

## UC Davis

### UC Davis Previously Published Works

#### Title

Ion channel molecular complexes in vascular smooth muscle

#### Permalink

<https://escholarship.org/uc/item/7jb0s8nq>

#### Authors

da Silva, Eric A Pereira  
Baudel, Miguel Martín-Aragón  
Navedo, Manuel F  
et al.

#### Publication Date

2022

#### DOI

10.3389/fphys.2022.999369

Peer reviewed



## OPEN ACCESS

## EDITED BY

Kevin J. Sampson,  
Columbia University, United States

## REVIEWED BY

Anna Bukiya,  
University of Tennessee Health Science  
Center (UTHSC), United States  
M. Teresa Perez-Garcia,  
University of Valladolid, Spain  
Jonathan H. Jaggar,  
University of Tennessee Health Science  
Center (UTHSC), United States

## \*CORRESPONDENCE

Madeline Nieves-Cintrón,  
mcnieves@ucdavis.edu

## SPECIALTY SECTION

This article was submitted to Membrane  
Physiology and Membrane Biophysics,  
a section of the journal  
Frontiers in Physiology

RECEIVED 21 July 2022

ACCEPTED 02 August 2022

PUBLISHED 26 August 2022

## CITATION

Pereira da Silva EA,  
Martín-Aragón Baudel M, Navedo MF  
and Nieves-Cintrón M (2022), Ion  
channel molecular complexes in  
vascular smooth muscle.  
*Front. Physiol.* 13:999369.  
doi: 10.3389/fphys.2022.999369

## COPYRIGHT

© 2022 Pereira da Silva, Martín-Aragón  
Baudel, Navedo and Nieves-Cintrón.  
This is an open-access article  
distributed under the terms of the  
[Creative Commons Attribution License  
\(CC BY\)](https://creativecommons.org/licenses/by/4.0/). The use, distribution or  
reproduction in other forums is  
permitted, provided the original  
author(s) and the copyright owner(s) are  
credited and that the original  
publication in this journal is cited, in  
accordance with accepted academic  
practice. No use, distribution or  
reproduction is permitted which does  
not comply with these terms.

# Ion channel molecular complexes in vascular smooth muscle

Eric A. Pereira da Silva, Miguel Martín-Aragón Baudel,  
Manuel F. Navedo and Madeline Nieves-Cintrón\*

Department of Pharmacology, University of California, Davis, Davis, CA, United States

Ion channels that influence membrane potential and intracellular calcium concentration control vascular smooth muscle excitability. Voltage-gated calcium channels (VGCC), transient receptor potential (TRP) channels, voltage ( $K_V$ ), and  $Ca^{2+}$ -activated  $K^+$  (BK) channels are key regulators of vascular smooth muscle excitability and contractility. These channels are regulated by various signaling cues, including protein kinases and phosphatases. The effects of these ubiquitous signaling molecules often depend on the formation of macromolecular complexes that provide a platform for targeting and compartmentalizing signaling events to specific substrates. This manuscript summarizes our current understanding of specific molecular complexes involving VGCC, TRP, and  $K_V$  and BK channels and their contribution to regulating vascular physiology.

## KEYWORDS

**L-type calcium channel, TRP channels, potassium channels, blood pressure, resistance arteries**

## Introduction

The diameter of small resistance arteries is a critical determinant of blood flow and tissue perfusion. The contractility of vascular smooth muscle (VSM) regulates arterial diameter. Multiple ion channels regulate VSM contraction by controlling membrane potential and the magnitude of intracellular calcium concentration  $[Ca^{2+}]_i$  (Knot and Nelson, 1995). In VSM, voltage-dependent L-type  $Ca_V1.2$  (LTCCs) channels are the main  $Ca^{2+}$  influx pathway for contraction (Ghosh et al., 2017), and a role for T-type channels regulation of VSM excitability is also described (Cribbs, 2006; Abd El-Rahman et al., 2013). Transient receptor potential (TRP) channels contribute to vascular function by regulating membrane potential, contraction, and myogenic tone development (Brayden et al., 2008). Voltage ( $K_V$ ) and  $Ca^{2+}$ -activated  $K^+$  (BK) channels provide a negative-feedback regulation of VGCC activity, hence  $Ca^{2+}$  influx and contraction, by modulating VSM membrane potential. Different stimuli within the body, including variations in pressure, vasoactive substances released from endothelial cells, and nerve terminals, modulate contraction by initiating cellular signaling that impinges on the function of these ion channels.

Signaling complexes permit efficient transduction of the many signals received with the specificity necessary to support function. VGCC,  $K^+$ , and TRP channels form complexes with key proteins (e.g., ion channels, GPCR, signaling molecules) that modulate their function and, in doing so, regulate VSM contractility. Scaffold proteins, such as AKAP5, have been shown to facilitate molecular complex formation with signaling and effectors proteins in VSM. For more extensive reviews on the different ion channels, readers are directed to (Earley and Brayden, 2015; Ghosh et al., 2017; Tykocki et al., 2017). Here we will summarize current knowledge on specific molecular complexes involving LTCC, TTCC, TRP,  $K_V$ , and BK channels and their contribution to regulating vascular physiology.

## Voltage-gated calcium channels

Voltage-gated  $Ca^{2+}$  channels (VGCC) are found in various cell types throughout the body. Changes in membrane potential activate these channels, leading to  $Ca^{2+}$  influx and the regulation of many physiological processes (Catterall, 2011). VGCC comprises a family of ten members, subdivided into three major subfamilies,  $Ca_V1$ ,  $Ca_V2$ , and  $Ca_V3$ , based on their biophysical properties (Catterall, 2000; Catterall, 2011). Members of the  $Ca_V1$  and  $Ca_V2$  families have been identified in VSM (Tykocki et al., 2017). The  $Ca_V1$  family activates at depolarized membrane potential and is characterized by large conductance and long openings, hence their name L-type  $Ca^{2+}$  channels (LTCC). Meanwhile, the  $Ca_V2$  or T-type (TTCC) family of VGCC has tiny currents that activate at more negative potentials than the LTCC and inactivate fast (Catterall, 2011). Of relevance to this review is the LTCC, which is necessary for the VSM myogenic contraction (Moosmang et al., 2003), and the TTCC that emerging data suggest participate in arterial tone regulation (Cribbs, 2006; Abd El-Rahman et al., 2013; Harraz et al., 2015a). In the following section, we summarize the contributions of these channels to VSM physiology, emphasizing their participation in the signaling domains that facilitate the regulation of VSM excitability.

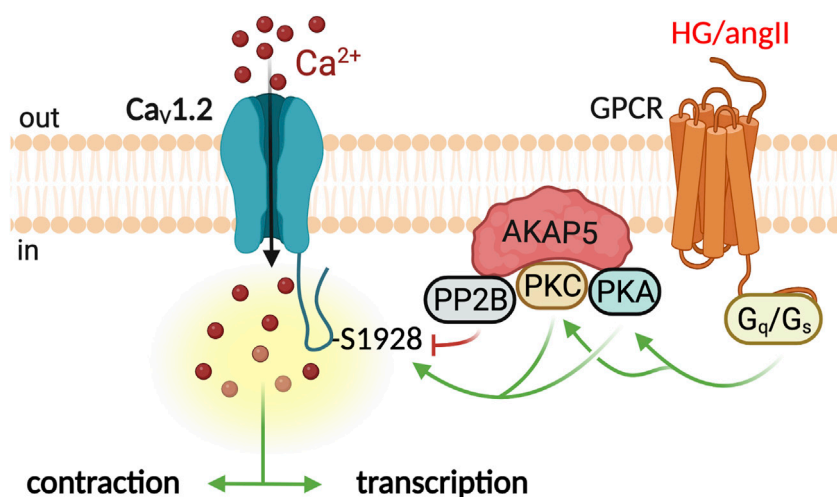
## $Ca_V1.2$ signaling complexes in VSM

L-type calcium channel  $Ca_V1.2$  is the main entryway for  $Ca^{2+}$  in vascular smooth muscle contributing  $Ca^{2+}$  for contraction and gene expression. Indeed,  $Ca^{2+}$  influx through  $Ca_V1.2$  is necessary for pressure-induced contraction (i.e., myogenic tone), and studies suggest that about 50% of phenylephrine-induced contraction is due to  $Ca^{2+}$  influx via these channels (Moosmang et al., 2003). Moreover,  $Ca^{2+}$  influx via  $Ca_V1.2$  channels is linked to transcriptional regulation in VSM (Wamhoff et al., 2004; Wamhoff et al., 2006;

Kudryavtseva et al., 2013). The altered function of the  $Ca_V1.2$  channels is associated with contractility and gene expression changes. For instance, studies associate changes in  $Ca_V1.2$  function with increased myogenic tone in conditions such as hypertension (Nieves-Cintrón et al., 2008; Navedo et al., 2010a) and diabetes (Navedo et al., 2010b; Nystoriak et al., 2014; Nystoriak et al., 2017a).

LTCCs are heteromeric complexes of  $\alpha1$ -,  $\beta$ , and  $\alpha2\delta$  subunits (Catterall, 2000). The  $\alpha1$ -subunit, which forms the ion conduction pore, consists of four homologous domains (I-IV), each domain having six transmembrane segments (S1-S6) linked by three intracellular loops, and intracellular amino and carboxy-terminal. The homologous transmembrane segment S6 and the loop between S5 and S6 of each homologous domain form the ion conduction pore. Voltage sensitivity is provided by transmembrane segment S1-S4 (Catterall, 2011).  $\beta$  and  $\alpha2\delta$  subunits are auxiliary subunits that have been shown to contribute to membrane trafficking and regulate the channel biophysics (De Jongh et al., 1990; Singer et al., 1991; Gao et al., 1999; Dolphin, 2003; Catterall, 2011). Among the four known subtypes ( $\beta1$  -  $\beta4$ ),  $\beta3$  is the principal subunit in VSM (Murakami et al., 2000; Kharade et al., 2013). The  $\alpha2\delta$  subunit arises from a single gene; subsequent posttranslational processing produces an extracellular  $\alpha2$  and membranous  $\delta$  subunits that associate via a disulfide bridge to form a functional subunit (Gurnett et al., 1996; Arikath and Campbell, 2003). Three different  $\alpha2\delta$  delta isoforms have been identified ( $\alpha2\delta1$  -  $\alpha2\delta3$ ) (Klugbauer et al., 1999). The  $\alpha2\delta1$  subunit was critical for membrane expression of the  $Ca_V1.2$   $\alpha1c$  in VSM from rat cerebral artery (Bannister et al., 2009).

