Regulation of transient receptor potential melastatin 4 channel by sarcoplasmic reticulum inositol trisphosphate receptors: Role in human detrusor smooth muscle function

Aaron Provence, Eric S. Rovner, and Georgi V. Petkov

Department of Drug Discovery and Biomedical Sciences, South Carolina College of Pharmacy, University of South Carolina, Columbia, SC, USA; Department of Urology, Medical University of South Carolina, Charleston, SC, USA; Department of Pharmaceutical Sciences, College of Pharmacy, University of Tennessee Health Science Center, Memphis, TN, USA

ABSTRACT

We recently reported key physiologic roles for Ca²⁺-activated transient receptor potential melastatin 4 (TRPM4) channels in detrusor smooth muscle (DSM). However, the Ca²⁺-signaling mechanisms governing TRPM4 channel activity in human DSM cells are unexplored. As the TRPM4 channels are activated by Ca²⁺, inositol 1,4,5-trisphosphate receptor (IP₃R)-mediated Ca²⁺ release from the sarcoplasmic reticulum represents a potential Ca²⁺ source for TRPM4 channel activation. We used clinically-characterized human DSM tissues to investigate the molecular and functional interactions of the IP₃Rs and TRPM4 channels. With in situ proximity ligation assay (PLA) and perforated patch-clamp electrophysiology, we tested the hypothesis that TRPM4 channels are tightly associated with the IP₃Rs and are activated by IP₃R-mediated Ca²⁺ release in human DSM. With in situ PLA, we demonstrated co-localization of the TRPM4 channels and IP₃Rs in human DSM cells. As the TRPM4 channels and IP₃Rs must be located within close apposition to functionally interact, these findings support the concept of a potential Ca²⁺-mediated TRPM4-IP₃R regulatory mechanism. To investigate IP₃R regulation of TRPM4 channel activity, we sought to determine the consequences of IP₃R pharmacological inhibition on TRPM4 channel-mediated transient inward cation currents (TICCs). In freshly-isolated human DSM cells, blocking the IP₃Rs with the selective IP₃R inhibitor xestospongin-C significantly decreased TICCs. The data suggest that IP₃Rs have a key role in mediating the Ca²⁺-dependent activation of TRPM4 channels in human DSM. The study provides novel insight into the molecular and cellular mechanisms regulating TRPM4 channels by revealing that TRPM4 channels and IP₃Rs are spatially and functionally coupled in human DSM.

Introduction

The functions of the urinary bladder—the principle organ responsible for the storage and periodic release of urine—are mediated by the activity of detrusor smooth muscle (DSM). In DSM cells, it is well-established that Ca²⁺ influx through L-type voltage-gated Ca²⁺ channels underlies the depolarization phase of spontaneous action potentials while the large-conductance voltage- and Ca²⁺-activated K⁺ (BK) channels, in addition to other voltage-gated Kv channels, work in opposition to L-type voltage-gated Ca²⁺ channel activity to promote membrane hyperpolarization.1-4 Albeit these mechanisms have been known for many years in DSM, recent studies from our group have identified an important role for an additional Ca²⁺-sensitive cationic conductance regulating DSM function in rodents and humans.5-8 Indeed, Ca²⁺-activated monovalent cation channels known as the transient receptor potential (TRP) melastatin 4 (TRPM4) channels are functionally expressed in rat, guinea pig, and human DSM.5-8

Of the 28 members that constitute the TRP channel superfamily, TRPM4 channels, in addition to TRPM5, are the only TRP channel members selective for monovalent cations (K⁺ and Na⁺) over Ca²⁺.9,10 TRPM4 channel functional roles in excitable cells and tissues, including rodent6-8 and human DSM,5-8 have begun to emerge in recent years following studies using the novel and selective TRMP4 channel inhibitor, 9-phenanthrol.6-8,11-13 We recently provided the first
detailed study demonstrating the expression and key functional roles for TRPM4 channels in human DSM excitability and contractility using the selective TRPM4 channel inhibitor 9-phenanthrol.5 Our novel study was the central topic of an editorial focus in the American Journal of Physiology – Cell Physiology,14 which highlighted the attractiveness of the TRPM4 channels as potential novel targets for the pharmacological control of the urinary bladder. Under pathophysiological conditions, DSM dysfunction is associated with some forms of overactive bladder.15 The TRPM4 channels, in addition to the cellular mechanisms regulating their activity, represent an exciting and novel therapeutic approach to the treatment of various bladder disorders and the collateral effects these conditions inflict on patient quality of life. Nonetheless, significant work still remains to ascertain the cellular mechanisms regulating TRPM4 channel activity in human DSM function to further validate their potential therapeutic role for bladder disorders.

