# Investigation of the functional role of endothelial $K_{Ca}$ channels in regulation of resistance artery diameter

by

Ran Wei

A thesis submitted in partial fulfillment of the requirements for the degree of

Doctor of Philosophy

Department of Pharmacology
University of Alberta

# Abstract

**Background:** The vascular endothelium regulates arterial diameter, and thus blood flow and pressure, through release of diffusible factors such as nitric oxide (NO), and spread of electrical charge to smooth muscle cells via myoendothelial gap junctions (MEGJs). Activation of these pathways is mediated by a rise in endothelial $Ca^{2+}$ levels leading to recruitment of downstream effectors. Two of the most important effectors are NO synthase, and $Ca^{2+}$ activated $K^+$ ($K_{Ca}$) channels which mediate hyperpolarization of the endothelial cell membrane potential. This hyperpolarization spreads to surrounding smooth muscle cells to limit contraction by reducing the open probability of voltage-operated $Ca^{2+}$ channels. The majority of research in this area has focused on activation of these mechanisms by stimuli acting directly on endothelial cells leading to the idea that $K_{Ca}$ channels and NO mediate distinct pathways for vasodilation. However, our lab has shown that in resistance arteries stimulation of smooth muscle cells by $\alpha_1$-adrenoceptor agonists activates the converse signaling pathway, termed myoendothelial feedback. Briefly, flux of inositol trisphosphate ($InsP_3$) from smooth muscle to endothelial cells elicits localized increases in $Ca^{2+}$, activation of intermediate conductance ($IK_{Ca}$) $K_{Ca}$ channels located at MEGJs and production of NO. Elucidation of the myoendothelial feedback pathway indicates that the role of endothelial $K_{Ca}$ channels and NO in modulating vasoconstriction may depend on the contractile stimulus, and rather than being independent, these vasorelaxant pathways may interact. Further support for this proposal comes from studies showing that blockers of endothelial small ($SK_{Ca}$) and $IK_{Ca}$ channels inhibit vasorelaxation mediated by endothelium-derived NO, and activators of endothelial $K_{Ca}$ channels can elicit vasodilation that is blocked by inhibitors of NO signaling.

My over-arching goal has been to further explore the functional role of $SK_{Ca}$ and $IK_{Ca}$ channels in regulating resistance artery diameter, and investigate potential interactions between

$K_{Ca}$ channels and NO in mediating vasodilation. To this end, I have addressed three hypotheses:

1. *The functional contribution of $InsP_3/IK_{Ca}$ channel-mediated myoendothelial feedback to limiting arterial diameter is determined by the ability of the vasoconstrictor stimulus to engage the endothelium.*
2. *Small molecule activators of endothelial $K_{Ca}$ channels modulate myogenic reactivity at least in part through endothelium-derived NO.*
3. *NO facilitates $K_{Ca}$ channel mediated, endothelium-dependent smooth muscle hyperpolarization.*

**Methods:** To test these hypotheses I have used a combination of functional (wire and pressure myography), electrophysiological (intracellular recording of endothelial and smooth muscle membrane potential using sharp electrodes), biochemical (immunohistochemical localization of $K^+$ channel proteins and qPCR to examine expression of mRNA for these channels) techniques to address 3 aims:

i. To investigate the contribution of myoendothelial feedback to endothelial modulation of resistance artery responses to contractile agonists, increases in pressure and stimulation of perivascular sympathetic nerves.

ii. To investigate the mechanisms underlying vasodilation to endothelial $K_{Ca}$ channel activators in myogenically active vessels.

iii. To determine if endothelium-dependent hyperpolarization of vascular smooth is modulated by NO.

**Results:** My data show firstly, a differential ability of vasoconstrictor stimuli to engage $IK_{Ca}$ channel-mediated myoendothelial feedback, and that in the presence of constant flow, shear stress-induced activation of $SK_{Ca}$ channels plays a dominant role in modulating resistance artery

vasoconstriction. Second, that pharmacological activators of endothelial $K_{Ca}$ channels can modulate myogenic reactivity, an effect dependent on inwardly rectifying $K^+$ channels, and possibly, NO. Finally, interactions between NO- and $K_{Ca}$ channel-dependent vasorelaxant pathways occur at the level of both the endothelium (dilation to an activator of $SK_{Ca}$ channels is dependent on NO) and smooth muscle (NO facilitates endothelium-dependent smooth muscle hyperpolarization)

**Conclusion:** To conclude, rather than separate pathways, endothelial modulation of resistance artery diameter results from the integrated activity of $SK_{Ca}$ and $IK_{Ca}$ channels, and NO to fine-tune the contractile state of smooth muscle cells in a stimulus- and context-dependent manner. Such integration of voltage-dependent and -independent pathways will contribute to coordinated spatial and temporal control of blood flow in resistance artery beds.

# Preface

A version of **Chapter 3** of this thesis has been published as **Ran Wei,** Stephanie Elizabeth Lunn, Raymond Tam, Stephen Lynn Gust, Boyd Classen, Paul Martin Kerr, and Frances Plane. (2018) Vasoconstrictor stimulus determines the functional distribution of myoendothelial feedback to mesenteric arterial tone. *J. Physiol*. doi:10.1113/JP274797. I was responsible for the majority of the data collection, data analysis, and manuscript composition (drafting text and preparing figures). R.T., S.E.L., S.L.G. and B.C. contributed to data collection and analysis as shown in the figure legends of this chapter, P.M.K. contributed to concept formation and F.P. was the supervisory author.

A version of Chapter 4 will be submitted for publication as **Ran Wei,** Stephanie Elizabeth Lunn, Paul Martin Kerr, and Frances Plane. Modulation of myogenic tone by $K_{Ca}$ channel activators is dependent on $K_{ir}$ channels. I was responsible for the majority of the data collection, data analysis, and will be responsible for manuscript composition. S.E.L. contributed to data collection as shown in the figure legends of this chapter. Together with P.M.K. and F.P., I contributed to concept formation and F.P. was the supervisory author.

A version of Chapter 5 will be submitted for publication as **Ran Wei,** Paul Martin Kerr, and Frances Plane. NO facilitates endothelium-dependent smooth muscle hyperpolarization in rat basilar artery. I was responsible for the majority of the data collection, data analysis, and will be responsible for manuscript composition. F.P. contributed to data collection as outlined in the figure legends of this chapter. Together with P.M.K. and F.P., I contributed to concept formation and F.P. was the supervisory author.

All animal care and experimental procedures were approved by the Animal Care and Use Committee (ACUC HS1; AUP 312) of the Faculty of Medicine and Dentistry at the University of Alberta, and performed in accordance with Canadian Council on Animal Care guidelines, and the principles and regulations as described by Grundy (2015).

# Acknowledgements

First and foremost, I would like to express my sincere gratitude to my supervisors Dr. Frances Plane and Dr. Paul Kerr. Thank you for taking me along on this wonderful journey in science. Without your guidance and patience, I would have not become the person I am today!

I would like to thank members of my supervisory committee Dr. Amy Tse and Dr. Paul Jurasz. Thank you for your expert advice, suggestions and encouragement during my study.

I would also like to thank Dr. Sean Marrelli and Dr. Harley Kurata for agreeing to be my examiners for my thesis defense.

I also thank Dr. Shaun Sandow for conducting the confocal imaging and Dr. Simonetta Sipione and Dr. Danny Galleguillos for their generous assistance with qPCR.

I am grateful to have my lab mate and wonderful friend Stephanie Lunn beside me in sharing this PhD experience. It would not have been the same without you.

I want to express my gratitude to my family, to my parents-in-law for all your support and encouragement; to mom and dad, for teaching me the real meaning of perseverance and always being there when I need you the most; to my grandma, who has always believed in me.

Finally, I would to thank Larry. Thank you for being an amazing husband, supporting and encouraging me to achieve my goal.

# Table of Contents

# List of Figures

## Chapter 1: Introduction



## Chapter 3: Vasoconstrictor stimulus determines the functional contribution of myoendothelial feedback to mesenteric arterial tone

## Chapter 4: Modulation of myogenic reactivity by $K_{Ca}$ channel activators: role of NO and $K_{ir}$ channels

## Chapter 5: NO facilitates endothelium-dependent smooth muscle hyperpolarization in rat basilar artery

# List of Tables

## Chapter 1: Introduction



## Chapter 2: Materials and methods



## Chapter 3: Vasoconstrictor stimulus determines the functional contribution of myoendothelial feedback to mesenteric arterial tone



## Chapter 5: NO facilitates endothelium-dependent smooth muscle hyperpolarization in rat basilar artery

# Abbreviations

| | |
|---|---|
| **1-EBIO** | 1-Ethyl-1,3-dihydro-2H-benzimidazol-2-one; 1-Ethylbenzimidazolinone |
| **5-HT** | 5-hydroxytryptamine (serotonin) |
| **ACh** | acetylcholine |
| **AKAP150** | A-kinase anchoring protein 150 |
| **ANOVA** | analysis of variance |
| **Ang II** | angiotensin II |
| **ATP** | adenosine triphosphate |
| **ADP** | adenosine diphosphate |
| **$Ba^{2+}$** | barium |
| **$BK_{Ca}$** | large conductance $Ca^{2+}$-activated $K^+$ channel |
| **CaCC** | $Ca^{2+}$-gated $Cl^-$ channel |
| **$[Ca^{2+}]_i$** | Intracellular $Ca^{2+}$ concentration |
| **CaM** | calmodulin |
| **Cav-1** | caveolin-1 |
| **cGMP** | cyclic guanosine monophosphate |
| **COX** | cyclooxygenase |
| **DAG** | diacylglycerol |
| **DEA NONOate** | diethylamine NONOate |
| **DMSO** | dimethyl sulfoxide |
| **EDH** | endothelium-dependent hyperpolarization |
| **$E_k$** | $K^+$ reversal potential |
| **eNOS** | endothelial nitric oxide synthase |
| **EP** | prostaglandin $E_2$ receptor |
| **ER** | endoplasmic reticulum |
| **FAK** | focal adhesion kinase |
| **GIRK** | G-protein gated inwardly rectifying $K^+$ channel |
| **GPCR** | G-protein coupled receptor |
| **IbTX** | iberiotoxin |
| **IEL** | internal elastic lamina |
| **$IK_{Ca}$** | intermediate conductance $Ca^{2+}$-activated $K^+$ channel |
| **$InsP_3$** | inositol 1,4,5-trisphosphate |
| **$K_{Ca}$** | $Ca^{2+}$-activated $K^+$ channel |
| **$K_{IR}$** | inwardly rectifying $K^+$ channel |
| **$K_V$** | voltage-gated $K^+$ channel |
| **LC** | light chain |
| **L-NOARG** | L-$N^G$-nitro arginine L-$N^G$-nitro arginine |
| **MEGJ** | myoendothelial gap junction |
| **MLC** | myosin light chain |
| **MLCK** | myosin light chain kinase |
| **MLCP** | myosin light chain phosphatase |
| **MYPT1** | myosin phosphatase target subunit 1 |
| **NO** | nitric oxide |

| | |
|---|---|
| **NOS** | nitric oxide synthase |
| **NPY** | neuropeptide Y |
| **ODQ** | 1H-[1,2, 4]oxadiazolo[4,3,-a]quinoxalin-1-one |
| **$P_{2X}$** | purinergic ionotropic receptor |
| **$P_{2Y}$** | G-protein coupled purinergic receptor |
| **PE** | phenylephrine |
| **$PGE_2$** | prostaglandin $E_2$ |
| **$PGI_2$** | prostacyclin |
| **$PIP_2$** | phosphatidylinositol 4,5-bisphosphate |
| **PKA** | protein kinase A |
| **PKC** | protein kinase C |
| **PKG** | protein kinase G |
| **$PLA_2$** | phospholipase $A_2$ |
| **PLC** | phospholipase C |
| **RCK** | regulator of $K^+$ conductance domains |
| **ROK** | rho-associated protein kinase |
| **RhoGEF** | rho guanine nucleotide exchange factor |
| **RyR** | ryanodine receptor |
| **SEM** | standard error of mean |
| **SFK** | src family kinase |
| **STIM** | stromal interaction molecule |
| **$SK_{Ca}$** | small conductance $Ca^{2+}$-activated $K^+$ channel |
| **SOCE** | store-operate calcium entry |
| **SR** | sarcoplasmic reticulum |
| **TP** | thromboxane $A_2$ receptor |
| **TRP** | transient receptor potential |
| **TRPA** | ankyrin transient receptor potential channel |
| **TRPC** | canonical transient receptor potential channel |
| **TRPM** | melastatin transient receptor potential channel |
| **TRPML** | mucolipin transient receptor potential channel |
| **TRPP** | polycystin transient receptor potential channel |
| **TRPV** | vanilloid transient receptor potential channel |
| **TTX** | tetrodotoxin |
| **$TXA_2$** | thromboxane$A_2$ |
| **VOCC** | voltage-operated $Ca^{2+}$ channel |

# Chapter 1: Introduction

## 1.1 Overview of blood vessel structure and function

Blood vessels are essential for the adequate perfusion of organs and tissues to provide oxygen and nutrients, and remove waste. Arteries and veins are two major types of blood vessels, which play different functional roles but share many structural similarities; an outermost layer composed of connective tissue called the tunica adventitia, a middle layer(s) of smooth muscle cells called the tunica media, and the tunica intima a single layer endothelial cells which line the lumen. The major structural difference between arteries and veins is that the former have more layers of smooth muscle cells which are necessary to change vessel diameter **(Figure 1.1)**.

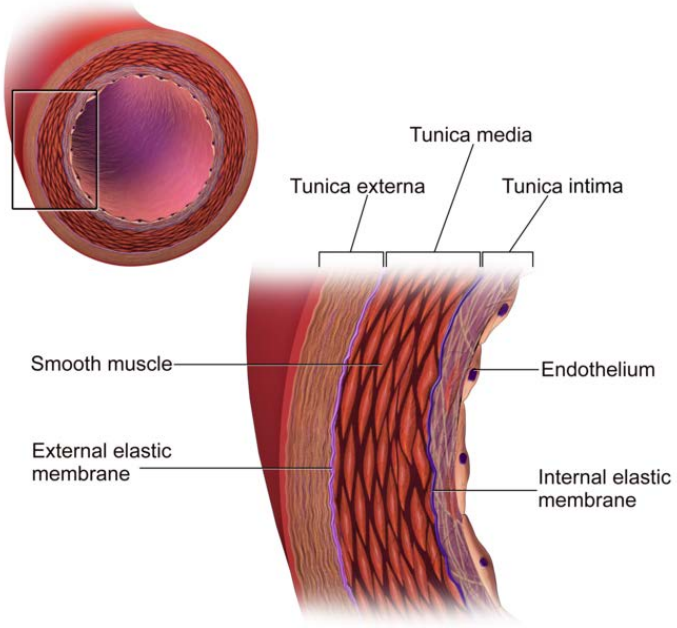



**Figure 1.1: The structure of blood vessels**. This diagram shows the three layers (tunica adventitia/externa, tunica media, and tunica media) of a typical artery wall (BruceBalus 2016).

In the body, the diameter of small resistance arteries is a major determinant of vascular resistance and thus, blood flow and blood pressure. Resistance artery diameter is determined by the contractile state of the smooth muscle cells in the tunica media, which in turn is the result of the integrated response to many factors including intrinsic mechanisms (e.g. myogenic reactivity)

and the actions of chemical mediators circulating in the blood and released from nerves and endothelial cells. Increases in vascular resistance due to changes in both resistance artery structure and function are a major contributor to the clinical manifestations of cardiovascular disease such as high blood pressure, stroke, heart attack and kidney failure. Therefore, detailed understanding how the contractile state of smooth muscle cells is regulated is crucial for exploring potential new therapeutic targets to prevent and treat cardiovascular diseases.

**1.2 Mechanisms for contraction of smooth muscle in resistance arteries**

The term resistance artery refers to a small artery or arteriole which contributes significantly to the creation of resistance to and regulation of blood flow. In rats, diameter of resistance arteries is between 100 µm to 400 µm in diameter (Mulvany and Aalkjaer 1990; Smaje et al. 1970; Bohlen et al. 1977; Chilian et al. 1986). However, it is the location of vessels (in the distal part of the circulation towards the capillaries) rather than their absolute size, that determines their role (reviewed by Martinez-Lemus 2012). According to the Hagen-Poiseuille law (Pfitzner 1976; $R = \frac{8\eta L}{\pi r^4}$ ; $R \propto \frac{1}{r^4}$; R is resistance, $\eta$ is varicosity, L is vessel length, r is arterial radius) the degree of vascular resistance is inversely proportional to the fourth power of the arterial radius and thus, what appear to be relatively small changes in arterial diameter can have a large effect on vascular resistance.

*1.2.1 $Ca^{2+}$-dependent contraction:* As mentioned above, resistance artery diameter is determined by the contractile state of the smooth muscle cells within the vessel wall. A smooth muscle cell is mainly composed of cytoskeleton and the contractile proteins, myosin and actin; myosin forms thick filaments which are surrounded by the thin filaments of actin. As in all muscle cells, vascular smooth muscle contraction requires adenosine triphosphate (ATP) and an increase in intracellular $Ca^{2+}$ concentration ($[Ca^{2+}]_i$) via entry through channels in the cell membrane or $Ca^{2+}$ release from intracellular stores (reviewed by Ghosh et al. 2017). $Ca^{2+}$ binds to calmodulin (CaM) to form a

$Ca^{2+}$-CaM complex which activates myosin light chain kinase (MLCK; Allen and Walsh 1994). MLCK is physically bound by its N-terminus to actin filaments and activation allows it to phosphorylate nearby myosin molecules (Kamm and Stull 2001). Myosin filaments are composed of hexameric myosin molecules, each made up of two heavy chains and two pairs of light chains ($LC_{17}$ and $LC_{20}$). Activated MLCK phosphorylates $LC_{20}$ at serine 19 to induce a conformational change that allows interaction between actin and the myosin, and an increase in the actin-activated MgATPase activity of myosin (Walsh 1991). Energy generated through hydrolysis of ATP then drives cross-bridge cycling and the contraction of the muscle cell (**Figure 1.2**; reviewed by Saddouk et al. 2017). To break this interaction, dephosphorylation of $LC_{20}$ needs to occur along with the removal of $Ca^{2+}$, primarily via CaATPases which pump $Ca^{2+}$ into the sarcoplasmic reticulum (SR; Raeymaekers and Wuytack 1993). MLCK is inactivated by dissociation of $Ca^{2+}$ from CaM and block of the active site of MLCK by an autoinhibitory domain. Dephosphorylation is then mediated by myosin light chain phosphatase (MLCP) resulting in disruption of myosin and actin binding and thus muscle relaxation (reviewed by Szasz and Webb 2017; Brozovich et al. 2016)). Vasorelaxant stimuli can activate MLCP via phosphorylation of kinases such as protein kinase G (PKG) whereas contractile stimuli can inhibit MLCP activity to enhance contractile force without further changes in $[Ca^{2+}]$i, a process known as $Ca^{2+}$ sensitization (reviewed by Somlyo and Somlyo 2003; see **Section 1.2.3**).

*1.2.2 $Ca^{2+}$ homeostasis in vascular smooth muscle cells:* Increases in $[Ca^{2+}]_i$ in vascular smooth muscle cells are fundamental to eliciting contraction in response to a wide range of stimuli, and also, through actions on $Ca^{2+}$-dependent transcription factors, regulate smooth muscle phenotype. $Ca^{2+}$ diffuses slowly within cells and a large flux is required to achieve the high $Ca^{2+}$ concentration necessary for activation of $Ca^{2+}$-dependent processes (Berridge 2008). $Ca^{2+}$ signaling within

smooth muscle cells, as in other cell types, displays compartmentalization; local increases in $Ca^{2+}$ can activate contractile mechanisms without influencing other $Ca^{2+}$-dependent signaling pathways (Billaud et al. 2014; Kargacin 1994; Hideaki 1989). Thus, to control the activation of $Ca^{2+}$-dependent functions, smooth muscle cells display a large range of $Ca^{2+}$ signaling patterns with different spatial and temporal patterns (reviewed by Amberg and Navedo 2013). However, for the purposes of this thesis I will focus on how changes in $[Ca^{2+}]_i$ are regulated to control vascular tone, and thus, appropriate regulation of blood flow and pressure. Increases in smooth muscle cell $[Ca^{2+}]_i$ in response to contractile stimuli can occur through release of $Ca^{2+}$ from intracellular stores or $Ca^{2+}$ entry through channels on the cell membrane.

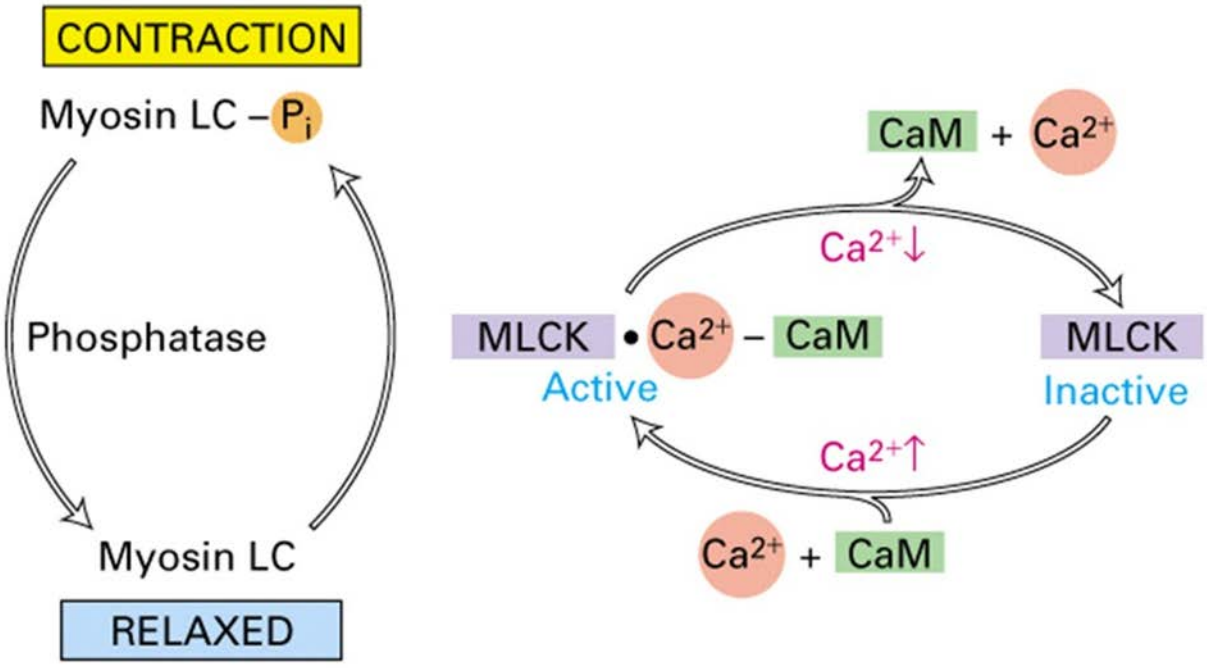



**Figure 1.2: Mechanism of smooth muscle contraction.** This diagram outlines the mechanisms underlying smooth muscle contraction elicited by a stimulus that increases $[Ca^{2+}]_i$ levels within smooth muscle cells. An increase in $[Ca^{2+}]_i$ leads to $Ca^{2+}$ binding to CaM which activates MLCK. MLCK phosphorylates $LC_{20}$ to increase the activity of the myosin ATPase which drives the cycling of actin-myosin crossbridges to create muscle tension.

There are two main $Ca^{2+}$ stores, the SR and mitochondria with SR being the primary $Ca^{2+}$

stores responsible for $Ca^{2+}$ release and uptake related to smooth muscle contraction (Dirksen 2009). To date, release of $Ca^{2+}$ from mitochondria has not been associated with contraction in smooth muscle (Amberg and Navedo 2013), although they may play a role in the re-uptake of $Ca^{2+}$ via mitochondrial $Ca^{2+}$ uniporter (Williams et al. 2013; Smith 1996; McCarron et al. 2013) in refilling of SR stores (Poburko et al. 2009).

*a) Release of $Ca^{2+}$ from intracellular stores:* $Ca^{2+}$ stored in the SR, a sub-type of endoplasmic reticulum, can be released via activation of both inositol 1,4,5-trisphosphate ($InsP_3$) and ryanodine (RyR) receptors (Laporte et al. 2004).

*$InsP_3$ receptors*: $InsP_3$ receptors are $Ca^{2+}$ release channels consisting of four membrane-spanning subunits, each of six transmembrane domains, surrounding a pore. $InsP_3$ binds to a domain on the cytosolic $NH_2$-terminus which also bears binding sites for ATP and $Ca^{2+}$ (Fan et al. 2015), as well as a coupling domain for physical interactions with TRPC channels (see below). There are 3 isoforms of $InsP_3$ receptors (Narayanan et al. 2012), with type 1 the predominant isoform in smooth muscle cells of small resistance arteries (Zhou et al. 2008). Agonists such as noradrenaline, angiotensin II (Ang II) and endothelin (ET-1) act on $G_{q/11}$-protein coupled receptors (GPCRs) to increase $InsP_3$ through cleavage of membrane bound phosphatidylinositol 4,5-bisphosphate ($PIP_2$) by phospholipase C (PLC; reviewed by Berridge 2008) and so elicit $InsP_3$-mediated $Ca^{2+}$ release. $InsP_3$ receptors can be inhibited by pharmacological agents such as xestospongin C (Gafni et al. 1997), and can be modulated by $[Ca^{2+}]_i$, luminal SR $Ca^{2+}$ load, ATP, protein kinases such as protein kinase A (PKA) and reactive oxygen species (Narayanan et al. 2012; Bezprozavanny et al. 1991).

Differences in the spatiotemporal pattern of $Ca^{2+}$ release though $InsP_3$ receptors can have different functional effects on smooth muscle cells. $Ca^{2+}$ puffs are localized events produced by

clusters of $InsP_3$ receptors (Parker and Smith 2010) which have been shown to promote opening of TRPM4 channels leading to pressure-induced membrane depolarization and contraction in cerebral arteries (Gonzales and Earley 2012). The initial release of $Ca^{2+}$ through $InsP_3$ receptors can lead to opening of further $InsP_3$ receptors through $Ca^{2+}$-induced $Ca^{2+}$ release giving rise to waves which can lead to contraction by directly increasing global $[Ca^{2+}]_i$ (Foskett et al. 2007).

*RyRs:* Like $InsP_3$ receptors, RyRs are channels that mediate $Ca^{2+}$ release from the SR. A functional channel is composed of four identical subunits and each subunit contains six transmembrane domains (Zalk et al. 2015). RyR also contains a large cytoplasmic domain and structural models predict both $NH_2$- and COOH-termini to be in the cytosol with $Ca^{2+}$ sensitivity imparted by EF-hand domains in the area connecting the COOH-terminus to the pore (Efremov et al. 2015). Three RyR isoforms have been identified (reviewed by Lanner et al. 2010), and detected in smooth muscle cells although they show differential expression between arteries and may play different roles (Westcott et al. 2012; Westcott and Jackson 2011). For example, in the skeletal muscle vascular bed, RyR2 is more highly expressed in feed artery smooth muscle cells as opposed to arteriolar smooth muscle cells, whereas RyR3 shows the opposite pattern of expression (Westcott et al. 2012). RyR2 has been shown to contribute to large, spatially-restricted $Ca^{2+}$ release events called sparks (Ji et al. 2004; see below). RyR activity can be modulated by caffeine, which depending on its concentration, can induce massive $Ca^{2+}$ release or increase the frequency of $Ca^{2+}$ sparks (Jaggar et al. 2000). RyRs can be inhibited in a concentration-dependent manner by ryanodine; at nanomolar concentration ryanodine can activate RyR, while at micromolar concentrations it inhibits the receptor (Lanner et al. 2010; Jaggar 2001).