Alterations in the subunit expression profiles have been linked to changes in physiology. For example, studies report increased expression of  $Ca_V1.2$   $\alpha1c$  subunit in VSM in models of high blood pressure, including genetic models of hypertension (Pratt et al., 2002; Pesic et al., 2004). Moreover, increased expression of  $\beta3$  subunit contributes to the upregulation of  $Ca_V1.2$   $\alpha1c$  subunit membrane expression in VSM from animal models of hypertension owing to its role in the channel trafficking (Kharade et al., 2013). Elevation of the  $\alpha2\delta1$  subunit expression during hypertension was also reported and linked with higher  $Ca_V1.2$  membrane surface expression and currents (Bannister et al., 2012). Changes in the expression of LTCC subunits have also been reported in a genetic model of hypertension (BPH mice) (Tajada et al., 2013). However, while several studies report increases in the expression of the  $Ca_V1.2$   $\alpha1c$  subunit in hypertension (Pratt et al., 2002; Pesic et al., 2004; Sonkusare et al., 2006), the study by Tajada et al. shows a decrease in  $Ca_V1.2$   $\alpha1c$  expression in mesenteric artery VSM from BPH mice relative to normotensive control mice (Tajada et al., 2013). Accordingly, mesenteric VSM from BPH mice showed a reduction in  $Ca_V1.2$   $\alpha1c$  and a change in the expression profile of the accessory subunits. The authors



**FIGURE 1**

Ca<sub>v</sub>1.2 molecular complex in the regulation of VSM contraction and transcription. Ca<sup>2+</sup> influx through Ca<sub>v</sub>1.2 channels is essential for the pressure-induced contraction of the VSM (Moosmang et al., 2003). Activation of G<sub>q</sub>/PKA signaling by high glucose or G<sub>q</sub>/PKC by angiotensin II potentiate Ca<sub>v</sub>1.2 activity (Navedo et al., 2008; Navedo et al., 2010b; Prada et al., 2019). The scaffolding protein AKAP5 facilitates PKA/PKC phosphorylation of Ca<sub>v</sub>1.2 channels at S1928 (Nystoriak et al., 2017a). The phosphatase PP2B opposes phosphorylation dampening Ca<sub>v</sub>1.2 potentiation. Graphics created with BioRender.com.

proposed that in normotensive BPN mice, Ca<sub>v</sub>1.2 currents were carried by  $\alpha 1c/\beta 3/\alpha 2\delta$ , whereas in BPH mice, the current was likely mediated by channels composed of  $\alpha 1c/\beta 2/\alpha 2\delta$  subunits. This discrepancy could be due to the hypertension model used (e.g., angiotensin-induced vs. genetic hypertension), the species (rat vs. mice), and vascular beds. Nevertheless, the studies highlight the relevance of the accessory subunits in regulating LTCC activity and VSM contractility.

In VSM, the Ca<sub>v</sub>1.2 channel forms molecular complexes with signaling components including receptors, enzymes, and effector proteins orchestrated by scaffolds proteins, such as the A-kinase anchoring protein 5 (AKAP5) (Tykocki et al., 2017). AKAPs are structurally diverse intracellular scaffolding proteins that bind PKA, PKC, calcineurin (PP2B), and Ca<sub>v</sub>1.2, thus facilitating regulation of the channel by these proteins (Colledge and Scott, 1999; Langeberg and Scott, 2005). Optical recording of Ca<sup>2+</sup> influx revealed that protein kinases and phosphatases modulate Ca<sub>v</sub>1.2 activity in an AKAP-dependent manner in VSM (M Nieves-Cintrón et al., 2018) (Figure 1). Fluorescent signals using TIRF microscopy. Contrary to expectation, not all Ca<sub>v</sub>1.2 channels had a similar open probability. Subpopulations of channels showed stochastic transient openings (low activity sparklets). In contrast, other channels displayed events with prolonged available time produced by activating two or more channels that generated areas of almost continuous calcium influx (Navedo et al., 2005). This high-activity mode, termed persistent sparklets, required PKC activity and AKAP5 expression (Navedo et al., 2006). Moreover,

the phosphatase PP2B is part of the molecular complex, and its activity limits Ca<sub>v</sub>1.2-persistent sparklets (Navedo et al., 2006). Persistent Ca<sub>v</sub>1.2-sparklets contributed to approximately 50% of Ca<sup>2+</sup> influx through LTCC (Amberg et al., 2007), highlighting the relevance of these events and the macromolecular complex that regulates them to VSM physiology.

Ca<sub>v</sub>1.2-sparklets events are of higher frequency and amplitude in VSM from hypertensive mice than in corresponding control mice. In the angiotensin II-induced hypertension model, higher Ca<sub>v</sub>1.2-sparklets required activation of PKC signaling (Navedo et al., 2008). Higher Ca<sub>v</sub>1.2 sparklets increased arterial wall Ca<sup>2+</sup>, tone, and blood pressure. Interestingly, angiotensin II failed to elicit an increase in Ca<sub>v</sub>1.2-sparklets in AKAP5 knockout mice (Navedo et al., 2008). Moreover, these mice did not develop hypertension in response to angiotensin infusion (Navedo et al., 2008). Thus, angiotensin II increases arterial tone and blood pressure by stimulating Ca<sub>v</sub>1.2 sparklets locally in an AKAP5-dependent manner. VSM from murine models of diabetes also shows elevated Ca<sup>2+</sup> sparklet activity, and AKAP5 is also central to the signaling events leading to higher Ca<sub>v</sub>1.2 sparklet activity during diabetic hyperglycemia (Navedo et al., 2010b; Nieves-Cintrón et al., 2021). AKAP5 orchestrates a signaling module that includes the purinergic receptor P2Y<sub>11</sub>, adenylyl cyclase 5, PKA, and the Ca<sub>v</sub>1.2 subunit (Prada et al., 2019; Syed et al., 2019; Prada et al., 2020) (Figure 1). Activating this signaling module increases Ca<sub>v</sub>1.2 activation and arterial contractility during diabetes. The fact that PKA was required for Ca<sub>v</sub>1.2-sparklets during diabetes is surprising as this kinase is associated with vasodilation. It also

highlights the exquisite level of signal specificity achieved by segregating receptors, signaling enzymes, and effector proteins (Colledge and Scott, 1999; Langeberg and Scott, 2005) into macromolecular complexes.

## T-type calcium channel signaling complex in VSM

TTCC  $\alpha 1$  subunit topology is similar to that of other VGCC. However, no auxiliary subunits have been co-purified with the TTCC, but studies suggest that TTCC activity may be modified by other proteins, including the LTCC auxiliary subunits (Perez-Reyes, 2006; Dolphin, 2016). TTCC subunits  $\text{Ca}_v3.1$ ,  $\text{Ca}_v3.2$ , and  $\text{Ca}_v3.3$  have been identified in VSM from several vascular beds, and emergent evidence points toward a contribution of these channels to the regulation of the VSM reactivity (Cribbs, 2006; Abd El-Rahman et al., 2013). TTCCs have been shown to contribute to vasoconstrictor responses in mesenteric arterioles (Gustafsson et al., 2001), skeletal muscle arteries (VanBavel et al., 2002), and retinal arteriolar (Fernandez et al., 2015) VSM. In cerebral VSM from rats and mice,  $\text{Ca}_v3.1$  and  $\text{Ca}_v3.2$  carry the nifedipine insensitive component of  $\text{Ba}^{2+}$  currents (Nikitina et al., 2007; Abd El-Rahman et al., 2013; Harraz et al., 2015b). Human cerebral arteries express  $\text{Ca}_v3.2$  however the  $\text{Ca}_v3.1$  described in rodents is replaced by the  $\text{Ca}_v3.3$  isoform in these arteries (Harraz et al., 2015a).  $\text{Ca}_v3.1$  and  $\text{Ca}_v3.3$  activity were found to contribute to the pressure-induced constriction (Harraz et al., 2014; Harraz et al., 2015b). Interestingly, selective inhibition of  $\text{Ca}_v3.2$  channels with micromolar  $\text{Ni}^{2+}$  concentration elicited depolarization and constriction of wild-type rat cerebral arteries (Harraz et al., 2014). Electron tomography of rat cerebral VSM showed microdomains consisting of caveolae and sarcoplasmic reticulum (SR). Immunogold labeling localized  $\text{Ca}_v3.2$  and RyR to these microdomains (Harraz et al., 2014). The studies proposed that caveolae facilitate a  $\text{Ca}^{2+}$  signaling network that enables the coupling of  $\text{Ca}_v3.2$  mediated  $\text{Ca}^{2+}$  influx with activation of RyR receptors leading to activation of  $\text{Ca}^{2+}$ -activated potassium channels contributing to membrane potential hyperpolarization (Harraz et al., 2014; Harraz et al., 2015a; Harraz et al., 2015b). Thus, the segregation into a microdomain allows  $\text{Ca}_v3.2$  to modulate arterial tone by regulating the RyR-BK channels axis (Harraz et al., 2014; Harraz et al., 2015a).

