

# Control of urinary bladder smooth muscle excitability by the TRPM4 channel modulator 9-phenanthrol

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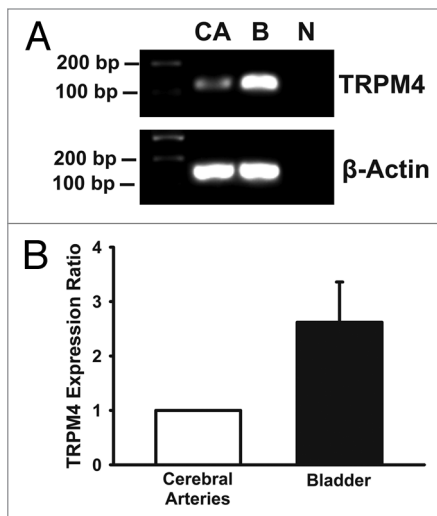
Smith AC, Hristov KL, Cheng Q, Xin W, Parajuli SP, Earley S, Malysz J, Petkov GV. Novel role for the transient potential receptor melastatin 4 channel in guinea pig detrusor smooth muscle physiology. *Am J Physiol Cell Physiol* 2013; 304:C467-77; PMID:23302778; <http://dx.doi.org/10.1152/ajpcell.00169.2012>

**T**he Ca<sup>2+</sup>-activated monovalent cation selective transient receptor potential melastatin 4 (TRPM4) channel has been recently identified in detrusor smooth muscle (DSM) of the urinary bladder. Two recent publications by our research group provide evidence in support of the novel hypothesis that TRPM4 channels enhance DSM excitability and contractility. This is a critical question as prior studies have primarily targeted hyperpolarizing currents facilitated by K<sup>+</sup> channels, but the depolarizing component in DSM cells is not well understood. For the first time, we utilized the selective TRPM4 channel inhibitor, 9-phenanthrol, to investigate TRPM4 channel functional effects in DSM at both cellular and tissue levels in rodents. Our new data presented here showed that in rat DSM cells, 9-phenanthrol attenuates spontaneous inward currents in the presence of the muscarinic receptor agonist, carbachol, thus reducing DSM cell excitability. In support of our original hypothesis, we found that TRPM4 channel mRNA levels are much higher in DSM vs. vascular smooth muscle and that inhibition of TRPM4 channels can potentially attenuate DSM excitability. Thus, we postulate the novel concept that selective pharmacological inhibition of TRPM4 channels can limit both excitability and contractility of DSM.

## Introduction

Contraction and relaxation of detrusor smooth muscle (DSM), which makes up

the wall of the urinary bladder, facilitates the storage and voiding of urine. Multiple ion channels that are expressed in DSM control the excitability and contractility of this tissue. However, the mechanisms by which ion channels regulate DSM function are yet to be completely elucidated. This lack of knowledge hinders the efforts aimed at identifying suitable ion channel targets and channel modulators for urinary bladder disorders. Recently, members of the transient receptor potential (TRP) superfamily of ion channels have been implicated in normal and pathologic bladder function.<sup>1-3</sup> Mammalian genomes encode 27 human and 28 rodent TRP channel members, respectively, subdivided into 6 subfamilies based on their sequence homology (TRPC, TRPV, TRPM, TRPA, TRPP, and TRPML).<sup>4,5</sup> TRP channels respond to physical and chemical stimuli such as temperature, pH, osmolality, pressure, stretch, light, alkaloids, as well as intracellular stimuli including Ca<sup>2+</sup>; and constitute a fundamental way by which cells perceive and respond to changes in the extracellular environment.<sup>5</sup> Until recently, several TRP members have been identified in the bladder urothelium and nerves, but not yet in DSM.<sup>1,3,6</sup> One such member is the transient receptor potential melastatin 4 (TRPM4) channel.<sup>7,8</sup> TRPM4 channel physiological significance in urothelium remains unknown,<sup>6</sup> and its functional role in DSM was recently described by our group when we reported the expression and function of TRPM4 channels in rat and guinea pig DSM.<sup>9,10</sup> The function



**Figure 1.** TRPM4 channel mRNA is expressed at higher levels in DSM compared with cerebral artery smooth muscle in rats. **(A)** Agarose gel of PCR products from either bladder (B) or cerebral artery (CA) cDNA for TRPM4 (117 bp) or  $\beta$ -actin (145 bp); no products were detected when cDNA template was absent (N). **(B)** Summary data showing that TRPM4 channel mRNA expression is  $2.6 \pm 0.7$  fold higher in DSM relative to cerebral arteries as determined by quantitative RT-PCR in Sprague Dawley rats ( $n = 3$ ,  $N = 3$ ;  $p < 0.05$ ).

of TRPM4 channels has been studied in non-DSM myocytes and the channel has been identified as an important mediator of smooth muscle cell excitability and contractility.<sup>11-16</sup> Novel data included in this addendum indicate that expression of TRPM4 channels is greater in DSM compared with cerebral arterial myocytes, suggesting that the channel may have greater impact in bladder function.