As mentioned above, a range $Ca^{2+}$ signals with differing spatial and temporal patterns account for the fine control of smooth muscle tone, and release of $Ca^{2+}$ from SR stores via $InsP_3$

and RyR receptors produces several types of $Ca^{2+}$ signal which serve different functions. Propagating $Ca^{2+}$ waves resulting from release of $Ca^{2+}$ via $InsP_3$ receptors and/or RyR (Amberg and Navedo 2013; Wray and Burdyga 2010) contributes to agonist-induced contraction and the myogenic response in small mesenteric, cerebral and skeletal muscle arteries (Westcott et al. 2012; Mufti et al. 2010b; Boittin et al. 1999; Zacharia et al. 2007; Lamont and Wier 2004). In contrast, the most-studied $Ca^{2+}$ release event in vascular smooth muscle, the RyR-mediated $Ca^{2+}$ spark, contributes to increases in global $[Ca^{2+}]_i$ (Santana and Navedo 2009), but also plays an important modulatory role due to the proximity of RyRs to plasma membrane ion channels (reviewed by Amberg and Navedo 2013). In cerebral resistance arteries, $Ca^{2+}$ sparks activate large conductance $Ca^{2+}$-activated $K^+$ ($BK_{Ca}$) channels to promote hyperpolarization and oppose vasoconstriction (Nelson et al. 1995; **Figure 1.3)**. This relationship has been proposed to underlie the actions of vasodilator and vasoconstrictor agonists as protein kinases such as PKG and protein kinase C (PKC) can act on RyRs to stimulate and inhibit $Ca^{2+}$ sparks activity, respectively (Jaggar et al. 2000; Bonev et al. 1997). Also, in animal models, decreased expression of $BK_{Ca}$ channel $\beta_1$ subunit reduces channel $Ca^{2+}$ sensitivity causing membrane depolarization, increases in arterial tone, and hypertension, illustrating the physiological importance of the $Ca^{2+}$ spark-$BK_{Ca}$ channel signaling unit in regulating resistance artery diameter and hence blood flow and pressure (Plüger et al. 2000; Amberg et al. 2003; Brenner et al. 2000).

Activation of $BK_{Ca}$ channels by $Ca^{2+}$ sparks can be recorded in isolated cerebral arterial smooth muscle cells as spontaneous transient outward currents (STOCs); STOC production, frequency and amplitude, is intimately tied to depolarization and driven by $Ca^{2+}$ sparks (Jaggar et al. 1998; Pérez et al. 2001). In both skeletal and cardiac muscle there is a tight physical and functional relationship between voltage-operated $Ca^{2+}$ channels (VOCCs) and RyRs (reviewed by

Eisner 2014). This tight relationship does not appear to be present in vascular smooth muscle cells to the same degree but the link between RyR-dependent generation of $Ca^{2+}$ sparks, STOCs and membrane potential indicate that in cerebral resistance arteries at least, VOCCs modulate RyRs mediated $Ca^{2+}$ sparks by contributing to global $[Ca^{2+}]_i$ and SR $Ca^{2+}$ load (Essin et al. 2007).

Initial interest focused on L-type VOCCs, the dominant $Ca^{2+}$ conductance pathway in] vascular smooth muscle cells (Jaggar 2001; see below). However, selective inhibition of T-type VOCCs (Cav3.2) also reduced $Ca^{2+}$ spark activity in mouse mesenteric arteries, and these channels were co-localized with RyR in specific microdomains within the cells (Harraz et al. 2014; Harraz, Brett, et al. 2015). These findings support the idea that L-and T-type VOCCs, which operate over different voltage ranges (see below), work together to modulate RyR-dependent $Ca^{2+}$ spark generation, STOCs and arterial diameter in cerebral arteries across a wider range of membrane potentials (Hashad et al. 2016) than would not be possible if only one channel sub-type were involved.

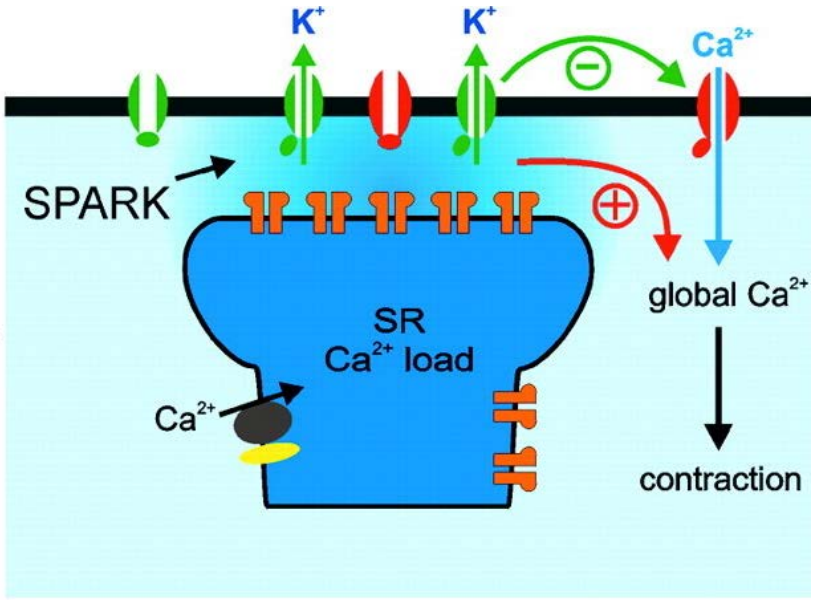



**Figure 1.3. A schematic illustrating activation of $BK_{Ca}$ channels by $Ca^{2+}$ sparks.** In smooth muscle cells release of $[Ca^{2+}]$ through RyR leads to the activation of $BK_{Ca}$ channels to induce hyperpolarization and so decrease the activity of voltage-operated $Ca^{2+}$ channels. $Ca^{2+}$ sparks can also contribute to increase in global $[Ca^{2+}]$ (Jaggar et al. 2000).

In rabbit and mouse portal vein smooth muscle cells, sparks are linked not to $BK_{Ca}$ channels but to opening of $Ca^{2+}$-gated $Cl^-$ channels (CaCCs) to depolarize the membrane potential and increase $Ca^{2+}$ influx through VOCCs (Leblanc et al. 2005; Saleh and Greenwood 2005). The physiological significance of coupling between sparks and CaCCs has received less attention than between sparks and $BK_{Ca}$ channels. However, in isolated rat retinal arteriole smooth cells, global increases in $[Ca^{2+}]_i$ evoked by ET-1 results in the activation of CaCCs to depolarize the membrane potential and cause vasoconstriction (Stewart et al. 2012). Furthermore, inhibition of the recently identified CaCC, transmembrane protein 16A (TMEM16A; Wu et al. 2014) by $T16A_{inh}$-A01, an Ano1 inhibitor, decreased myogenic reactivity in rat cerebral arteries (Bulley et al. 2012) and agonist-evoked responses in rat mesenteric arteries (Dam et al. 2014). TMEM16A has also been shown to be involved in Ang II-induced constriction via the Rho/Rho-associated protein kinase (ROCK) signaling pathway in rat basilar arteries (Li et al. 2016).

*b) $Ca^{2+}$ influx pathways: Store-operated $Ca^{2+}$ entry (SOCE):* Upon depletion of $Ca^{2+}$ from intracellular stores by activation of $InsP_3$ receptors, the SR $Ca^{2+}$ sensor protein STIM (stromal interaction molecule; first identified in 2005 (Liou et al. 2005; Roos et al. 2005)) undergoes spatial reorganization to aggregate into clusters that relocate to the SR-plasmalemmal junction where they physically interact with and activate $Ca^{2+}$ selective Orai channels ($Ca^{2+}$-release activated $Ca^{2+}$ channel, reviewed by Trebak 2012).

There are two subtypes of STIM, STIM1 and STIM2, with STIM1 being largely associated with initiation of SOCE by binding to Orai channels (Gruszczynska-Biegala et al. 2011). STIM proteins are single-transmembrane proteins located in the SR that sense alterations in SR luminal $[Ca^{2+}]_i$ via $NH_2$ terminal EF-hand domains; depletion of SR $Ca^{2+}$ leads to dissociation of $Ca^{2+}$ from these domains, allowing STIM proteins to unfold and interact with Orai channels. Orai

channels are heteromeric channels consisting of 4–6 Orai subunits (Hou et al. 2012). Each Orai subunit consists of cytosolic $NH_2$ and COOH-termini, which contain sites for functional regulation by $Ca^{2+}$/CaM, PKC, and STIM proteins (Frischauf et al. 2009; Hooper et al. 2015; Mullins et al. 2009). The activation of Orai-mediated $Ca^{2+}$ influx by STIM requires a COOH-terminus coiled-coil interaction (Muik et al. 2008). Under normal conditions, STIM/Orai are expressed at a relatively low level but in vascular smooth muscle of spontaneously hypertensive rats (SHRs), their expression is upregulated and depletion of SR stores lead to greater $Ca^{2+}$ entry (Giachini et al. 2009).

Orai may not be the only channel responsible for SOCE as TRPC channels can also be activated by STIM after store depletion (Zeng et al. 2008). But, their contribution to SOCE appears to be variable (reviewed by Beech 2012) and whether there is a physical interaction between TRPC and Orai is currently unknown. TRP channels exhibit a wide range of gating properties (see below) and have been implicated in a number of signalling pathways including SOCE (Albert and Large 2002; Earley and Brayden 2015) For example, in a number of cell types including smooth muscle cells, TRPC6 can mediate both SOCE and receptor-operated $Ca^{2+}$ influx, as well as being activated by mechanical stimuli (Dietrich and Gudermann 2014). TRPC1 can mediate $Ca^{2+}$ influx after store depletion with thapsigargin in vascular smooth muscle cells isolated from a range of sources (Xu and Beech 2001).

*Voltage-dependent $Ca^{2+}$channels:* Many contractile stimuli elicit depolarization of the membrane potential of vascular smooth muscle cells and so increase the open probability of VOCCs encoded by pore-forming $\alpha_1$ (Cav) subunits. For example, the myogenic response, the intrinsic ability of resistance arteries to constrict to elevated pressure first described by Bayliss in 1902 (Bayliss 1902), is dependent on smooth muscle depolarization and activation of VOCCs.

There are 10 $\alpha_1$ pore–forming subtypes which make up VOCCs (Cav1.x, 2.x and 3.x), among which $Ca_V1.2$ channel (L-type) predominates in terms of mediating vascular smooth muscle contractility (reviewed by Catterall 2011; Zamponi et al. 2015).

L-type VOCCs are made up of pore-forming $\alpha_{1c}$ together with modulatory β, α2δ, and γ subunits **(Figure 1.4)**. The Cav1.2$\alpha_{1c}$ sub-unit contains the voltage sensor, gating apparatus, and $Ca^{2+}$-permeable pore, and is made up of four domains (I, II, III, IV), each of which is composed of six transmembrane segments (S1–S4 make up the voltage-sensor, and S5 and S6 form the pore) and intracellular $NH_2$- and COOH-termini (Bezanilla 2008). Diversity in structure, function and expression is generally due to alternative splicing of the $\alpha_{1c}$ transcript in combination with differential expression of the modulatory subunits. Evidence for the functional role of L-type VOCCs in regulation of arterial diameter has largely come from studies using dihydropyridine antagonists (e.g. nifedipine) that selectively inhibit $\alpha_{1c}$ activity. For example, block of L-type VOCCs abolished myogenic reactivity in isolated rat cerebral arteries (Knot and Nelson 1998), whereas dihydropyridine agonists.

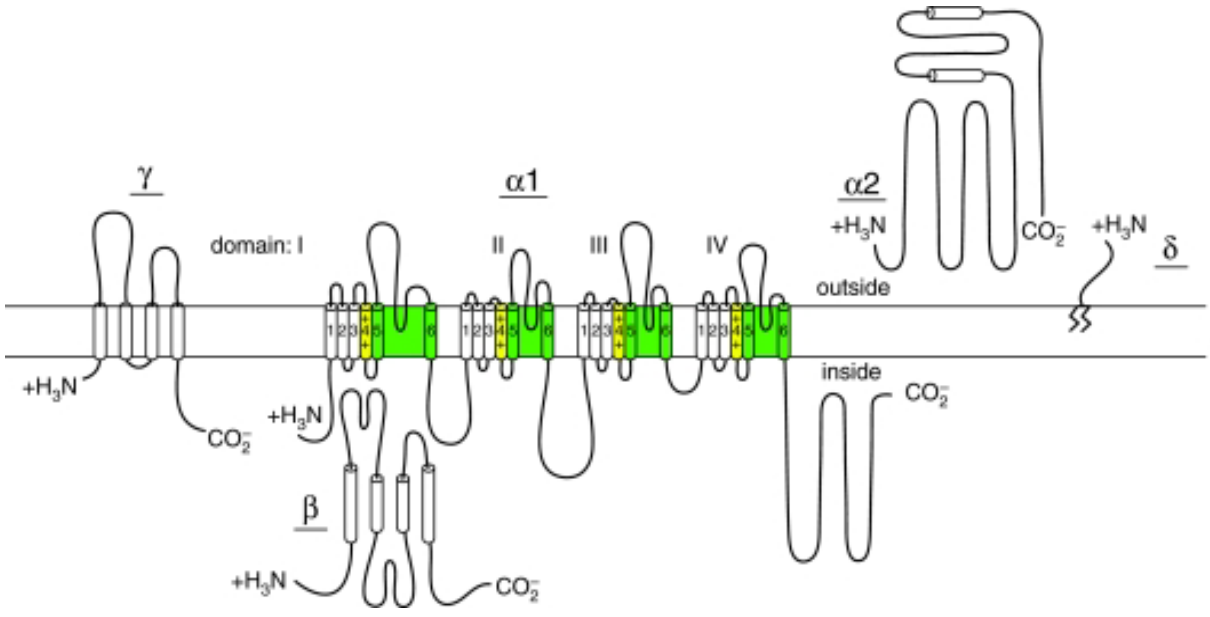



**Figure 1.4: Components of VOCCs.** VOCCs are made up of pore-forming $\alpha_{1c}$ subunits together with modulatory β, α2δ, and γ subunits (Catterall 2011).

that stimulate the activity of L-type VOCCs enhanced the myogenic response in rabbit ear arteries (Hwa and Bevan 1986). Membrane depolarization leads to increased opening of L-type VOCCs and global influx of $Ca^{2+}$ into smooth muscle cells to cause contraction. However, localized increases in $[Ca^{2+}]_i$ mediated by these channels, termed sparklets (Amberg et al. 2007), have also been resolved which can be modulated by protein kinases (Navedo et al. 2006; Navedo et al. 2008; Santana and Navedo 2009). This regulation is facilitated by association of a sub-population of channels with the scaffolding protein A-kinase anchoring protein 150 (AKAP150) in a complex with PKCα, PKA and the phosphatase calcineurin (Navedo et al. 2008; Navedo, Takeda, et al. 2010; Oliveria et al. 2007). Increased sparklet activity has been associated with activation of PKC (Navedo et al. 2006). A large body of functional and electrophysiological data demonstrates that activation of PKC by phorbol esters and agonists acting at GPCRs (e.g., Ang II, ET-1), potentiates the activity of L-type VOCCs and vasoconstriction (reviewed by Weiss and Dascal 2015) supporting the idea that sparklets can contribute to smooth muscle contraction. However, agonists that stimulate PKA trigger vasodilation, and yet activation of this kinase has also been reported to increase sparklets activity (Navedo, Cheng, et al. 2010) and to inhibit, potentiate, or have no effect on channel activity (see review by Keef, Hume, and Zhong 2001). Thus, rather than contributing to global changes in $[Ca^{2+}]_i$, these AKAP-targeted signaling complexes may allow for localized activation of L-type VOCCs that is differentially regulated to modulate vascular tone development (Navedo and Amberg 2013; **Figure 1.5)**.

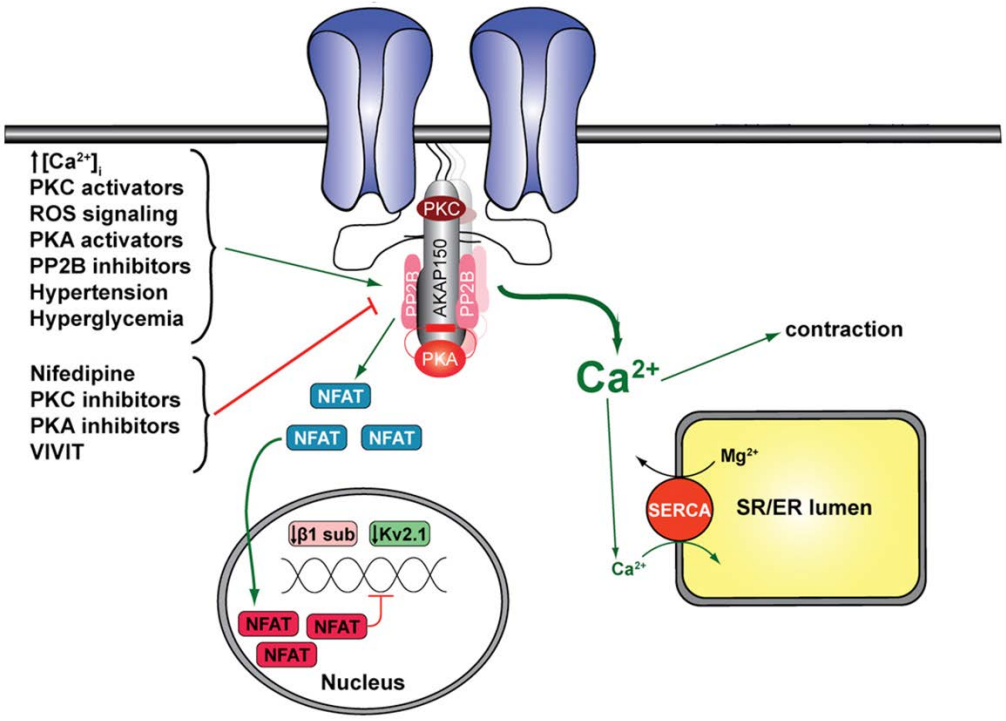



**Figure 1.5 Model for localized, heterogeneous modulation of L-type VOCCs.** A sub-population of L-type VOCCs can associate with the scaffold protein AKAP150 which provides the basis for a regulatory signaling complex composed of PKA, PKC, and calcineurin to allow localized modulation of channel activity (Modified from Navedo and Amberg 2013).

Although the majority of research has focused on L-type VOCCs, as mentioned above, recent evidence indicates that T-type VOCCs (Cav3.x) can also contribute to vascular smooth muscle contractility. T-type currents are activated at more hyperpolarized potentials (positive to −45 mV) as compared to L-type currents (positive to ~-20 mV; Tsien et al. 1988). Their functional role in regulation of arterial diameter has largely been explored using blockers such as mibefradil and nickel, and the fact that they are largely resistant to dihydropyridines such as nifedipine at concentrations below the micromolar range (Akaike et al. 1989). Transcript and protein for Cav3.1 and Cav3.2 have been found in vascular smooth muscle cells from rat cerebral resistance arteries (Harraz, Brett, et al. 2015; Abd El-Rahman et al. 2013; Kuo et al. 2010), and Cav3.3 has also been

identified in human cerebral artery cells (Harraz et al. 2015). Electrophysiological recordings have identified Cav3.1 and Cav3.2 channels as being responsible for the nifedipine-insensitive component of $Ca^{2+}$ current in rat and mouse cerebral vascular smooth muscle cells (Abd El-Rahman et al. 2013; Harraz et al. 2014).

Differences in the voltage dependence of activation of L and T-type VOCCs mean that acting together, these channels can mediate $Ca^{2+}$ influx over a wider range of membrane potentials than one channel alone. As an example, in cerebral arteries, Cav3.1/Cav3.3 T-type channels predominantly contribute to myogenic tone at lower intraluminal pressures (e.g., 20–40 mmHg), in which smooth muscle membrane potential ~−50 mV, and Cav1.2 L-type function at the more depolarized membrane potentials (~−45 to −36 mV) observed at higher intraluminal pressures (Harraz et al. 2014; Harraz et al. 2015). Co-localization of Cav3.2 with RyR in specific microdomains allows these channels to mediate negative feedback regulation of pressure-induced tone by modulating the RyR–$BK_{Ca}$ channel axis (Harraz et al. 2014).

*TRP channels:* TRP channels, first identified in *Drosophila* (Spradling et al. 1982), are a superfamily of cationic channels (28 encoding genes, 27 in humans) which mediate $Ca^{2+}$ influx in response to a wide range of stimuli. Multiple TRP channels have been shown to be expressed in both endothelial and vascular smooth muscle cells of resistance arteries from different species (reviewed by Yue et al. 2015), and have been proposed to contribute to regulation of membrane potential, contraction, and development of myogenic tone (Earley and Brayden 2015; Nilius and Szallasi 2014).

The TRP channel super family can be divided into six subfamilies: TRPC (canonical), TRPV (vanilloid), TRPM (melastatin), TRPP (polycystin), TRPA (ankyrin), and TRPML (mucolipin; Alonso-Carbajo et al. 2017; S. Earley and Brayden 2015; Yue et al. 2015). TRP

channel subunits consist of six membrane-spanning helices (S1–S6; S5 and S6 forming the pore) with intracellular $NH_2$- and COOH-termini, four of these subunits (homomers or heteromers) coming together to form a functional channel (**Figure 1.6**). All TRP channels are permeable to $Ca^{2+}$ with the exception of TRPM4 and TRPM5, which are $Ca^{2+}$ activated, but not $Ca^{2+}$ permeable (Gonzales and Earley 2012; Launay et al. 2002; Prawitt et al. 2003). Unlike VOCCs, TRP channels are not gated by voltage (Ramsey et al. 2006), but can respond to a 0range of thermal, mechanical, chemical, nociceptive, and local cellular environmental stimuli. TRPV1-V4 and TRPM3 are activated by high temperatures, whereas TRPM8, TRPA1, and TRPC5 are activated by low temperatures (details of TRP channel temperature sensitivity in **Table 1.1** Nilius 2007; Zimmermann et al. 2011). TRPC channel activity is dependent on the PLC pathway as these channels are activated either directly by diaglycerol (DAG; TRPC2, TRPC3, TRPC6, and TRPC7), or indirectly through a DAG-independent mechanism (TRPC1, TRPC4, and TRPC5; Schleifer et al. 2012; Trebak et al. 2003; Venkatachalam et al. 2003). A rise in $[Ca^{2+}]_i$ is necessary to activate several TRP channels (TRPM4, TRPM5, TRPM2, and TRPA1; Du et al 2009; Launay et al. 2002; Prawitt et al. 2003; Yue et al. 2015; Zurborg et al. 2007) and TRPM3 is activated by hypo-osmotic cell swelling, rises in $[Ca^{2+}]_i$, and high temperatures (Grimm et al. 2003; riens et al. 2011). Next I will briefly discuss the functional contribution of TRP channels to contractility of vascular smooth muscle cells.

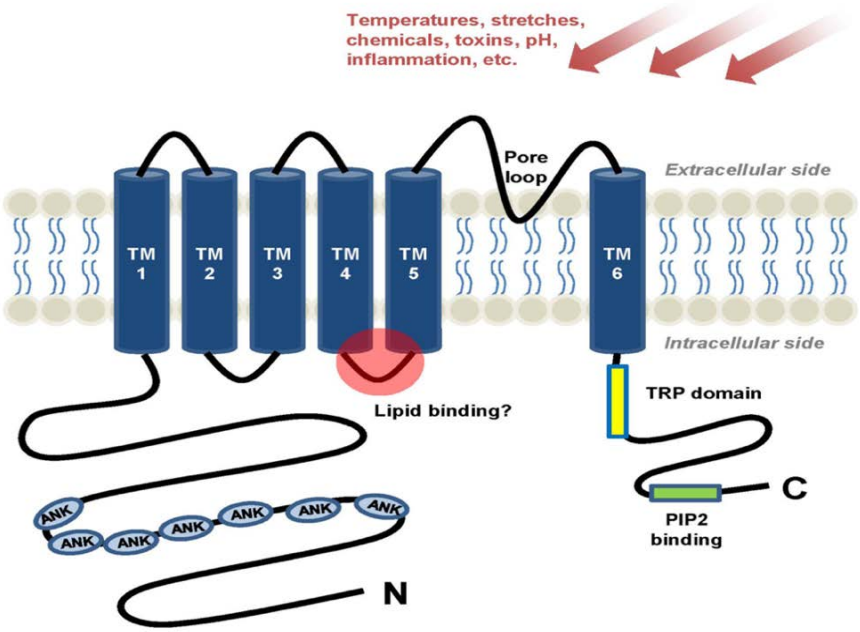



**Figure 1.6: Schematic representation of the structure of TRP channels.** Each subunit consists of six transmembrane domains (TM), a pore domain between TM5 and TM6 domain (Yoo et al. 2014).

| TRP channel subtypes | Temperature ($^0$C) |
|---|---|
| TRPV1 | >42 |
| TRPV2 | >53 |
| TRPV3 | >30-33 |
| TRPV4 | ~24-42 |
| TRPM3 | >25 |
| TRPM8 | <22-26 |
| TRPA1 | <17 |
| TRPC5 | 25-37 |

**Table 1.1 Sensitivity of TRP channels to temperature** (Zimmermann et al. 2011; reviewed by Nilius 2007; Dhaka, Viswanath, and Patapoutian 2006; Pertusa et al. 2012)

With their activation dependent on the PLC pathway, TRPC channels have been proposed as mediators of vasoconstriction to agonists acting at G-protein coupled receptors, although different TRPCs may be activated by different agonists (reviewed by Grayson et al. 2017). TRPC6 is responsible for $\alpha_1$-adrenoceptor-induced $Ca^{2+}$ entry in rabbit portal vein smooth muscle cells (Inoue et al. 2001) and is activated by low concentrations of Ang II in mesenteric artery myocytes (Saleh et al. 2006). However, TRPC6-KO mice exhibit elevated blood pressure and enhanced

agonist-induced vasoconstriction, likely due to upregulation of TRPC3 (Dietrich et al. 2005). ET-1 activates TRPC7 in rabbit coronary artery myocytes (Saleh et al. 2006) whereas TRPC3 is involved in agonist-induced smooth muscle depolarization and vasoconstriction of rat cerebral arteries (Reading et al. 2005). The dependence of resistance artery myogenic reactivity on VOCCs is well described but how changes in intraluminal pressure lead to depolarization of smooth muscle cells has remained ill-defined. Several mechano-sensitive TRP channels, including, TRPC6 (Welsh et al. 2002), TRPV2 (Muraki et al. 2003), TRPM4 (Earley et al. 2004), and TRPP2 (Sharif-Naeini et al. 2009), have been suggested to play a role in pressure-induced depolarization (reviewed by Inoue et al. 2009). For example, the use of pharmacological tools implicated TRPV2 channels in myogenic reactivity in rat retinal arteries (McGahon et al. 2016) whereas the use of siRNA demonstrated that both TRPC6 and TRPM4 are necessary for the response of rat cerebral arteries to increases in pressure (Gonzales et al. 2014).

*1.2.3 $Ca^{2+}$-sensitization:* Although $Ca^{2+}$ is required to initiate force generation within vascular smooth muscle cells, as discussed in **Section 1.2.1**, decreases in MLCP activity following protein kinase phosphorylation can enhance contractile force without further change in $[Ca^{2+}]_i$, a process known as $Ca^{2+}$ sensitization (reviewed by Somlyo and Somlyo 2003; Goulopoulou and Webb 2014). This process was first proposed to explain the observation that a higher degree of contractile force can be generated in isolated arteries by GPCR agonists in comparison to high levels of extracellular $K^+$ (to induce depolarization), even when the increase in $[Ca^{2+}]_i$ is comparable (Himpens and Casteels 1990; Bradley and Morgan 1987; Hideaki 1989). The pathways by which GPCR by agonists cause sensitization has been widely studied, and currently the consensus is that it is due to activation of Rho-associated kinase (ROK; Swärd et al. 2003; Wilson et al. 2005). Agonist-induced activation of the small GTPase RhoA via the $G_{12/13}$ family of heterotrimeric G-

proteins and a GEF (guanine nucleotide-exchange factor; Somlyo and Somlyo 2003) leads to activation of ROK (Swärd et al. 2003), which inhibits MLCP activity by phosphorylation of myosin phosphatase target subunit 1 (MYPT1; **Figure 1.7**; Brozovich et al. 2016; Murányi et al. 2005). Furthermore, protein kinases such as ROK and PKC can also phosphorylate C-kinase potentiated protein phosphatase-1 inhibitor (Walsh et al. 2007), an endogenous inhibitor of MLCP, which when phosphorylated binds the catalytic site of MLCP (El-Yazbi and Abd-Elrahman 2017; reviewed by Brozovich et al. 2016; El-Yazbi and Abd-Elrahman 2017). In addition to mediating vasocontraction to agonists, sensitization can contribute to pressure-induced constriction of resistance arteries as described in **Section 1.3.3**.