## TRP channels signaling complexes in VSM

TRP channels are a superfamily of cation channels encoded by 28 genes. The superfamily is divided into six subfamilies based on sequence homology, which include TRPC (canonical), TRPV (vanilloid), TRPM (melastatin), TRPP (polycystin), TRPA

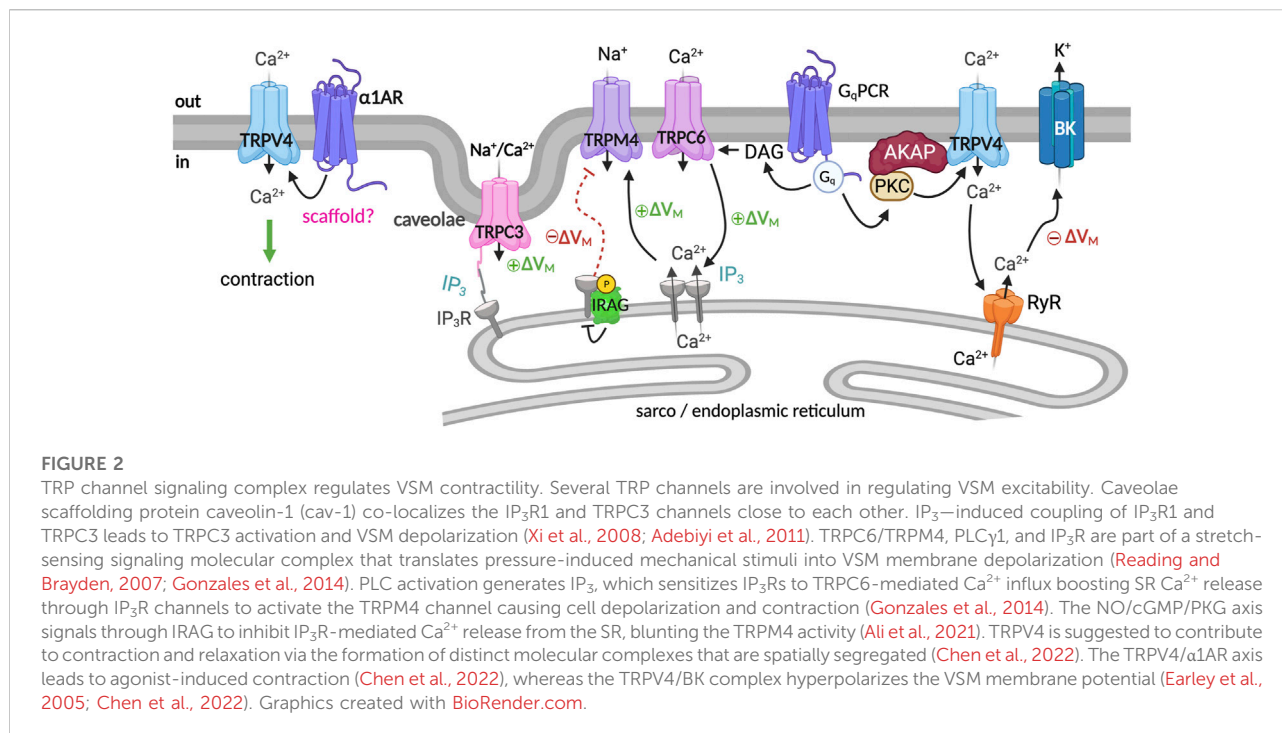
(ankyrin), and TRPML (mucolipin) channels (Earley and Brayden, 2015). TRP channels comprise six membrane-spanning segments (S1-S6) with intracellular amino and carboxy terminals. Electron cryomicroscopy studies of the TRPV1 channel suggest a symmetrical four-fold arrangement with the S5 and S6 loops forming the ion path (Liao et al., 2013). VSM cells express several TRP channels, which contribute to regulating membrane potential, contraction, and myogenic tone development. Some TRP channels translate mechanosensitive G-coupled signaling into changes in membrane potential in VSM cells (Earley and Brayden, 2015), thus contributing to artery autoregulation. Recent studies uncovered a role for specific TRP channels in signaling networks and molecular complexes that contribute to regulating contractile state. Here we summarize these studies, emphasizing well-characterized TRP channel signaling complexes contributing to the regulation of VSM excitability.

## TRPC3 & TRPC6

In VSM cells, the plasmalemmal canonical transient receptor potential 3 (TRPC3) channels decode G-protein coupled receptor (GPCR) signaling into a cation current that elicits cell depolarization (Earley and Brayden, 2015). Indeed, in VSM, TRPC3 channels are implicated in cerebral artery constriction in response to GPCR agonists such as angiotensin II and endothelin-1 (ET-1) (Reading et al., 2005; Xi et al., 2008; Earley and Brayden, 2015). Intriguingly, TRPC3 channels in VSM are activated by GPCR-phospholipase C (PLC) signaling independently of SR  $\text{Ca}^{2+}$  release and PKC (Xi et al., 2008). The agonist  $\text{IP}_3$  was shown to constrict rat (Sprague-Dawley (SD), male and female) cerebral arteries by facilitating coupling between type 1 inositol 1,4,5-trisphosphate receptor type 1 ( $\text{IP}_3\text{R1}$ ) and TRPC3 channels (Xi et al., 2008). Further studies showed that in cerebral artery (SD, male) VSM, the caveolae scaffolding protein caveolin-1 (cav-1) co-localizes the  $\text{IP}_3\text{R1}$  and TRPC3 channels close to each other (Adebiyi et al., 2011). This signaling complex allows  $\text{IP}_3$ -induced coupling of  $\text{IP}_3\text{R1}$  and TRPC3, leading to TRPC3 activation and generation of cation current that depolarizes VSM, thus eliciting contraction (Adebiyi et al., 2011) (Figure 2).

TRPC6 channels also contribute to regulating pressure-induced VSM contraction (Welsh et al., 2002). Like TRPC3, TRPC6 channels are activated by GPCR-PLC agonists such as angiotensin II via mechanisms requiring diacylglycerol but independent of PKC (Helliwell and Large, 1997; Saleh et al., 2006). Studies using cerebral arteries from male SD rats suggest that TRPC6 channels are part of a stretch-sensing signaling network, including  $\text{PLC}\gamma 1$ , TRPM4, and  $\text{IP}_3\text{Rs}$  that translate pressure-induced mechanical stimuli into membrane depolarization of cerebral VSM (Gonzales et al., 2014) (Figure 2). According to the proposed model, initiation of





PLCγ1 signaling generates IP<sub>3</sub>, which sensitizes IP<sub>3</sub>Rs to TRPC6-mediated Ca<sup>2+</sup> influx. This process boosts SR Ca<sup>2+</sup> release through IP<sub>3</sub>R, activating TRPM4 channels, cell depolarization, and artery constriction (Gonzales et al., 2014).

## TRPM4

Increases in intraluminal pressure depolarize VSM and constrict cerebral arteries partly via the activation of the TRPM4 channel (Brayden et al., 2008). TRPM4 channels are selective for monovalent cations. Accordingly, TRPM4 channels are activated by Ca<sup>2+</sup> but do not permeate this ion. Calmodulin binding sites have been identified on the C-terminus of the TRPM4 channel, which influences the sensitivity to Ca<sup>2+</sup> (Nilius et al., 2005). Moreover, TRPM4 Ca<sup>2+</sup> sensitivity is regulated by ATP and PKC-mediated phosphorylation of putative serine residues in the carboxy-terminal (Nilius et al., 2005). Suppressing TRPM4 expression in rat pial arteries compromises the ability of the arteries to regulate blood flow in response to changes in the mean arterial pressure (MAP) (Reading and Brayden, 2007), highlighting the physiological relevance of the channel for blood flow autoregulation. In rat's cerebral VSM, TRPM4 channels are activated by local IP<sub>3</sub>R-mediated increase in [Ca<sup>2+</sup>]<sub>i</sub> (Gonzales et al., 2010). As mentioned above, with TRPC6, TRPM4 channels are part of a mechanosensitive signaling complex whereby IP<sub>3</sub> is generated by activating GPCR-PLCγ1 signaling, which sensitizes IP<sub>3</sub>Rs to

TRPC6-Ca<sup>2+</sup> influx boosting IP<sub>3</sub>R-SR Ca<sup>2+</sup> release to activate TRPM4 (Gonzales et al., 2014) (Figure 2). By regulating TRPM4 activity, this signaling complex may regulate pressure-induced constriction in cerebral arteries.

Intriguingly, a recent study suggests that the tissue soluble gaseous vasodilator nitric oxide could relax arteries by inhibiting the activity of TRPM4 channels (Ali et al., 2021) (Figure 2). NO is produced by endothelial cells and exerts its vasodilatory action by increasing soluble cyclic guanosine monophosphate (cGMP) and stimulating the activity of cGMP-activated protein kinase (PKG) (Lincoln et al., 1985; Carvajal et al., 2000). Using male and female C57BL/6J cerebral arteries, a mechanism was described involving the formation of a signaling complex on the SR of VSM comprised of PKG, IP<sub>3</sub>Rs, and IP<sub>3</sub>R-associated cGMP-kinase substrate (IRAG) (Ali et al., 2021). IRAG is a regulator of IP<sub>3</sub>-triggered Ca<sup>2+</sup> release found to be complex with IP<sub>3</sub>Rs in the VSM (Geisshoringer et al., 2004). Phosphorylation of IRAG by activation of the NO/cGMP/PKG axis was found to inhibit IP<sub>3</sub>R-mediated Ca<sup>2+</sup> release from the SR (Geisshoringer et al., 2004; Schlossmann and Desch, 2011). As a result of IP<sub>3</sub>R inhibition and concomitant SR Ca<sup>2+</sup> release, TRPM4 channel activity is blunted. This leads to a reduction in TRPM4-mediated membrane depolarization and voltage-dependent Ca<sup>2+</sup> channel activity leading to vasodilation (Ali et al., 2021). The formation of this NO/cGMP/PKG/IRAG signaling complex allows for distinct regulation of TRPM4 channel activity and, therefore, VSM membrane potential and contractile state.

## TRPV4

VSM cells express transient receptor potential vanilloid 4 (TRPV4) channels. TRPV4 are  $\text{Ca}^{2+}$  permeable, nonselective cation channels, and their activation results in  $\text{Ca}^{2+}$  influx (Brayden et al., 2008). Interestingly, although the magnitude of the calcium influx through TRPV4 channels is significant, TRPV4 activity is linked to VSM relaxation (Earley et al., 2005). This has been attributed to relatively low basal TRPV4 activity and the formation of a signaling complex with ryanodine receptors (RyRs) and large-conductance  $\text{Ca}^{2+}$ -activated potassium (BK) channels (Earley et al., 2005) (Figure 2). In cerebral and cerebellar arteries from male SD rats, activation of TRPV4 by endothelium-derived signals elicits  $\text{Ca}^{2+}$  influx that activates RyRs (Earley et al., 2005). RyR activation results in SR  $\text{Ca}^{2+}$  release (i.e.,  $\text{Ca}^{2+}$ -spark), coupled with the opening of BK channels at the plasma membrane of VSM cells. Activating the BK channels leads to VSM membrane hyperpolarization, decreased voltage-dependent  $\text{Ca}^{2+}$  channel activity, and intracellular  $\text{Ca}^{2+}$  concentration  $[\text{Ca}^{2+}]_i$ , thereby causing VSM relaxation (Knot et al., 1998).