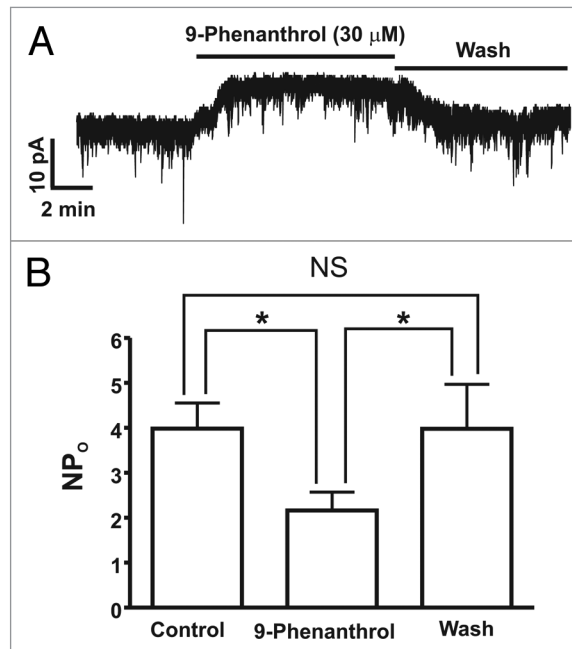
TRPM4, and the related TRPM5 channel, display atypical biophysical properties. TRPM4 is a  $\text{Ca}^{2+}$ -activated cation channel highly selective for monovalent cations with the rank order of  $\text{Na}^+ - \text{K}^+ > \text{Cs}^+ > \text{Li}^+$ , but impermeable to anions and divalent cations such as  $\text{Ca}^{2+}$ .<sup>16</sup> TRPM4 channels exhibit  $\text{Ca}^{2+}$  dependency, have single channel conductance  $\sim 25$  pS, and are voltage-dependent.<sup>17-20</sup> Activation of TRPM4 channels is thought to induce cell depolarization via the net entry of  $\text{Na}^+$  into the cell which in turn activates the L-type voltage-dependent  $\text{Ca}^{2+}$  channels (VDCC) favoring  $\text{Ca}^{2+}$  entry, thus modulating  $\text{Ca}^{2+}$  signaling<sup>13,21</sup> and eventually DSM contractility. Collectively,

these properties and fundamental roles make TRPM4 channels potentially useful pharmacological targets to control DSM function. However, previous investigations of TRPM4 channel function have been hampered by the lack of selective pharmacological modulators. Recently, a novel selective TRPM4 channel inhibitor, 9-phenanthrol, has been described.<sup>15,22,23</sup> Thus, 9-phenanthrol is an important pharmacological tool that can be used to investigate the functional role of TRPM4 channels in DSM excitation-contraction coupling. In this addendum, we provide further evidence for a physiological role of TRPM4 channels in rat DSM using its modulator 9-phenanthrol.

## Results

### Expression levels of TRPM4 channel mRNA are greater in DSM compared with cerebral artery smooth muscle

Quantitative RT-PCR experiments showed that TRPM4 channel mRNA expression level is greater in rat DSM compared with cerebral artery smooth muscle



**Figure 2.** Reduction of spontaneous inward current by the TRPM4 channel inhibitor 9-phenanthrol in freshly-isolated rat DSM cells. **(A)** An original recording illustrating the inhibitory effect of 9-phenanthrol ( $30 \mu\text{M}$ ) on spontaneous inward current recorded at  $-60$  mV in a single DSM cell using the perforated whole cell patch-clamp technique. **(B)** Summary data for the inhibitory effect of 9-phenanthrol on TRPM4 spontaneous inward current, presented as  $\text{NP}_0$  before (control) and after its application at  $30 \mu\text{M}$  ( $n = 13$ ,  $N = 6$ ;  $*p < 0.05$ ), and following the washout of 9-phenanthrol ( $n = 8$ ,  $N = 5$ ;  $p > 0.05$  washout vs control); NS = non-significant for the comparison shown.

( $2.6 \pm 0.7$  fold greater;  $n = 3$ ,  $N = 3$ ;  $p < 0.05$ , Fig. 1), suggesting that TRPM4 channels may have a more important functional impact in the bladder as compared with vasculature.

### TRPM4 channel blocker 9-phenanthrol reduces spontaneous inward current in freshly-isolated rat DSM cells

We used the amphotericin-B perforated whole cell patch-clamp technique and the selective inhibitor of TRPM4 channels, 9-phenanthrol, to evaluate the functional role of TRPM4 channels in the regulation of DSM cell excitability. As shown in Figure 2, freshly-isolated DSM cells at a holding potential of  $-60$  mV exhibited spontaneous basic activity along with numerous inward currents as originally reported in rat DSM cells at  $-70$  mV.<sup>10</sup> 9-phenanthrol ( $30 \mu\text{M}$ ) significantly attenuated the spontaneous inward current from  $\text{NP}_0$  of  $4.0 \pm 0.6$  under control conditions to  $2.2 \pm 0.4$  in the presence of  $30 \mu\text{M}$  9-phenanthrol ( $n = 13$ ,  $N = 6$ ;  $p < 0.05$ , Fig. 2). The inhibitory effect of 9-phenanthrol on spontaneous inward currents completely recovered to the

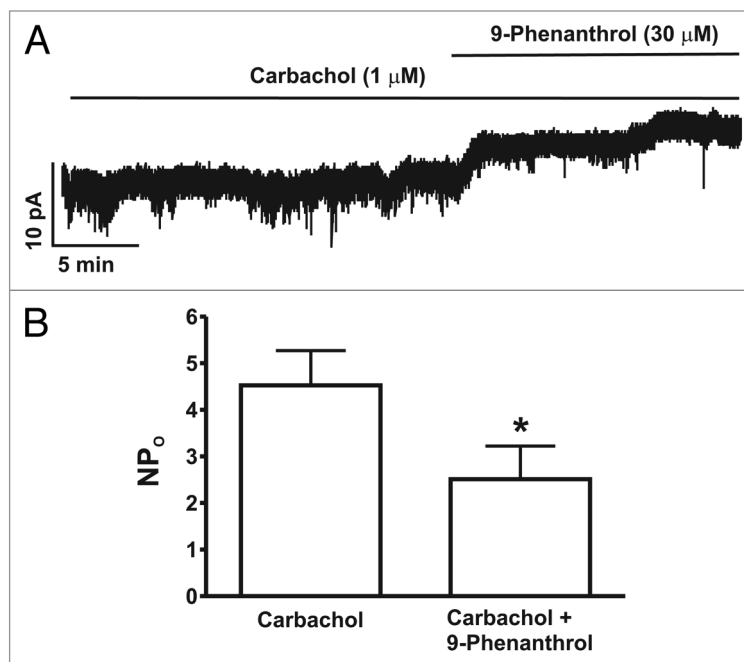
control level upon the washout of 9-phenanthrol from the bath solution ( $NP_o = 4.0 \pm 1.0$ ;  $n = 8$ ,  $N = 5$ ;  $p > 0.05$  washout vs. control; Fig. 2). Our experimental data confirms the concept that TRPM4 channels are tonically active under physiological conditions and are key regulators of DSM cell excitability.

#### TRPM4 channel blocker 9-phenanthrol inhibits spontaneous inward current in freshly-isolated rat DSM cells in the presence of muscarinic receptor agonist carbachol

In the next experimental series, we sought to evaluate whether pharmacological inhibition of TRPM4 channels with 9-phenanthrol affects the spontaneous inward current in freshly-isolated DSM cells in the presence of  $1 \mu\text{M}$  carbachol. As illustrated in Figure 3, in the presence of  $1 \mu\text{M}$  carbachol, spontaneous basic current activity along with numerous inward currents at a holding potential of  $-70 \text{ mV}$  were measured. Treatment of DSM cells with  $30 \mu\text{M}$  9-phenanthrol significantly suppressed the spontaneous inward currents from  $NP_o$  of  $4.5 \pm 0.7$  in the presence of  $1 \mu\text{M}$  carbachol alone to  $2.5 \pm 0.7$  in the presence of both  $1 \mu\text{M}$  carbachol and  $30 \mu\text{M}$  9-phenanthrol ( $n = 11$ ,  $N = 6$ ;  $p < 0.05$ , Fig. 3). Taken together, our results show that the TRPM4 channel inhibitor 9-phenanthrol attenuates the inward currents in the presence of the muscarinic receptor agonist, carbachol, thus regulating DSM cell excitability.

### Discussion

Aside from our just published reports,<sup>9,10</sup> the functional role of the TRPM4 channel in DSM cell excitability has not been studied. Recently, a novel selective TRPM4 channel inhibitor, 9-phenanthrol, has been described.<sup>9,10,15,22,23</sup> Thus, 9-phenanthrol is an important pharmacological tool that could be used to investigate the TRPM4 channel physiological role in DSM excitability.<sup>9,10</sup> Use of this novel selective pharmacological inhibitor has allowed us to show that TRPM4 channels contribute to the electrical activity of freshly-isolated rat and guinea pig DSM cells.<sup>9,10</sup> These studies provide novel mechanistic insights and better our understanding of the functional roles of



**Figure 3.** Reduction of spontaneous inward current by the TRPM4 channel inhibitor 9-phenanthrol in the presence of the muscarinic receptor agonist carbachol in freshly-isolated rat DSM cells. (A) A representative recording illustrating the inhibitory effect of 9-phenanthrol ( $30 \mu\text{M}$ ) on spontaneous inward current recorded in the presence of carbachol ( $1 \mu\text{M}$ ) at  $-70 \text{ mV}$  in a single DSM cell using the perforated whole cell patch-clamp technique. (B) Summary data for the inhibitory effect of 9-phenanthrol on TRPM4 spontaneous inward current in the presence of carbachol ( $1 \mu\text{M}$ ), presented as  $NP_o$  before (control) and after application of  $30 \mu\text{M}$  9-phenanthrol ( $n = 11$ ,  $N = 6$ ;  $*p < 0.05$ ).

TRPM4 channels in urinary bladder. Our recent papers<sup>9,10</sup> provide strong, initial evidence for a key role of the TRPM4 channels in regulating DSM excitability and contractility. Major strengths of our studies include the use of only native, freshly-isolated (not cultured) DSM cells, and a combination of experimental approaches encompassing TRPM4 mRNA and protein detection, patch-clamp electrophysiology, and tissue contractility.

New data presented here provide a significant rationale for further study of the role of TRPM4 channels in bladder function. This question is critical as prior studies have primarily targeted hyperpolarizing currents, such as  $K^+$  currents,<sup>24</sup> but the depolarizing component in DSM cells is not completely understood. In support of our original hypothesis, we found that inhibition of TRPM4 channels with 9-phenanthrol diminished DSM excitability in the presence or absence of the muscarinic receptor agonist, carbachol (Figs. 2 and 3), complementing our prior experimental evidence.<sup>9,10</sup> Most importantly, the

new quantitative RT-PCR data showed much higher TRPM4 channel mRNA expression levels in DSM compared with vascular smooth muscle in rat (Fig. 1). This key finding suggests TRPM4 channels might have a more prominent physiological role in DSM compared with the vasculature, and therefore, could be novel pharmacological targets for bladder dysfunction with minimal vascular effects.

Bladder dysfunction, such as overactive bladder (OAB), is a significant medical problem that affects ~17% of the Western population.<sup>25-27</sup> The current pharmacological treatment for OAB is based primarily on antimuscarinics.<sup>25,28</sup> The clinical use of these drugs is associated with dose-related side effects including dry mouth, dry eyes, constipation, and tachycardia.<sup>25,28</sup> Thus, there is a significant need to identify novel therapeutic treatments for OAB, which directly target DSM while minimizing unwanted side effects. A critical step in the development of more effective therapies for OAB involves a better understanding of the basic mechanisms that

control DSM excitability and contractility under normal and OAB conditions. We hypothesize that TRPM4 channels are major determinants of DSM excitability and contractility, and therefore, TRPM4 channels represent novel therapeutic targets for OAB. However, there are substantial gaps in our knowledge regarding the functional roles of TRPM4 channels in DSM regulation, especially in humans. Future investigations in this area may lead to the development of selective pharmacological therapies for OAB.<sup>24</sup> Targeting TRPM4 channels with novel highly selective inhibitors has the potential to decrease DSM contractility, and thus may have clinical application for the treatment of OAB, which drives the significance of future studies in this area.

## Materials and Methods

Patch-clamp electrophysiology was performed as previously described.<sup>9,10,29</sup> Quantitative RT-PCR experiments were performed as previously described.<sup>29,30</sup> **N** is the number of animals; **n** is the number of individual samples/cells.

## Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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