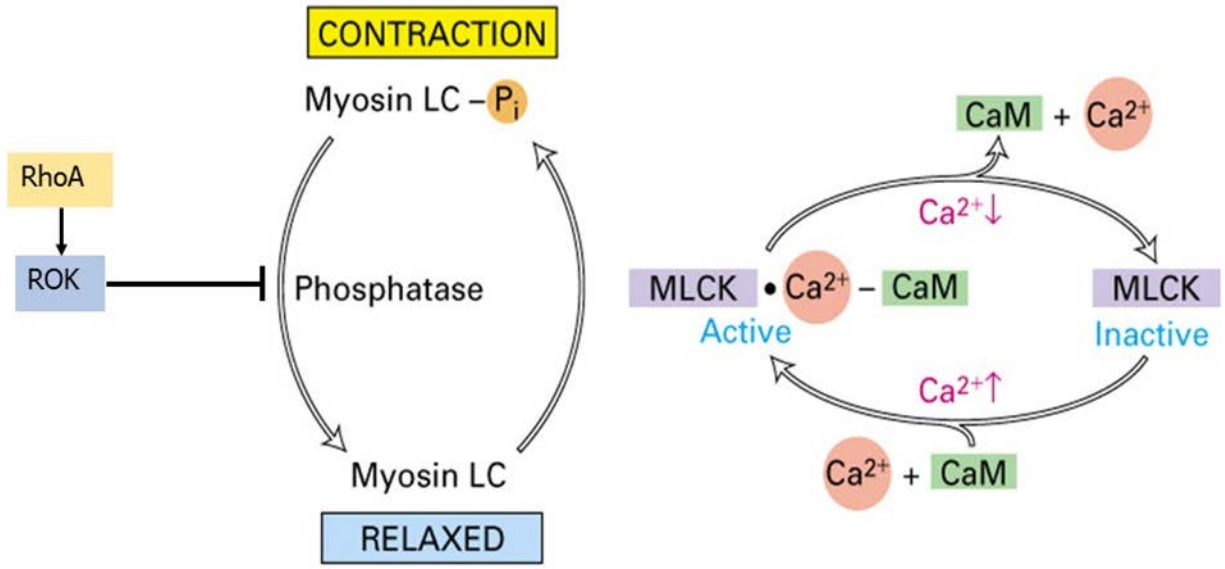



**Figure 1.7: Mechanism of smooth muscle contraction due to $Ca^{2+}$ sensitization.** Activation of the RhoA-ROK pathway leads to inhibition of MLCP activity so that contractile force can be increased without further change in $[Ca^{2+}]_i$.

*1.2.4. Modulation of vascular smooth muscle contraction by $K^+$ channels:* $K^+$ channels provide the major ionic conductance in vascular smooth muscle cells, and thus set and regulate membrane potential (Jackson 2000; Nelson and Quayle 1995; Nelson 1992, 1993; Tykocki et al. 2017). Membrane potential controls the open probability of VOCCs, a major source of $Ca^{2+}$ for

contraction of vascular smooth muscle cells (see **Section 1.2.2**). Vascular smooth muscle cells express a range of different types of $K^+$ channel: $BK_{Ca}$ channels, voltage-gated $K^+$ (Kv) channels, ATP-sensitive $K^+$ ($K_{ATP}$) channels, inward-rectifier $K^+$ ($K_{ir}$) channels, and members of the two-pore $K^+$ channel family of $K^+$ channels (Sepulveda et al. 2015); recently reviewed by Jackson 2016). In physiological conditions (3–5 mM $K^+$ outside of the cell and 140 mM $K^+$ inside), the driving force for movement of $K^+$ is outward and so opening of a $K^+$ conducting channel leads to loss of positive charge, and membrane hyperpolarization (Nelson et al. 1990), whereas closure of open $K^+$ channels will result in membrane depolarization. As membrane resistance is high, opening of a few $K^+$ channels can have a big impact on smooth muscle membrane potential and thus on the activity of VOCCs and cell contractility.

Here, I will briefly review the structure and function of $BK_{Ca}$ channels, Kv channels and $K_{ir}$ channels in regulating smooth muscle contractility and therefore arterial diameter in resistance arteries.

*a) $BK_{Ca}$ channels:* $BK_{Ca}$ channels (encoded by *KCNMA1*) are composed of homotetramers pore-forming α subunits together with regulatory β- and γ subunits **(Figure 1.8)**. The α subunit is composed of 7 transmembrane domains with the pore region in between S5 and S6(Hoshi et al. 2013). Two regulator of $K^+$ conductance domains (RCK1 and RCK2) located in the COOH-terminus contain $Ca^{2+}$ sensors (Ledoux et al. 2006) and positively charged residues in S2-4 serve as voltage sensors (Hoshi et al. 2013). These channels are fundamentally voltage-gated channels for which binding of $Ca^{2+}$ increases the sensitivity to voltage; $Ca^{2+}$ enhances voltage-dependent activation so that channel opening will be greater at any given membrane potential with increasing concentrations of $[Ca^{2+}]_i$. Auxiliary subunits β1 and γ are involved in modulation of channel activity. There are four β subunits (encoded by *KCNMβ1–4*) with β1 the dominant form in vascular

smooth muscle and can affect channel gating, sensitivity to $Ca^{2+}$ and voltage (Mcmanus et al. 1995; Meera et al. 1996) as well as contributing to the trafficking of α subunits to the plasma membrane (Leo et al. 2014). The γ subunit is associated with increasing channel sensitivity to voltage (Evanson et al. 2014). $BK_{Ca}$ channels have a large single channel conductance (150-250 pS), are highly expressed in vascular smooth muscle cells, but are not present in freshly isolated endothelial cells (Sandow et al. 2009).

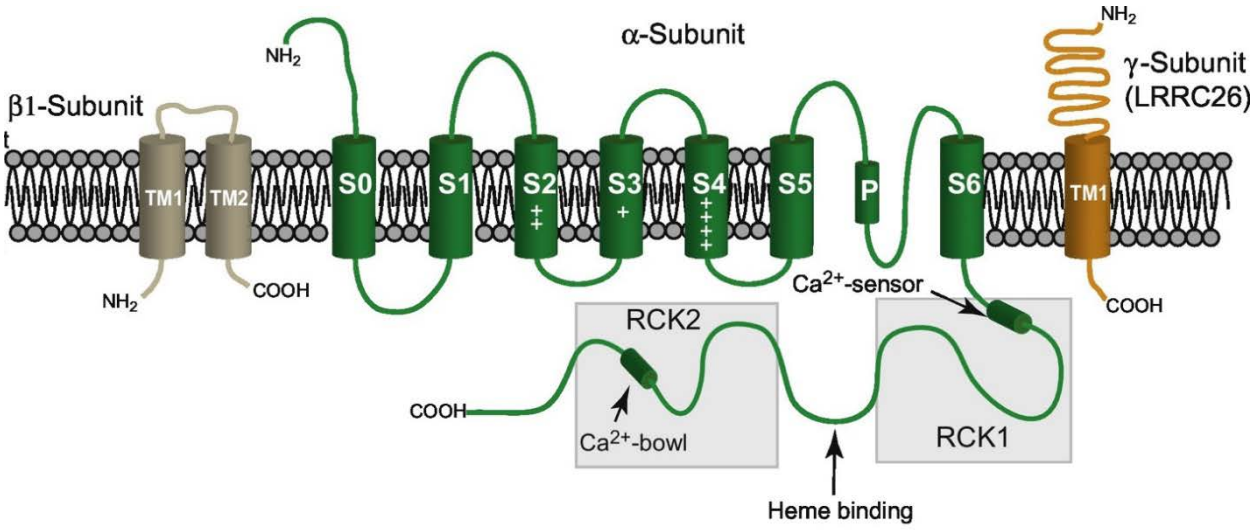



**Figure 1.8: Schematic showing the structure of $BK_{Ca}$ channels**. $BK_{Ca}$ channels are composed of pore-forming α, and modulatory ß1- and γ subunits. The α-subunit is made up of 7 transmembrane domains, S2-4 being the voltage sensor and the pore region located between S5 and S6, and 4 cytoplasmic domains within the COOH-terminus tail. Two regulator of $K^+$ conductance domains (RCK1 and RCK2) located in the cytoplasmic COOH-terminus contain channel's $Ca^{2+}$bindings sites. The auxiliary subunits ß1 and γ subunits are consist of 2 transmembrane domains and 1 transmembrane domain respectively (Jackson 2016).

As mentioned above (**Figure 1.3**), activation of $BK_{Ca}$ channels by RyR-derived $Ca^{2+}$ sparks regulates diameter in cerebral resistance arteries (reviewed by Jackson 2016)) but in other vessels, such as hamster and mouse cremaster arterioles, $Ca^{2+}$ entry through VOCCs may contribute to $BK_{Ca}$ channel activation (Westcott et al. 2012; Westcott and Jackson 2011). $BK_{Ca}$ channels can also be targeted by vasodilators. Both the cyclic adenosine monophosphate (cAMP)-PKA and

cGMP-PKG pathways have been shown to increases $BK_{Ca}$ channel activity through numerous mechanisms including modulation of $Ca^{2+}$ sparks (Jewell et al. 2004) and direct phosphorylation (Alioua et al. 1995) of channels.

*b) Kv channels:* Kv channels are made up of homo- or heterotetramers of pore-forming α-subunits with six transmembrane domains and the P-loop between S5 and S6 forming the pore (**Figure 1.9**). Positively charged residues in S2-4 serve as voltage sensors (Jan and Jan 1992). Accessory subunits affect membrane expression, gating kinetics, and voltage sensitivity (reviewed by Gutman et al. 2005).

Forty genes give rise to 12 families (Kv1-12) and functional diversity is increased by the formation of heteromultimers with a range of accessory subunits (for review see Jackson 2018). Many different Kv channels have been reported in vascular smooth muscle with the Kv1, 2 and 7 families being particularly prominent (Plane et al. 2005; Greenwood and Ohya 2009). Activation by membrane depolarization means that along with $BK_{Ca}$ channels, Kv channels can provide negative feedback regulation of contractility (Jackson 2018). Also, vasoconstrictors, such as Ang II (Aiello et al. 1996; Clément-Chomienne et al. 1996) inhibit delayed rectifier $K^+$ channels in vascular smooth muscle cells through PKC whereas activation of the cAMP-PKA pathway (Alejandro-Aiello et al. 1998) by vasodilator stimuli increases Kv channel activity.

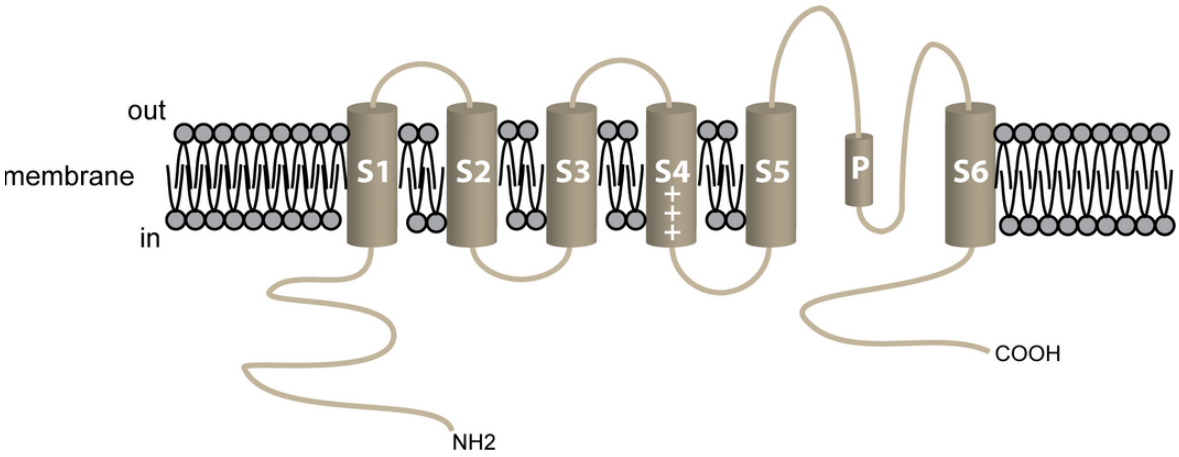



**Figure 1.9: Schematic showing the structure of pore-forming α subunits of Kv channels.** An α subunit of a $K_V$ channel is made of 6 transmembrane domains. Transmembrane domain S4 serves as a voltage-sensor and pore-region is located in between S5 and S6(William F. Jackson 2018).

*c) $K_{ir}$ channels:* The superfamily of 15 $K_{ir}$ channels contains 7 subfamilies, $K_{ir}1$ to $K_{ir}7$. $K_{ir}3$ channels are coupled to GPCRs and therefore called G-protein-gated inwardly rectifying $K^+$ (GIRK) channels, and $K_{ir}1$ and 7 are weak inward rectifier $K^+$ channels. Apart from the $K_{ir}6$ subtype, $K_{ir}$ channels are composed of homo- or heterotetramers of α-subunits. For example, heterotetrameric co-assembly of $K_{ir}2.1$ and $K_{ir}2.2$ was identified in rabbit cardiomyocytes using adenovirus-mediated overexpression of dominant-negative construct of each gene (Zobel et al. 2003) and functional channels can be assembled from $K_{ir}4.1$ and $K_{ir}5.1$ subunits in HEK cells (Tanemoto et al. 2000). The formation of $K_{ir}4.1/K_{ir}5.1$ heterotetramers significantly impacts both the biophysical properties of the channels and sensitivity to block by $Ba^{2+}$ as compared to homotetrameric channels (Goto et al. 2004). Each α-subunit contains two transmembrane domains (M1 and M2) connected by the pore-forming region with intracellular COOH and $NH_2$ termini (**Figure 1.10**; Quayle et al. 1997; Bichet et al. 2003).

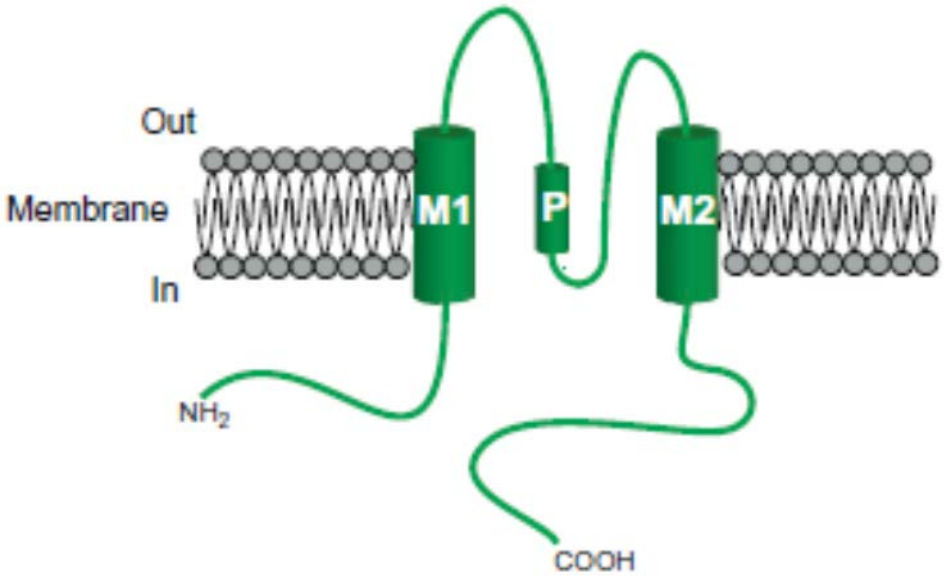



**Figure 1.10: Structure of $K_{ir}$ channels.** Schematic drawing of a $K_{ir}$ channel α–subunit. Each subunit comprises two transmembrane helices (M1 and M2), a pore-forming region containing the pore-helix (P), and $NH_2$ and COOH termini (Jackson 2016).

The inward rectification property is due to the voltage-dependent block of the pore by cytoplasmic $Mg^{2+}$ and polyamines (Matsuda et al. 1987; Lopatin et al. 1994). This reversible, competitive block of the pore happens when $Mg^{2+}$ and polyamines interact with basic residues in M2 and the COOH terminus when membrane potential is positive to the $K^+$ reversal potential (~-90 mV in 5 mM extracellular $K^+$; $[K^+]_o$), $E_K$, limiting outward movement of $K^+$. However, when membrane potential is negative to $E_K$ the block is relieved to allow $K^+$ move into the cell. The lack of voltage-sensing transmembrane domain on $K_{ir}$ channels means they are intrinsically voltage-insensitive. But, as the degree of $Mg^{2+}$ and polyamine block is dependent on membrane potential, this confers a voltage-dependence on channel activity.

The physiological importance of $K_{ir}$ channels is due to their negative slope conductance (Edwards et al. 1988), the small outward "hump" in the current-voltage relationship (Smith et al. 2008; Thorneloe and Nelson 2005; **Figure 1.11**). This means that hyperpolarization from resting

membrane potential increases outward $K_{ir}$ current and so amplifies the original hyperpolarization (Longden and Nelson 2015; Smith et al. 2008; Sonkusare et al. 2016). As shown in **Figure 1.11**, increasing $[K^+]_o$ to 15 mM $K^+$ will shift the $E_K$ to -60 mV. Under these conditions there is an elevated outward $K^+$ current at the original resting membrane potential which will hyperpolarize the membrane potential to lead to vasodilation (Doyle et al. 1998; Krapivinsky et al. 1998). These properties mean that $K_{ir}$ channels can mediate vasodilation to small increases in $[K^+]_o$ and may "boost" the vasodilation caused by agents such as acetylcholine (ACh) and pinacidil which open other potassium channels (Smith et al. 2008; Sonkusare et al. 2016).

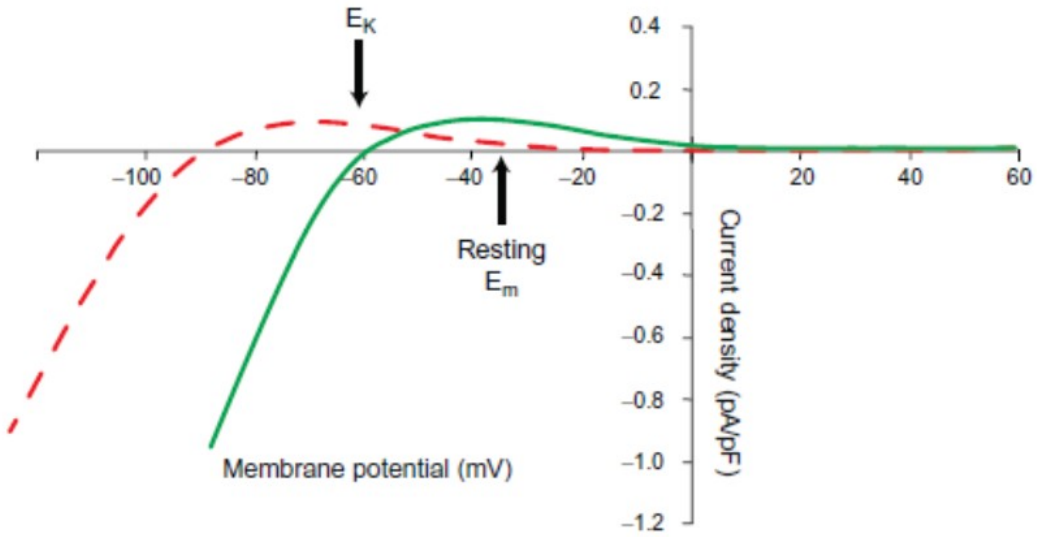



**Figure 1.11: Voltage-dependency of $K^+$ flux through $K_{ir}$ channels.** Red line shows idealized current-voltage (I-V) relationship for $Ba^{2+}$-sensitive currents (5 mM $[K^+]_o$ and 140 mM $K^+$ intracellular concentrations). At membrane potentials negative to $E_K$ (-90 mV) $K^+$ moves into the cells (inward rectification) but at membrane potentials positive to $E_K$, $K^+$ moves outwards. The outward currents decrease as the membrane potential becomes more positive producing a region of "negative slope conductance" where hyperpolarization increases outward current. Green line shows the effect of increasing $[K^+]_o$ to 15 mM $K^+$ to shift the $E_K$ to -60 mV. Under these conditions there is an elevated outward $K^+$ current at the original resting membrane potential which will hyperpolarize the membrane potential to lead to vasodilation. (Modified from Jackson 2016)

Different subtypes of $K_{ir}$ channel exhibit different degrees of inward rectification depending on the binding affinity of the blocking molecules for the channel. This binding affinity is determined by the presence of different amino acids in the channel pore. For example, there is a negatively charged aspartic acid residue at location 172 of the second transmembrane domain of $K_{ir}2$ series channels which has been identified as an important binding site for the blocking ions (Stanfield et al. 1994).

In weakly inward rectifying $K_{ir}1$ series channels there is an uncharged asparagine at location 171 but changing this to an aspartic acid increases the rectifying behavior of the channel (Lu and MacKinnon 1994; Stanfield et al. 1994; Wible et al. 1994; Yang et al. 1995). In addition, membrane bound $PIP_2$ can also regulate the activity and gating of $K_{ir}$ channels. This is accomplished through $PIP_2$ binding to both the cytoplasmic side of the transmembrane domain and gating machinery which is essential for channel activation (Suh and Hille 2008).

The small outward hyperpolarizing $K^+$ current conducted by $K_{ir}$ channels when membrane potential is positive to $E_K$ is physiologically important in coupling metabolism to blood flow. Moderate rises in $[K^+]_o$, from 3-5 mM to more than 10 mM, are commonly associated with hypoxia, ischemia and hypoglycemia in the cerebral circulation, and thus $K^+$-induced vasodilation is critical for maintaining the supply of oxygen and nutrients to tissues under those conditions (Sieber et al. 1993; Somjen 1979) as well as coupling of blood flow to neuronal activity (Longden et al. 2017; Longden and Nelson 2015). In other settings, reductions in $[K^+]_o$ below the physiological range (3-5 mM) or increases above 20 mM, can result in vasoconstriction (Haddy and Scott 1968; Chen et al. 1972; Sybertz et al. 1983; Haddy 1983).

The molecular identity of vascular $K_{ir}$ channels appears to show vessel and species variation (**Table 1.1**) but the $K_{ir}2$ series have received most attention. Expression of mRNA for

$K_{ir}$ 2.1 have been identified in rat mesenteric whole arteries (Goto et al. 2004; Tajada et al. 2012), vascular smooth muscle cells (Smith et al. 2008; Bradley et al. 1999; Kim et al. 2005) and endothelial cells (Sonkusare et al. 2016). In rat cerebral arteries, $K_{ir}$2.1 are expressed in whole artery (Wu et al. 2007) and smooth muscle cells (Bradley et al. 1999; Smith et al. 2008; Wu et al. 2007). In coronary arteries, $K_{ir}$2.1 have only been identified in smooth muscle cells (Bradley et al. 1999; Smith et al. 2008; refer to **Table 1.2-1.5** for expression of $K_{ir}$ in other species; no evidence of $K_{ir}$ 3.4 and 7.1 expression was found in arteries listed in the table). In addition, mRNA for $K_{ir}$2.2 has been shown in freshly isolated rat cerebral, coronary and mesenteric vascular smooth muscle cells (Goto et al. 2004; Smith et al. 2008). Also, currents attributed to $K_{ir}$2 series channels have been recorded from isolated vascular smooth muscle cells and show a conductance of around 20 pS (Park et al. 2005). There is limited evidence for $K_{ir}$3 series channels playing a functional role in vascular smooth muscle but melatonin-induced vasorelaxation in rat aorta (Weekley 1991) may dependent on the activation of $K_{ir}$3 series channels (Nelson et al. 1996).

At any point in time, the contractile state of resistance artery smooth muscle cells and thus, arterial diameter, is determined by their integrated response to surrounding physical and chemical influences. Therefore, I will now discuss how resistance artery diameter is modulated by the three most physiological important factors; intravascular pressure (myogenic reactivity), sympathetic nerve activity, and chemical and electrical signals from endothelial cells.

| | Mesentery | | |
|---|---|---|---|
| **$K_{ir}$** | **SMC** | **EC** | **whole vessel** |
| **2.1** | rat (Bradley et al. 1999; Smith et al. 2008; Kim et al. 2005) | rat (Sonkusare et al. 2016) | rat (Goto et al. 2004)<br>mice (Tajada et al. 2012) |
| **2.2** | rat (Smith et al. 2008)<br>rat (Bradley et al. 1999) | | rat (Goto et al. 2004) |
| **2.3** | rat (Bradley et al. 1999; Smith et al. 2008) | | |
| **2.4** | rat (Smith et al. 2008) | | rat (Goto et al. 2004) |
| **3.1** | mice (Tajada et al. 2012) | | mice (Tajada et al. 2012) |

**Table 1.2: Expression of $K_{ir}$ subunits identified in mesenteric arteries.** Font in black indicates expression of $K_{ir}$ subunits and font in red show absence of expression of the subunit in freshly isolated smooth muscle or endothelial cells. Empty boxes indicate no data available.

| **$K_{ir}$** | Coronary | |
|---|---|---|
| | **SMC** | **EC** |
| **2.1** | rat (Bradley et al. 1999; Smith et al. 2008)<br>rabbit (Park et al. 2005) | |
| **2.2** | rat (Smith et al. 2008)<br>rat (Bradley et al. 1999)<br>rabbit (Park et al. 2005) | |
| **2.3** | rat (Bradley et al. 1999; Smith et al. 2008)<br>rabbit (Park et al. 2005) | |
| **2.4** | rat ( Bradley et al. 1999; Smith et al. 2008) | |

**Table 1.3: Expression of $K_{ir}$ subunits identified in coronary arteries.** Font in black indicates expression of $K_{ir}$ subunits and font in red show absence of expression of the subunit in freshly isolated smooth muscle or endothelial cells. Empty boxes indicate no data available.

| $K_{ir}$ | Cerebral | | |
|---|---|---|---|
| | **SMC** | **EC** | **whole vessel** |
| **2.1** | rat (Bradley et al. 1999; Smith et al. 2008; Wu et al. 2007)<br>mice (Zaritsky et al. 2000)<br>hamster (Sancho et al. 2017) | mice (Longden et al. 2017)<br>hamster (Sancho et al. 2017) | rat (Wu et al. 2007)<br>hamster (Sancho et al. 2017) |
| **2.2** | Rat (Smith et al. 2008; Wu et al. 2007)<br>hamster (Sancho et al. 2017)<br>rat (Bradley et al. 1999) | hamster (Sancho et al. 2017) | rat (Wu et al. 2007)<br>hamster (Sancho et al. 2017) |
| **2.3** | rat (Bradley et al. 1999; Smith et al. 2008; Wu et al. 2007) | | rat (Wu et al. 2007) |
| **2.4** | rat (Smith et al. 2008) | rat (Wu et al. 2007) | rat (Wu et al. 2007) |

**Table 1.4: Expression of $K_{ir}$ subunits identified in cerebral arteries.** Font in black indicates expression of $K_{ir}$ subunits and font in red show absence of expression of the subunit in freshly isolated smooth muscle or endothelial cells. Empty boxes indicate no data available.

| $K_{ir}$ | Tail | | Pulmonary | | Renal |
|---|---|---|---|---|---|
| | **SMC** | **EC** | **SMC** | **EC** | **whole vessel** |
| **2.1** | rat (Schubert et al. 2004) | rat (Schubert et al. 2004) | | *calf (Qu et al. 2015) | rat (Chilton et al. 2008) |
| **2.2** | rat (Schubert et al. 2004) | rat (Schubert et al. 2004) | | *calf (Qu et al. 2015) | |
| **1.1** | | | | *calf (Qu et al. 2015) | |

**Table 1.5: Expression of $K_{ir}$ subunits identified in tail, pulmonary and renal arteries.** Font in black indicates expression of $K_{ir}$ subunits and font in red show absence of expression of the subunit. Empty boxes indicate no data available. * denotes cultured endothelial cells. Other studies used freshly isolated smooth muscle or endothelial cells.