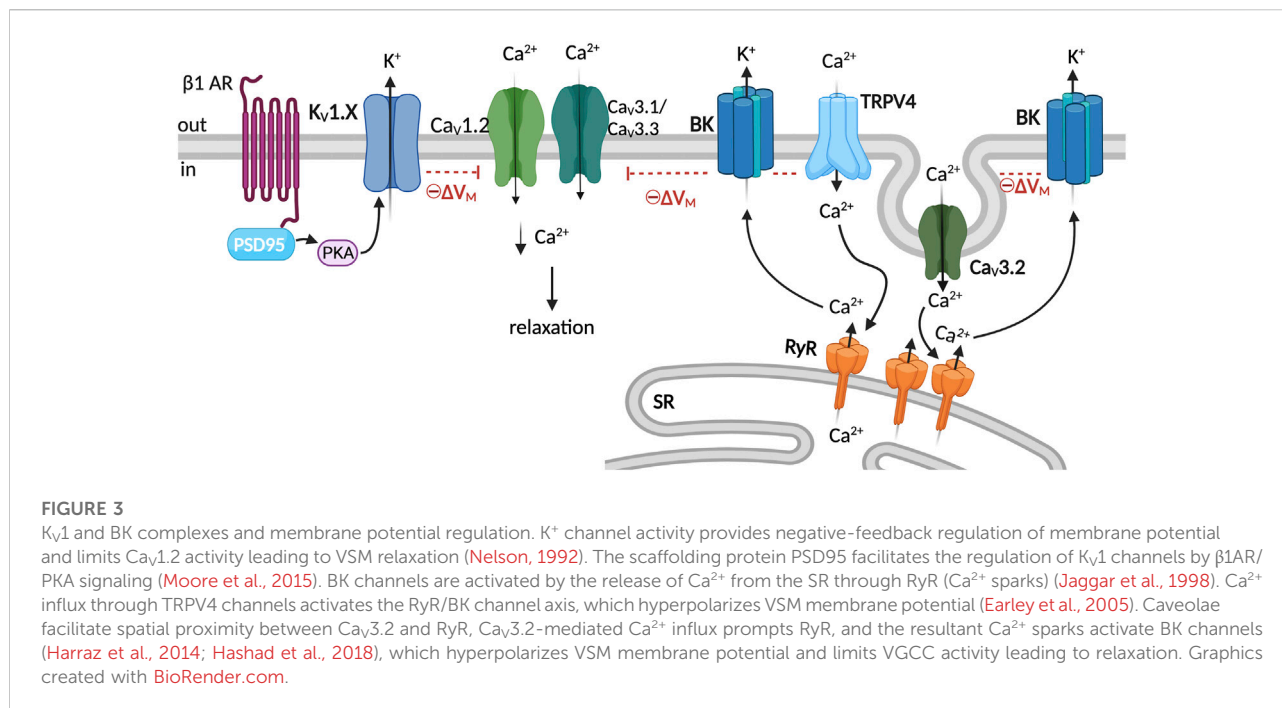
Studies using cerebral arteries from rats, C57BL/6J, and AKAP150<sup>-/-</sup> mice suggest that in VSM, AKAP5 targeted PKC $\alpha$  facilitates regulation of TRPV4 channels by GqPCR signaling (Mercado et al., 2014; Tajada et al., 2017). Interestingly, a new study using mice mesenteric and human paraspinal muscle arteries submits that TRPV4 channels form spatially separated complexes with  $\alpha 1$  adrenergic receptor ( $\alpha 1\text{AR}$ ) and the BK channel, allowing them to respond differentially to different physiological stimuli (Chen et al., 2022). In this model, TRPV4 channels in proximity to the  $\alpha 1\text{AR}$  are activated by  $\alpha 1\text{AR}/\text{GqPCR}$  signaling and contribute to agonist-induced vasoconstriction. In contrast, pressure-induced activation of the TRPV4/BK channel axis opposes contraction (Chen et al., 2022) (Figure 2). The TRPV4/ $\alpha 1\text{AR}$  signaling axis was elevated in hypertension, whereas the TRPV4/BK complex was decreased, highlighting a potential new therapeutic target for hypertension. It will be important to determine if different scaffolds are responsible for the distinct spatial segregation of signaling components. The referenced studies suggest that forming distinct signaling complexes allows a diversity of the regulation and function of plasmalemmal TRP activity with profound consequences for VSM membrane potential and contractile state (Figure 2).

## K<sup>+</sup> channels

K<sub>V</sub> channels are a varied group of membrane proteins comprising at least 12 families, namely K<sub>V</sub>1-K<sub>V</sub>12 (Gutman et al., 2005). Structurally, K<sub>V</sub> channels are formed by tetrameric assembly of alpha subunits, which form the ion conduction pore and auxiliary beta subunits (Choe et al., 1999). The tetramer's subunit composition and inclusion of auxiliary beta subunits

provide functional and pharmacological diversity (Zhong et al., 2010a; Kilfoil et al., 2013; Tykocki et al., 2017). VSM expresses multiple K<sub>V</sub> channel subunits, including members of the K<sub>V</sub>1 (K<sub>V</sub>1.1, K<sub>V</sub>1.2, K<sub>V</sub>1.3, K<sub>V</sub>1.5, K<sub>V</sub>1.6) (Cheong et al., 2001; Albarwani et al., 2003; Amberg et al., 2003; Tykocki et al., 2017), and K<sub>V</sub>2 (K<sub>V</sub>2.1) (Amberg and Santana, 2006; Tykocki et al., 2017) families. Members of the K<sub>V</sub>7 (K<sub>V</sub>7.1–5) (Zhong et al., 2010b) and K<sub>V</sub>9 (K<sub>V</sub>9.3) (Zhong et al., 2010a; Tykocki et al., 2017) have also been identified. Homomeric and heteromeric channels consisting of an assembly of distinct alpha subunits with auxiliary K<sub>V</sub> $\beta$  have been found. For example, heteromultimeric channels consisting of K<sub>V</sub>1.2-K<sub>V</sub>1.5 subunits have been shown to contribute to vascular K<sub>V</sub> currents in VSM (Kerr et al., 2001; Albarwani et al., 2003; Plane et al., 2005). Heteromers of K<sub>V</sub>2.1 and silent K<sub>V</sub>9.3 subunits have been identified in VSM from rat cerebral arteries that contribute to regulating arterial diameter (Zhong et al., 2010a). Expression of K<sub>V</sub>6.3 silent subunit was reported in mesenteric VSM from hypertensive mice (Moreno-Dominguez et al., 2009). Studies in murine coronary microvasculature show an association between K<sub>V</sub>1.5 and auxiliary K<sub>V</sub> $\beta$ 1 and K<sub>V</sub> $\beta$ 2 (Nystoriak et al., 2017b). The study suggests that K<sub>V</sub> $\beta$ 2 facilitates K<sub>V</sub>1.5 membrane trafficking in coronary VSM (Nystoriak et al., 2017b). K<sub>V</sub> channels control VSM contraction by regulating membrane potential and the magnitude of the VGCC-mediated  $\text{Ca}^{2+}$  influx (Knot and Nelson, 1995; Nystoriak and Navedo, 2017). Thus, mechanisms that regulate K<sub>V</sub> activity will impact VSM membrane potential, contractility, and ultimately arterial tone and diameter.

VSM K<sub>V</sub> channel activity is modulated by intracellular signaling (Ko et al., 2010). For example, several vasoconstrictors are shown to partially constrict arteries via PKC-dependent inhibition of K<sub>V</sub> channel activity (Aiello et al., 1996; Clement-Chomienne et al., 1996; Hayabuchi et al., 2001). In addition to PKC, the Src tyrosine kinase pathway has been shown to blunt K<sub>V</sub> activity and contract rat mesenteric arteries (Sung et al., 2013). On the other hand, protein kinase A (PKA) is associated with K<sub>V</sub> potentiation and vasodilation (Tykocki et al., 2017). For instance,  $\beta$ -adrenergic receptor agonists that engage adenylyl cyclase (AC)/cAMP/PKA signaling promote 4-AP-sensitive K<sub>V</sub> channel activity in VSM and vasodilation (Aiello et al., 1995; Cole et al., 1996; Satake et al., 1996; Berwick et al., 2010). Despite the evidence of intracellular signaling regulation of K<sub>V</sub> activity and VSM contractility, the formation of macromolecular complexes that could facilitate this regulation is relatively unknown. Recent evidence, however, supports the role of the scaffolding protein postsynaptic density 95 (PSD95) in facilitating the regulation of K<sub>V</sub> channels by  $\beta$ -adrenergic signaling (Joseph et al., 2011; Moore et al., 2014; Moore et al., 2015). As its name implies, PSD95 is found at the postsynaptic density, where it facilitates the formation of macromolecular complexes between receptors, ion channels, and signaling molecules (Won et al., 2017). PSD95 was found to colocalize with the  $\beta 1$ -adrenergic



receptor in VSM and to enable phosphorylation of K<sub>v</sub>1 channels by PKA in rat cerebral VSM (Joseph et al., 2011; Moore et al., 2014). Thus, in cerebral VSM, PSD95 facilitates β-adrenergic-mediated vasodilatory regulation via the formation of a macromolecular complex with K<sub>v</sub>1 channels (Moore et al., 2015; Rhee and Rusch, 2018) (Figure 3). Whether other scaffolds are involved in K<sub>v</sub> channel regulation in VSM is an area for further research development in VSM.

## BK-channels

Large-conductance Ca<sup>2+</sup>-activated potassium channels (BK) are critical regulators of VSM contractility by tonically regulating membrane potential (Jaggar et al., 1998). BK channels are activated by calcium and membrane depolarization (Latorre et al., 2017). The channel comprises tetrameric assemblies of pore-forming alpha subunits, which harbor the voltage sensor and cytosolic calcium-binding sites (Ko et al., 2008; Latorre et al., 2017). The BKα subunit assembles with auxiliary beta and gamma subunits. Four β (β1-β4) and four γ isoforms (γ1-γ4) have been identified so far (Li and Yan, 2016). The β1 subunit is the main β subunit isoform expressed in VSM cells; it increases BK calcium sensitivity and alters the biophysical properties of the channel (Brenner et al., 2000). The γ1 subunit has been reported in rat cerebral VSM (male SD) cells and suggested to increase the BK channel voltage sensitivity (Evanson et al., 2014). BK channels are activated by the simultaneous release of SR Ca<sup>2+</sup> via RyRs in close apposition to VSM plasmalemma BK channels

(Nelson et al., 1995) (Figure 3). Functional coupling between BK channels and RyR has been extensively characterized in VSM as a critical mechanism regulating arterial diameter (Nelson et al., 1995; Perez et al., 1999; Fan et al., 2019). Moreover, studies in mice mesenteric and human cerebral VSM suggest that BK channels are part of a Ca<sup>2+</sup> signaling microdomain that includes caveolae, T-type Ca<sub>v</sub>3.2, and RyRs (Harraz et al., 2014). Caveolae facilitates the coupling of Ca<sub>v</sub>3.2 channel activity with RyR activation (Hashad et al., 2018), and BK channels within the signaling domain are activated by the Ca<sup>2+</sup> sparks (Figure 3). As described above, BK channels are also suggested to be in a signaling complex with TRPV4 and RyRs, which promote BK channel activation in response to changes in the intraluminal pressure (Earley et al., 2005).

## Conclusion

Small resistance arteries respond to different stimuli by adjusting their diameter to meet the tissue perfusion needs. The adjustments in the arterial diameter of small resistance arteries and arterioles are largely determined by the contractile state of VSM lining the walls of arteries (Knot and Nelson, 1995; Knot and Nelson, 1998; Cole et al., 2005). VSM contractility is dependent on the interplay of different ionic conductance that controls membrane potential and the level of intracellular [Ca<sup>2+</sup>]<sub>i</sub>. Different stimuli within the body engage signaling mechanisms that regulate these ion channels and, in turn, modulate vascular function. Here, we have provided an overview of our current



knowledge of specific molecular complexes involving VGCC, TRP, and  $K_V$  and BK channels emphasizing well-characterized signaling complexes contributing to the regulation of VSM excitability. The formation of macromolecular complexes could provide a way for ion channels to respond differently to specific stimuli by bringing signaling generators and effectors within proximity to discrete regions of the cell. Determining whether similar complexes exist in different vascular beds and their contribution to VSM contractility in health and disease is an area of opportunity in the vascular field. Understanding the specific mechanism that governs the formation of these molecular complexes will provide novel strategies for developing therapeutics with enhanced specificity in the fight against cardiovascular disease.

## Author contributions

Each author wrote a section of the manuscript. ES gathered contributions and generated figures. MC integrated all the contributions and provided overall supervision and direction to the project. All authors revised and approved the final version.

## References

- Abd El-Rahman, R. R., Harraz, O. F., Brett, S. E., Anfinogenova, Y., Mufti, R. E., Goldman, D., et al. (2013). Identification of L- and T-type  $Ca^{2+}$  channels in rat cerebral arteries: Role in myogenic tone development. *Am. J. Physiol. Heart Circ. Physiol.* 304 (1), H58–H71. doi:10.1152/ajpheart.00476.2012
- Adebiji, A., Narayanan, D., and Jaggar, J. H. (2011). Caveolin-1 assembles type 1 inositol 1, 4, 5-trisphosphate receptors and canonical transient receptor potential 3 channels into a functional signaling complex in arterial smooth muscle cells. *J. Biol. Chem.* 286 (6), 4341–4348. doi:10.1074/jbc.M110.179747
- Aiello, E. A., Clement-Chomienne, O., Sontag, D. P., Walsh, M. P., and Cole, W. C. (1996). Protein kinase C inhibits delayed rectifier  $K^+$  current in rabbit vascular smooth muscle cells. *Am. J. Physiol.* 271 (12), H109–H119. doi:10.1152/ajpheart.1996.271.1.H109
- Aiello, E. A., Walsh, M. P., and Cole, W. C. (1995). Phosphorylation by protein kinase A enhances delayed rectifier  $K^+$  current in rabbit vascular smooth muscle cells. *Am. J. Physiol.* 268 (22), H926–H934. doi:10.1152/ajpheart.1995.268.2.H926
- Albarwani, S., Nemetz, L. T., Madden, J. A., Tobin, A. A., England, S. K., Pratt, P. F., et al. (2003). Voltage-gated  $K^+$  channels in rat small cerebral arteries: Molecular identity of the functional channels. *J. Physiol.* 551, 751–763. doi:10.1113/jphysiol.2003.040014
- Ali, S., Solano, A. S., Gonzales, A. L., Thakore, P., Krishnan, V., Yamasaki, E., et al. (2021). Nitric oxide signals through IRAG to inhibit TRPM4 channels and dilate cerebral arteries. *Function* 2 (6), zqab051. doi:10.1093/function/zqab051
- Amberg, G. C., Koh, S. D., Imaizumi, Y., Ohya, S., and Sanders, K. M. (2003). A-type potassium currents in smooth muscle. *Am. J. Physiol. Cell Physiol.* 284 (3), C583–C595. doi:10.1152/ajpcell.00301.2002
- Amberg, G. C., Navedo, M. F., Nieves-Cintrón, M., Molkentin, J. D., and Santana, L. F. (2007). Calcium sparklets regulate local and global calcium in murine arterial smooth muscle. *J. Physiol.* 579 (1), 187–201. doi:10.1113/jphysiol.2006.124420
- Amberg, G. C., and Santana, L. F. (2006).  $Kv2$  channels oppose myogenic constriction of rat cerebral arteries. *Am. J. Physiol. Cell Physiol.* 291 (2), C348–C356. doi:10.1152/ajpcell.00086.2006
- Arikath, J., and Campbell, K. P. (2003). Auxiliary subunits: Essential components of the voltage-gated calcium channel complex. *Curr. Opin. Neurobiol.* 13 (3), 298–307. doi:10.1016/s0959-4388(03)00066-7
- Bannister, J. P., Adebiji, A., Zhao, G., Narayanan, D., Thomas, C. M., Feng, J. Y., et al. (2009). Smooth muscle cell  $\alpha 2\delta 1$  subunits are essential for

## Funding

This work was supported by American Heart Association grants 852984 (MC.), 830626 (MB), and R01HL149127 and 161872 (MN).

## Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

## Publisher's note

All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

vasoregulation by  $CaV1.2$  channels. *Circ. Res.* 105 (10), 948–955. doi:10.1161/CIRCRESAHA.109.203620

Bannister, J. P., Bulley, S., Narayanan, D., Thomas-Gatewood, C., Luzny, P., Pachau, J., et al. (2012). Transcriptional upregulation of  $\alpha 2\delta 1$  elevates arterial smooth muscle cell voltage-dependent  $Ca^{2+}$  channel surface expression and cerebrovascular constriction in genetic hypertension. *Hypertension* 60 (4), 1006–1015. doi:10.1161/HYPERTENSIONAHA.112.199661

Berwick, Z. C., Payne, G. A., Lynch, B., Dick, G. M., Sturek, M., and Tune, J. D. (2010). Contribution of adenosine A(2A) and A(2B) receptors to ischemic coronary dilation: Role of  $K(V)$  and  $K(ATP)$  channels. *Microcirculation* 17 (8), 600–607. doi:10.1111/j.1549-8719.2010.00054.x

Brayden, J. E., Earley, S., Nelson, M. T., and Reading, S. (2008). Transient receptor potential (TRP) channels, vascular tone and autoregulation of cerebral blood flow. *Clin. Exp. Pharmacol. Physiol.* 35 (9), 1116–1120. doi:10.1111/j.1440-1681.2007.04855.x

Brenner, R., Perez, G. J., Bonev, A. D., Eckman, D. M., Kosek, J. C., Wiler, S. W., et al. (2000). Vasoregulation by the  $\beta 1$  subunit of the calcium-activated potassium channel. *Nature* 407 (6806), 870–876. doi:10.1038/35038011

Carvajal, J. A., Germain, A. M., Huidobro-Toro, J. P., and Weiner, C. P. (2000). Molecular mechanism of cGMP-mediated smooth muscle relaxation. *J. Cell. Physiol.* 184 (3), 409–420. doi:10.1002/1097-4652(200009)184:3<409:AID-JCP16>3.0.CO;2-K

Catterall, W. A. (2000). Structure and regulation of voltage-gated  $Ca^{2+}$  channels. *Annu. Rev. Cell Dev. Biol.* 16, 521–555. doi:10.1146/annurev.cellbio.16.1.521

Catterall, W. A. (2011). Voltage-gated calcium channels. *Cold Spring Harb. Perspect. Biol.* 3 (8), a003947. doi:10.1101/cshperspect.a003947

Chen, Y. L., Daneva, Z., Kuppusamy, M., Ottolini, M., Baker, T. M., Klimentova, E., et al. (2022). Novel smooth muscle  $Ca^{2+}$ -signaling nanodomains in blood pressure regulation. *Circulation*, 101161CIRCULATIONAHA121058607. Epub ahead of print. doi:10.1161/CIRCULATIONAHA.121.05860

Cheong, A., Dedman, A. M., Xu, S. Z., and Beech, D. J. (2001).  $K(V)$   $\alpha$  channels in murine arterioles: Differential cellular expression and regulation of diameter. *Am. J. Physiol. Heart Circ. Physiol.* 281 (3), H1057–H1065. doi:10.1152/ajpheart.2001.281.3.H1057