It has been suggested that Ca$^{2+}$ release from the sarcoplasmic reticulum (SR) inositol 1,4,5-trisphosphate (IP$_3$) receptors (IP$_3$Rs) may constitute a primary Ca$^{2+}$ source for TRPM4 channel activation.16 The concept of IP$_3$R-mediated Ca$^{2+}$ release serving as critically important Ca$^{2+}$ source for TRPM4 channel activation, as opposed to Ca$^{2+}$ influx through L-type voltage-gated Ca$^{2+}$ channels, was supported by the observation that removal of extracellular Ca$^{2+}$ had no acute effects on TRPM4 channel activity.16 Whether this TRPM4-IP$_3$R regulatory mechanism exists in human DSM, however, is completely unknown.

Therefore, the current study sought to investigate the molecular and functional interaction of the TRPM4 channels and IP$_3$Rs in human DSM. This was achieved via a combined experimental approach using in situ proximity ligation assays (PLA) and perforated patch-clamp electrophysiology in combination with pharmacological tools. This study reveals the novel mechanism whereby SR IP$_3$Rs regulate the excitability of human DSM through molecular and functional interactions with TRPM4 channels.

Materials and methods

Human DSM tissue acquisition

Human DSM tissues were acquired from donor patients undergoing routine open bladder surgeries as described previously.5,17,18 All procedures attendant upon the collection of human DSM tissues have been reviewed and approved by the Institutional Review Board of the Medical University of South Carolina (protocol number Pro00045232). 6 Caucasian patients (3 men and 3 women; average age 64.5 ± 11.2 years) without preoperative histories of overactive bladder and American Urological Association symptom scores <8 were used for this study. Following surgeries, human DSM tissues were stored on ice in a container containing HEPES-buffered dissection solution (see Solutions and Drugs) for immediate transport to the laboratory for DSM cell isolation.

DSM cell isolation

Human DSM single cells were enzymatically isolated using a combination of papain and collagenase as described previously.5,17,18 Freshly-isolated DSM cells were used for in situ PLA or electrophysiological experiments within 12 h following isolation.

In situ proximity ligation assay (PLA)

In situ PLA was performed on freshly-isolated DSM cells using the Duolink® in situ orange starter kit goat/rabbit (Cat. No: DUO92106, Sigma-Aldrich, St. Louis, MO) according to the manufacturer’s instructions. DSM cells underwent fixation in 2% paraformaldehyde (10 min, 37°C) followed by 2 15-min washes in phosphate buffered saline. DSM cells were blocked in Duolink blocking solution (30 min, 37°C). Subsequently, DSM cells were incubated with the anti-rabbit IP$_3$R and goat anti-TRPM4 channel antibodies (Santa Cruz Biotechnology, Dallas, TX; catalog numbers SC-27540 TRPM4; SC-28614 IP$_3$R), respectively, in Duolink antibody diluent solution overnight at 4°C, followed by 2 × 5 min washes in Duolink 1x wash buffer A solution. The rabbit anti-Kv7.5 antibody was also obtained from Santa Cruz (catalog number SC-50416). DSM cells were then incubated with the Duolink PLA probe anti-rabbit MINUS and anti-goat PLUS oligonucleotide-conjugated secondary antibodies (1 h, 37°C), followed by 2 × 5 min washes in Duolink 1x wash buffer A solution. DSM cells then underwent incubation in ligase-lation solution (30 min, 37°C), which hybridizes the 2 PLA MINUS and PLUS probes when in close proximity (< 40 nm). Following ligation, DSM cells were washed 2 × 2-min in Duolink 1x wash buffer A solution. Finally, DSM cells were incubated in...
amplification-polymerase solution (100 min, 37°C) containing nucleotides and fluorescently-labeled oligonucleotides. The oligonucleotide arm of one PLA probe is a primer for rolling-circle amplification. The fluorescently-labeled oligonucleotides hybridize to the rolling-circle amplification product thus permitting detection. Following amplification, DSM cells were washed 2 × 10 min in 1x wash buffer B and then a 1 min wash in 0.01x wash buffer B. Slides were then mounted using minimal volume of Duolink in situ mounting medium with 4',6-diamidino-2-phenylindole (DAPI). PLA signals (Duolink In Situ Detection Reagents Orange (λ_exitation/emission = 554/576 nm)) were detected using laser scanning LSM 700 confocal microscope (Carl Zeiss, Germany) with a 63x oil immersion objective.

**Data analysis and statistics**

The TICCs were analyzed as open channel probability (NPo) as described previously. Data are summarized as means ± SEM for n (the number of DSM cells) isolated from N (the number of patients). Data were compared using a paired Student’s t-test. A P value <0.05 was considered statistically significant.

**Results**

**TRPM4 channels are colocalized with the IP3Rs in human DSM cells**

In order for the discrete Ca^{2+} release events from SR IP3Rs to regulate TRPM4 channel activity in human DSM, the IP3Rs and TRPM4 channels must be located within close apposition. To determine TRPM4 channel colocalization with IP3Rs, we conducted Duolink in situ PLA on freshly-isolated human DSM cells. To elucidate TRPM4/IP3R colocalization, human DSM cells were incubated with the anti-TRPM4 goat and anti-IP3R rabbit antibodies, respectively. As shown in Fig. 1A, co-incubation of TRPM4 and IP3R antibodies positively confirmed that TRPM4 channels and IP3Rs are located in close proximity (< 40 nm) to the cell membrane as indicated by the bright red fluorescence. Fluorescent detection was not observed when the anti-TRPM4 goat antibody was omitted (Fig. 1B). As a negative control, we used a rabbit antibody specific for the voltage-gated Kv7.5 channel subtype, which is expressed in human DSM, but not expected to interact with TRPM4 channels. Incubation of human DSM cells with anti-TRPM4 and anti-Kv7.5 rabbit antibodies, respectively, resulted in no observable fluorescent signal (Fig. 1). Results were confirmed in at least 3 human DSM cells.

**IP3R pharmacological inhibition with xestospongin-C decreases transient inward cation current activity in human DSM cells**

In non-DSM cells, TRPM4 channels have been shown to be regulated by Ca^{2+} release events originating from the SR IP3Rs. Therefore, SR IP3R Ca^{2+} release activity may constitute a fundamentally important cellular mechanism regulating TRPM4 channel activity in human DSM. To ascertain whether SR IP3Rs...
potentially regulate TRPM4 channel activity in human DSM, we examined the physiologic consequences of IP₃R pharmacological inhibition on TRPM4 channel-mediated TICCs. At the holding potential of -70 mV, where BK channel activity is negligible, pharmacological inhibition of the IP₃Rs with the selective inhibitor xestospongin-C (1 μM) significantly reduced TICC activity (NPₒ) from 2.0 ± 1.1 in control conditions to 1.3 ± 1.0 in the presence of 1 μM xestospongin-C (n = 6, N = 4; P<0.05; Fig. 2). These results support the concept that Ca²⁺ release events from SR IP₃Rs are involved in the regulation of TRPM4 channel-mediated TICCs in human DSM cells (Fig. 3).

Discussion

This study sought to determine whether IP₃Rs of the SR regulate TRPM4 channel activity in human DSM function. This was achieved by elucidating IP₃R-TRPM4 channel spatial and functional interactions in human DSM by using in situ PLA and perforated patch-clamp electrophysiology in combination with the IP₃R inhibitor xestospongin-C. In situ PLA confirmed co-localization between IP₃Rs and TRPM4 channels in human DSM isolated cells. Further, IP₃R pharmacological inhibition with xestospongin-C led to the attenuation of TICCs. This study established the novel finding that TRPM4 channels and SR IP₃Rs are physically and functionally coupled in the regulation of human DSM excitability.