## 1.3 Myogenic reactivity

Small resistance arteries constrict in response to increases in intravascular pressure, a response termed myogenic reactivity or the myogenic response. As mentioned earlier, this phenomena was first described by Bayliss in 1902 who showed that in the rabbit hind limb circulation, increases in intravascular pressure resulted in a reduction in arterial lumen diameter, and is therefore also known as the Bayliss effect (Bayliss 1902). The myogenic reactivity is now known to be a crucial autoregulatory mechanism to maintain constant blood flow within tissues and organs in the face of changes in blood pressure (Davis and Hill 1999). When blood pressure is low, resistance arteries passively dilate but when pressure is increased, they will constrict to increase resistance and prevent excessive blood flow and damage to downstream arterioles and capillaries (Davis 2012). This biphasic pattern (**Figure 1.12**) has been widely reported in the literature as is particularly well-defined in skeletal and renal vascular beds with active tone development beginning at intravascular pressures of around 60 mmHg (Loutzenhiser, Bidani, and Chilton 2002; Davis 1993).

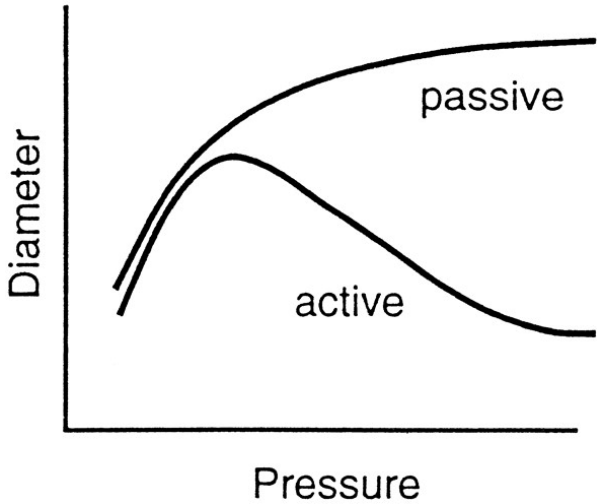



**Figure 1.12: A diagram illustrating the biphasic nature of myogenic reactivity. A** myogenically active artery will passively dilate when the intravascular pressure is low, and will constrict when pressure is increased. In the absence of extracellular $Ca^{2+}$ or in the presence of inhibitors of VOCCs the artery passively dilates as pressure increases illustrating the requirement of $Ca^{2+}$ influx for active tone development.

The ability of small arteries to develop myogenic tone has been demonstrated using vessels from many vascular beds such as the cerebral, renal, skeletal uterine and coronary vasculature ( Davis and Hill 1999; Sandow and Hill 2000; Hill et al. 2001; Hill et al. 2006; Hemmings et al. 2005; Hill and Meininger 1994). These studies have shown that the degree of myogenic reactivity shown by arteries is inversely proportional to their diameter (Davis 1993) and that there is variation in the profile of the response between arteries from different vascular beds. For example, the biphasic nature of the myogenic reactivity described in skeletal and renal arteries is less evident in cerebral arteries in which active tone development occurs at intravascular pressures from 20 mmHg to 100mmHg.

Myogenic reactivity is a major factor in determining total peripheral vascular resistance and so contributes to arterial blood pressure (Johnson 1986), and impairment of the myogenic reactivity has been proposed to contribute to the development of vascular and end-organ damage associated with conditions such as obesity, hypertension, and preeclampsia (Haddock et al. 2011; Rummery and Hill 2004; Belik 1995; VanWijk et al. 2002; Hu et al. 2012).

*1.3.1 Role of smooth muscle membrane potential and VOCC-mediated $Ca^{2+}$ influx:* The development of myogenic tone is associated with depolarization of the smooth muscle cell membrane potential and $Ca^{2+}$ influx through VOCCs (Knot and Nelson 1998; Harder 1984; McCarron et al. 1989) to activate contractile mechanisms (see **Section 1.2**). In isolated rat cerebral arteries there is a strong correlation between intraluminal pressure, membrane depolarization, increased $[Ca^{2+}]_i$, and reductions in diameter (Knot and Nelson 1998).

This pressure-dependent membrane depolarization is thought to be activated by a pressure sensor in the smooth muscle cell membrane (Davis 2012). This sensor and how it is activated has yet to be identified but "*the wall tension hypothesis*" proposed by Paul Johnson in 1981 (Burrows

and Johnson 1981) suggests that increases in wall tension is the trigger for pressure-induced vasoconstriction. Wall tension is calculated by the LaPlace relationship $T = P \cdot r$ which $T$ is wall tension, $P$ is transmural pressure and $r$ is vessel radius (von Anrep 1912). Increase in wall tension lead to raise of the $[Ca^{2+}]_i$ in rat cremaster vascular smooth muscles and development of myogenic reactivity (Burrows and Johnson 1981). Furthermore, it has been shown that increase in wall tension not only increase $[Ca^{2+}]_i$ but also induce the phosphorylation of MLC in smooth muscles cells to enhance contractility and set basal arterial tone[183]. Overall, there is strong evidence linking increases in wall tension to the development of myogenic tone. However, it is still unclear which cellular components are responsible for the conversion of mechanical stimuli to biochemical signals.

*1.3.2 The mechanosensor:* Proposed candidates for the mechanosensor involved in development of myogenic tone include integrins, stretch-activated ion channels, and GPCRs (reviewed by El-Yazbi and Abd-Elrahman 2017). Integrins are transmembrane proteins that form a link between the smooth muscle cytoskeleton and extracellular matrix that is suitable for bi-directional signal transfer (Davis et al. 2001; Vuori 1998). One model proposes that increased wall tension caused by intraluminal pressure deforms extracellular matrix proteins to expose integrin-interacting motifs that initiate downstream signaling resulting in constriction (Ross et al. 2013; Schwartz 2010; Osol 1995; Hill and Meininger 2012). In support of this proposal, tripeptide integrin-binding sequences, result in a transient constriction of isolated rat cremaster arterioles(Mogford et al. 1996; Davis et al. 2001). In another model, stretch-activated TRP channels act as a mechanosensor to elicit membrane depolarization and subsequent $Ca^{2+}$ entry through VOCCs (Carlson and Beard 2011; Sharif-Naeini et al. 2008). Inhibition of TRPM4 channels with 9-phenanthrol abolished the myogenic response in rat cerebral arteries (Gonzales et al. 2010) and knockdown of the channels

with oligodeoxynucleotides resulted in a decrease of 70% to 85% myogenic constriction in the same vessels (Reading and Brayden 2007), leading to the suggestion that they could act as a pressure sensor. However, TRPM4 channels are not directly activated by membrane stretch but in rat cerebral arterioles they contribute to smooth muscle membrane depolarization and myogenic constriction through mechanical activation of purinergic GPCRs (Li et al. 2014); molecular suppression of $P_{2Y}$ receptors using antisense oligodeoxynucleotides reduced the myogenic response by about 45% (Brayden et al. 2013; Li et al. 2014). The Ang II ($AT_1$) receptor has also been proposed as a mechanosensor as the receptor can switch to an active conformation in response to cell stretch in a ligand-independent manner (Yasuda et al. 2008) and the same receptor can contribute to myogenic reactivity in coronary, mesenteric, skeletal and renal arterioles via coupling to $G_{q/11}$ (Schleifenbaum et al. 2014) and possibly activation of $Ca^{2+}$ influx via TRPC6 (Schnitzler et al. 2008; Schnitzler et al. 2011; Schleifenbaum et al. 2014). In freshly isolated pulmonary artery smooth muscle cells a mechanosensitive cation channel with similar properties to TRPC channels was identified (Park et al. 2003).

In cultured endothelial cells overexpressing TRPC6 channels, pressure induced cation influx leads to membrane depolarization(Spassova et al. 2006; Suchyna et al. 2000) and together with the observation that acute knockdown of TRPC6 channels in rat cerebral arteries resulted in a significant reduction in myogenic constriction (Welsh et al. 2002) supports a role for TRPC6 in myogenic reactivity. However, activation of TRPC channels is dependent on the PLC pathway (Hill-Eubanks et al. 2014; Hofmann et al. 1999), indicating that although they may play a role in the response of resistance arteries to pressure, and may contribute to the $Ca^{2+}$ influx required for myogenic constriction, they are not the mechanosensor necessary for the development of myogenic tone.

*1.3.3 Contribution of $Ca^{2+}$-independent mechanisms to myogenic reactivity:* In contrast to the findings of Knot and Nelson (Knot and Nelson 1998) of a strong correlation between intraluminal pressure, membrane depolarization, increased $[Ca^{2+}]_i$, and vasoconstriction, other studies have suggested that $Ca^{2+}$-mediated MLCK activation and $LC_{20}$ phosphorylation are not the only mechanism mediating force generation during the myogenic reactivity (VanBavel, Van der Meulen, and Spaan 2001; Gokina et al. 2005). For example, membrane depolarization and $[Ca^{2+}]_i$ did not change with increased force generation at pressures between 60–140 mmHg in cerebral arteries (Osol et al. 2002). Thus, $Ca^{2+}$-sensitization, mediated by inhibition of MLCP inhibition to shift the equilibrium toward the accumulation of phosphorylated $LC_{20}$ without an associated increase in $Ca^{2+}$-induced MLCK activity (Somlyo and Somlyo 2003; **Section 1.2.3**), has been proposed to contribute to myogenic reactivity.

Currently, the weight of evidence supports the role of ROK in $Ca^{2+}$-independent myogenic reactivity (**Figure 1.13;** El-Yazbi and Abd-Elrahman 2017). As described in **Section 1.2.3**, ROK-dependent sensitization is mediated by phosphorylation of the myosin targeting subunit of MYPT1. Phosphorylation occurs at two threonine residues; T697 which inhibits MLCP catalytic activity, and T855 to inhibit both catalytic activity and myosin binding (Murányi et al. 2005). The link between pressure-evoked ROK activation and downstream effectors was made by the demonstration that increased intravascular pressure leads to a graded increase in ROK-mediated MYPT1 phosphorylation, MLCP inhibition, and increased $LC_{20}$ phosphorylation in rat cerebral and skeletal muscle arterioles (Johnson et al. 2009; Moreno-Domínguez et al. 2013). Further experiments by the same group provided evidence for a reduction in G-actin (unpolymerized) during myogenic vasoconstriction (Johnson et al. 2009; Moreno-Domínguez et al. 2013), and demonstrated that at higher pressures, actin cytoskeleton reorganization was the major mechanism

for force generation (Moreno-Domínguez et al. 2014). In these experiments, both the pressure-evoked constriction and G-actin pool reduction were sensitive to ROK inhibition supporting the idea that dynamic ROK-mediated actin reorganization contribute to myogenic reactivity. Investigation of the link between increases in intravascular pressure and activation of ROK-mediated processes in rat cerebral arteries has led to a model in which stimulation of integrins leads to focal adhesion kinase (FAK) activation by autophosphorylation to permit Src family kinase (SFK) binding and its subsequent autophosphorylation and activation (Colinas et al. 2015). SFK in turn phosphorylates FAK to further enhance its catalytic activity, leading to activation of Rho guanine nucleotide exchange factors (RhoGEFs) to exchange GTP for GDP of RhoA. RhoA-GTP is the active form of RhoA that activates ROK to phosphorylate MYPT1-T855 and suppress MLCP activity, and to regulate actin dynamics (El-Yazbi and Abd-Elrahman 2017). In a physiological context where resistance arteries are faced with a maintained intravascular pressure, and the ability to elicit contraction without large fluctuations in $[Ca^{2+}]_i$ would allow conservation of $Ca^{2+}$ signaling (Schubert et al. 2008).

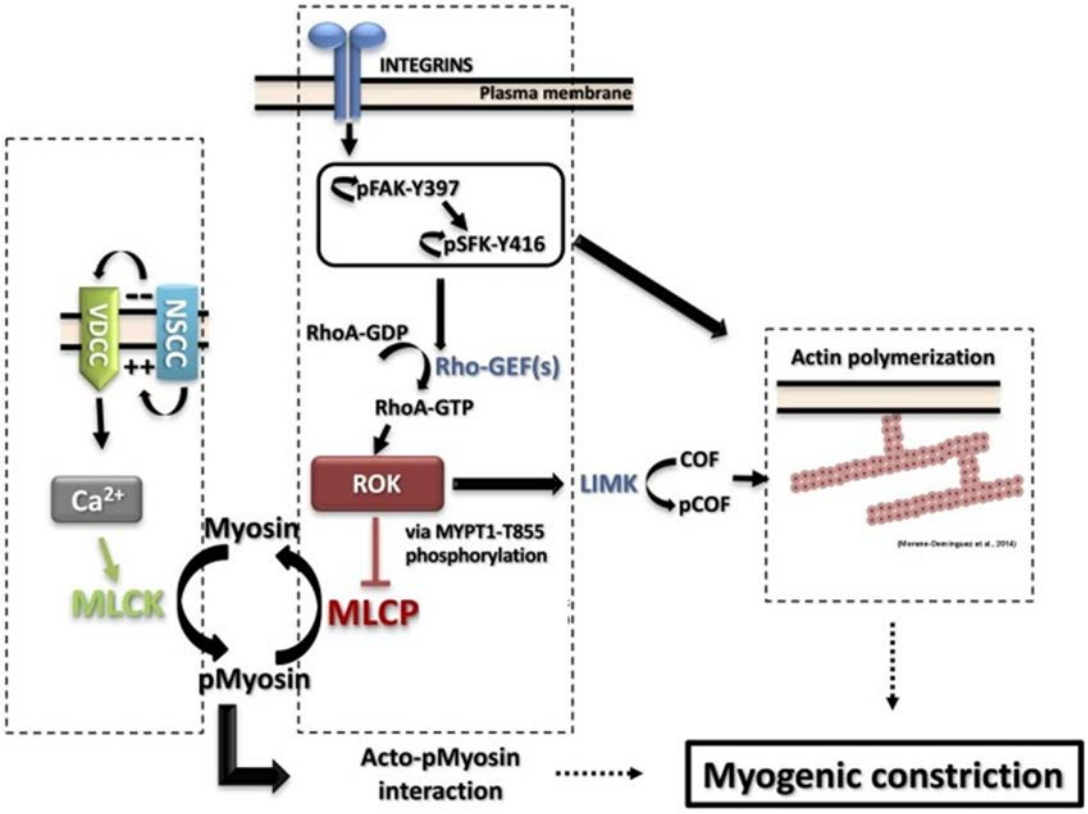



**Figure 1.13: Mechanisms contributing to myogenic reactivity in resistance arteries.** Depolarization of the smooth muscle cell membrane potential and $Ca^{2+}$ influx through VOCCs (Knot and Nelson 1998; Harder 1984; McCarron et al. 1989) to activate MLCK which phosphorylates $LC_{20}$ to increase the activity of the myosin ATPase and drive the cycling of actin-myosin crossbridges to create muscle tension. Stimulation of integrins leads to phosphorylation of FAK, activation of RhoGEFs, RhoA subsequently activates ROK to phosphorylate MYPT1 and suppress MLCP activity. ROK also mediates reorganization of actin. Modified from (El-Yazbi and Abd-Elrahman 2017).

*1.3.4. Endothelial modulation of myogenic reactivity:* The ability of small arteries to contract in response to increases in pressure is an intrinsic property of the vessel smooth muscle cells (Davis and Hill 1999). The vascular endothelium is not required for development of myogenic reactivity (Falcone et al. 1991; Falcone, Granger, and Meininger 1993; Hill et al. 2006; Kuo et al 1990), although it may be able to exert a modulatory influence (Meininger and Davis 1992; Palomares and Cipolla 2014). In mesenteric arteries from spontaneously hypertensive rats, endothelium

removal enhanced myogenic constriction, possibly due to the loss of endothelium-derived nitric oxide (NO; Garcia et al. 1997). Also, inhibitors of NO synthase (NOS) potentiated pressure-induced constriction of hamster cremaster arterioles in vivo (Wit et al. 1998), and isolated mouse mesenteric and rat coronary arteries (Garcia and Bund 1998; Scotland et al. 2001). Endothelial modulation of myogenic reactivity in rat mesenteric arteries is explored in **Chapter 4**.

*1.3.5: Myogenic reactivity in disease states:* Changes in myogenic reactivity, both attenuation and enhancement, have been associated with a number of pathological conditions. Loss of myogenic reactivity has been documented in middle cerebral arteries of stroke prone hypertensive and hypertensive-mono-arthritic rats (Daneshtalab and Smeda 2010; Randell et al. 2016; Smeda and King 2000; Smeda and Daneshtalab 2011). The ability of the middle cerebral artery to constrict in responses to increases in pressure is essential for appropriate and controlled perfusion of smaller cerebral arteries and so loss of this response facilitates over-perfusion, elevated microvascular pressures and cerebral haemorrhage in these animals. Similarly, loss of myogenic reactivity in retinal arteries has been associated with retinopathy in diabetic rats (Ito et al. 2006) and humans (Blum et al. 2006). In mesenteric arteries from a rat model of chronic renal failure (Vettoretti et al. 2006), the threshold for the development of myogenic constriction was shifted to a higher pressure range and the magnitude of the response was reduced, possibly as a compensatory mechanism to counteract the increase in peripheral resistance. However, in the kidney, the myogenic reactivity of renal afferent arterioles is essential for preventing increased glomerular capillary pressure and consequent nephropathy. Attenuation of this myogenic response in streptozotocin-treated diabetic (Hayashi et al. 1992), Fawn Hooded Hypertensive (Burke et al. 2013) and Dahl salt-sensitive (Azar et al. 1979) rats is associated with an increase renal (Bell et al.

2006) blood flow, glomerular capillary hypertension and ultimately glomerular damage and proteinuria (Scholey and Meyer 1989).

Conversely, increases in both the magnitude and pressure sensitivity of the myogenic response have been suggested to contribute to elevated vascular resistance in animal models of hypertension although a causal role has not been demonstrated. The myogenic response of skeletal muscle, cerebral, renal and mesenteric resistance vessels from hypertensive rats was enhanced ( Dunn et al. 1998; Falcone and Meininger 1999; Harder, Smeda, and Lombard 1985; Hayashi, Epstein, and Loutzenhiser 1992; Huang and Koller 1996, 1997; Huang et al. 1993; Izzard et al. 1996; Osol and Halpern 1985; i.e., greater constriction for a given increase in pressure). The skeletal muscle vessels were also shown to be able to maintain constriction at higher pressures than those from normotensive animals without any apparent wall thickening (Falcone et al. 1991). Although the endothelium is not necessary for the development of myogenic tone (Falcone et al. 1991; Falcone, et al. 1993; Hill et al. 2006), changes in endothelial function (e.g. loss of NO and/or increased production of an endothelium-derived constricting factor (Falcone and Meininger 1999; Huang and Koller 1996, 1997; Huang, Sun, and Koller 1993) have been reported to contribute to enhanced myogenic reactivity in rat skeletal muscle resistance arteries during chronic hypertension. Physiologically, enhancement of myogenic reactivity in resistance arteries may well provide a protective mechanism to maintain constant pressure and/or flow in downstream capillaries in hypertensive states. However, it could ultimately contribute to increases in peripheral vascular resistance causing a further rise in arterial blood pressure and so exacerbate the hypertension.

## 1.4 Modulation of resistance artery diameter by perivascular sympathetic nerves

The sympathetic nervous system regulates total peripheral resistance and is a key regulator of arterial diameter in resistance arteries (Fenger-Gron et al. 1995; Matheson et al. 2000). Sensory,

sympathetic and nitrergic (release NO) perivascular nerves have also been identified but for the purposes of this thesis I will focus on sympathetic innervation as sympathetic nerves account for the largest proportion of nerves in resistance arteries (reviewed by Westcott and Segal 2013).

Perivascular sympathetic nerves arise from postganglionic efferent axons, their cell bodies being located in the paravertebral ganglia (McLachlan 2003). In contrast to classical, structurally well-defined neuromuscular junctions in skeletal muscle, sympathetic nerve fibres (Hirst and Edwards 1989) do not penetrate into the smooth muscle layers of the artery wall and neurotransmitter is released from varicosities in the axons as they run across the adventitia (**Figure 1.14**). Stimulation of sympathetic nerves releases noradrenaline, co-transmitter ATP and neuromodulator neuropeptide Y (NPY; Geoffrey Burnstock 2007). Noradrenaline acts on post-synaptic $\alpha_1$-adrenoceptors to cause vasoconstriction (Furness and Marshall 1974) via a number of mechanisms including $InsP_3$-mediated release of $Ca^{2+}$ from SR stores, membrane depolarization to increase $Ca^{2+}$ influx through VOCCs and $Ca^{2+}$-sensitization (Mauban et al. 2001; Villalba et al. 2007). Neurotransmitter release is modulated by noradrenaline acting at pre-junctional $\alpha_2$-adrenoceptors which stimulate reuptake as well as reducing its own release (Vanhoutte et al. 1981).

ATP activates post-synaptic $P_{2X}$ receptors to cause an influx of $Na^+$ and $Ca^{2+}$ ions that excites the smooth muscle and creates an excitatory junction potential (Evans et al. 1996). Activation of post-synaptic $P_{2Y}$ receptors coupled to Gq-protein also causes smooth muscle excitation and narrowing of arterial diameter by uridine nucleotides (Panhwar et al. 2015). However, ATP released from perivascular sympathetic nerves acts predominantly on $P_{2X}$ receptors on smooth muscle cells to induce vasoconstriction (reviewed by Burnstock and Ralevic 2013). The relative contribution of ATP to sympathetic vasoconstriction varies between arteries and species. For example in rabbit jejunal artery, ATP and noradrenaline each account around 50% of the nerve-

evoked constriction (Evans and Cunnane 1992) whereas in the rat and porcine mesenteric bed, a purinergic component of sympathetic neurotransmission was only observed when perfusion pressure was elevated with ET-1 or thromboxane $A_2$ ($TXA_2$; Pakdeechote et al. 2007; Shatarat, Dunn, and Ralevic 2014).

NPY binds to post-synaptic Y1 (Redrobe et al. 1999) or Y2 receptors (Chu et al. 2003), GPCRs linked to $InsP_3$-mediated release of $Ca^{2+}$ from the SR (Brain and Cox 2009) but rather than actually eliciting direct smooth muscle contraction, the role of NPY appears to be to potentiate the noradrenaline-evoked vasoconstriction (Edvinsson et al. 1984). NPY can also act at pre-junctional Y2 receptors to attenuate noradrenaline release and so inhibit sympathetic vasoconstriction (Yang and Chiba 2002). The contribution of NPY to nerve-mediated responses shows significant variation between vascular beds and species so its role in control of resistance artery remains unclear.

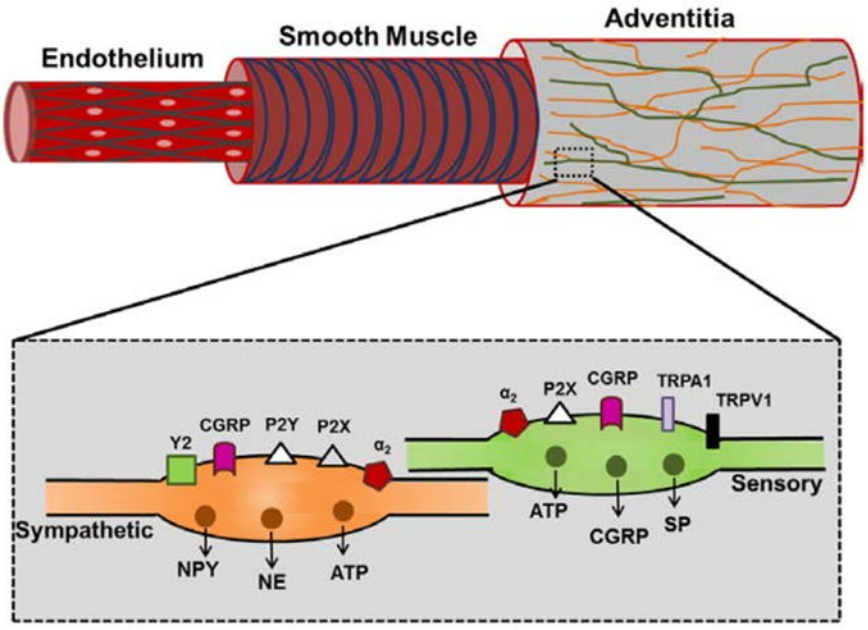



**Figure 1.14: Schematic representation of perivascular nerves.** Varicosities along sympathetic and sensory axons release multiple neurotransmitters that can act on both pre- and post-synaptic receptors to elicit vasoconstriction or dilation, or modulate neurotransmitter release (Westcott and Segal 2013).

*1.4.1. Adrenoceptors in resistance arteries:* Although $\alpha_1$-adrenoceptors mediate sympathetic vasoconstriction in all arteries, the expression and relative contribution of post-junctional $\alpha_1$ versus $\alpha_2$-adrenoceptors to sympathetic vasoconstriction varies between vascular beds, arterial branches within vascular beds, and species (reviewed by Piascik et al. 1996). For example, in rat (Ohyanagi et al. 1991) and mouse (Moore, Jackson, and Segal 2010) cremaster muscle bed, $\alpha_1$-adrenoceptors alone mediate sympathetic vasoconstriction of first-order vessels, while $\alpha_2$-adrenoceptors contribute more to constriction of second- and third-order vessels. Further heterogeneity in sympathetic signaling is provided by different $\alpha_1$-adrenoceptor sub-types; $\alpha_{1a}$-adrenoceptors appear to be the dominant isoform in rat (Zacharia et al. 2004) and human (Jarajapu et al. 2001) skeletal muscle arteries but $\alpha_{1D}$-adrenoceptors predominate in rat mesenteric arteries (Hussain and Marshall 1997) and both sub-types contribute to sympathetic constriction in rat retinal arterioles (Mori et al. 2011).

In contrast to α-adrenoceptors, β-adrenoceptors mediate vasodilation of resistance arteries. β-adrenoceptors are GPCRs expressed on vascular smooth muscle cells (Briones et al. 2005) and mediate relaxation via activation of adenylyl cyclase, and possibly $K_{ATP}$ channels, to reduce $[Ca^{2+}]_i$ (Garland et al. 2011; Kornfeld et al. 2000). $\beta_2$-adrenoceptors have been identified on endothelial cells and activation of these receptors appears to evoke NO-dependent smooth muscle relaxation (Brawley et al. 2000; Gray and Marshall 1992; Kamata et al. 1989). However, the majority of studies were conducted in large blood vessels and so the physiological role of β-adrenoceptors in modulating resistance artery diameter is not clear as stimulation of sympathetic nerves evokes frequency-dependent vasoconstriction in resistance arteries which is not affected by block of β-adrenoceptors (Moore, Jackson, and Segal 2010).

Sympathetic nerve activity does not act in isolation and the resulting effect on resistance

artery tone is due to integration of the actions of the neurotransmitters with endothelium-derived signals against a background of myogenic reactivity. The interaction between myogenic tone and sympathetic nerve activity have received little attention. Enhanced resistance artery sensitivity to noradrenaline in isolated rabbit and rat mesenteric arteries with myogenic tone, as compared with arteries mounted under isometric condition, has been reported (Dunn et al. 1994; Falloon et al. 1995). Also, the contribution of ATP to neurovascular transmission was shown to increase when intravascular pressure was raised from 30 to 90 mmHg but these experiments were conducted in second order rat mesenteric arteries and the increase in pressure did not elicit myogenic reactivity (Rummery et al. 2007). However, the impact of myogenic reactivity on responses of resistance arteries to sympathetic nerve stimulation has not been assessed. In contrast, interactions between the endothelium and sympathetic nerve activity has been investigated as described below.

## 1.5 Endothelium-dependent modulation of resistance artery diameter

The endothelium was long thought of as just a barrier between the arterial muscle wall and vessel lumen. However, the discovery that endothelial cells can release diffusible factors NO and cyclooxygenase (COX)-derived prostaglandins such as prostacyclin ($PGI_2$) which can modulate smooth muscle contractility and hence arterial diameter, opened up a wide field of research. Today, the vascular endothelium is considered as a complex endocrine organ which not only regulates arterial diameter and thus, blood pressure and flow, but also plays a vital role in hemostasis, inflammation and vascular growth and remodeling in the cardiovascular system (reviewed by Förstermann and Sessa 2012 and Salvemini et al 2013). However, for the purposes of this thesis I will focus on the role of the endothelium in regulating the contractile state of vascular smooth muscle cells (**Figure 1.15**).

