Characterization of the functional efficacy of KB-1754, a novel Kv7 activator, on smooth muscle contractility

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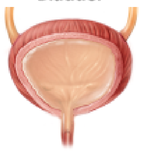
Introduction

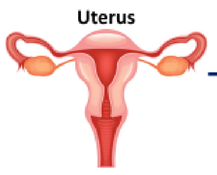
Smooth muscle cells (SMCs) form the outer lining of the:

Bladder



Artery

Uterus



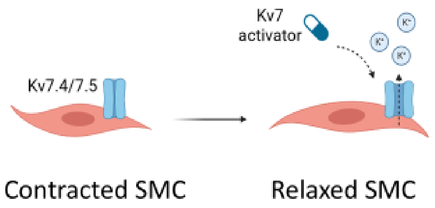
Hyper-contractility of SMCs can lead to disorders such as:

Urinary Incontinence (Overactive Bladder)

Ischemic Heart Disease

Pre-term Labour

Kv7.4 & Kv7.5 are especially abundant on SMCs:



Would a Kv7.4 activator be an effective relaxant of SMCs?

Gap

Selective Kv7.4 activators have not been extensively reported or studied in SMCs. Given the differential abundance of this channel on SMCs, it is unknown if activation of Kv7.4 would be effective in counteracting various conditions of smooth muscle hypercontractility.

Hypothesis

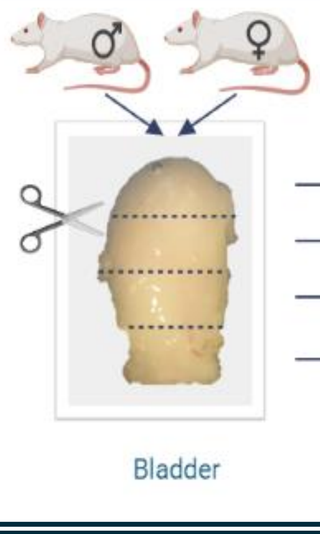
We hypothesize that a Kv7.4 activator will be an effective relaxant in various smooth muscle tissues, like the bladder, mesenteric arteries and uterus.

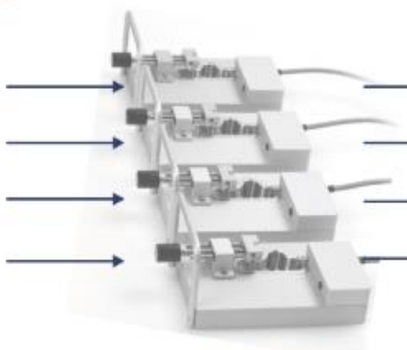
Aims

The present study aimed to characterize the functional efficacy of a Kv7.4 activator, KB-1754, in different tissues and under various conditions like concentration, time and sex. In addition, we compared its efficacy to retigabine.

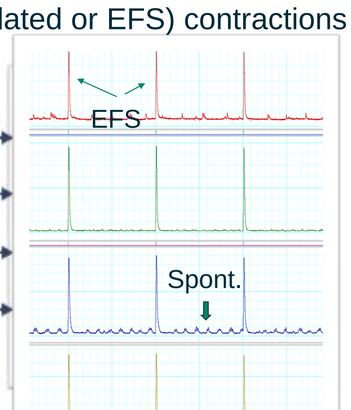
Methods

Whole-cell, voltage-clamp experiments were performed on the Qube®, an automated patch-clamp system. Compounds were tested at 10 µM. Peak inward tail currents were measured at 0 mV after activating pulses (-110 to +30 mV). Mean data were fit with a Boltzmann sigmoid equation to calculate the half-maximal voltage (Vhalf) and max tail current. Strips of bladder from male and female Wistar rats were mounted in a 4 chamber myograph to measure and display contractile amplitudes of the tissues. Functional studies of KB-1754 were conducted by the addition of the drug to the tissue chambers of the myograph. Changes to contractile amplitude (in milli-Newtons) were measured and plotted using GraphPad Prism software. Expression studies were conducted using reverse transcription quantitative polymerase chain reaction (RT-qPCR).