Choe, S., Kreuzsch, A., and Pfaffinger, P. J. (1999). Towards the three-dimensional structure of voltage-gated potassium channels. *Trends biochem. Sci.* 24 (9), 345–349. doi:10.1016/s0968-0004(99)01440-1

- Clement-Chomienne, O., Walsh, M. P., and Cole, W. C. (1996). Angiotensin II activation of protein kinase C decreases delayed rectifier K<sup>+</sup> current in rabbit vascular myocytes. *J. Physiol.* 495 (3), 689–700. doi:10.1113/jphysiol.1996.sp021626
- Cole, W. C., Chen, T. T., and Clement-Chomienne, O. (2005). Myogenic regulation of arterial diameter: Role of potassium channels with a focus on delayed rectifier potassium current. *Can. J. Physiol. Pharmacol.* 83 (8-9), 755–765. doi:10.1139/y05-082
- Cole, W. C., Clement-Chomienne, O., and Aiello, E. A. (1996). Regulation of 4-aminopyridine-sensitive, delayed rectifier K<sup>+</sup> channels in vascular smooth muscle by phosphorylation. *Biochem. Cell Biol.* 74 (4), 439–447. doi:10.1139/o96-048
- Colledge, M., and Scott, J. D. (1999). AKAPs: From structure to function. *Trends Cell Biol.* 9 (6), 216–221. doi:10.1016/s0962-8924(99)01558-5
- Cribbs, L. L. (2006). T-Type Ca<sup>2+</sup> channels in vascular smooth muscle: Multiple functions. *Cell Calcium* 40 (2), 221–230. doi:10.1016/j.ceca.2006.04.026
- De Jongh, K. S., Warner, C., and Catterall, W. A. (1990). Subunits of purified calcium channels. Alpha 2 and delta are encoded by the same gene. *J. Biol. Chem.* 265 (25), 14738–14741. doi:10.1016/s0021-9258(18)77174-3
- Dolphin, A. C. (2003). Beta subunits of voltage-gated calcium channels. *J. Bioenerg. Biomembr.* 35 (6), 599–620. doi:10.1023/b:jobb.0000008026.37790.5a
- Dolphin, A. C. (2016). Voltage-gated calcium channels and their auxiliary subunits: Physiology and pathophysiology and pharmacology. *J. Physiol.* 594 (19), 5369–5390. doi:10.1113/jp227262
- Earley, S., and Brayden, J. E. (2015). Transient receptor potential channels in the vasculature. *Physiol. Rev.* 95 (2), 645–690. doi:10.1152/physrev.00026.2014
- Earley, S., Heppner, T. J., Nelson, M. T., and Brayden, J. E. (2005). TRPV4 forms a novel Ca<sup>2+</sup> signaling complex with ryanodine receptors and BKCa channels. *Circ. Res.* 97 (12), 1270–1279. doi:10.1161/01.RES.0000194321.60300.d6
- Evanson, K., Bannister, J. P., Leo, M. D., and Jaggar, J. H. (2014). LRRc26 is a functional BK channel auxiliary gamma subunit in arterial smooth muscle cells. *Circ. Res.* 115, 423–431. doi:10.1161/CIRCRESAHA.115.303407
- Fan, G., Cui, Y., Gollasch, M., and Kassmann, M. (2019). Elementary calcium signaling in arterial smooth muscle. *Channels (Austin)* 13 (1), 505–519. doi:10.1080/19336950.2019.1688910
- Fernandez, J. A., McGahon, M. K., McGeown, J. G., and Curtis, T. M. (2015). CaV3.1 T-Type Ca<sup>2+</sup> channels contribute to myogenic signaling in rat retinal arterioles. *Invest. Ophthalmol. Vis. Sci.* 56 (9), 5125–5132. doi:10.1167/iovs.15-17299
- Gao, T., Chien, A. J., and Hosey, M. M. (1999). Complexes of the alpha1C and beta subunits generate the necessary signal for membrane targeting of class C L-type calcium channels. *J. Biol. Chem.* 274 (4), 2137–2144. doi:10.1074/jbc.274.4.2137
- Geiselhoringer, A., Werner, M., Sigl, K., Smital, P., Wornor, R., Acheo, L., et al. (2004). IRAG is essential for relaxation of receptor-triggered smooth muscle contraction by cGMP kinase. *EMBO J.* 23 (21), 4222–4231. doi:10.1038/sj.emboj.7600440
- Ghosh, D., Syed, A. U., Prada, M. P., Nystoriak, M. A., Santana, L. F., Nieves-Cintrón, M., et al. (2017). Calcium channels in vascular smooth muscle. *Adv. Pharmacol.* 78, 49–87. doi:10.1016/bs.apha.2016.08.002
- Gonzales, A. L., Amberg, G. C., and Earley, S. (2010). Ca<sup>2+</sup> release from the sarcoplasmic reticulum is required for sustained TRPM4 activity in cerebral artery smooth muscle cells. *Am. J. Physiol. Cell Physiol.* 299 (2), C279–C288. doi:10.1152/ajpcell.00550.2009
- Gonzales, A. L., Yang, Y., Sullivan, M. N., Sanders, L., Dabertrand, F., Hill-Eubanks, D. C., et al. (2014). A PLCγ1-dependent, force-sensitive signaling network in the myogenic constriction of cerebral arteries. *Sci. Signal.* 7 (327), ra49. doi:10.1126/scisignal.2004732
- Gurnett, C. A., De Waard, M., and Campbell, K. P. (1996). Dual function of the voltage-dependent Ca<sup>2+</sup> channel alpha 2 delta subunit in current stimulation and subunit interaction. *Neuron* 16 (2), 431–440. doi:10.1016/s0896-6273(00)80061-6
- Gustafsson, F., Andreasen, D., Salomonsson, M., Jensen, B. L., and Holstein-Rathlou, N. (2001). Conducted vasoconstriction in rat mesenteric arterioles: Role for dihydropyridine-insensitive Ca(2+) channels. *Am. J. Physiol. Heart Circ. Physiol.* 280 (2), H582–H590. doi:10.1152/ajpheart.2001.280.2.H582
- Gutman, G. A., Chandy, K. G., Grissmer, S., Lazdunski, M., McKinnon, D., Pardo, L. A., et al. (2005). International Union of Pharmacology. LIII. Nomenclature and molecular relationships of voltage-gated potassium channels. *Pharmacol. Rev.* 57 (4), 473–508. doi:10.1124/pr.57.4.10
- Harraz, O. F., Abd El-Rahman, R. R., Bigdely-Shamloo, K., Wilson, S. M., Brett, S. E., Romero, M., et al. (2014). Ca(V)3.2 channels and the induction of negative feedback in cerebral arteries. *Circ. Res.* 115 (7), 650–661. doi:10.1161/CIRCRESAHA.114.304056
- Harraz, O. F., Brett, S. E., Zechariah, A., Romero, M., Puglisi, J. L., Wilson, S. M., et al. (2015). Genetic ablation of CaV3.2 channels enhances the arterial myogenic response by modulating the RyR-BKCa axis. *Arterioscler. Thromb. Vasc. Biol.* 35 (8), 1843–1851. doi:10.1161/ATVBAHA.115.305736
- Harraz, O. F., Visser, F., Brett, S. E., Goldman, D., Zechariah, A., Hashad, A. M., et al. (2015). CaV1.2/CaV3.x channels mediate divergent vasomotor responses in human cerebral arteries. *J. Gen. Physiol.* 145 (5), 405–418. doi:10.1085/jgp.201511361
- Hashad, A. M., Harraz, O. F., Brett, S. E., Romero, M., Kassmann, M., Puglisi, J. L., et al. (2018). Caveolae link CaV3.2 channels to BKCa-mediated feedback in vascular smooth muscle. *Arterioscler. Thromb. Vasc. Biol.* 38 (10), 2371–2381. doi:10.1161/ATVBAHA.118.311394
- Hayabuchi, Y., Standen, N. B., and Davies, N. W. (2001). Angiotensin II inhibits and alters kinetics of voltage-gated K(+) channels of rat arterial smooth muscle. *Am. J. Physiol. Heart Circ. Physiol.* 281 (6), H2480–H2489. doi:10.1152/ajpheart.2001.281.6.H2480
- Helliwell, R. M., and Large, W. A. (1997). Alpha 1-adrenoceptor activation of a non-selective cation current in rabbit portal vein by 1, 2-diacyl-sn-glycerol. *J. Physiol.* 499 (2), 417–428. doi:10.1113/jphysiol.1997.sp021938
- Jaggar, J. H., Wellman, G. C., Heppner, T. J., Porter, V. A., Perez, G. J., Gollasch, M., et al. (1998). Ca<sup>2+</sup> channels, ryanodine receptors and Ca<sup>2+</sup>-activated K<sup>+</sup> channels: A functional unit for regulating arterial tone. *Acta Physiol. Scand.* 164 (4), 577–587. doi:10.1046/j.1365-201X.1998.00462.x
- Joseph, B. K., Thakali, K. M., Pathan, A. R., Kang, E., Rusch, N. J., and Rhee, S. W. (2011). Postsynaptic density-95 scaffolding of Shaker-type K<sup>+</sup> channels in smooth muscle cells regulates the diameter of cerebral arteries. *J. Physiol.* 589 (21), 5143–5152. doi:10.1113/jphysiol.2011.213843
- Kerr, P. M., Clement-Chomienne, O., Thorneloe, K. S., Chen, T. T., Ishii, K., Sontag, D. P., et al. (2001). Heteromultimeric Kv1.2-Kv1.5 channels underlie 4-aminopyridine-sensitive delayed rectifier K<sup>+</sup> current of rabbit vascular myocytes. *Circ. Res.* 89 (11), 1038–1044. doi:10.1161/hh2301.100803
- Kharade, S. V., Sonkusare, S. K., Srivastava, A. K., Thakali, K. M., Fletcher, T. W., Rhee, S. W., et al. (2013). The β3 subunit contributes to vascular calcium channel upregulation and hypertension in angiotensin II-infused C57BL/6 mice. *Hypertension* 61 (1), 137–142. doi:10.1161/HYPERTENSIONAHA.112.197863
- Kilfoil, P. J., Tipparaju, S. M., Barski, O. A., and Bhatnagar, A. (2013). Regulation of ion channels by pyridine nucleotides. *Circ. Res.* 112 (4), 721–741. doi:10.1161/CIRCRESAHA.111.247940
- Klugbauer, N., Lacinova, L., Marais, E., Hobom, M., and Hofmann, F. (1999). Molecular diversity of the calcium channel α<sub>2</sub>δ subunit. *J. Neurosci.* 19 (2), 684–691. doi:10.1523/jneurosci.19-02-00684.1999
- Knot, H. J., and Nelson, M. T. (1998). Regulation of arterial diameter and wall [Ca<sup>2+</sup>] in cerebral arteries of rat by membrane potential and intravascular pressure. *J. Physiol.* 508 (1), 199–209. doi:10.1111/j.1469-7793.1998.199br.x
- Knot, H. J., and Nelson, M. T. (1995). Regulation of membrane potential and diameter by voltage-dependent K<sup>+</sup> channels in rabbit myogenic cerebral arteries. *Am. J. Physiol.* 269 (12), H348–H355. doi:10.1152/ajpheart.1995.269.1.H348
- Knot, H. J., Standen, N. B., and Nelson, M. T. (1998). Ryanodine receptors regulate arterial diameter and wall [Ca<sup>2+</sup>] in cerebral arteries of rat via Ca<sup>2+</sup>-dependent K<sup>+</sup> channels. *J. Physiol.* 508 (1), 211–221. doi:10.1111/j.1469-7793.1998.211br.x
- Ko, E. A., Han, J., Jung, I. D., and Park, W. S. (2008). Physiological roles of K<sup>+</sup> channels in vascular smooth muscle cells. *J. Smooth Muscle Res.* 44 (2), 65–81. doi:10.1540/jsmr.44.65
- Ko, E. A., Park, W. S., Firth, A. L., Kim, N., Yuan, J. X., and Han, J. (2010). Pathophysiology of voltage-gated K<sup>+</sup> channels in vascular smooth muscle cells: Modulation by protein kinases. *Prog. Biophys. Mol. Biol.* 103 (1), 95–101. doi:10.1016/j.pbiomolbio.2009.10.001
- Kudryavtseva, O., Aalkjaer, C., and Matchkov, V. V. (2013). Vascular smooth muscle cell phenotype is defined by Ca<sup>2+</sup>-dependent transcription factors. *FEBS J.* 280 (21), 5488–5499. doi:10.1111/febs.12414
- Langeberg, L. K., and Scott, J. D. (2005). A-kinase-anchoring proteins. *J. Cell Sci.* 118 (15), 3217–3220. doi:10.1242/jcs.02416
- Latorre, R., Castillo, K., Carrasquel-Ursulaez, W., Sepulveda, R. V., Gonzalez-Nilo, F., Gonzalez, C., et al. (2017). Molecular determinants of BK channel functional diversity and functioning. *Physiol. Rev.* 97 (1), 39–87. doi:10.1152/physrev.00001.2016
- Li, Q., and Yan, J. (2016). Modulation of BK channel function by auxiliary beta and gamma subunits. *Int. Rev. Neurobiol.* 128, 51–90. doi:10.1016/bs.irm.2016.03.015