In addition to release of diffusible factors, stimulation of the endothelium by activation of GPCRs or, most importantly in the physiological setting, by mechanical stimuli such as increases

in shear stress (Sessa 2005; Mayer and Hemmens 1997) results in activation of endothelial $Ca^{2+}$-activated $K^+$ ($K_{Ca}$) channels (Brakemeier et al. 2003; Jalil 2008; Plane et al. 2013; Sun et al. 2001). Opening of these channels causes hyperpolarization of the endothelial cell membrane potential which spreads to the underlying smooth muscle cells via myoendothelial gap junctions (MEGJs; Figueroa and Duling 2009; Garland and Dora 2016; de Wit, Boettcher, and Schmidt 2008) to reduce opening of VOCCs, decrease $Ca^{2+}$ influx and so cause relaxation. This pathway is termed endothelium-dependent hyperpolarization (EDH). The NO, COX and EDH pathways are all dependent on an increase in endothelial $[Ca^{2+}]_i$. Thus, I will briefly discuss endothelial $Ca^{2+}$ signaling and how each of these pathways can regulate smooth muscle contractility to control resistance artery diameter.

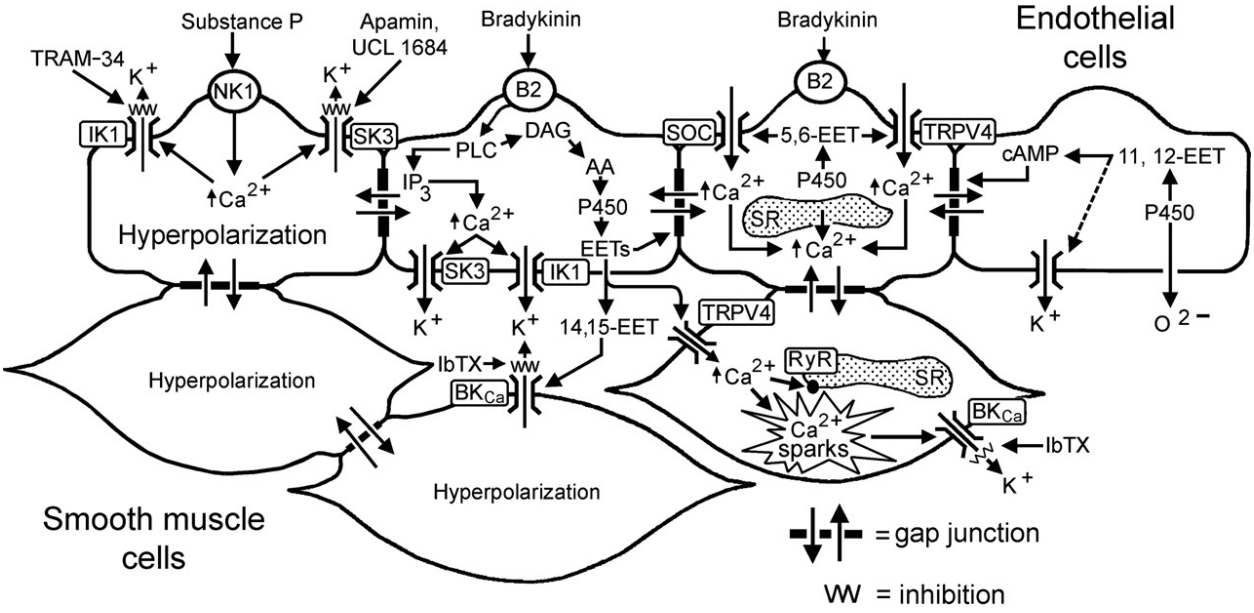



**Figure 1.15: Overview of endothelium-dependent control of vascular smooth muscle contractility** (Félétou and Vanhoutte 2006).

*1.5.1 $Ca^{2+}$signaling in vascular endothelial cells:* Each of the endothelium-dependent pathways for regulation of smooth muscle contractility described above share a common feature in that they are dependent on a rise in $[Ca^{2+}]_i$ in endothelial cells. Stimulated increases in $[Ca^{2+}]_i$ are elicited through release from endoplasmic reticulum (ER) stores or via $Ca^{2+}$ influx through TRP channels. It is noteworthy that there are no VOCCs in fresh isolated endothelial cells (Muraki et al. 2000; Uchida et al. 1999). I will briefly discuss the mechanisms underlying increases in endothelial $[Ca^{2+}]_i$ elicited by receptor agonists and increases in shear stress. I will also discuss the role of TRP channels in mediating $Ca^{2+}$ entry in endothelial cells.

*Receptor agonists:* GPCR agonists such as ACh which acts on $M_3$ muscarinic receptors in most blood vessels (Gericke et al. 2011), stimulate $InsP_3$-mediated release of $Ca^{2+}$ from ER stores (Himmel et al. 1993) which, as described above for smooth muscle cells, leads to SOCE mediated by both Orai1 (Abdullaev et al. 2008; Hirano et al. 2009) and TRP channels (Ruhle and Trebak 2013; Yue et al. 2015). The rise in $[Ca^{2+}]_i$ activates $Ca^{2+}$-dependent enzymes such as NOS, as well as small ($SK_{Ca}$) and intermediate ($IK_{Ca}$) conductance $K_{Ca}$ channels to elicit endothelial hyperpolarization (Chen et al. 1988). The contribution of these mechanisms to endothelium-dependent dilation shows significant variation between arteries but in general, EDH plays a larger role in resistance arteries. The identity of the channel mediating receptor-linked SOCE has remained unclear and may vary between stimuli, arteries and species, but recent evidence suggest a role for TRPV4 channels in ACh-evoked $Ca^{2+}$ entry in mouse mesenteric (Zhang et al. 2009) and carotid arteries ( Earley et al. 2009), and TRPC4 in the same responses in aortae from a knockout mouse model (Freichel et al. 2001). A receptor-operated $Ca^{2+}$ entry mechanism may also be involved mediated with TRPC and TRPV subtypes activated by DAG.

*Shear stress:* Increases in blood flow in arteries result in increases in the shear stress across the endothelial cell surface which activate vasodilator pathways (e.g. NOS) by increasing endothelial $[Ca^{2+}]_i$ (Ando et al. 1988). Compared to agonist evoked responses, less is known about how this occurs but the increase is dependent upon $Ca^{2+}$ entry and possibly SOCE (Liu et al. 2006). Mechano-sensitive TRP channels may be responsible for the $Ca^{2+}$ entry, and recent studies indicate a role for TRPV4 in mouse arteries (Mendoza et al. 2010). Limited evidence has also been provided that flow-induced increases in endothelial $[Ca^{2+}]_i$ are mediated by the release of ACh or ATP which then act back on their receptors (Parnavelas et al. 1985; Yamamoto et al. 2006). Also, over time, shear stress can stimulate NOS through the PI3 kinase/Akt pathway in the absence of any change in $[Ca^{2+}]_i$ (Fisslthaler et al. 2000).

*1.5.2 Endothelium-derived NO:* NO plays a critical regulatory and protective role in the cardiovascular system. Endothelium-derived NO elicits relaxation of surrounding smooth muscle cells to cause vasodilation, regulates local cell growth and protects blood vessels from the damaging consequences of platelet aggregation and activation of inflammatory responses. In pathological conditions such as diabetes and hypertension, decreased NO synthesis and increased scavenging of NO by superoxide anions ($O_2^-$) disrupts these processes leading to enhanced vasoconstriction, clot formation and inflammation within the vascular system (Bryan et al. 2005; Sessa 2005; Vanhoutte 2003).

NO is synthesized from L-arginine by endothelial NOS, a $Ca^{2+}$-CaM-dependent enzyme activated by dissociation from caveolin-1 (cav-1; Garcia et al. 1997; Jennings 2009; Lincoln et al. 2001; Sessa 2005). L-arginine enters endothelial cells via a cationic amino acid transporters (Zani and Bohlen 2005), the activity of which can be regulated by multiple factors such as endothelial membrane potential (Bogle et al. 1996; Guidotti and Gazzola 1987; Zharikov et al. 1997), agonists

(Bogle et al. 1996), and even NO itself through S-nitrosylation (Zhou et al. 2010). NOS is activated by $Ca^{2+}$-CaM when the endothelial $[Ca^{2+}]_i$ is increased (half-maximal activity between 200 and 400 nM), either through $Ca^{2+}$ influx through non-selective cation (TRP) channels, or through the release of $Ca^{2+}$ from intracellular stores (Kochukov et al. 2014; Lin et al. 2000).

The NOS enzyme exists in three isoforms: neuronal nNOS (NOS I), inducible iNOS (NOS II), and endothelial eNOS (NOS III; Förstermann and Sessa 2012). eNOS and nNOS are constitutively expressed and highly regulated by transcriptional, post-transcriptional (including microRNA), and post-translational mechanisms, whereas iNOS, is mainly regulated through gene transcription in inflammatory settings. All isoforms of NOS utilize L-arginine as a substrate, and $O_2$ and reduced nicotinamide-adenine-dinucleotide phosphate (NADPH) as co-substrates. Flavin adenine dinucleotide (FAD), flavin mononucleotide (FMN), and 5,6,7,8-tetrahydro-l-biopterin ($BH_4$) are cofactors of all isozymes. Functional eNOS is a dimer that consists of a C-reductase domain (binds NADPH, FAD and FMN), which is linked to the N-terminal oxygenase domain (binds $BH_4$, $O_2$, L-arginine and heme). A CaM-recognition site is essential for the linkage between the reductase and oxygenase domains and allows dimerization. Catalysis occurs when electrons are transferred from NADPH, via the flavins FAD and FMN in the carboxy-terminal reductase domain, to the heme in the amino-terminal oxygenase domain. At the heme site, the electrons are used to reduce and activate $O_2$ and to oxidize L-arginine to L-citrulline and NO (Förstermann and Sessa 2012; reviewed by Roszer 2012; Vanhoutte et al. 2017; Vanhoutte et al. 2016). Binding of $BH_4$ in the dimer interface is required for the stabilization of the eNOS dimer and 'coupled' NOS activity. Without $BH_4$, NOS is uncoupled, electrons are transferred directly to $O_2$, and superoxide anions $O_2^-$ are generated (Xia et al. 1998). $Ca^{2+}$-CaM is essential for eNOS activity (reviewed by Hemmens and Mayer 1998) but several other proteins also interact with eNOS to regulate its

activity. For example, heat shock protein 90 (hsp90; García-Cardeña et al. 1998; Pritchard et al. 2001) serves as a positive allosteric modulator and the caveolae coat protein, cav-1 is a tonic inhibitor (Drab et al. 2001) of eNOS activity (Drab et al. 2001). Recruitment of $Ca^{2+}$-CaM and hsp90 to eNOS can displace cav-1 from the enzyme thereby activating it (Gratton et al. 2000).

Once released from endothelial cells, NO causes relaxation of smooth muscle cells via activation of soluble guanylyl cyclase to increases production of cyclic guanosine monophosphate (cGMP), which subsequently activates PKG (Triggle et al. 2012; reviewed by Denninger and Marletta 1999; Tsai and Kass 2009). PKG phosphorylates a number of downstream target proteins including MLCP, phospholamban and $BK_{Ca}$ channels (Lincoln et al. 2001; Rees et al. 1990; Sawada et al. 2001; Schlossmann et al. 2003), to reduce $[Ca^{2+}]_i$ and thus evoke vasodilation (Lincoln et al. 2001; **Figure 1.16).** The half-life of NO and therefore its biological activity is determined by its interaction with $O_2^-$. This free radical rapidly reacts with NO to form the highly reactive intermediate peroxynitrite ($ONOO^-$) in a reaction approximately 10 times faster than the dismutation of $O_2^-$ by the superoxide dismutase: $\mathbf{O_2^- + NO \rightarrow ONOO^-}$**.** In disease states, increases in oxidative stress lead to increased inactivation of NO by $O_2^-$ and to eNOS uncoupling as described above. Potential mechanisms underlying this change include oxidation of the cofactor $BH_4$ (Landmesser et al. 2003; Vásquez-Vivar et al. 1998; Werner-Felmayer et al. 1993) and depletion of L-arginine (Rossitch et al. 1991; Vanhoutte et al. 2016). The importance of NO in regulating arterial diameter, blood flow and pressure was facilitated by the discovery that structural analogues of L-arginine such as L-$N^G$-nitro arginine (L-NOARG) and L-$N^G$-nitroarginine methylester (L-NAME) act as selective, competitive inhibitors of NOS ( Moore et al. 1990; Pfeiffer et al. 1996). For example, infusion or oral administration of L-NAME causes hypertension in rats (Gardiner et al. 1990, 1992) and L-NOARG inhibits endothelium-dependent relaxation in

isolated rat aorta (Moore et al. 1990). The demonstration that deletion of NOS leads to hypertension in mice was also an advance in demonstrating the physiological importance of this molecule (Huang et al. 1995).

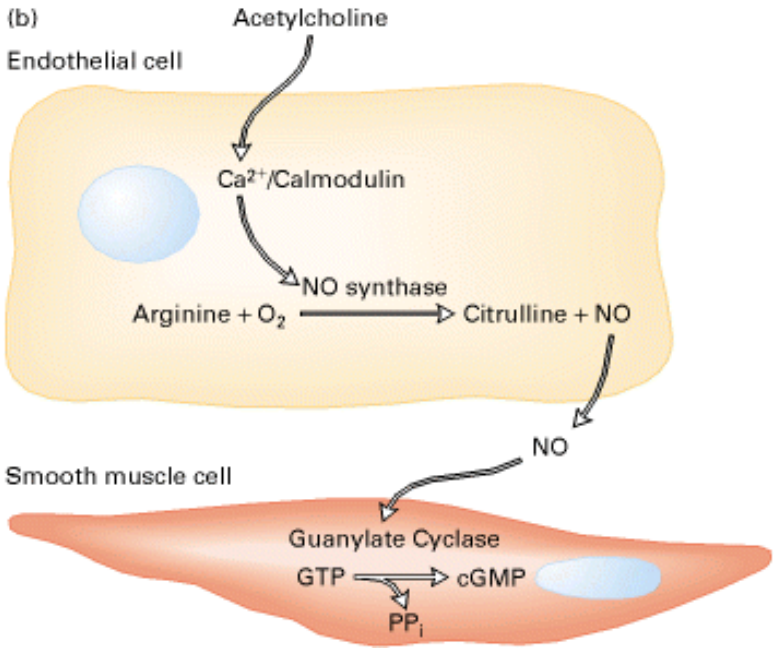



**Figure 1.16: Schematic diagram illustrating the pathway for NO-mediated relaxation of smooth muscle** (Harvey et al. 2000).

*1.5.3 Endothelium-derived COX products:* COX, also known as prostaglandin-endoperoxide synthase exists in two isoforms, constitutively expressed COX-1 and inducible COX-2, thought to be involved in inflammation. In most blood vessels, only COX-1 is expressed in the endothelium but recently COX-2 has also been found to be present in some arteries and activated by mechanical stimuli such as shear stress (Funk and FitzGerald 2007). Arachidonic acid, the substrate for COX enzyme, is released from cell membrane phospholipids by the action of phospholipase $A_2$ ($PLA_2$) which is expressed constitutively and activated by sub-micromolar $[Ca^{2+}]_i$ (Kudo and Murakami 2002; Smith and Marnett 1991). COX generates $PGH_2$, the substrate for a number of enzymes such as thromboxane synthase, which generates the vasoconstrictor $TXA_2$, and prostacyclin

synthase which produces the vasodilator $PGI_2$ (Fitzpatrick 2004; Kim 2014; Majed and Khalil 2012; Ricciotti and Fitzgerald 2011; **Figure 1.17**). The fate of $PGH_2$ in terms of whether it is converted

to a vasodilator or vasoconstrictor product is determined by the expression profile of the enzymes which utilizes it and by local stimuli. In most blood vessels under normal conditions, the prominent expression of prostacyclin synthase means that $PGI_2$, which has anti-platelet as well as vasodilator actions and so is regarded as being vasculoprotective, is the major COX product produced by endothelial cells (Mitchell et al. 2008) although *in vivo* data have shown that $PGI_2$ may have little

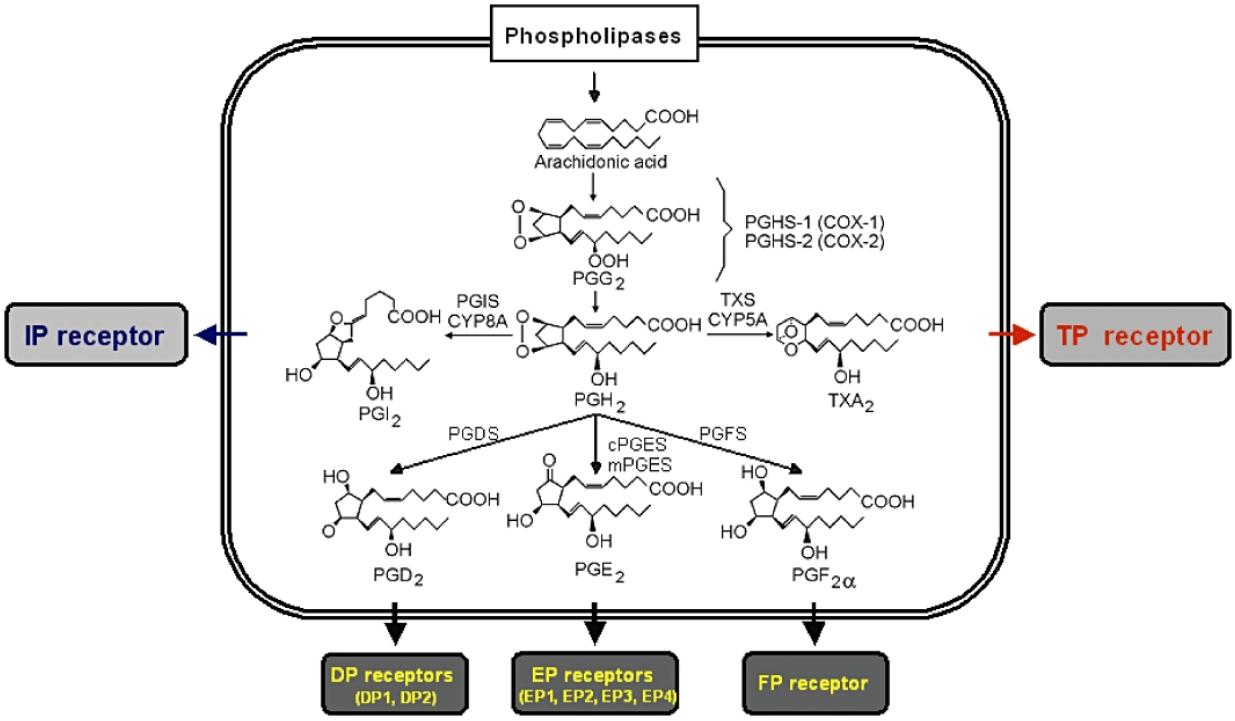



**Figure 1.17. Schematic diagram illustrating arachidonic acid metabolism.** (See text for more details)**.**

role in the regulation of BP compared with NO (Parkington et al. 2004). However, in pathological conditions such as hypertension and atherosclerosis, expression of thromboxane synthase is increased, shifting the balance toward increase in the production of the vasoconstrictor and stimulator of platelet aggregation, $TXA_2$ (Yuhki et al. 2010).

The effects of both $PGI_2$ and $TXA_2$ on arterial diameter are mediated by IP and TP GPCRs on smooth muscle cells. $PGI_2$ acts on IP2 and IP4 receptors which couple to Gs to mediate increases in cAMP, activation of PKA and smooth muscle relaxation (Lim and Dey 2002; Haynes et al. 1992) $TXA_2$ binds to TP receptors (Cyrus et al. 2010), GPCRs linked to $G_q$ or $G_{12/13}$ and vasoconstriction. Activation of the $G_q$ pathway leads to activation of $InsP_3$-mediated release of $Ca^{2+}$ and phosphorylation of MLCK (Hirata et al. 1991; Tosun et al. 1998; Cogolludo et al. 2003), whereas the $G_{12/13}$ pathways causes ROK-mediated inhibition of MLCP and thus $Ca^{2+}$ sensitization (Smyth et al. 2009; Smyth 2010; Worzfeld et al. 2008).

*1.5.4 Endothelium-dependent hyperpolarization (EDH):* EDH plays a more prominent role in endothelium-dependent dilation of resistance arteries than in large vessels and so is an important determinant of local tissue perfusion (Bény 1997; Busse et al. 2002; McGuire et al. 2001). Inhibition of the EDH pathway contributes to cardiovascular complications associated with diabetes and pregnancy (Gokina et al. 2015; Li et al. 2017; Armstrong et al 2013). EDH was initially thought to be mediated by a diffusible factor, EDHF, but current consensus is that direct electrical coupling of endothelial and smooth muscle cells accounts for EDH responses (Bény 1997; Busse et al. 2002; McGuire, Ding, and Triggle 2001; reviewed by Bryan et al. 2005). By reducing influx of $Ca^{2+}$ through VOCCs, EDH of vascular smooth muscle causes vasodilation[47].

The defining characteristic of EDH-mediated vasodilation is that it persists in the presence of inhibitors of eNOS and COX and, under these conditions, is abolished by blockers of endothelial $SK_{Ca}$ and $IK_{Ca}$ channels, apamin and TRAM-34 or charybdotoxin (reviewed by Ledoux et al. 2006). These channels are expressed on all endothelial cells but not normal vascular smooth muscle cells (Burnham et al. 2002; Cipolla et al. 2009), although expression of $IK_{Ca}$ channels has been shown in proliferating vascular smooth muscles (Neylon et al. 1999).

Endothelial $SK_{Ca}$ and $IK_{Ca}$ channels, are encoded by the *KCNN* gene family; the three subtypes of $SK_{Ca}$ channels are SK1, SK2 and SK3 and $IK_{Ca}$ channels are denoted as SK4 with SK3 and SK4 expressed in endothelial cells (see reviews Adelman et al. 2012; Stocker 2004). Both $SK_{Ca}$ and $IK_{Ca}$ channels have 6 transmembrane domains with a pore region between S5 and S6 (Christophersen and Wulff 2015). Unlike $BK_{Ca}$ channels found in vascular smooth muscle cells, they are voltage-independent channels that are sensitive to $[Ca^{2+}]_i$ in the sub-micromolar range; $EC_{50}$ values ranging from 95 to 350 nM (Xia et al. 1998). $Ca^{2+}$ binding to CaM which is constitutively bound to the COOH-terminus of the channels (Xia et al. 1998), results in channel activation/opening **(Figure 1.18**; Schumacher et al. 2001; Schumacher et al 2004; Fanger et al. 1999). Native and expressed $SK_{Ca}$ channels have a conductance of around 10-40 pS (Sakai 1990; Muraki et al. 1997; Groschner et al. 1992) and $IK_{Ca}$ channels have a conductance of around 30-80 pS (Nilius et al. 2001; Sauvé et al. 1990). Although both $SK_{Ca}$ and $IK_{Ca}$ channels are expressed in endothelial cells, they are located in spatially distinct microdomains and participate in different signaling pathways (Kerr et al. 2012; Sandow et al. 2006). Flux of $InsP_3$ from smooth muscle cells to endothelial cell projections stimulated by GPCRs which leads to release of $Ca^{2+}$ from ER or $Ca^{2+}$ entry through TRPC3 channels subsequently activate $IK_{Ca}$ channels in endothelial cells is a process termed myoendothelial feedback (see below; Kerr et al. 2015; Ledoux et al. 2008; Tran et al. 2012). $SK_{Ca}$ are located at inter-endothelial junctions and co-localize in caveolae with TRP channels (Saliez et al. 2008) where they may respond to local $Ca^{2+}$ increases evoked by increases in shear stress-induced activation of TRPV4 channels (Brähler et al. 2009). Although they are part of different signaling pathways, activation of either channel initiates EDH and vasodilation (Dalsgaard et al. 2010; Eichler et al. 2003; Köhler et al. 2010). However, in isolated arteries block of both $SK_{Ca}$ and $IK_{Ca}$ channels is required to inhibit EDH, the reason for this being unclear

(Parkington et al. 1995). The physiological importance of endothelial $K_{Ca}$ channels *in vivo* is highlighted by the increased vascular reactivity and raised arterial blood pressure recorded in mice lacking one or both of the channels (Si 2006; Taylor et al. 2003).

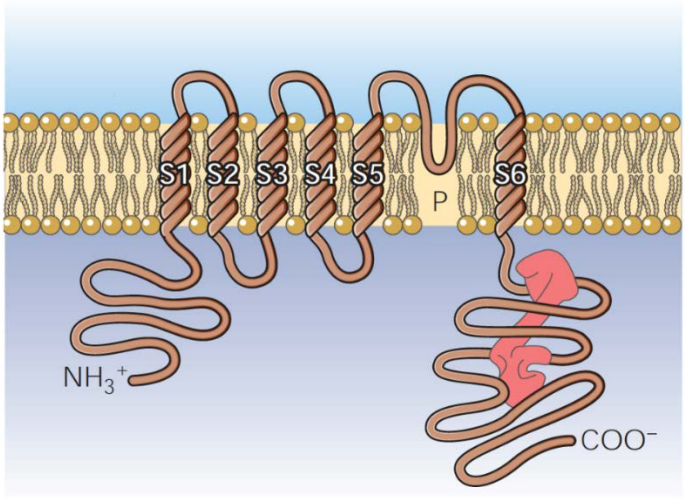



**Figure 1.18**. **Schematic of a $SK_{Ca}$/$IK_{Ca}$ channel subunit**. Sub-unit consists of six transmembrane domains with a pore region (P) between S5 and S6. CaM interacts with the channel's intracellular COOH terminus (Ledoux et al. 2006).

Compared to other ion channels, $SK_{Ca}$ and $IK_{Ca}$ channels have a well-developed pharmacology which has aided investigation of their physiological functions. The "classical" blockers are charybdotoxin for $IK_{Ca}$ channels and the bee venom apamin for $SK_{Ca}$ channels (Wulff et al. 2007). However, charybdotoxin also inhibits $BK_{Ca}$ and Kv1.3 channels and so has been replaced by 1-[(2-chlorophenyl)diphenyl-methyl]1H pyrazole (TRAM-34), which binds to Thr250 and Val275 in the inner pore, and the benzothiazinone NS 6180 which binds to the same place and blocks the channel with an $IC_{50}$ of 11 nM. Up to a concentration of 1 µM NS 6180 and 5 µM TRAM-34 are highly selective $IK_{Ca}$ channel blockers and show no effect on the T-cell $Ca^{2+}$ entry and a range of Kv, sodium and TRP channels. Apamin is a highly selective blocker of $SK_{Ca}$ channels, with an $IC_{50}$ value for SK3 of 1-13 nM. Apamin inhibits the channels by an allosteric

mechanism involving residues in the S3-S4 extracellular loop (Weatherall et al. 2011).

A number of positive and negative modulators are available that apparently make $SK_{Ca}$ and $IK_{Ca}$ channels more or less sensitive to $Ca^{2+}$. Benzimidazolone-type activators like 1-EBIO, do not differentiate between $SK_{Ca}$ and $IK_{Ca}$ channels, most likely as the binding site is on the COOH-terminus (Pedarzani et al. 2001). In contrast, N-Cyclohexyl-N-[2-(3,5-dimethyl-pyrazol-1-yl)-6-methyl-4-pyrimidinamine (CyPPA) activates SK3 channels with an $EC_{50}$ value 140 nM, but has no effect on $IK_{Ca}$ channels (Kasumu et al. 2012). $IK_{Ca}$ naphtha[1,2-d]thiazol-2-ylamine (SKA-31; Sankaranarayanan et al. 2009) is a mixed $SK_{Ca}$ and $IK_{Ca}$ channel activator, with ~10-fold selectivity for $IK_{Ca}$ channels (Sankaranarayanan et al. 2009). Both of these drugs act by increasing the sensitivity of the channels to $Ca^{2+}$ (Sankaranarayanan et al. 2009; Hougaard et al. 2007).