Spontaneous & nerve-evoked (electric field stimulated or EFS) contractions

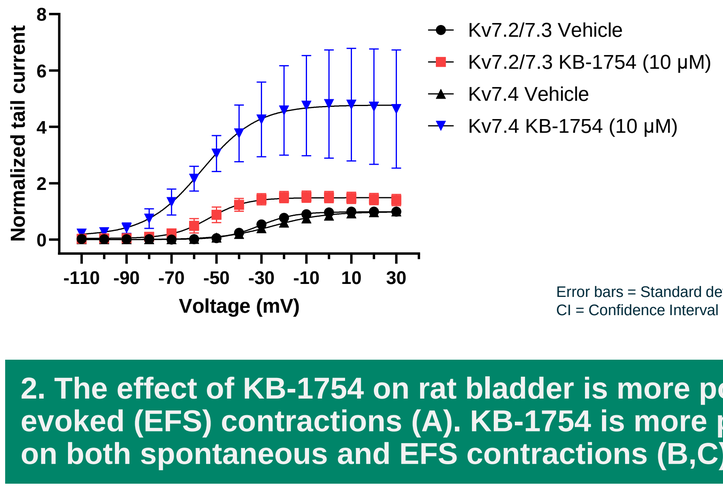


Conclusion

KB-1754 preferentially activates Kv7.4 when tested in CHO cells stably expressing Kv7.4 or Kv7.2/7.3. KB-1754 relaxed the detrusor, uterus and mesenteric arteries in a concentration-dependent manner and exerted significantly greater relaxation in bladders from males compared to proestrous females. The effects of KB-1754 were maintained in conditions of hypercontractility in both the detrusor and uterus. KB-1754 is an effective Kv7.4 prototype activator to better understand the role of this channel in smooth muscle contractility.

Results

1. KB-1754 preferentially activates Kv7.4 as shown by electrophysiological studies.



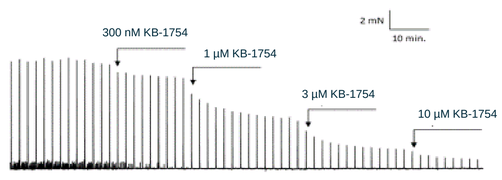
Delta Vhalf = Vhalf compound – Vhalf vehicle
Normalized tail = Tail compound – Tail vehicle

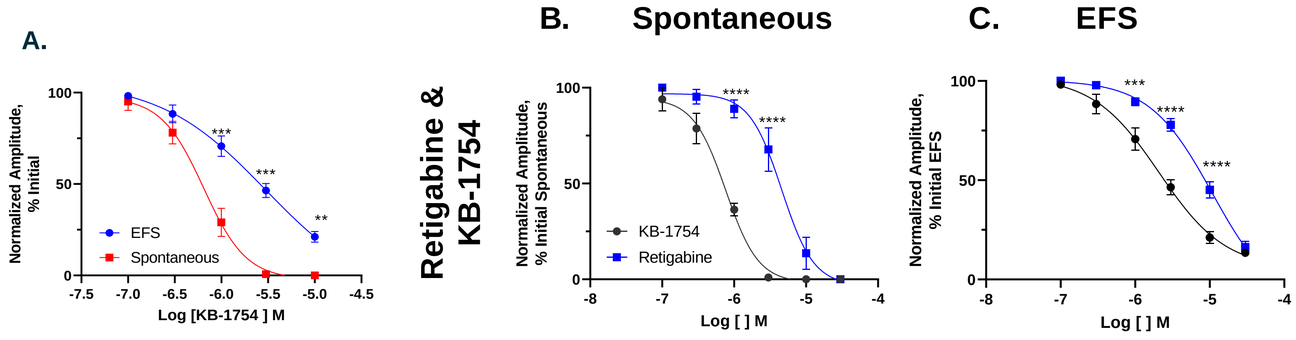
Kv7.2/7.3	n	Delta Vhalf (mV), 95% CI	Normalized Tail, 95% CI
KB-1754	24	-22 (-24 to -21)	1.5 (1.5 to 1.5)
Retigabine	108	-33 (-34 to -32)	1.1 (1.1 to 1.2)