- Liao, M., Cao, E., Julius, D., and Cheng, Y. (2013). Structure of the TRPV1 ion channel determined by electron cryo-microscopy. *Nature* 504 (7478), 107–112. doi:10.1038/nature12822
- Lincoln, T. M., Dey, N., and Sellak, H. (1985). Invited review: cGMP-dependent protein kinase signaling mechanisms in smooth muscle: From the regulation of tone to gene expression. *J. Appl. Physiol.* 91 (3), 1421–1430. doi:10.1152/jappl.2001.91.3.1421
- M Nieves-Cintrón, S. T., Santana, L. F., and Navedo, M. F. (2018). *Methods in signal transduction: Vascular smooth muscle*. 1st Edition ed. Boca Raton, FL: CRC Press, 87–103. Total internal reflection fluorescent microscopy in vascular smooth muscle
- Mercado, J., Baylie, R., Navedo, M. F., Yuan, C., Scott, J. D., Nelson, M. T., et al. (2014). Local control of TRPV4 channels by AKAP150-targeted PKC in arterial smooth muscle. *J. Gen. Physiol.* 143 (5), 559–575. doi:10.1085/jgp.201311050
- Moore, C. L., McClenahan, S. J., Hanvey, H. M., Jang, D. S., Nelson, P. L., Joseph, B. K., et al. (2015). Beta1-adrenergic receptor-mediated dilation of rat cerebral artery requires Shaker-type KV1 channels on PSD95 scaffold. *J. Cereb. Blood Flow. Metab.* 35 (9), 1537–1546. doi:10.1038/jcbfm.2015.91
- Moore, C. L., Nelson, P. L., Parelkar, N. K., Rusch, N. J., and Rhee, S. W. (2014). Protein kinase A-phosphorylated KV1 channels in PSD95 signaling complex contribute to the resting membrane potential and diameter of cerebral arteries. *Circ. Res.* 114 (8), 1258–1267. doi:10.1161/CIRCRESAHA.114.303167
- Moosmang, S., Schulla, V., Welling, A., Feil, R., Feil, S., Wegener, J. W., et al. (2003). Dominant role of smooth muscle L-type calcium channel Cav1.2 for blood pressure regulation. *Embo J.* 22 (22), 6027–6034. doi:10.1093/emboj/cdg583
- Moreno-Dominguez, A., Ciudad, P., Miguel-Velado, E., Lopez-Lopez, J. R., and Perez-Garcia, M. T. (2009). De novo expression of Kv6.3 contributes to changes in vascular smooth muscle cell excitability in a hypertensive mice strain. *J. Physiol.* 587 (3), 625–640. doi:10.1113/jphysiol.2008.165217
- Murakami, M., Yamamura, H., Murakami, A., Okamura, T., Nunoki, K., Mitui-Saito, M., et al. (2000). Conserved smooth muscle contractility and blood pressure increase in response to high-salt diet in mice lacking the beta3 subunit of the voltage-dependent calcium channel. *J. Cardiovasc. Pharmacol.* 36 (2), S69–S73. doi:10.1097/00005344-200000006-00015
- Navedo, M. F., Amberg, G., Votaw, S. V., and Santana, L. F. (2005). Constitutively active L-type Ca<sup>2+</sup> channels. *Proc. Natl. Acad. Sci. U. S. A.* 102 (31), 11112–11117. doi:10.1073/pnas.0500360102
- Navedo, M. F., Amberg, G. C., Nieves, M., Molkentin, J. D., and Santana, L. F. (2006). Mechanisms underlying heterogeneous Ca<sup>2+</sup> sparklet activity in arterial smooth muscle. *J. Gen. Physiol.* 127 (6), 611–622. doi:10.1085/jgp.200609519
- Navedo, M. F., Cheng, E. P., Yuan, C., Votaw, S., Molkentin, J. D., Scott, J. D., et al. (2010). Increased coupled gating of L-type Ca<sup>2+</sup> channels during hypertension and Timothy syndrome. *Circ. Res.* 106 (4), 748–756. doi:10.1161/CIRCRESAHA.109.213363
- Navedo, M. F., Nieves-Cintrón, M., Amberg, G. C., Yuan, C., Votaw, V. S., Lederer, W. J., et al. (2008). AKAP150 is required for stuttering persistent Ca<sup>2+</sup> sparklets and angiotensin II-induced hypertension. *Circ. Res.* 102 (2), e1–e11. doi:10.1161/CIRCRESAHA.107.167809
- Navedo, M. F., Takeda, Y., Nieves-Cintrón, M., Molkentin, J. D., and Santana, L. F. (2010). Elevated Ca<sup>2+</sup> sparklet activity during acute hyperglycemia and diabetes in cerebral arterial smooth muscle cells. *Am. J. Physiol. Cell Physiol.* 298 (2), C211–C220. doi:10.1152/ajpcell.00267.2009
- Nelson, M. T., Cheng, H., Rubart, M., Santana, L. F., Bonev, A. D., Knot, H. J., et al. (1995). Relaxation of arterial smooth muscle by calcium sparks. *Science* 270 (5236), 633–637. doi:10.1126/science.270.5236.633
- Nelson, M. T. (1992). Regulation of arterial tone by potassium channels. *Jpn. J. Pharmacol.* 58 (2), 238P–42P. doi:10.1016/s0021-5198(19)59920-6
- Nieves-Cintrón, M., Amberg, G. C., Navedo, M. F., Molkentin, J. D., and Santana, L. F. (2008). The control of Ca<sup>2+</sup> influx and NFATc3 signaling in arterial smooth muscle during hypertension. *Proc. Natl. Acad. Sci. U. S. A.* 105 (40), 15623–15628. doi:10.1073/pnas.0808759105
- Nieves-Cintrón, M., Flores-Tamez, V. A., Le, T., Baudel, M. M., and Navedo, M. F. (2021). Cellular and molecular effects of hyperglycemia on ion channels in vascular smooth muscle. *Cell. Mol. Life Sci.* 78 (1), 31–61. doi:10.1007/s00018-020-03582-z
- Nikitina, E., Zhang, Z. D., Kawashima, A., Jahromi, B. S., Bourry, V. A., Takahashi, M., et al. (2007). Voltage-dependent calcium channels of dog basilar artery. *J. Physiol.* 580 (2), 523–541. doi:10.1113/jphysiol.2006.126128
- Nilius, B., Prenen, J., Tang, J., Wang, C., Owsanik, G., Janssens, A., et al. (2005). Regulation of the Ca<sup>2+</sup> sensitivity of the nonselective cation channel TRPM4. *J. Biol. Chem.* 280 (8), 6423–6433. doi:10.1074/jbc.M411089200
- Nystoriak, M. A., and Navedo, M. F. (2017). Regulation of microvascular function by voltage-gated potassium channels: New tricks for an "ancient" dog. *Microcirculation* 25, 12345. doi:10.1111/micc.12435
- Nystoriak, M. A., Nieves-Cintrón, M., Nygren, P. J., Hinke, S. A., Nichols, C. B., Chen, C. Y., et al. (2014). AKAP150 contributes to enhanced vascular tone by facilitating large-conductance Ca<sup>2+</sup>-activated K<sup>+</sup> channel remodeling in hyperglycemia and diabetes mellitus. *Circ. Res.* 114 (4), 607–615. doi:10.1161/CIRCRESAHA.114.302168
- Nystoriak, M. A., Nieves-Cintrón, M., Patriarchi, T., Buonarati, O. R., Prada, M. P., Morotti, S., et al. (2017). Ser1928 phosphorylation by PKA stimulates the L-type Ca<sup>2+</sup> channel CaV1.2 and vasoconstriction during acute hyperglycemia and diabetes. *Sci. Signal.* 10 (463), eaaf9647. doi:10.1126/scisignal.aaf9647
- Nystoriak, M. A., Zhang, D., Jagatheesan, G., and Bhatnagar, A. (2017). Heteromeric complexes of aldo-keto reductase auxiliary KVβ subunits (AKR6A) regulate sarcolemmal localization of KV1.5 in coronary arterial myocytes. *Chem. Biol. Interact.* 276, 210–217. doi:10.1016/j.cbi.2017.03.011
- Perez, G. J., Bonev, A. D., Patlak, J. B., and Nelson, M. T. (1999). Functional coupling of ryanodine receptors to KCa channels in smooth muscle cells from rat cerebral arteries. *J. Gen. Physiol.* 113 (2), 229–238. doi:10.1085/jgp.113.2.229
- Perez-Reyes, E. (2006). Molecular characterization of T-type calcium channels. *Cell Calcium* 40 (2), 89–96. doi:10.1016/j.ceca.2006.04.012
- Pesic, A., Madden, J. A., Pesic, M., and Rusch, N. J. (2004). High blood pressure upregulates arterial L-type Ca<sup>2+</sup> channels. Is membrane depolarization the signal? *Circ. Res.* 94, e97–104. doi:10.1161/01.RES.0000131495.93500.3c
- Plane, F., Johnson, R., Kerr, P., Wiehler, W., Thorneloe, K., Ishii, K., et al. (2005). Heteromultimeric Kv1 channels contribute to myogenic control of arterial diameter. *Circ. Res.* 96 (2), 216–224. doi:10.1161/01.RES.0000154070.06421.25
- Prada, M. P., Syed, A. U., Buonarati, O. R., Reddy, G. R., Nystoriak, M. A., Ghosh, D., et al. (2019). A Gs-coupled purinergic receptor boosts Ca<sup>2+</sup> influx and vascular contractility during diabetic hyperglycemia. *eLife* 8, e42214. doi:10.7554/eLife.42214
- Prada, M. P., Syed, A. U., Reddy, G. R., Martin-Aragon Baudel, M., Flores-Tamez, V. A., Sasse, K. C., et al. (2020). AKAP5 complex facilitates purinergic modulation of vascular L-type Ca(2+) channel CaV1.2. *Nat. Commun.* 11 (1), 5303. doi:10.1038/s41467-020-18947-y
- Pratt, P. F., Bonnet, S., Ludwig, L. M., Bonnet, P., and Rusch, N. J. (2002). Upregulation of L-type Ca<sup>2+</sup> channels in mesenteric and skeletal arteries of SHR. *Hypertension* 40 (2), 214–219. doi:10.1161/01.hyp.0000025877.23309.36
- Reading, S. A., and Brayden, J. E. (2007). Central role of TRPM4 channels in cerebral blood flow regulation. *Stroke* 38 (8), 2322–2328. doi:10.1161/STROKEAHA.107.483404
- Reading, S. A., Earley, S., Waldron, B. J., Welsh, D. G., and Brayden, J. E. (2005). TRPC3 mediates pyrimidine receptor-induced depolarization of cerebral arteries. *Am. J. Physiol. Heart Circ. Physiol.* 288 (5), H2055–H2061. doi:10.1152/ajpheart.00861.2004
- Rhee, S. W., and Rusch, N. J. (2018). Molecular determinants of beta-adrenergic signaling to voltage-gated K(+) channels in the cerebral circulation. *Microcirculation* 25 (1), e12425. doi:10.1111/micc.12425
- Saleh, S. N., Albert, A. P., Peppiatt, C. M., and Large, W. A. (2006). Angiotensin II activates two cation conductances with distinct TRPC1 and TRPC6 channel properties in rabbit mesenteric artery myocytes. *J. Physiol.* 577 (2), 479–495. doi:10.1113/jphysiol.2006.119305
- Satake, N., Shibata, M., and Shibata, S. (1996). The inhibitory effects of iberiotoxin and 4-aminopyridine on the relaxation induced by beta 1- and beta 2-adrenoceptor activation in rat aortic rings. *Br. J. Pharmacol.* 119 (3), 505–510. doi:10.1111/j.1476-5381.1996.tb15700.x
- Schlossmann, J., and Desch, M. (2011). IRAG and novel PKG targeting in the cardiovascular system. *Am. J. Physiol. Heart Circ. Physiol.* 301 (3), H672–H682. doi:10.1152/ajpheart.00198.2011
- Singer, D., Biel, M., Lotan, I., Flockerzi, V., Hofmann, F., and Dascal, N. (1991). The roles of the subunits in the function of the calcium channel. *Science* 253 (5027), 1553–1557. doi:10.1126/science.1716787
- Sonkusare, S., Palade, P. T., Marsh, J. D., Telemaque, S., Pesic, A., and Rusch, N. J. (2006). Vascular calcium channels and high blood pressure: Pathophysiology and therapeutic implications. *Vasc. Pharmacol.* 44 (3), 131–142. doi:10.1016/j.vph.2005.10.005
- Sung, D. J., Noh, H. J., Kim, J. G., Park, S. W., Kim, B., Cho, H., et al. (2013). Serotonin contracts the rat mesenteric artery by inhibiting 4-aminopyridine-sensitive Kv channels via the 5-HT2A receptor and Src tyrosine kinase. *Exp. Mol. Med.* 45, e67. doi:10.1038/emmm.2013.116
- Syed, A. U., Reddy, G. R., Ghosh, D., Prada, M. P., Nystoriak, M. A., Morotti, S., et al. (2019). Adenylyl cyclase 5-generated cAMP controls cerebral vascular reactivity during diabetic hyperglycemia. *J. Clin. Invest.* 129 (8), 3140–3152. doi:10.1172/JCI124705