*1.5.5 NO and EDH – two aspects of one pathway?* To date, the majority of research has focused on activation of these endothelial mechanisms by stimuli acting directly on endothelial cells leading to the idea that $K_{Ca}$ channels and NO mediate distinct pathways for vasodilation. Our lab has shown that in resistance arteries, stimulation of smooth muscle cells by $\alpha_1$-adrenoceptor agonists activates the converse signaling pathway, termed myoendothelial feedback (**Figure 1.19)**. Briefly, flux of $InsP_3$ from smooth muscle to endothelial cells elicits localized increases in $Ca^{2+}$, activation of $IK_{Ca}$ channels located at MEGJs and production of NO (Kerr et al. 2012, 2015; Tran et al. 2012). In addition to demonstrating that endothelial-smooth muscle communication can be bi-directional, this work supports the idea that rather than being independent, the NO and EDH pathways may act in an integrated manner to regulate arterial diameter. However, activation of this pathway has only been demonstrated in response to bath application of $\alpha_1$-adrenoceptor agonists. Thus, in **Chapter 3** I have investigated whether the $InsP_3/IK_{Ca}$ channel-mediated

myoendothelial feedback can also modulate vascular smooth muscle contraction elicited by physiological stimuli, sympathetic nerve activity and increases in intravascular pressure.

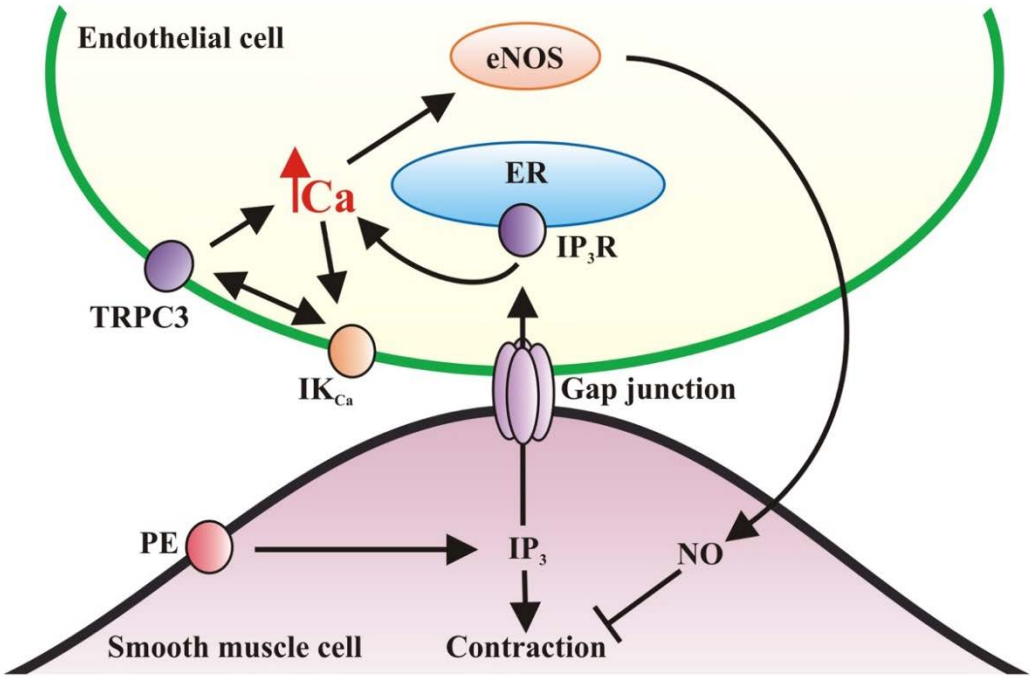



**Figure 1.19: Myoendothelial feedback**. Contractile agonists increase $InsP_3$ levels within smooth muscle cells. Flux of $InsP_3$ through myoendothelial gap junctions gives rise to localized increases in $Ca^{2+}$ and subsequent activation of a discrete pool of $IK_{Ca}$ channels and eNOS localized within spatially restricted myoendothelial projections. The resulting hyperpolarization and/or NO feeds back to the surrounding smooth muscle cells to attenuate agonist-induced depolarization and contraction.

Further support for the idea of a link between NO and EDH-mediated modulation of arterial diameter comes from a number of studies showing that activation of $K_{Ca}$ channels can modulate the release of NO (Sheng and Braun 2007) and that $K_{Ca}$ channel blockers are able to attenuate relaxation mediated by endothelium-derived NO (Stankevicus et al. 2011; Kerr et al. 2012). Pharmacological activation of $K_{Ca}$ channels modulates the release of NO from cultured endothelial cells and in rat cremaster arterioles have shown that 6,7-dichloro-1H-indole-2,3-dione 3-oxime (NS309) and 5,6-dichloro- 1-ethyl-1,3-dihydro-2H-benzimidazol-2-one (DCEBIO), activators of

$SK_{Ca}/IK_{Ca}$ channels, enhanced ATP-induced hyperpolarization, increase in cytosolic $Ca^{2+}$ concentration and NO synthesis (Sheng and Braun 2007; Sheng et al. 2009). In large conduit arteries, such as basilar and superior mesenteric artery, agonist-induced endothelium-dependent vasorelaxation is primarily mediated through the release of NO (Vanhoutte et al. 2017; Shimokawa 2014; Takaki et al. 2008). Blocking $K_{Ca}$ channels in these arteries resulted in the reduction of both relaxation and smooth muscle hyperpolarization (Plane and Cole 2006; Chen and Cheung 1997; Stankevicius et al. 2006), relaxation (Plane and Cole 2006; Chen and Cheung 1997; Stankevicius et al. 2006) and NO release (Stankevicius et al. 2006). NS309 also induced NO- dependent dilation and NO production was blocked by apamin and TRAM-34 in rat superior mesenteric artery. Together, these data indicate that there may be a facilitatory relationship between endothelial $SK_{Ca}$ and $IK_{Ca}$ channel activity and NO, a relationship I have further explored in **Chapters 4** and **5**.

The basis for the link between $K_{Ca}$ channel-mediated hyperpolarization and NO release has not been established. It is known that activation of $K_{Ca}$ channels and release of NO both rely on the increase in endothelial $[Ca^{2+}]_i$; the $[Ca^{2+}]_i$ threshold for NO-dependent dilation is 220 nM, whereas for EDH-mediated dilation it is 340 nM in rat middle cerebral arteries (Marrelli 2001). Early studies showed that membrane depolarization inhibits both agonist-induced increases in $[Ca^{2+}]_i$ and NO release from cultured endothelial cells (Adams et al. 1989; A. Lückhoff and Busse 1990; Andreas Lückhoff and Busse 1990), and in rat isolated basilar arteries, endothelial depolarization was associated with a reduction in NO-mediated relaxation to ACh (Allen et al. 2002). These findings supported the idea that as endothelial cells lack VOCCs and $Ca^{2+}$ influx is mediated by TRP channels, membrane hyperpolarization is required to maintain an appropriate electrochemical driving force for agonist-induced $Ca^{2+}$ influx. It may also prevent $Ca^{2+}$ entry by channel inactivation and/or reduction in unitary conductance, as suggested by the fact that the

decline in $Ca^{2+}$ influx with depolarization positive to ~-5 mV exceeds that which can be explained by a reduction in driving force (Oike et al. 1994; Wang and Breemen 1999). However, the ability of hyperpolarization to regulate $Ca^{2+}$ entry by increasing the electrical driving force has been controversial (Dora and Garland 2013), particularly as there is very big concentration gradient of ~20,000-fold from the extracellular space to the inside of the cell (Clapham 2007). Numerous studies using cultured cells and intact vessels have subsequently concluded that changes in endothelial $[Ca^{2+}]_i$ are independent of changes membrane potential (Marrelli et al. 2003; McSherry et al. 2005; Takano et al. 2004). But recent work with endothelial cells tubes isolated from resistance arteries support hyperpolarization-induced $Ca^{2+}$ entry contributing to agonist-evoked increases in endothelial $[Ca^{2+}]_i$ (Behringer and Segal 2015) and indicate that pharmacological activation of $SK_{Ca}$ and $IK_{Ca}$ channel would not only enhance $Ca^{2+}$ entry to further amplify $K_{Ca}$ channel activity, but also boost NO production (Behringer 2017).

## 1.6 Hypothesis and Aims

The vascular endothelium plays a crucial role in regulating resistance artery diameter and thus blood flow and pressure. This is accomplished through release of NO and $K_{Ca}$ channel-mediated hyperpolarization which spreads to smooth muscle cells via MEGJs. Although long thought of as distinct mechanisms for vasodilation, recent evidence suggests that there may be a link between these two pathways; for example, $IK_{Ca}$ channel-mediated myoendothelial feedback leads to release of NO and block of $K_{Ca}$ channels can inhibit NO-mediated vasorelaxation. My over-arching goal has been to further explore the functional role of $SK_{Ca}$ and $IK_{Ca}$ channels in regulating resistance artery diameter, and investigate potential interactions between $K_{Ca}$ channels and NO in mediating vasodilation. To this end, in this thesis I have addressed three hypotheses:

*1. The functional contribution of $InsP_3/IK_{Ca}$ channel-mediated myoendothelial feedback to limiting arterial diameter is determined by the ability of the vasoconstrictor stimulus to engage the endothelium.*

*2. Small molecule activators of endothelial $K_{Ca}$ channels modulate myogenic reactivity at least in part through endothelium-derived NO.*

*3. NO facilitates $K_{Ca}$ channel mediated, endothelium-dependent smooth muscle hyperpolarization.*

# Chapter 2: Materials and Methods

## 2.1 Ethical approval

All animal care and experimental procedures were approved by the Animal Care and Use Committee (ACUC HS1; AUP 312) of the Faculty of Medicine and Dentistry at the University of Alberta, and performed in accordance with Canadian Council on Animal Care guidelines, and the principles and regulations as described by Grundy (Grundy 2015).

## 2.2 Animal care and use

Male Sprague-Dawley rats (250-300g; from Science Animal Support Services, University of Alberta) were housed in an enriched environment maintained on a 12:12 h light–dark cycle at ~23°C with fresh tap water and standard chow available ad libitum. Rats were euthanized by inhalation of isoflurane followed by decapitation. The mesenteric bed and brain were removed and placed in cold Kreb's buffer containing (mM): NaCl 119.0, $NaHCO_3$ 25.0, KCl 4.7, $MgSO_4$ 1.2, $KH_2PO_4$ 1.18, glucose 11, and $CaCl_2$ 2.5. For experiments requiring a nominally $Ca^{2+}$-free solution $CaCl_2$ was omitted from the above composition.

## 2.3 Wire myography

Basilar and third order mesenteric arteries were cleaned of adhering tissue and cut into segments (~2 mm in length). Arterial segments were mounted between two gold-plated tungsten wires (20 μm diameter) in a Mulvany-Halpern myograph (model 400A, J.P. Trading, Denmark). Changes in isometric tension were recorded via a PowerLab using Chart 5.0 or 8.0 software (AD Instruments, Colorado, USA). Tissues were maintained in Krebs' buffer gassed with 95% $O_2$/5% $CO_2$ at 37°C (pH 7.4) and set to a pre-determined optimal resting tension of 5 mN for mesenteric arteries and 2 mN for basilar arteries (these values were determined from active length-tension curves). In some experiments, the endothelium was removed. For basilar arteries, this was accomplished by gently rubbing the lumen with a hair, and for mesenteric arteries the endothelium was removed by flushing of the intact mesenteric bed with 0.5% Triton X-100 in water for 30 secs

followed by rapid washout with Krebs buffer. After an equilibration period of 30 mins, endothelial function was assessed as % relaxation to ACh (10 μM) following pre-stimulation with phenylephrine (PE; 3 μM; 75% of maximal tone; 13.89 ± 0.69 mN, n=22) for mesenteric arteries or 5-hydroxytrypatmine (5-HT; 3 μM; 75% of maximal tone; 8.9 ± 1.7 mN, n=24) for basilar arteries. Arteries in which ACh induced >90% reversal of agonist-induced tone were designated as endothelium-intact, and tissues in which the response to ACh was <10% were deemed to be endothelium-denuded. Arteries in which the % reversal of PE- or 5-HT-induced tone elicited by ACh fell between these values were discarded.

*2.3.1 Cumulative concentration-response curves to agonists.* Cumulative concentration-response curves to vasorelaxant stimuli (e.g. ACh, KCl, diethylamine NONOate (DEA NONOate)) were constructed in arterial segments pre-stimulated with 5-HT (3 μM; basilar arteries) or PE (3 μM; mesenteric arteries) and results expressed as % reversal of agonist-induced tone. In mesenteric arteries, cumulative concentration-response curves to PE and the $TXA_2$ mimetic U46619 were constructed and results expressed as % maximal response.

*2.3.2 Stimulation of perivascular nerves in isolated arteries.* Electrical field stimulation of perivascular nerves was applied via two platinum electrodes (AD Instruments, Colorado) placed in parallel on either side of the arterial segments and connected to a Digitimer D330 stimulator (Digitimer, Welwyn Garden City, UK). Following an equilibration period of 30 mins, frequency-response curves were constructed by stimulating the preparation at 0.5-20 Hz (90 V, pulse width 2 msecs, 20 secs) at 5 min intervals. Three repeated frequency-response curves could be constructed with 30 mins between them without a significant change in the peak size and sensitivity of the evoked increases in tone (**Figure 3.5b**) although the duration of responses often appeared to decrease (**Figure 3.5a**). Nerve-evoked responses recorded in isolated arteries mounted

in the wire myograph are expressed as absolute values for changes in isometric tension. In all wire myograph experiments in which inhibitors were used, the inhibitors were applied for 15 mins prior to the beginning of the second frequency-response curve, with the exception of L-NAME which was incubated with the tissues for 30 mins.

**2.4 Pressure myography**

Leak-free segments of 3$^{rd}$ to 4$^{th}$ order mesenteric artery (2-3 mm in length) were cleaned of adhering connective tissue and mounted between two glass cannulae in an arteriograph chamber (Living Systems Instrumentation, Burlington, VT) under conditions of no luminal flow. In some experiments, the endothelium was removed by flushing the intact mesenteric bed with 0.5% Triton X-100 in water for 30 secs followed by rapid washout with Krebs before isolation of individual arteries.

Vessels were bathed in Kreb's buffer at 37$^{o}$C (pH 7.4) constantly gassed with compressed air (4.94% $CO_2$, 20.96% 92% $O_2$, balance $N_2$) and intravascular pressure was maintained via a pressure servo-control system (PS200, Living Systems Instrumentation, Burlington, VT). Arteries were viewed through a Nikon TMS inverted microscope, and measurements of the internal diameter were made via an automated video dimension analyzer (VDA10, Living Systems Instrumentation, Burlington, VT). The glass cannulae (borosilicate glass with OD of 1.2 mm and ID of 0.69 mm) were pulled using a Model P87 Flaming/Brown micropipette puller (Sutter Instruments, Novato, USA). Pressure and diameter measurements were recorded via a PowerLab (AD Instruments, Colorado, USA) using Chart 5.0 software (AD Instruments, Colorado). In some experiments, the endothelium was removed by gently rubbing the lumen with a hair.

*2.4.1 Responses to increases in intravascular pressure.* Arteries which did not develop myogenic reactivity or a displayed a reduction in diameter of less than 50 μm during the initial equilibration period of 30-40 mins at 80 mm Hg, were discarded. This pressure was used as it approximates to

the mean arterial blood pressure of a rat *in vivo*. Following the equilibration period, intravascular pressure was reduced to 20 mmHg and a pressure ramp was then applied by increase the pressure from 20 mmHg to 120 mmHg in increments of 20 mmHg. Each pressure step was held for 2-3 mins or until the vessel diameter stabilized. All drugs were applied to the bath while the vessels held at an intravascular pressure of 20 mmHg for 15 mins prior to applying the same pressure ramp. The one exception was L-NAME which was incubated for 20 mins. At the end of each experiment, arteries were bathed in $Ca^{2+}$-free Krebs solution to reveal the maximum passive diameter at each pressure step. Myogenic reactivity is expressed as the percentage difference in active diameter versus passive diameter (% myogenic tone = ((passive diameter – activate diameter)/(passive diameter))*100%).

*2.4.2 Cumulative concentration-response curves to vasorelaxants.* After an equilibration period of 30 mins, endothelial function was assessed as % relaxation to acetylcholine (ACh; 3 μM) following pre-stimulation with PE (3 μM) while the intravascular pressure is held constantly at 60 mmHg. Arteries in which ACh induced >90% reversal of agonist-induced tone were designated as endothelium-intact, and tissues in which the response to ACh was <10% were deemed to be endothelium-denuded. Arteries in which the % reversal of PE-induced tone elicited by ACh fell between these values were discarded. Cumulative concentration-response curves to vasorelaxant stimuli (e.g. CyPPA and SKA-31) were constructed in arterial segments pre-stimulated PE and results expressed as % reversal of agonist-induced tone. Cumulative concentration-response curves CyPPA and SKA-31 were constructed and results expressed as % maximal response.

**2.5 Perfused mesenteric vascular bed**

The mesenteric bed was perfused via the superior mesenteric artery as previously described(Narang et al. 2014). Briefly, the mesenteric vascular bed was separated from the intestine and the superior mesenteric artery cleaned of connective tissue, cannulated with a blunted

hypodermic needle (20 G) and flushed with Krebs buffer to remove blood. In some experiments, the endothelium was removed by flushing the bed with 0.5% Triton X-100 in water for 30 secs followed by rapid washout with Krebs. The vascular bed was placed on a wire mesh in a warm chamber and perfused with oxygenated Krebs buffer at a constant flow rate of 5 $mlmin^{-1}$ (37°C). Vasoconstrictor responses were measured as increases in perfusion pressure which were monitored via a pressure transducer and recorded via a PowerLab data acquisition system using Chart 5.0 software (AD Instruments, Colorado) and displayed as normalized values. Endothelial function was assessed as the response to ACh (1 μM) following vasoconstriction with the $\alpha_1$-adrenoceptor agonist methoxamine (1 μM). Tissues in which ACh elicited >80% reversal of constriction were deemed to be endothelium-intact and <25% were deemed to be endothelium-denuded. Preparations in which the % reversal of methoxamine-induced constriction elicited by ACh fell between these values were discarded.

*2.5.1 Responses to stimulation of perivascular nerves.* Electrodes were attached to the cannulating needle and to the wire mesh to allow electrical field stimulation using a Grass SD9 stimulator (Grass Technologies, USA). Following an equilibration period of 30 mins, a single stimulation (30Hz, 90V, pulse width 1 msec, 30 secs) was applied to assess the viability of the preparation. After a further 10 mins, a frequency-response curve was constructed by stimulating the preparation at 1-40 Hz (90 V, pulse width 1 msec, 30 secs) at 10 min intervals (Pakdeechote et al. 2007). The effects of agents on nerve-evoked vasoconstriction were assessed by perfusing the drugs through the lumen of the preparation for 20 mins prior to constructing a second frequency-response curve. In some experiments, a third frequency-response curve was constructed following washout of the drugs.

## 2.6 Intracellular recording of smooth muscle membrane potential in intact arteries

For measurement of smooth muscle membrane potential basilar or 2nd order mesenteric arteries were cut open longitudinally and pinned to the bottom of a Sylgard chamber as previously described(T. Allen et al. 2002)·(Kerr et al. 2015). This method limits movement of the tissue to allow for recording of more sustained responses. The arterial segments were orientated with the endothelial surface uppermost for recording of endothelial cell membrane potential or endothelial surface downwards for recording of smooth muscle membrane potential. Tissues were maintained at 37°C and constantly superfused with warmed oxygenated Krebs buffer at a rate of 5 $mlmin^{-1}$. Measurements of membrane potential were made with sharp glass microelectrodes, back-filled with 3 M KCl and with resistances of 60-100 MΩ. The criteria for successful cell impalement included a sharp negative deflection upon entry and a sharp return to baseline upon electrode removal. Data were recorded via a PowerLab data acquisition system using Chart 5.0 software (AD Instruments, Colorado). Drugs were added to the superfusate or as a bolus dose to the bath as indicated and the membrane potential values quoted were recorded with 1-2 mins of drug addition. All membrane potential measurements are shown as absolute values in mV. In endothelium-denuded rat basilar and mesenteric arteries, hyperpolarization to ACh was not observed.

## 2.7 Real-time reverse-transcription PCR (qRT-PCR)

Expression levels of $K_{ir}2.1$, $K_{ir}$ 2.2, $K_{ir}$ 2.3, $K_{ir}$ 2.4, $K_{ir}$ 3.1, $K_{ir}$ 3.4, $K_{ir}$ 1.1, and $K_{ir}7.1$ mRNA were determined in rat mesenteric arteries using real-time PCR analysis. For each channel transcript, data was obtained from mesenteric arteries isolated from 4 different rats.

*2.7.1 RNA extraction and cDNA synthesis.* Total RNA was extracted from freshly isolated rat mesenteric arteries without adhering tissue and stored in RNAlater® Solution (Ambion, Life Technologies Corporation) at -20°C. RNA extraction was performed using RNeasy Plus Micro Kit (QIAGEN CAT # 74034) following the manufacturer's protocol. The concentration of RNA

extracted was assessed base on the amount of light absorbed by the sample when exposed to UV light at a wavelength of 260 nm using a spectrophotometer (NanoDrop® ND-10000). Aliquot of RNA extracted were run on a 1.5% agarose gel by electrophoresis to examine RNA integrity. For each transcript, 150 ng of extracted RNA was used to synthesize cDNA in a total volume of 20 µl using QScript cDNA SuperMix (Quantabio CAT # 95048) as recommended by the manufacturer. qPCR experiments were performed using two different protocols:

a) *SYBR green dye method:*

Reactions were performed in a total volume of 10 µl:

- 5 µl DyNAmo ColorFlash SYBR Green Master mix (ThermoFisher Scientific CAT# F416)
- 1 µl primers (1 µM) listed in **Table 2.1** (0.5 µl forward and 0.5 µl reverse)
- 2 µl cDNA sample
- 2 µl nuclease-free water

Cycling conditions (used to detect KCNJ genes):

- Hold: 50°C for 2 mins
- Hold: 95°C for 10 mins
- 40 cycles of:
    - Denature: 95°C for 15 secs
    - Anneal/extend: 60°C for 30 secs
- Melt Curve

Cycling conditions (used to detect KCNN genes):

- Hold: 95°C for 15 mins
-40 cycles of:
    -Denature: 94°C for 15 secs
    -Anneal: 58.2°C for 30 secs
    -Extend: 72°C for 30 secs
-Melt Curve

| Primers for qPCR (SYBR green dye) | | |
|---|---|---|
| **Species/Gene** | **Primer ID/sequence** | **Manufacturer** |
| Rat-KCNJ12 ($K_{ir}$2.2) | F 5'-gttggggactctgctggag-3'<br>R 5'-tgcccaaattaaaaaccaatg-3' | Integrated DNA Technologies |
| Rat-KCNJ14 ($K_{ir}$2.4) | F 5'-ggagccaggaaacagtcg-3'<br>R 5'-tttttgacgaagcgtccac-3' | Integrated DNA Technologies |
| Rat-KCNJ3 ($K_{ir}$3.1) | F 5'-gagggacggaaaactcactct-3'<br>R 5'-ctcaggtgtctgccgagatt-3' | Integrated DNA Technologies |
| Rat-KCNJ5 ($K_{ir}$3.4) | F 5'-ctgtcggtacgtaggcaatg-3'<br>R 5'-cctagaatcaccggccatag-3' | Integrated DNA Technologies |
| Rat-KCNJ1 ($K_{ir}$1.1) | F 5'-aacggagtgtgttcagagtgc-3'<br>R 5'-accatcttcggaggtgtttg-3' | Integrated DNA Technologies |
| Rat-ACTB | F 5'-agattactgccctggctcct-3'<br>R 5'-actcctgcttgctgatccac-3' | Integrated DNA Technologies |
| Rat-KCNN1 ($K_{Ca}$2.1) | F 5'-gtgaagattgaacaagggaagg-3'<br>R 5'-ggcctccaactcctcctg-3' | Thermo Fischer Scientific |
| Rat-KCNN2 ($K_{Ca}$2.2) | F 5'-accatcagacagcagcaaaggg-3'<br>R 5'-gaccgccgcctcctggac-3' | Thermo Fischer Scientific |
| Rat-KCNN3 ($K_{Ca}$2.3) | F 5'-gccaactccaccgccatc-3'<br>R 5'-ggctgtggaacttggagag-3' | Thermo Fischer Scientific |
| Rat-KCNN4 ($K_{Ca}$ 3.1) | F 5'-atgctgctacgtctctac-3'<br>R 5'-gaatcggacttggttgag-3' | Thermo Fischer Scientific |

**Table 2.1:** Primers used for the amplification of KCNJ12, KCNJ14, KCNJ1, KCNJ3, KCNJ5 and KCNN1-4 for qPCR.

Final reaction products were electrophoresed on a 2% agarose gel, extracted using QIAquick Gel Extraction Kit (Qiagen CAT# 28704) and sequenced at the Applied Genomics Core (University of Alberta).

b) *TaqMan assay method*:

Reactions were performed in a total volume of 20 µl:

- 10 µl TaqMan® Fast Advanced Master Mix (2X) (CAT# 4444556)

- 1 µl TaqMan® assay listed in **Table 2.2** (0.5 µL forward and 0.5 µL reverse)
- 2 µl cDNA sample
- 7 µl distilled water

Cycling conditions:

- Hold: 50°C for 2 mins
- Hold: 95°C for 20 secs
- 40 cycles of:
    - Denature: 95°C for 1 secs
    - Anneal/extend: 60°C for 20 secs

| **Primers for qPCR (TaqMan assays)** | | |
|---|---|---|
| **Species/Gene** | **Primer ID/sequence** | **Manufacturer** |
| Rat-KCNJ2 ($K_{ir}$2.1) | Assay ID: Rn00568808_s1 | ThermoFisher Scientific |
| Rat-KCNJ4 ($K_{ir}$2.3) | Assay ID: Rn01502359_m1 | ThermoFisher Scientific |
| Rat-KCNJ13 ($K_{ir}$7.1) | Assay ID: Rn00586020_m1 | ThermoFisher Scientific |
| Rat-ACTB | Assay ID: Rn00667869_m1 | ThermoFisher Scientific |

**Table 2.2:** TaqMan assays used for the amplification of KCNJ2, KCNJ4, and KCNJ13 for qPCR.

Final reaction products were electrophoresed on a 2% agarose gel to confirm the sizes of product.

mRNA expression of KCNJ and KCNN subtypes are compared to the expression of ß-actin using comparative quantification algorithms $\Delta C_t$ ($\Delta C_t$ is the difference between the $C_t$ values obtained for the expression of the gene of interest and ß-actin); fold difference is expressed as $2^{\wedge -\Delta C_t}$, $\Delta C_t = C_{t\,(gene)} - C_{t\,(ß\text{-}actin)}$.

## 2.8 Immunohistochemical localization of $SK_{Ca}$, $IK_{Ca}$, $K_{ir}$2.1 and 2.2 channel proteins in intact arteries

Protein expression of $SK_{Ca}$, $IK_{Ca}$, $K_{ir}$ 2.1 and 2.2 channels was localized in intact arteries using confocal immunohistochemistry in collaboration with Dr. Shaun Sandow (University of the Sunshine Coast, Australia) using methods described by Chadha et al., (2010)(Chadha et al. 2010). Briefly, arteries were fixed with 2% paraformaldehyde in 0.1 M PBS, cut along the lateral plane and pinned out as a flat sheet with the intima uppermost onto Sylgard blocks. Whole-mount tissues were incubated in blocking buffer (PBS containing 1% bovine serum albumin, 0.2% Tween 20) for 2 hrs at room temperature, rinsed in PBS (3 × 5 mins), and incubated in primary antibody ($IK_{Ca}$, as IK, 1:100, Mark Chen, GlaxoSmithKline, Stevenage, United Kingdom, M75; $SK_{Ca}$ as SK3, M75 1:100, Craig Neylon, University of Melbourne, Australia; $K_{ir}$2.1 as KCNJ2, 1:100, Alomone, APC-026; $K_{ir}$2.2 as KCNJ12, 1:100, Alomone, APC-042) in blocking buffer for 18 hrs at 4°C. Tissue was then rinsed in PBS (3 × 5 mins) and incubated in secondary antibody (Alexa Fluor 633; Invitrogen) diluted in 0.01% Tween 20 for 2 hrs. It was then rinsed in PBS (3 × 5 mins), mounted intima uppermost in anti-fade glycerol, and examined with a confocal microscope (FV1000; Olympus, Tokyo, Japan) using uniform settings. Controls for antibody specificity involved abolition of staining after pre-incubation of SK3 (M75) antibodies with their respective antigenic peptides, omission of the primary antibody, and the use of transfected cells, and positive controls for SK3, KCNJ2 and KCNJ12.