Kv7.4	n	Delta Vhalf (mV), 95% CI	Normalized Tail, 95% CI
KB-1754	20	-32 (-37 to -27)	4.8 (4.5 to 5.1)
Retigabine	109	-9 (-10 to -7)	2.8 (2.7 to 2.8)

Error bars = Standard deviations
CI = Confidence Interval

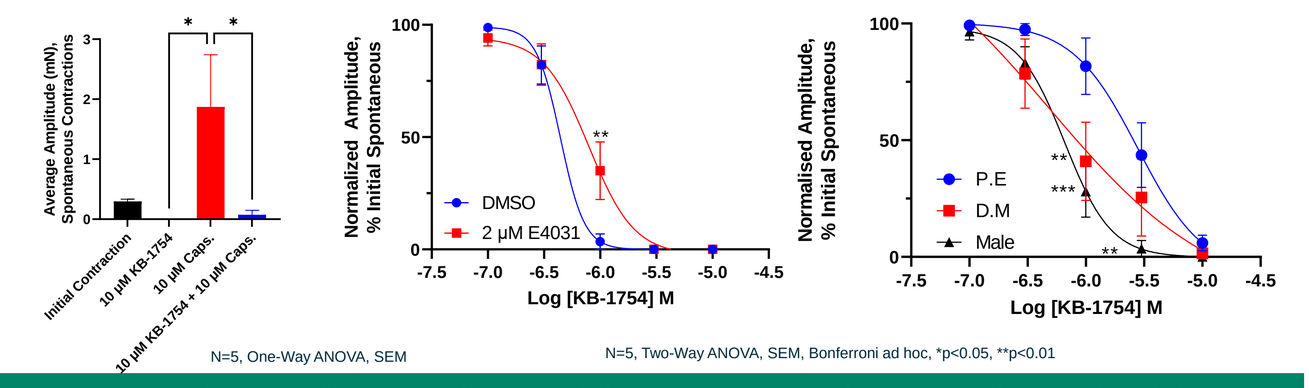
2. The effect of KB-1754 on rat bladder is more potent on spontaneous versus nerve-evoked (EFS) contractions (A). KB-1754 is more potent than the Kv7 activator, retigabine, on both spontaneous and EFS contractions (B,C).





N=5, Two-Way ANOVA, SEM, Bonferroni ad hoc, *p<0.05, **p<0.01,***p<0.0001,****p<0.0001