- Tajada, S., Cidad, P., Colinas, O., Santana, L. F., Lopez-Lopez, J. R., and Perez-Garcia, M. T. (2013). Down-regulation of CaV1.2 channels during hypertension: How fewer CaV1.2 channels allow more Ca(2+) into hypertensive arterial smooth muscle. *J. Physiol.* 591 (24), 6175–6191. doi:10.1113/jphysiol.2013.265751
- Tajada, S., Moreno, C. M., O'Dwyer, S., Woods, S., Sato, D., Navedo, M. F., et al. (2017). Distance constraints on activation of TRPV4 channels by AKAP150-bound PKCa in arterial myocytes. *J. Gen. Physiol.* 149 (6), 639–659. doi:10.1085/jgp.201611709
- Tykocki, N. R., Boerman, E. M., and Jackson, W. F. (2017). Smooth muscle ion channels and regulation of vascular tone in resistance arteries and arterioles. *Compr. Physiol.* 7 (2), 485–581. doi:10.1002/cphy.c160011
- VanBavel, E., Sorop, O., Andreasen, D., Pfaffendorf, M., and Jensen, B. L. (2002). Role of T-type calcium channels in myogenic tone of skeletal muscle resistance arteries. *Am. J. Physiol. Heart Circ. Physiol.* 283 (6), H2239–H2243. doi:10.1152/ajpheart.00531.2002
- Wamhoff, B. R., Bowles, D. K., McDonald, O. G., Sinha, S., Somlyo, A. P., Somlyo, A. V., et al. (2004). L-type voltage-gated Ca2+ channels modulate expression of smooth muscle differentiation marker genes via a rho kinase/myocardin/SRF-dependent mechanism. *Circ. Res.* 95 (4), 406–414. doi:10.1161/01.RES.0000138582.36921.9e
- Wamhoff, B. R., Bowles, D. K., and Owens, G. K. (2006). Excitation-transcription coupling in arterial smooth muscle. *Circ. Res.* 98 (7), 868–878. doi:10.1161/01.RES.0000216596.73005.3c
- Welsh, D. G., Morielli, A. D., Nelson, M. T., and Brayden, J. E. (2002). Transient receptor potential channels regulate myogenic tone of resistance arteries. *Circ. Res.* 90 (3), 248–250. doi:10.1161/hh0302.105662
- Won, S., Levy, J. M., Nicoll, R. A., and Roche, K. W. (2017). MAGUKs: Multifaceted synaptic organizers. *Curr. Opin. Neurobiol.* 43, 94–101. doi:10.1016/j.conb.2017.01.006
- Xi, Q., Adebisi, A., Zhao, G., Chapman, K. E., Waters, C. M., Hassid, A., et al. (2008). IP3 constricts cerebral arteries via IP3 receptor-mediated TRPC3 channel activation and independently of sarcoplasmic reticulum Ca2+ release. *Circ. Res.* 102 (9), 1118–1126. doi:10.1161/CIRCRESAHA.108.173948
- Zhong, X. Z., Abd-Elrahman, K. S., Liao, C. H., El-Yazbi, A. F., Walsh, E. J., Walsh, M. P., et al. (2010). Stromatoxin-sensitive, heteromultimeric Kv2.1/Kv9.3 channels contribute to myogenic control of cerebral arterial diameter. *J. Physiol.* 588 (22), 4519–4537. doi:10.1113/jphysiol.2010.196618
- Zhong, X. Z., Harhun, M. I., Olesen, S. P., Ohya, S., Moffatt, J. D., Cole, W. C., et al. (2010). Participation of KCNQ (Kv7) potassium channels in myogenic control of cerebral arterial diameter. *J. Physiol.* 588 (17), 3277–3293. doi:10.1113/jphysiol.2010.192823