## 2.9 Drugs and chemicals

All salts were purchased from Sigma-Aldrich. All other chemicals, their source, mechanism of action, and the solvent used are listed in Table 2.3.

| Drug | Company | Mechanism of action | Solvent |
|---|---|---|---|
| 1-EBIO | Sigma | $SK_{Ca}$ and $IK_{Ca}$ channel activator (Devor et al. 1996) | DMSO |
| 5-HT | Sigma | 5-HT receptor agonist (Berger et al. 2009) | $H_2O$ |
| Acetylcholine | Sigma | acetylcholine receptor agonist (Wessler et al. 1999) | $H_2O$ |
| Apamin | Tocris | $SK_{Ca}$ channel antagonist (van der Staay et al. 1999) | $H_2O$ |
| Barium chloride | Sigma | $K_{ir}$ channel inhibitor (Robertson et al. 1996) | $H_2O$ |
| Capsaicin | Sigma | inhibitor of sensory nerve function via activation of TRPV1(Bevan et al. 1992) | Ethanol |
| CyPPA | Tocris | $SK_{Ca}$ channel activator (Hougaard et al. 2007) | DMSO |
| DEA NONOate | Sigma | NO donor (Keefer et al. 1996) | Degassed Krebs |
| IbTX | Tocris | $BK_{Ca}$ channel inhibitor (Kunz et al. 2002) | $H_2O$ |
| Indomethacin | Sigma | cyclooxygenase (COX) inhibitor (Ferreira et al. 1971) | $NaHCO_3$ |
| L-NAME | Sigma | NOS inhibitor (Pfeiffer et al. 1996) | $H_2O$ |
| ML-133 hydrochloride | Tocris | $K_{ir}2$ channel inhibitor (Wu et al. 2010) | DMSO |
| Nifedipine | Sigma | L-type VOCC blocker (Shen et al. 2000) | DMSO |
| NS309 | Sigma | $SK_{Ca}$ and $IK_{Ca}$ channel activator (Strøbæk et al. 2004) | DMSO |
| NS 6180 | Tocris | $IK_{Ca}$ channel antagonist (Strøbaek et al. 2013) | DMSO |
| ODQ | Sigma | soluble guanylyl cyclase inhibitor (Garthwaite et al. 1995) | DMSO |
| Phenylephrine | Sigma | $\alpha_1$-adrenoceptor agonist (Minneman et al. 1994) | $H_2O$ |
| Prazosin | Sigma | $\alpha_1$-adrenoceptor antagonist | $H_2O$ |

| | | | |
|---|---|---|---|
| | | (Skomedal et al. 1980) | |
| Propranolol | Sigma | β-adrenoceptor antagonist(Bond 1967) | $H_2O$ |
| Pyr3 | Tocris | TRPC3 channel antagonist (Kiyonaka et al. 2009) | DMSO |
| SKA-31 | Tocris | $IK_{Ca}$ channel activator (Sankaranarayanan et al. 2009) | DMSO |
| Tertiapin | Tocris | $K_{ir}$3.1/3.4 inhibitor (Jin and Lu 1999) | $H_2O$ |
| Tetrodotoxin | Sigma | Voltage-gated $Na^+$ channel inhibitor (Chong and Ruben 2008) | $H_2O$ |
| TRAM- 34 | Tocris | $IK_{Ca}$ channel antagonist (Wulff et al. 2000) | DMSO |
| U46619 | Sigma | $TXA_2$ receptor agonist (Coleman et al. 1981) | DMSO |
| U73122 | Sigma | Phospholipase C inhibitor (Smallridge et al. 1992) | DMSO |
| U73343 | Sigma | Inactive analog of U73122 (negative control; Muto, Nagao, and Urushidani 1997) | DMSO |
| VU 590 | Tocris | Inhibitor of Kir1.1 and $K_{ir}$7.1 (Lewis et al. 2009) | DMSO |
| Xestospongin C | Tocris | $InsP_3$ receptor inhibitor (Gafni et al. 1997) | DMSO |

**Table 2.3:** Drugs used in the experiments described in this thesis. * For each of these drugs control experiments were carried out using appropriate concentrations of DMSO, $NaHCO_3$, degassed Krebs and ethanol.

## 2.10 Data analysis and statistics

Summary data are presented as mean ± SEM (n) where n indicates the number of animals. Unless otherwise indicated, responses in the absence and presence of drugs were paired (i.e. obtained from the same tissues). Normalized agonist concentration-response curves were fitted to a sigmoidal curve with a variable slope using four parameters logistic equation in GraphPad Prism 6.0 (GraphPad Software, La Jolla, CA). For the purposes of statistical comparison, agonist $EC_{50}$ values were converted to $pEC_{50}$ values. Comparisons of means between control and drug-treatments (with one independent variable) were performed using Student's t-test. One-way ANOVA was used when comparing between control and drug-treatments when there are more

than two groups in total and Post hoc Tukey test was used for individual comparison. Two-way repeated measures ANOVA was used when there were two independent variables. Post hoc Holm-Sidak test was used for individual comparison when there are two categorical groups and post hoc Tukey test was used when there are more than two categorical groups for individual comparisons. Statistical analyses were performed using GraphPad Prism 6.0 with $P<0.05$ considered to be statically significant.

# Chapter 3: Vasoconstrictor stimulus determines the functional contribution of myoendothelial feedback to mesenteric arterial tone

## 3.1 Introduction

As described in **Chapter 1**, the diameter of resistance arteries, a key determinant of tissue perfusion and blood pressure, is tightly regulated through the coordinated activity of endothelial cells, sympathetic nerves and smooth muscle cells (Kerr et al. 2012; Westcott and Segal 2013). Endothelial cells modulate the contractile state of surrounding smooth muscle cells via direct electrical coupling through MEGJs and release of diffusible factors such as NO (For review see Ellinsworth et al. 2016 and Vanhoutte et al. 2017). A vast literature describes how these mechanisms mediate endothelium-dependent vasodilation to agents such as ACh (for reviews see Busse et al. 2002, Edwards et al. 2010; Edwards, Félétou, and Weston 2010; Vanhoutte et al. 2017) but recent studies have focused on addressing the question of how agonist-evoked constriction of isolated resistance arteries is limited by reciprocal activation of the endothelium. This work has led to the current model for myoendothelial feedback in which movement of $InsP_3$ from smooth muscle to endothelial cells via MEGJs, generates localized $InsP_3$-dependent $Ca^{2+}$ transients that activate $IK_{Ca}$ channels within myoendothelial projections. The resulting hyperpolarization of the endothelial membrane potential then feeds back to the smooth muscle cells to limit further reductions in vessel diameter (Kerr et al. 2015; Tran et al. 2012; Nagaraja et al. 2013; for review see Kerr et al. 2012 and Segal 2015; see **Section 1.5.5** for details).

This model arose from experiments employing global application of pharmacological concentrations of $\alpha_1$-adrenoceptor agonists to elicit smooth muscle depolarization, increased $Ca^{2+}$ influx through L-type VOCCs, and generation of $InsP_3$ by PLC in a large number, if not all, smooth muscle cells (Kerr et al. 2015; Tran et al. 2012). However, this situation may not be replicated when other vasoconstrictor stimuli are employed. Agonists and physical stimuli can utilize a

diverse range of contractile mechanisms to contract smooth muscle cells, not all of which involve generation of $InsP_3$ (reviewed by Brozovich et al. 2016) and sympathetic nerve activity results in the release of quanta of noradrenaline to act on clusters of $\alpha_1$-adrenoceptors within spatially restricted post-synaptic regions on a limited number of smooth muscle cells.

Furthermore, for myoendothelial feedback to limit vasoconstriction the endothelial mechanisms engaged must be able to effectively inhibit tone development. In hamster skeletal muscle feed arteries, feedback of $IK_{Ca}$ channel-mediated hyperpolarization to limit smooth muscle depolarization fully accounts for endothelium-dependent modulation of constriction to the $\alpha_1$-adrenoceptor agonist PE (Tran et al. 2012). But, in rat mesenteric and basilar arteries, $InsP_3/IK_{Ca}$ mediated myoendothelial feedback is linked to both hyperpolarization and release of NO (Kerr et al. 2015). Smooth muscle cell hyperpolarization is primarily effective against depolarization-induced contraction whereas NO can inhibit vasoconstriction through a range of mechanisms such as decreasing the $Ca^{2+}$ sensitivity of contractile proteins (Carvajal et al. 2000), inhibiting $InsP_3$-induced $Ca^{2+}$ release (Ji et al. 1998), and activating $K^+$ channels (Bolotina et al. 1994; Van Hove et al. 2009; Mistry and Garland 1998). So, as shown previously for ACh-evoked relaxations (Plane and Garland 1996), the relative contribution of hyperpolarization and NO to myoendothelial feedback may reflect variations in the contribution of electrical and non-electrical pathways to vasoconstriction.

Thus, I hypothesized that *the functional contribution of $InsP_3/IK_{Ca}$ channel-mediated myoendothelial feedback to limiting arterial diameter is influenced by the ability of the vasoconstrictor stimulus to engage the endothelium.* To test this hypothesis, I have investigated the functional role of the $IK_{Ca}$ channel-mediated myoendothelial feedback in modulating contraction to the $TXA_2$ agonist U46619, increases in intravascular pressure and stimulation of

perivascular sympathetic nerves in mesenteric arteries. I chose to use mesenteric arteries for this study as our lab has previously described the role of the myoendothelial feedback pathway in modulating increases in tone elicited by $\alpha_1$-adrenoceptor agonists in these vessels (Kerr et al. 2015) as shown in **Figure 3.1**.

## 3.2: Methods

A full descriptions of methods are provided in Chapter 2.

## 3.3 Results

*3.3.1 Myoendothelial feedback modulates noradrenaline-but not U46619-induced tone in isolated mesenteric arteries:* The role of $IK_{Ca}$ channel-mediated myoendothelial feedback in modulating responses to bath-applied noradrenaline (1 nM-30 μM) and U46619 (1 nM-10 μM) was investigated by constructing concentration-response curves to these agonists in the presence and absence of the $IK_{Ca}$ channels inhibitor TRAM-34 (1 μM). As previous work has shown a role for NO in myoendothelial feedback in mesenteric arteries (Kerr et al. 2015), concentration-response curves were also conducted in the presence of the NOS inhibitor L-NAME (100 μM) alone, or with TRAM-34.

Noradrenaline-evoked increases in tone were significantly enhanced by either TRAM-34 or L-NAME alone, and in the presence of a combination of the two inhibitors, the degree of potentiation was the same as that seen with TRAM-34 alone (**Figure 3.1**). In contrast, responses to U46619 were potentiated by L-NAME but unaffected by application of TRAM-34, and in the presence of a combination of the two inhibitors, the degree of potentiation was the same as that seen with L-NAME alone (**Figure 3.1** and **Table 3.1**). Apamin (50 nM), an inhibitor of $SK_{Ca}$ channels, did not affect responses to either agonist (**Table 3.1**). Increases in tone elicited by noradrenaline were abolished by the $\alpha_1$-adrenoceptor antagonist prazosin (1 μM; n=5) but unaffected by propranolol (0.3 μM; **Table 3.1**).

| | Endothelium-intact arteries | |
|---|---|---|
| | **Noradrenaline** | **U46619** |
| Control | 5.95 ± 0.04 | 7.34 ± 0.14 |
| L-NAME | 6.41 ± 0.02* | 8.20 ± 0.13* |
| TRAM-34 | 7.02 ± 0.04*$ | 7.23 ± 0.16 |
| L-NAME + TRAM-34 | 7.05 ± 0.05*$ | 8.13 ± 0.15* |
| Apamin | 6.1 ± 0.04 | 7.2 ± 0.19 |
| Propranolol | 5.93 ± 0.03 | - |

**Table 3.1: $pEC_{50}$ values for noradrenaline- and U46619-induced tone in the presence and absence of inhibitors.** Values are shown as mean ± SEM (n =6). * denotes significantly different to control values. $ denotes significantly different to L-NAME (*Data with apamin contributed by Stephen Gust*).

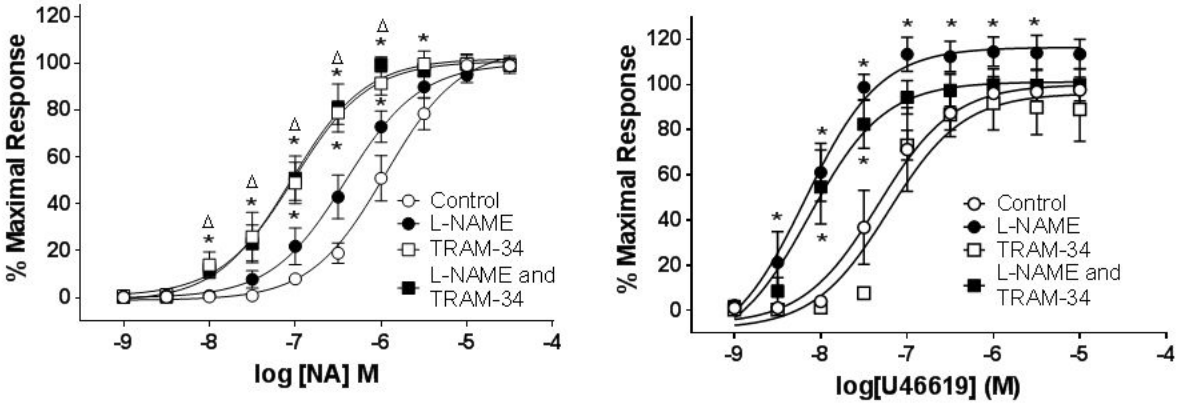



**Figure 3.1: Noradrenaline- and U46619-evoked increases in tone in mesenteric arteries: role of myoendothelial feedback**. **a)** Mean cumulative concentration-response curves for noradrenaline (NA) in the presence and absence of L-NAME (100 μM) and/or TRAM-34 (1 μM) in endothelium-intact segments of rat mesenteric artery (n=6). **b)** Mean cumulative concentration-response curves for U46619 in the presence and absence of L-NAME (100 μM) and/or TRAM-34 (1 μM) in endothelium-intact segments of rat mesenteric artery (n=4; *data contributed by Stephen Gust*). Data is shown as mean ± SEM. Two-way ANOVA was performed. * denotes significantly different to control values ($p<0.05$). Δ denotes significantly different to L-NAME alone ($p<0.05$).

The differential effects of TRAM-34 on noradrenaline and U46619-evoked changes in tone in mesenteric arteries were mirrored by its effects on changes in smooth muscle cell membrane potential elicited by these agonists. In endothelium-intact arteries, TRAM-34 (1 μM) caused a

significant depolarization of resting membrane potential, enhanced noradrenaline (10 μmoles)-evoked smooth muscle depolarization, but was without effect on the response to U46619 (1 μmole; **Figure 3.2**). Noradrenaline-evoked smooth muscle depolarization was abolished by the $\alpha_1$-adrenoceptor antagonist prazosin (1 μM; n=4). Thus, activation of $\alpha_1$-adrenoceptors by noradrenaline leads to activation of $IK_{Ca}$ channels to modulate smooth muscle depolarization but U46619 does not elicit the same type of feedback.

In the current model of myoendothelial feedback it is generation of $InsP_3$ within smooth muscle cells that leads to activation of $IK_{Ca}$ channels. Therefore, we used U73122 (10 μM), an inhibitor of PLC, and xestospongin C (10 μM), an inhibitor of $InsP_3$ receptors, to investigate the contribution of $InsP_3$ to noradrenaline (0.5 μM) and U46619 (0.1 μM) responses in endothelium-intact arteries mounted in a wire myograph. Pre-incubation of arteries with U73122 for 10 mins significantly inhibited responses to both noradrenaline and U46619 (**Figure 3.3a**) but U73343, an inactive analogue of U73122, was without effect. In contrast, exposure to xestospongin C for 10 mins significantly inhibited noradrenaline-evoked increases in tone but did not affect responses to U46619 (**Figure 3.3b**). Thus, although both agonists act through PLC, only responses to noradrenaline are dependent, at least partially, on that activation of $InsP_3$ receptor to elicit the release of $Ca^{2+}$ which can activate $IK_{Ca}$ channel-mediated myoendothelial feedback pathway.

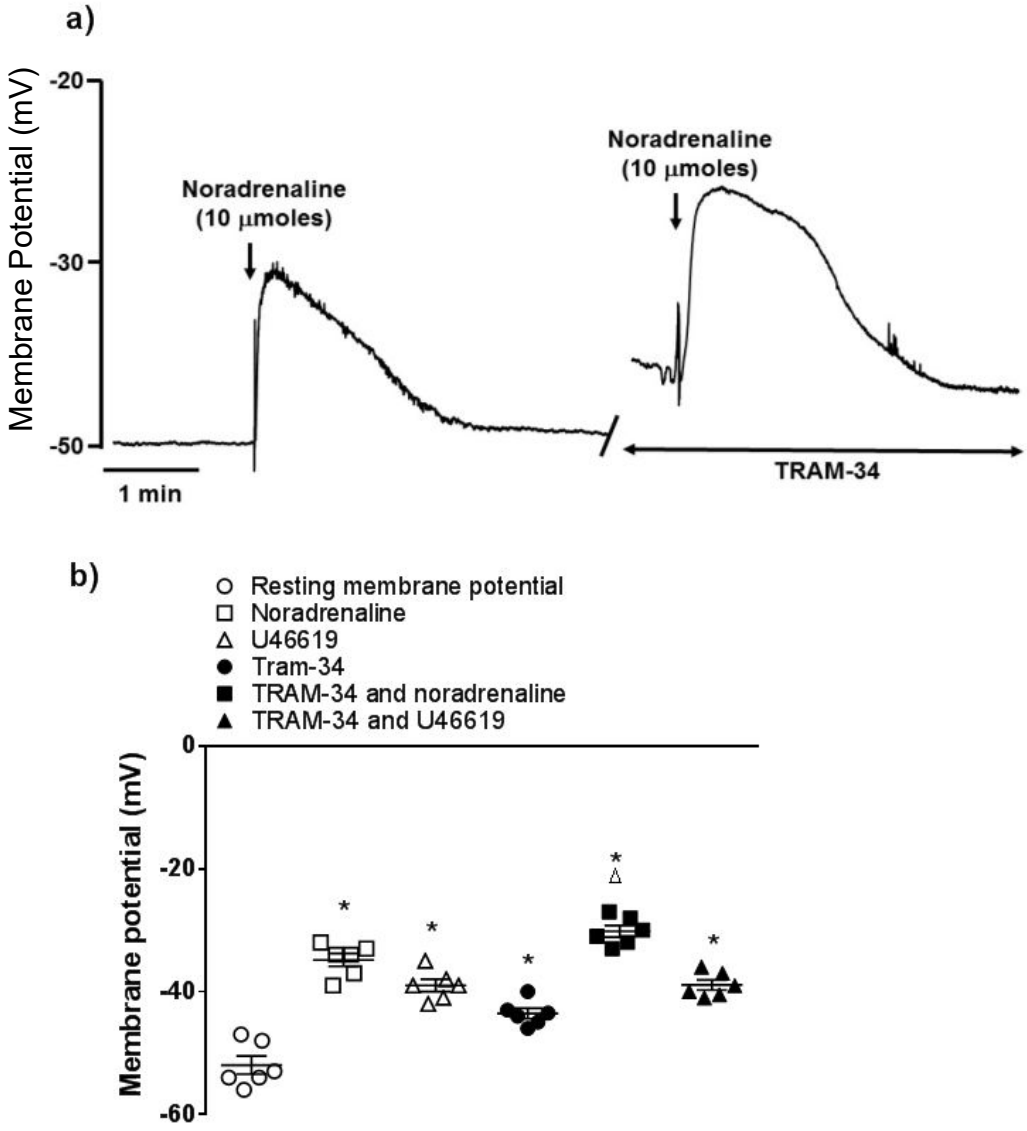



**Figure 3.2: Block of $IK_{Ca}$ channels potentiates smooth muscle depolarization to noradrenaline but not U46619. a)** Representative traces showing smooth muscle depolarization to bolus doses of noradrenaline (10 μmoles) in endothelium-intact segments of rat mesenteric artery in the presence and absence of TRAM-34 (1 μM). **b)** Mean data showing changes in smooth muscle membrane potential evoked by noradrenaline and U46619 in the presence and absence of TRAM-34 (n=6). Data is shown as mean ± SEM. Two-way ANOVA was performed. * denotes significantly different from resting membrane potential ($p<0.05$). $^{\Delta}$ denotes significantly different to noradrenaline alone ($p<0.05$).

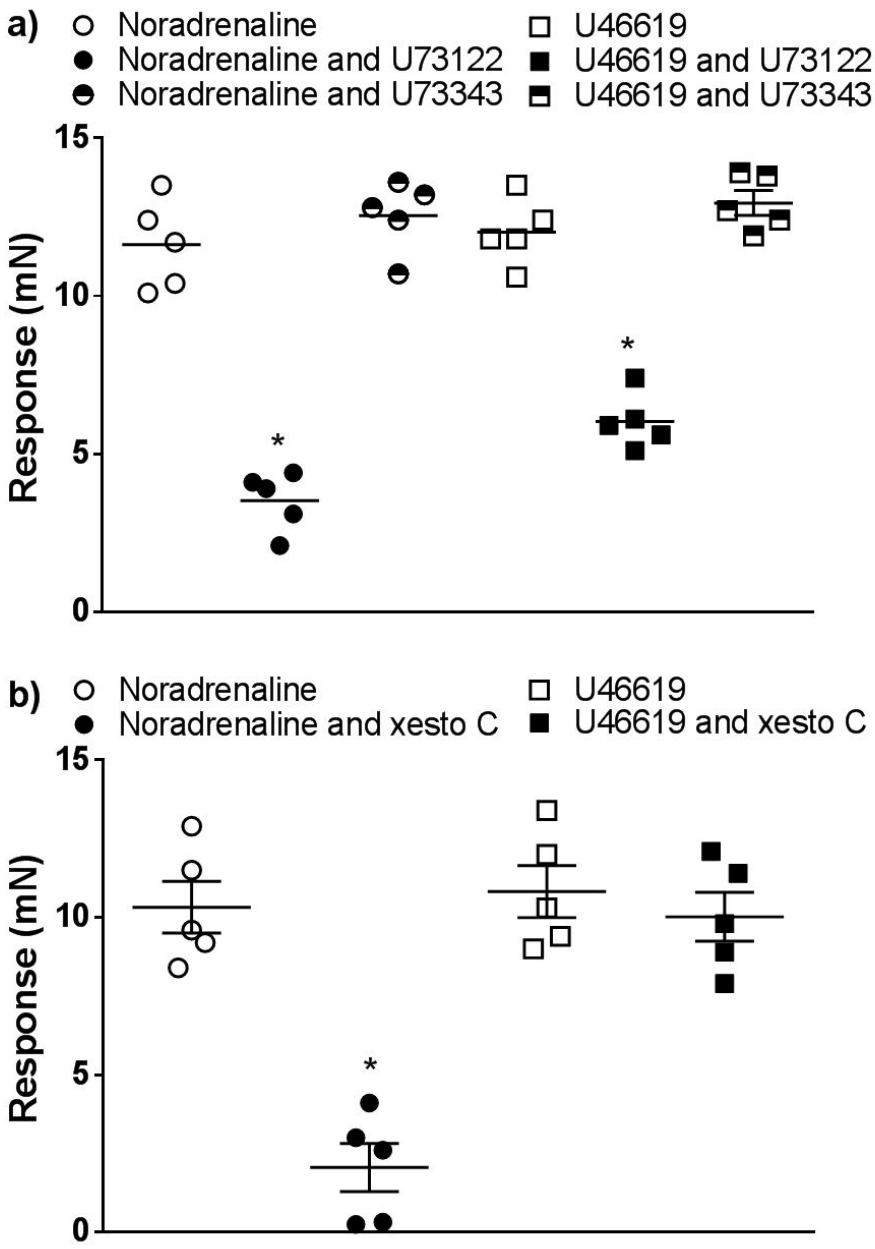



**Figure 3.3: Differential role of $InsP_3$ receptors in noradrenaline- and U46619-induced increases in tone.** Mean responses to noradrenaline (3 μM) and U46619 (0.1 μM) in the presence and absence of **a)** U73122 and U73343 (both 10 μM), and **b)** xestospongin C (10 μM)) in endothelium-intact segments of rat mesenteric artery (n=5). Two-way ANOVA was performed. Data is shown as mean ± SEM. * denotes significantly different control values ($p<0.05$).

*3.3.2 Myoendothelial feedback does not modulate myogenic reactivity:* Mesenteric arteries were mounted in a pressure myograph and subjected to step-wise increases in intraluminal pressure (20 to 120 mmHg). Arteries maintained their diameter across the whole range whereas in $Ca^{2+}$-free buffer, passive dilation was observed at each step (**Figure 3.4a**). Active tone development was not significantly altered by removal of the endothelium (**Figure 3.4b**), nor by the $IK_{Ca}$ channel inhibitor TRAM-34 (1 μM), or the NOS inhibitor L-NAME (100 μM; **Figures 3.4c** and **d**). To determine whether the lack of effect of TRAM-34 reflected an inability of $IK_{Ca}$ channels to modulate myogenic reactivity, we applied pressure ramps to endothelium-intact arteries in the presence of SKA-31(5 μM), an activator of endothelial $IK_{Ca}$ channels. The presence of SKA-31 resulted in loss of myogenic reactivity such that at intravascular pressure of 60 mmHg and above, arterial diameters in the presence of this agent were not significantly different from passive diameters obtained in $Ca^{2+}$-free buffer (**Figure 3.4e**). Furthermore, application of the $InsP_3$ receptor antagonist xestospongin C (10 μM) to myogenically active arteries held at 80 mmHg significantly inhibited subsequent constriction to the adrenoceptor agonists PE and noradrenaline (3 μM) but did not alter the background myogenic tone. The mean baseline diameter of the arteries held at 80 mmHg in the absence and presence of xestospongin C was 247.6 ± 12.7 μm and 245.9 ± 17.1 ($n=10$; $p>0.05$). Addition of either PE or noradrenaline alone to these arteries reduced the diameter to 196 ± 11.5μm ($n=5$; $p<0.05$ compared to baseline) and 183 ± 10.5μm ($n=5$; $p<0.05$ compared to baseline), respectively. However, in the presence of xestospongin C, the mean arterial diameter following application of these agonists was 228 ± 13.8 μm ($n=5$; $p<0.05$) and 235 ± 19.9 μm ($n=5$; $p<0.05$). Thus, the inability of myoendothelial feedback to modulate myogenic reactivity may reflect the lack of a role for $InsP_3$ in mediating the response to changes in intravascular pressure in these arteries.