Several studies from our laboratory have revealed key roles for the TRPM4 channels in regulating the excitability and contractility of DSM in rodents. More recently, we further explored the translational implications of our findings in rodents by investigating the physiologic roles for the TRPM4 channels in clinically-characterized human DSM. As summarized in a recent editorial article in the *American Journal of Physiology Cell Physiology*, our group revealed critical roles for the TRPM4 channels in regulating DSM function. TRPM4 channel expression is abundant in human DSM and species-related differences in the...
The functional roles of the TRPM4 channels have been reported between human and rodents. This previous report by our group provided compelling evidence at the cellular level in human DSM demonstrating the TRPM4 channel inhibitor 9-phenanthrol had a substantially greater inhibitory effect on DSM cell excitability in human versus rodent DSM cells.

Utilizing the selective TRPM4 channel inhibitor 9-phenanthrol, we reported that the pharmacological inhibition of the TRPM4 channels hyperpolarized the membrane potential in human DSM cells and caused inhibition of spontaneous phasic, pharmacologically-induced, and nerve-evoked contractions in human DSM isolated strips. Among the mechanisms underlying the inhibitory effects of 9-phenanthrol on human DSM excitability and contractility includes the attenuation of TICCs. TICCs are caused by Na\(^+\) influx following Ca\(^{2+}\)-dependent TRPM4 channel activation which, under physiologic conditions, functions to enhance DSM cell excitability. As TRPM4 channels are activated by Ca\(^{2+}\), it was critically important to determine the specific Ca\(^{2+}\)-dependent molecular and cellular mechanisms involved in the regulation of TRPM4 channel activity. Although well-known, there are 2 key sources for the elevation of intracellular Ca\(^{2+}\) concentrations, including: 1) Ca\(^{2+}\) entry through L-type voltage-gated Ca\(^{2+}\) channels at the cell membrane, and 2) Ca\(^{2+}\) release events through SR ryanodine receptors or IP\(_3\)Rs.

![Diagram of IP\(_3\)R inhibition with xestospongin-(C) decreased TICC activity in human DSM cells.](image)

**Figure 2.** IP\(_3\)R inhibition with xestospongin-(C) decreased TICC activity in human DSM cells. (A) An original recording showing the inhibitory effect of xestospongin-C (1 \(\mu\)M) on TICCs in a human DSM single cell recorded at a holding potential of -70 mV. (B) Summary data illustrating the inhibitory effects of xestospongin-C (1 \(\mu\)M) on TICCs, analyzed as single channel open probability (NPo) \((n = 6, N = 4; ^*P < 0.05)\).

![Diagram of IP\(_3\)R regulatory role of TRPM4 channels in human DSM function.](image)

**Figure 3.** Illustration of the IP\(_3\)R regulatory role of TRPM4 channels in human DSM function. TRPM4 channels conduct monovalent cations (primarily Na\(^+\) under resting conditions) and control human DSM membrane potential. TRPM4 channels are controlled by IP\(_3\)R-mediated Ca\(^{2+}\) release from the sarcoplasmic reticulum as inhibition of the IP\(_3\)Rs with xestospongin-C reduces TRPM4 channel activity. Pharmacological blockade of TRPM4 channels with 9-phenanthrol hyperpolarizes the DSM cell membrane, inhibits the voltage-gated Ca\(^{2+}\) channels, reduces intracellular Ca\(^{2+}\) levels and promotes DSM relaxation.
emerging evidence from non-DSM cell types has shown that members of the TRP channel family, including TRPM4, can be activated by intracellular Ca\(^{2+}\) released from SR IP\(_3\)Rs. IP\(_3\)-mediated activation of adjacent TRPM4 channels, as has been reported in non-DSM cell types,\(^{16,25}\) would lead to depolarization of the cell membrane potential, activation of L-type voltage-gated Ca\(^{2+}\) channels, an increase in global intracellular Ca\(^{2+}\) concentrations, and thus the activation of DSM contractions. In fact, in cerebral artery myocytes, sustained TRPM4 channel activity was shown to be dependent on Ca\(^{2+}\) released from the SR IP\(_3\)Rs.\(^{26}\)

Given the critically important role for the TRPM4 channels in human DSM excitability and contractility, we sought to examine whether IP\(_3\)Rs and TRPM4 channels functionally interact to regulate human DSM function. Our results demonstrated that xestospongin-C, a selective IP\(_3\)R inhibitor, significantly decreased TICC activity in human DSM cells (Fig. 2). These results are consistent with IP\(_3\)R-dependent regulation of TRPM4 channels in human DSM by a similar mechanism as was suggested for cerebral artery myocytes. Ca\(^{2+}\) released from the SR IP\(_3\)Rs are localized and transient events as has been demonstrated in murine colonic myocytes and rabbit portal vein myocytes.\(^{12,27}\) Hence, localized Ca\(^{2+}\) release events for the SR IP\(_3\)Rs must be in close apposition to the plasma-lemmal TRPM4 channels in order for IP\(_3\)R-TRPM4 channel functional coupling. Thus, to ascertain the potential co-localization for the TRPM4 channels and IP\(_3\)Rs, we used the Duolink \textit{in situ} PLA technique in combination with TRPM4 channel and IP\(_3\)R-specific antibodies. As illustrated by Fig. 1, \textit{in situ} PLA experiments demonstrated the co-localization of IP\(_3\)Rs and TRPM4 channels in freshly-isolated human DSM cells within the vicinity of the cell membrane. These results are in contrast to findings from arterial myocytes, where IP\(_3\)R co-localization was confirmed with TRPC3 channels, while no co-localization with either the TRPM4 or TRPC6 channels was found.\(^{28}\) The existence of IP\(_3\)R co-localization with TRPM4 channels in DSM, but not in arterial myocytes, may suggest TRPM4 channel-IP\(_3\)R spatial interactions follow, to an extent, a certain level of tissue-specific expression.

While our current data provide strong evidence to support the spatial and functional coupling of the IP\(_3\)Rs and TRPM4 channels in human DSM (Figs. 1-2), we cannot exclude potential involvement of alternative signaling pathways. IP\(_3\) and Ca\(^{2+}\) are the 2 central modulators of SR IP\(_3\)R activity.\(^{29}\) Indeed, IP\(_3\)R activity has been shown to be regulated by IP\(_3\) generated downstream from the activation of G\(_{q/11}\)-coupled muscarinic receptor pathways involving phospholipase C hydrolysis of phosphatidylinositol bisphosphate (PIP\(_2\)).\(^{29}\) Further, more recent studies have shown Ca\(^{2+}\)-permeable TRPC6 channels are spatially and functionally coupled with the SR IP\(_3\)Rs in cerebral arterial myocytes.\(^{30}\) Thus, TRPC6 activation of the IP\(_3\)Rs leads to Ca\(^{2+}\) release events from the SR IP\(_3\)Rs, which then activate adjacent TRPM4 channels at the cell membrane.\(^{30}\) Also, in the vasculature, protein kinase C was shown to modulate vascular myogenic tone in a TRPM4 channel-dependent manner.\(^{31,32}\) Consistently, in a separate study on HEK-293 cells expressing recombinant TRPM4 channels, the sensitivity for TRPM4 channels to Ca\(^{2+}\)-dependent activation is enhanced by PKC phosphorylation.\(^{33}\) Whether these potential TRPM4 channel regulatory mechanisms exist in human DSM is yet to be explored. Our data confirm IP\(_3\)R-TRPM4 channel functional coupling, and this work represents a foundational study in the human urinary bladder and provides a solid platform for subsequent mechanistic studies in human DSM (Fig. 3).

The key functional role of the TRPM4 channels in promoting DSM excitability has provided an intriguing opportunity for potential therapeutic exploitation in the context of overactive bladder syndrome. Specifically, pharmacological modulators of the TRPM4 channels or mechanisms controlling their activity may represent a promising novel approach for treatment of bladder dysfunction. Given the critical role of the TRPM4 channels in human DSM function and dysfunction, further efforts to ascertain TRPM4 channel regulatory mechanisms are critical for confirming and validating their potential therapeutic utility. The current study has provided novel mechanistic insight into TRPM4 channel regulation by SR IP\(_3\)Rs in human DSM. As current treatments of overactive bladder lack desired efficacy, research efforts to identify novel therapeutic modalities are urgently needed and examining TRPM4 channel physiologic roles and mechanisms of regulation is of scientific and clinical relevance.

\textbf{Disclosure of potential conflicts of interest}

No potential conflicts of interest were disclosed.
Acknowledgments

We would like to thank Drs. Kiril L. Hristov and Shankar P. Parajuli for their help with the patch-clamp experiments. We thank the Medical University of South Carolina (MUSC) Urology staff surgeons: Drs. Thomas Keane, Harry Clarke, Stephen Savage, Ross Rames, Sandip Prasad, and Jonathan Picard, as well as the MUSC Urology Residents: Ryan Levey, Austin Younger, Mark Carrin, Nima Baradaran, Olugbemisola McCoy, Alyssa Greiman, Sarah Starosta, Bryce Wyatt and Tracy Tipton for their help with human tissue collection. We also thank Drs. Whitney Kellett and Damiano Angoli for the critical evaluation of the manuscript.

Funding

This study was supported by a grant from the National Institutes of Health R01 DK106964 to Georgi V. Petkov. Aaron McCoy, Alyssa Greiman, Sarah Starosta, Bryce Wyatt and Tracy Tipton for their help with human tissue collection. We also thank Drs. Whitney Kellett and Damiano Angoli for the critical evaluation of the manuscript.

References

[1] 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; 140:159-69; PMID:12967945; https://doi.org/10.1038/sj.bjp.0705320
[2] Petkov GV. Role of potassium ion channels in detrusor smooth muscle function and dysfunction. Nat Rev Urol 2012; 9:30-40; PMID:22158596; https://doi.org/10.1038/nrruro.2011.194
[3] Hashitani H, Brading AF. Electrical properties of detrusor smooth muscles from the pig and human urinary bladder. Br J Pharmacol 2003; 140:146-58; PMID:12967944; https://doi.org/10.1038/sj.bjp.0705319
[4] Petkov GV. Central role of the BK channel in urinary bladder smooth muscle physiology and pathophysiology. Am J Physiol Regul Integr Comp Physiol 2014; 307:R571-84; PMID:24990859; https://doi.org/10.1152/ajpregu.00142.2014
[5] Hristov KL, Smith AC, Parajuli SP, Malyss J, Rovner ES, Petkov GV. Novel regulatory mechanism in human urinary bladder: Central role of transient receptor potential melastatin 4 channels in detrusor smooth muscle function. Am J Physiol Cell Physiol 2016; 310(C):C903-12; PMID:21697543; https://doi.org/10.1152/ajpcell.00169.2010
[6] Hristov KL, Afeli SA, Parajuli SP, Cheng Q, Rovner ES, Petkov GV. Neurogenic detrusor overactivity is associated with decreased expression and function of the large-conductance voltage- and Ca2+-activated K+ channels. Am J Physiol Cell Physiol 2013; 304:C467-77; PMID:23302778; https://doi.org/10.1152/ajpcell.00169.2012
[7] Smith AC, Hristov KL, Cheng Q, Xin W, Parajuli SP, Earley S, Malyss J, Petkov GV. Novel role for the transient receptor potential melastatin 4 channel in guinea pig detrusor smooth muscle physiology. Am J Physiol Cell Physiol 2013; 304:C467-77; PMID:23302778; https://doi.org/10.1152/ajpcell.00169.2012
[20] Provence A, Hristov KL, Parajuli SP, Rovner ES, Petkov GV. Voltage-gated KCNQ channels in human detrusor smooth muscle contractility: A novel target for the pharmacological treatment of overactive bladder. J Urol 2015; 193:e188; https://doi.org/10.1016/j.juro.2015.02.912