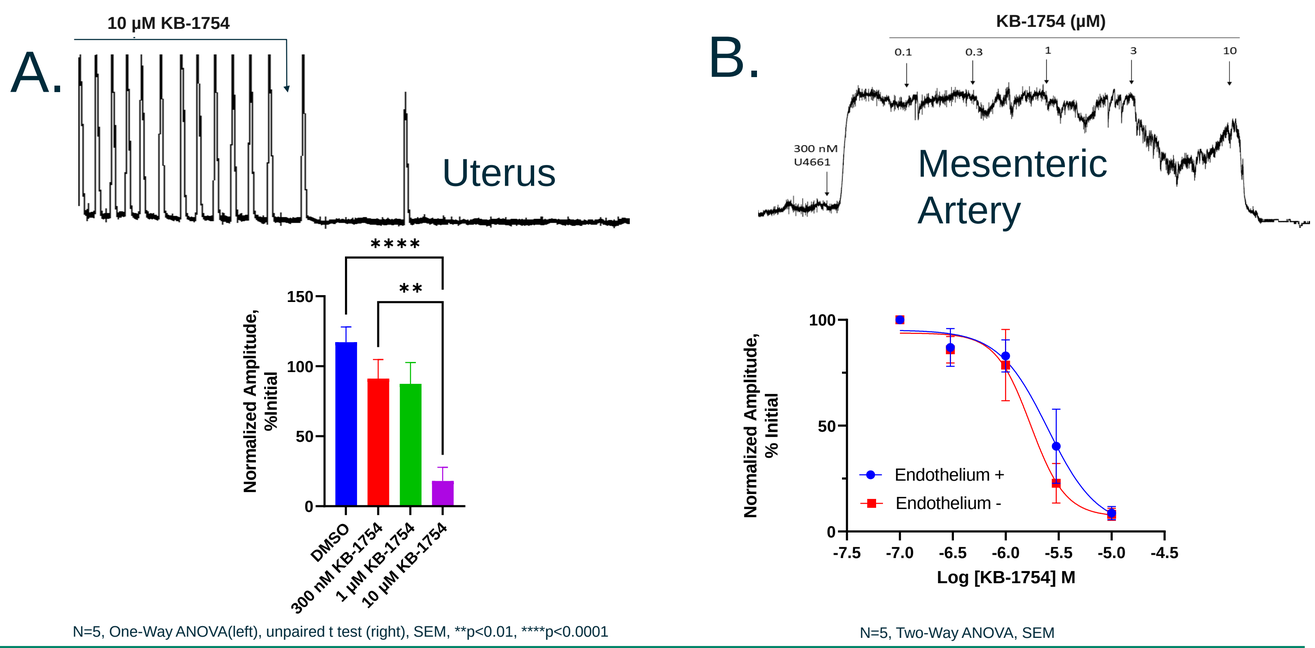
3. KB-1754 is effective in hypercontractile conditions, including those induced by capsaicin (A) and ERG channel blockade E4031 (B). KB-1754 was least potent in bladders from proestrous and oestrous females (PE) compared to males and D.M females (C).



N=5, One-Way ANOVA, SEM

N=5, Two-Way ANOVA, SEM, Bonferroni ad hoc, *p<0.05, **p<0.01

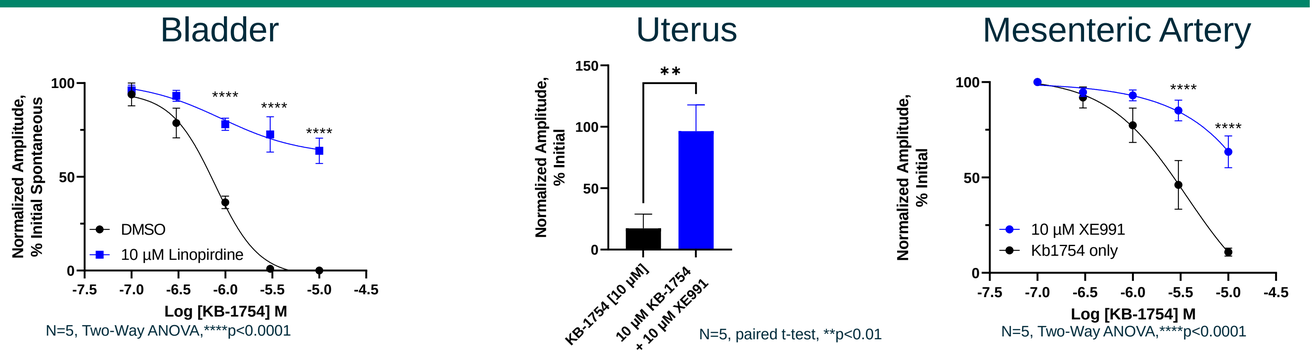
4. KB-1754 is also an effective relaxant of the uterus (A) and mesenteric arteries (B).



N=5, One-Way ANOVA(left), unpaired t test (right), SEM, **p<0.01, ****p<0.0001

N=5, Two-Way ANOVA, SEM

5. The effect of KB-1754 is inhibited in the presence of non-selective Kv7 inhibitors, Linopirdine and XE991.



N=5, Two-Way ANOVA,****p<0.0001

N=5, paired t-test, **p<0.01

N=5, Two-Way ANOVA,****p<0.0001