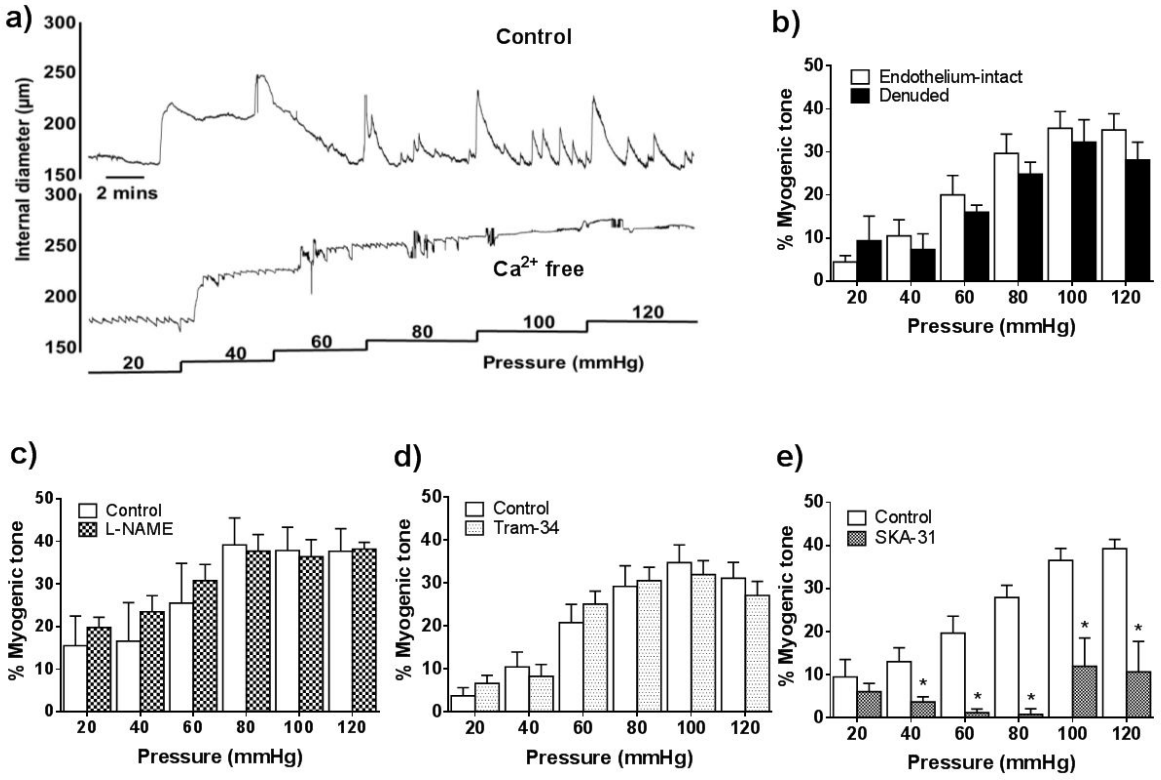



**Figure 3.4: Myogenic reactivity of mesenteric resistance arteries is not modulated by myoendothelial feedback. a)** Representative traces of pressure-diameter relationships in an endothelium-intact mesenteric artery in the presence and absence of extracellular $Ca^{2+}$. **b)** Mean % myogenic tone in endothelium-intact (n=11) and denuded (n=6) arteries. Mean % myogenic tone in endothelium-intact arteries in the absence and presence of **c)** L-NAME (100 µM; n=8), **d)** TRAM-34 (1 µM; n=6), and **e)** SKA-31 (5 µM; n=5). Data is shown as mean ± SEM. Two-way ANOVA was performed. *denotes significantly different to control values ($p<0.5$).

*3.3.3 Frequency-dependence of the contribution of myoendothelial-feedback to nerve-evoked increases in tone in isolated mesenteric arteries*: To investigate the ability of $IK_{Ca}$ channel-mediated myoendothelial feedback in modulating responses to sympathetic nerve activity, we applied transmural nerve stimulation (0.25-20Hz) to isolated segments of endothelium-intact artery mounted in a wire myograph (**Figure 3.5a**). The resulting frequency-dependent increases in tension were abolished by the $\alpha_1$-adrenoceptor antagonist prazosin (0.1 µM) and by tetrodotoxin

(TTX; 0.5 μM) a blocker of neuronal $Na^+$ channels, but were not affected by capsaicin (10 μM; **Table 3.2**) an inhibitor of sensory nerve function. Therefore, these responses are predominantly mediated by release of noradrenaline from nerves and not due to direct electrical stimulation of smooth muscle cells. Application of repeated frequency-response curves showed that nerve-evoked responses did not significantly change over the time course of experiments (**Figure 3.5b**).

| | **Endothelium-intact arteries (mN)** |
|---|---|
| Control | 11.0 ± 3.8 |
| Prazosin | 1.8 ± 0.5* |
| TTX | 1.3 ± 0.2 * |
| Capsaicin | 9.9 ± 3.2 |

**Table 3.2: Responses of isolated mesenteric arteries to stimulation of perivascular nerves at 12 Hz**. Values are shown as mean ± SEM (n =6). T-test was performed, * denotes significantly different to control values ($P<0.05$).

To investigate modulation of nerve-evoked responses by myoendothelial feedback, frequency-response curves were constructed in the presence of L-NAME (100 μM) and/or TRAM-34 (1 μM). Neither agent alone had an effect on nerve-evoked responses. But, the combination of L-NAME and TRAM-34 together significantly increased nerve-evoked increases in tone at frequencies of 12 Hz and above (**Figure 3.5c**), an effect that was abolished by the removal of the endothelium (**Figure 3.5d**).

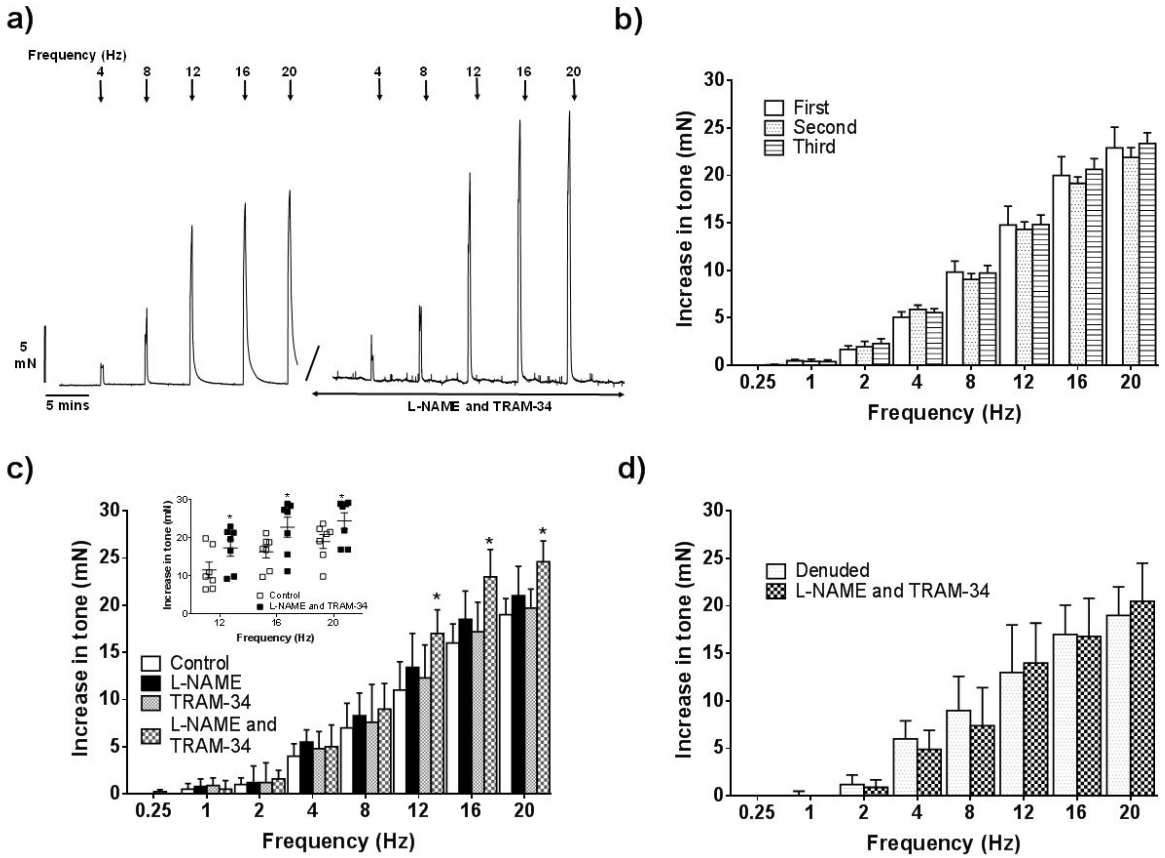



**Figure 3.5: Endothelium-dependent modulation of nerve-evoked increases in tone in isolated rat mesenteric arteries. a)** Representative traces of frequency-dependent increases in tone in an endothelium-intact segment of rat mesenteric artery in the absence and presence of L-NAME and TRAM-34. **b)** Sequential mean frequency-response relationships obtained in endothelium-intact arteries. Mean frequency-response relationships obtained in the presence of L-NAME (100 μM) and/or TRAM-34 (1 μM) in **c)** endothelium-intact (n=7) and **d)** endothelium-denuded arteries (n=6; *data contributed by Raymond Tam*). All values are presented as mean ± SEM. Two-way ANOVA was performed. * denotes significant different compared to control ($P<0.05$). A scatter plot is included as an inset in **c)** showing the data points from control and L-NAME and TRAM-34 groups at 12-20 Hz.

The apparent frequency-dependence of the ability of the endothelium to modulate increases in tone evoked by nerve stimulation then led us to investigate the relative contribution of voltage-dependent and $InsP_3$-dependent mechanisms to these responses in vessels mounted in a wire myograph. The L-type VOCC blocker nifedipine (1 μM) showed frequency-dependence in the

extent of inhibition of nerve-evoked increases in tone; nifedipine abolished responses at lower frequencies but at 8 Hz and above, nifedipine-resistant responses persisted. These nifedipine-resistant responses were abolished by addition of xestospongin C (10 μM; **Figure 3.6**). Thus, it appears that at lower frequencies, nerve-evoked increases in tone can be fully accounted for by depolarization leading to increased $Ca^{2+}$ influx though L-type VOCCs, but at higher frequencies there is an increasing contribution of $InsP_3$. This pattern mirrors the ability of myoendothelial feedback to modulate responses to higher frequencies of nerve stimulation.

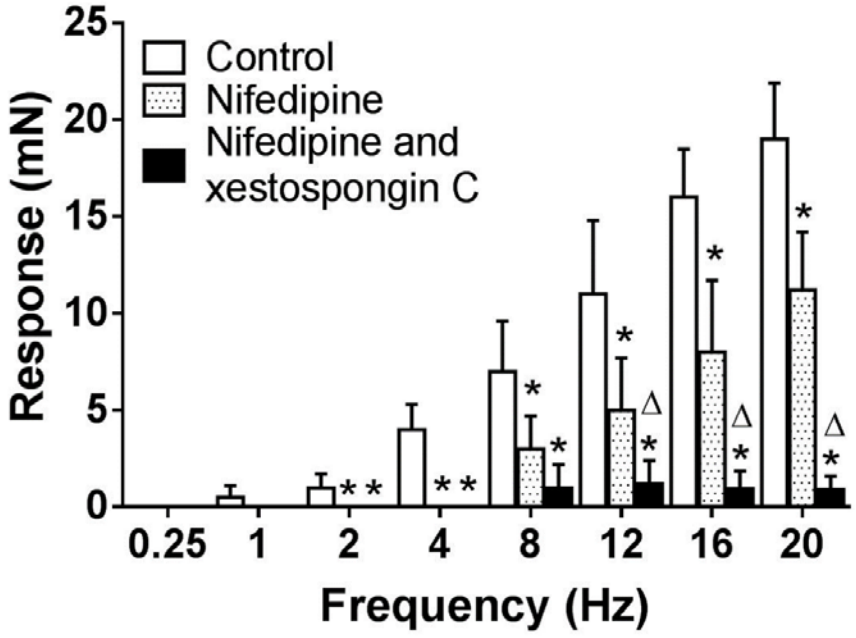



**Figure 3.6: Frequency-dependent changes in the contribution of L-type VOCCs and $InsP_3$ to nerve-evoked increases in tone in rat isolated mesenteric arteries.** Mean frequency-response relationships obtained in endothelium-intact arteries mounted in a wire myograph in the presence and absence of nifedipine (1 μM) or nifedipine and xestospongin C (10 μM). All values are presented as mean ± SEM (n=6). Two-way ANOVA was performed. * denotes significant different compared to control ($P<0.05$), $^{\Delta}$ denotes significantly different compared to nifedipine alone ($P<0.05$).

*3.3.4 Endothelial modulation of nerve-evoked vasoconstriction in the rat perfused mesenteric bed:*

Following on from the experiments in isolated arteries, I sought to determine whether myoendothelial feedback makes a significant contribution to limiting vasoconstriction in the intact mesenteric bed. As in isolated arteries, responses of the mesenteric bed to infusion of noradrenaline (15 μM) were significantly enhanced by block of $IK_{Ca}$ channels with TRAM-34 (1 μM) indicating that $IK_{Ca}$-mediated myoendothelial feedback can occur in this preparation, but responses to U46619 (1 μM) were unaffected (**Table 3.3**). However, in contrast to isolated arteries, block of $SK_{Ca}$ channels by apamin caused significant enhancement of responses to both noradrenaline and U46619. The responses to both noradrenaline and U46619 in the presence of apamin were partially additive with L-NAME, indicating that another mechanism contributes to endothelial modulation of tone in the presence of flow (**Table 3.3**).

| | **Noradrenaline** | **U46619** |
|---|---|---|
| TRAM-34 | 134.2 ± 9.4* | 103.5 ± 5.7 |
| Apamin | 154.9 ± 11.5* | 136.9 ± 8.2* |
| L-NAME | 170 ± 12.9* | 156.3 ± 15.6 |
| Apamin and L-NAME | 195 ± 8.5* | 189 ± 7.3* |

**Table 3.3: Responses of perfused mesenteric bed to noradrenaline and U46619 in the presence of TRAM-34, apamin and L-NAME** are shown as mean ± SEM (n =5) of percentage of control values. Paired t-test was performed. *denotes significantly different to control values ($P<0.05$; *data with noradrenaline contributed by Boyd Classen*).

Next, I examined the role of the endothelium in modulating nerve-evoked vasoconstriction in the perfused bed by constructing frequency-response curves to transmural stimulation (1-40 Hz; **Figure 3.7a**). The $\alpha_1$-adrenoceptor antagonist prazosin (0.1 μM) significantly inhibited nerve-evoked increases in perfusion pressure reducing the mean response to 40 Hz stimulation in endothelium-intact preparations to 7.9 ± 2.0 % (n=5; $p<0.05$) of control values. Capsaicin was

without effect and in its presence the mean response to 40 Hz stimulation was 104.1 ± 10.7 % (n=5) of control.

Modulation of nerve-evoked vasoconstriction by $IK_{Ca}$ channel-mediated myoendothelial feedback was then investigated by perfusing endothelium-intact vascular beds with TRAM-34 (1 μM) and/or L-NAME (100 μM). Block of $IK_{Ca}$ channels with TRAM-34 significantly potentiated responses to only to the highest level of stimulation (40 Hz), indicating that $IK_{Ca}$ channel-mediated myoendothelial feedback does not play a dominant role in regulating vascular tone in the perfused mesenteric bed. In contrast, L-NAME significantly enhanced increases in perfusion pressure at all frequencies, and the combination of TRAM-34 and L-NAME did not further increase responses beyond those seen in the presence of L-NAME alone (**Figure 3.7b**).

Constriction of arteries in the presence of constant flow will increase shear stress, which has been linked to activation of $SK_{Ca}$ channels located on the luminal surface of endothelial cells (Brähler et al. 2009) and release of NO (Macedo and Lautt 1996; Macedo et al. 2011). Thus, we investigated the possibility that $SK_{Ca}$ channels, rather than $IK_{Ca}$ channels, are responsible for engaging the endothelium to modulate nerve-evoked constriction in the perfused mesenteric bed. Infusion of apamin (50 nM), an inhibitor of $SK_{Ca}$ channels, significantly increased nerve-evoked responses (**Figure 3.7c**), an effect dependent on the endothelium (**Figure 3.7d**). Inhibition of NOS by L-NAME also significantly enhanced responses to nerve stimulation in an endothelium-dependent manner (**Figure 3.7d**), an effect that was partially additive with that of apamin. Thus, in the intact perfused bed, under conditions of constant flow, it appears that activation of $SK_{Ca}$ channels and release of NO in response to increases in shear stress, myoendothelial feedback, are the predominant mediators of endothelial modulation of vasoconstriction.

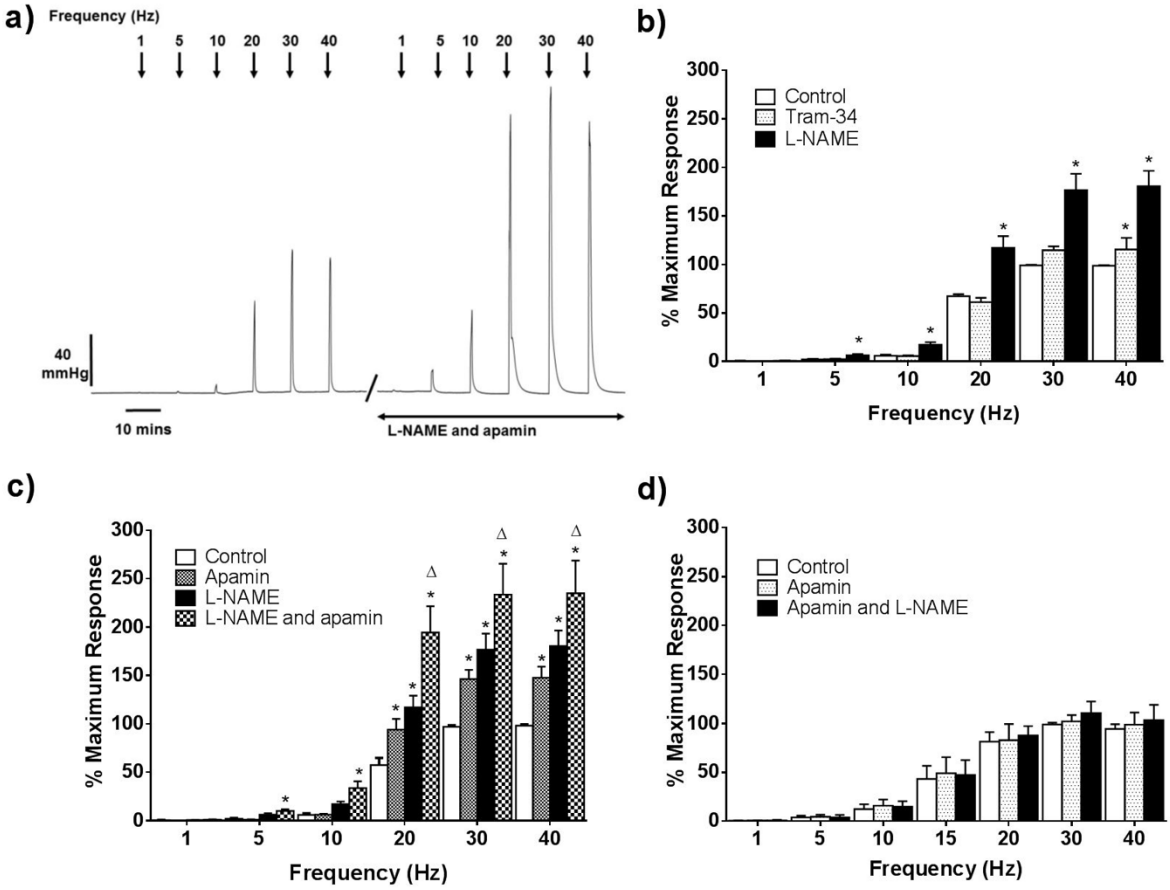



**Figure 3.7: Endothelium-dependent modulation of nerve-evoked increases in perfusion pressure in rat mesenteric vascular bed. a)** Representative trace of frequency-dependent increases in perfusion pressure in an endothelium-intact mesenteric bed perfused at a constant flow rate in the absence and presence of L-NAME and apamin. Mean frequency-response relationships obtained in endothelium-intact mesenteric beds in the absence and presence of **b)** TRAM-34 (1 μM) or L-NAME (100 μM; *data contributed by Stephanie Lunn*), **c)** apamin (50 nM) and/or L-NAME (100 μM; *data contributed by Stephanie Lunn*), **d)** Mean frequency-response relationships obtained in endothelium-denuded mesenteric beds in the absence and presence of apamin and L-NAME. All values are presented as mean ± SEM (n=4-6). Two-way ANOVA was performed. * denotes $P<0.05$ compared to control. $^{\Delta}$ denotes $P<0.05$ compared to L-NAME alone.

## 3.4 Discussion

The data presented supports my hypothesis as I show that in isolated arteries, responses to noradrenaline and stimulation of sympathetic nerves, but not to U46619 and increases in intravascular pressure, were modulated by $IK_{Ca}$ channel-dependent myoendothelial feedback. However, in the intact mesenteric bed perfused under conditions of constant flow, shear stress-induced release of NO and activation of endothelial $SK_{Ca}$ channels appeared to be the primary mediators of endothelial modulation of vasoconstriction to both agonists and nerve stimulation. Thus, I propose that myoendothelial feedback may contribute to local control of diameter within arterial segments, but at the level of the intact vascular bed, increases in shear stress may be the major stimulus for engagement of the endothelium during vasoconstriction. Furthermore, these data indicate that in addition to the nature of the vasoconstrictor stimulus involved, the biological context in which that stimulus is operating also determine how the endothelium is engaged to limit vasoconstriction.

As previously described for the $\alpha_1$-adrenoceptor agonist PE (Lamboley et al. 2005; Kansui et al. 2008; Tran et al. 2012; Kerr et al. 2015) , application of noradrenaline to endothelium-intact mesenteric arteries evoked increases in tone and smooth muscle depolarization mediated by $\alpha_1$-adrenoceptors, and limited by $InsP_3/IK_{Ca}$ channel-dependent myoendothelial feedback. Three subtypes of $InsP_3$ receptor are expressed within endothelial cells and show a heterogeneous distribution (Grayson et al. 2004; Toussaint et al. 2015) which may suggest that distinct isoforms contribute to different aspects of endothelial signaling (Mountian et al. 1999; Moccia et al. 2012). This is yet to be fully explored and the $InsP_3$ receptor inhibitor xestospongin C used in this study does not discriminate between sub-types. However, responses to PE were unchanged in arteries from mice lacking endothelial type 1 $InsP_3$ receptors (Yuan et al. 2016), potentially indicating a role for types 2 and 3 in myoendothelial feedback.

In contrast to noradrenaline, the $TXA_2$ analogue U46619 did not engage the $InsP_3/IK_{Ca}$ channel-dependent myoendothelial feedback pathway and may directly activate endothelial thromboxane receptors to stimulate NO release (Kent et al. 1993). Multiple mechanisms have been proposed to underlie the contractions to U46619 including activation of PLC (Suzuki et al. 2012), $InsP_3$-mediated $Ca^{2+}$ release (Yamagishi et al. 1992), PKC (Nobe and Paul 2001), $Ca^{2+}$ influx through voltage-dependent and -independent channels (Tosun et al. 1998), and rho kinase (ROCK)-mediated sensitization of contractile filaments to $Ca^{2+}$ (Wilson et al. 2005) with their relative contributions showing significant variation between arteries and species. For example, in rat caudal artery contraction to U46619 is accounted for by activation of both the $Ca^{2+}$/calmodulin/myosin light chain kinase pathway and the RhoA/ROCK pathway (Wilson et al. 2005) whereas in rat aorta the response is due to DAG-mediated activation of voltage-dependent and -independent $Ca^{2+}$ channels (Suzuki et al. 2012). Responses to U46619 in rat mesenteric arteries have not been extensively studied but ROK, PKC, p38 MAPK, VOCCs and cyclic nucleotide-gated channels have all been implicated (Bolla et al. 2002; Shaw et al. 2004; Leung et al. 2010). Our finding that responses to U46619, whilst sensitive to inhibition of PLC, are resistant to pharmacological block of $InsP_3$ receptors supports the proposal that the lack of engagement of myoendothelial feedback could be due to the absence of a role for $InsP_3$ in U46619-evoked responses in these vessels. Smooth muscle-specific deletion of $InsP_3$ receptor sub-types in mice led to a depression of contraction to U46619 in mesenteric arteries (Lin et al. 2016) but, as far as I can ascertain, there is currently no evidence supporting a role of $InsP_3$ in responses to U46619 in rat mesenteric arteries. Responses to U46619 in mouse coronary arteries are less-sensitive to block of VOCCs than the responses in equivalent arteries from rats (Yang et al.) and thus the difference between our data and that of Lin et al. may be due to a species difference.

Myogenic reactivity is a crucial autoregulatory mechanism to allow organ blood flow to remain constant over a range of blood pressures (Hill et al. 2009). Development of myogenic reactivity is not dependent on the endothelium (Falcone et al. 1991; Hill et al. 2006) but a modulatory role has been proposed in cerebral arteries (Palomares and Cipolla 2014). I show that although the $IK_{Ca}$ channel activator SKA-31 dilates myogenically active mesenteric arteries, myoendothelial feedback does not modulate myogenic reactivity, possibly due to the absence of a role for $InsP_3$ generation in the response to intravascular pressure in these vessels; the $InsP_3$ receptor antagonist xestospongin C did not alter myogenic tone but inhibited constriction to the adrenoceptor agonists PE and noradrenaline. Thus, the inability of myoendothelial feedback to modulate myogenic reactivity may reflect the lack of a role for $InsP_3$ in mediating the response to changes in intravascular pressure in these arteries. A large body of published work shows that myogenic responses are dependent on smooth muscle depolarization(Knot and Nelson 1998; Knot et al. 1998; Vanbavel et al. 1998) but our understanding of the contribution of other contractile mechanisms is incomplete. PLC and its products, $InsP_3$ and DAG, may contribute to myogenic reactivity in rat cerebral arteries (Osol et al. 1989; Jarajapu and Knot 2002; Mufti et al. 2010a). However, our findings are in agreement with recent data from mouse mesenteric arteries showing that although PLC is essential, it is DAG rather than $InsP_3$ which promotes pressure-induced increases in smooth muscle $Ca^{2+}$ and myogenic vasoconstriction (Mauban et al. 2015).

Sympathetic nerves are key regulators of resistance artery diameter (Westcott and Segal 2013), and therefore blood flow, through the release of ATP and noradrenaline to act on arterial $P_{2X}$ receptors and $\alpha_1$-adrenoceptors, respectively (Sneddon and Burnstock 1984; von Kügelgen and Starke 1985). Nerve-evoked responses in both isolated mesenteric arteries and the perfused mesenteric bed were abolished by prazosin indicating that under our experimental conditions, these

responses are mediated by noradrenaline acting on $\alpha_1$-adrenoceptors with little contribution from ATP. This is in line with a previous reports that the relative importance of ATP as a functional sympathetic neurotransmitter in the rat and porcine mesenteric beds is revealed only when the level of preexisting vascular tone or pressure is increased (Pakdeechote et al. 2007; Rummery et al. 2007; Shatarat et al. 2014).

Acting on $\alpha_1$-adrenoceptors, noradrenaline activates PLC, elevates DAG and $InsP_3$, and causes membrane depolarization to increase $Ca^{2+}$-influx through L-type VOCCs (Neild and Kotecha 1987; Nilsson et al. 1998; Seager et al. 1994) but the relative contribution of these mechanisms to contraction of isolated arteries depends on how the agonist is applied. For example, bath application of the $\alpha_1$-adrenoceptor agonist PE (0.1 μM) to isolated hamster skeletal muscle arteries evoked sustained constrictions largely dependent on $Ca^{2+}$ influx through VOCCs, whereas focal application of a high concentration (1 mM) of the same agonist in the same preparation generated a local constriction entirely dependent on $InsP_3$ (Tran et al. 2009). The rationale for such observations is that bath application of an agonist stimulates most if not all smooth muscles in an arterial segment to generate sufficient charge for electromechanical coupling to occur, whereas focal stimulation of a limited number of smooth muscle cells generates insufficient current to initiate depolarization, partly due to dissipation of charge to neighboring unstimulated cells via gap junctions (Tran et al. 2009). As discussed above, responses of isolated mesenteric arteries to bath applied noradrenaline are modulated by myoendothelial feedback, but as release of noradrenaline from perivascular nerves may result in activation of discrete populations of cells, I sought to determine whether electrically evoked responses are able to engage the myoendothelial feedback pathway.

At lower frequencies (≤8 Hz), nerve-evoked responses of isolated mesenteric arteries mounted in a wire myograph were completely dependent on voltage-dependent mechanisms, as evidenced by their abolition by nifedipine, and hence were not modulated by myoendothelial feedback. