(B)**, muscle force **(C)**, duration **(D)**, frequency **(E)**, and muscle tone **(F)**. A summary of potency and maximum efficacy values is given **(G)**.



G	IC50, Mean (95% CI) Max Inhibition, Mean ± SEM		
Phasic Contraction Parameter	Retigabine (n=6, N=5)	ICA-069673 (n=10, N=5)	ML213 (n=13, N=5)
Amplitude	15.4 (7.5 - 32.3) μM 100 ± 0 %	11.5 (9.0 - 13.9) μM 97.6 ± 2.4 %	2.0 (1.4 - 3.0) μM 100 ± 0 %
Muscle Force	17.3 (7.7 - 42.0) μM 100 ± 0 %	12.6 (8.4 - 14.8) μM 98.0 ± 2.0 %	2.0 (1.3 - 3.0) μM 100 ± 0 %
Duration	36.5 (16.0 - 107.9) μM 100 ± 0 %	23.7 (14.4 – 35.2) μM 93.4 ± 6.6 %	2.5 (1.7 - 3.7) μM 100 ± 0 %
Frequency	15.3 (9.9 - 23.9) μM 100 ± 0 %	15.3 (9.8 – 24.1) μM 91.0 ± 9.0 %	1.8 (1.3 - 2.4) μM 100 ± 0 %
Muscle Tone	5.8 (3.1 - 11.0) μM 40.1 ± 3.7 %	6.2 (3.0 - 13.5) μM 52.8 ± 7.0 %	0.9 (0.4 - 2.0) μM 38.9 ± 5.5 %

Figure 7. Activation of K_V7 channels attenuates 1 μM carbachol-induced phasic UBSM contractions. **A)** Representative isometric tension recording of response to increasing concentrations of retigabine are shown here. **B-F)** Cumulative concentration-response curves for the inhibitory effects of retigabine (100 nM–100 μM) , ICA-069673 (30 nM–30 μM), and ML213 (10 nM–30 μM) on UBSM phasic contractions are presented for: amplitude **(B)**, muscle force **(C)**, duration **(D)**, frequency **(E)**, and muscle tone **(F)**. A summary of potency and maximum efficacy values is given **(G)**.



F	IC50, Mean (95% CI) Max Inhibition, Mean ± SEM		
Phasic Contraction Parameter	Retigabine (n=9, N=5)	ICA-069673 (n=9, N=5)	ML213 (n=7, N=4)
Amplitude	13.8 (9.6 - 20.0) μM 97.8 ± 1.4 %	20.6 (8.9 - 76.3) μM 62.9 ± 5.8 %	1.4 (1.1 - 1.8) μM 99.2 ± 0.8 %
Muscle Force	11.4 (7.7 - 17) μM 98.2 ± 0.9 %	13.1 (4.9 - 52) μM 61.5 ± 7.7 %	1.3 (1.0 - 1.7) μM 99.0 ± 1.0 %
Duration	104.4 (14.3 - 152.4) μM 59.9 ± 16.4 %	N/A -1.2 ± 7.2 %#	17.4 (7.1 - 73.0) μM 85.7 ± 14.3 %
Muscle Tone	163.5 (12.8 - 287.0) μM 42.1 ± 12.3 %	158.3 (5.3 - 379.2) μM 54.4 ± 37.1 %	3.4 (0.4 - 94.1) μM 25.7 ± 7.4 %

Figure 8. Activation of K_V7 channels attenuates 10 Hz EFS-induced contractions. **A)** Representative isometric tension recording of response to increasing concentrations of retigabine are shown here. **B-E)** Cumulative concentration-response curves for the inhibitory effects of retigabine (100 nM–100 μM), ICA-069673 (30 nM–30 μM), and ML213 (10 nM–30 μM) on UBSM phasic contractions are presented: amplitude **(B)**, muscle force **(C)**, duration **(D)**, and muscle tone **(E)**. A summary of potency and maximum efficacy values is given **(F)**.
#Negative value describes increase in contractility

Summary and Conclusions

- Rat UBSM expresses K_V7 channel proteins, specifically K_V7.4 and K_V7.5 channel subtypes.
- Freshly isolated single cells confirmed by the presence of SM actin, express K_V7.4 channels proteins.
- Activation of UBSM K_V7 channels leads to membrane potential hyperpolarization, reducing UBSM single cell excitability.
- Reduction in UBSM single cell excitability by UBSM K_V7 channel activation results in a decrease in UBSM contractility.
- Activation of K_V7 channels attenuates UBSM contractility for spontaneous phasic, 20 mM KCl-induced, carbachol induced, and electrical field stimulated contraction protocols.
- **K_V7 channels play a critical role In urinary bladder smooth muscle function.**

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Conflicts of interest: None

1. Robbins J (2001) Pharmacol Ther 90:1-19.
2. Malysz J & Petkov GV (2020) *Front Physiol* 11:1004.
3. Provence A *et al.* (2015) *J Pharmacol Exp Ther* 354: 290-301.
4. Afeli SA *et al.*(2013) *PLOS One* 8: e75873.
5. Provence, A *et al.* (2018) *J Pharmacol Exp Ther* 364: 131-144.