[21] Provence A, Angoli D, Rovner ES, Petkov GV. KV7 channel pharmacological modulation in human detrusor: A promising two-way street for the potential treatment of overactive and underactive bladder. J Urol 2017; 197: e1353; https://doi.org/10.1016/j.juro.2017.02.3162

[22] Gonzales AL, Earley S. Regulation of cerebral artery smooth muscle membrane potential by Ca\textsuperscript{2+}-activated cation channels. Microcirculation 2013; 20:337-47; PMID:23116477; https://doi.org/10.1111/micc.12023

[23] Fliegert R, Glassmeier G, Schmid F, Cornils K, Genisyuerek S, Harneit A, Schwarz JR, Guse AH. Modulation of Ca\textsuperscript{2+} entry and plasma membrane potential by human TRPM4b. FEBS J 2007; 274:704-13; PMID:17288552; https://doi.org/10.1111/j.1742-4658.2006.05614.x

[24] Amberg GC, Navedo MF. Calcium dynamics in vascular smooth muscle. Microcirculation 2013; 20:281-9; PMID:23384444; https://doi.org/10.1111/micc.12046

[25] Xi Q, Adebiyi A, Zhao G, Chapman KE, Waters CM, Hassid A, Jaggar JH. IP\textsubscript{3} constrains cerebral arteries via IP\textsubscript{3} receptor-mediated TRPC3 channel activation and independently of sarcoplasmic reticulum Ca\textsuperscript{2+} release. Circ Res 2008; 102:1118-26; PMID:18388325; https://doi.org/10.1161/CIRCRESAHA.108.173948

[26] Gonzales AL, Amberg GC, Earley S. Ca\textsuperscript{2+} release from the sarcoplasmic reticulum is required for sustained TRPM4 activity in cerebral artery smooth muscle cells. Am J Physiol Cell Physiol 2010; 299:C279-88; PMID:20427713; https://doi.org/10.1152/ajpcell.00550.2009

[27] Gordienko DV, Bolton TB. Crosstalk between ryanodine receptors and IP\textsubscript{3} receptors as a factor shaping spontaneous Ca\textsuperscript{2+}-release events in rabbit portal vein myocytes. J Physiol 2002; 542:743-62; PMID:12154176; https://doi.org/10.1113/jphysiol.2001.015966

[28] Adebiyi A, Zhao G, Narayanan D, Thomas-Gatewood CM, Bannister JP, Jaggar JH. Isoform-selective physical coupling of TRPC3 channels to IP\textsubscript{3} receptors in smooth muscle cells regulates arterial contractility. Circ Res 2010; 106:1603-12; PMID:20378853; https://doi.org/10.1161/CIRCRESAHA.110.216804

[29] Hill-Eubanks DC, Werner ME, Heppner TJ, Nelson MT. Calcium signaling in smooth muscle. Cold Spring Harb Perspect Biol 2011; 3:a004549; PMID:21709182; https://doi.org/10.1101/cshperspect.a004549

[30] Gonzales AL, Yang Y, Sullivan MN, Sanders L, Dabertrand F, Hill-Eubanks DC, Nelson MT, Earley S. A PLCgamma1-dependent, force-sensitive signaling network in the myogenic constriction of cerebral arteries. Sci Signal 2014; 7:ra49; PMID:24866019; https://doi.org/10.1126/scisignal.2004732

[31] Earley S, Straub SV, Brayden JE. Protein kinase C regulates vascular myogenic tone through activation of TRPM4. Am J Physiol Heart Circ Physiol 2007; 292: H2613-22; PMID:17293488; https://doi.org/10.1152/ajpheart.01286.2006

[32] Earley S, Waldron BJ, Brayden JE. Critical role for transient receptor potential channel TRPM4 in myogenic constriction of cerebral arteries. Circ Res 2004; 95:922-9; PMID:15472118; https://doi.org/10.1161/01.RES.0000147311.54833.03

[33] Nilius B, Prener J, Tang J, Wang C, Owsiianik G, Janssens A, Voets T, Zhu MX. Regulation of the Ca\textsuperscript{2+} sensitivity of the nonselective cation channel TRPM4. J Biol Chem 2005; 280:6423-33; PMID:15590641; https://doi.org/10.1074/jbc.M411089200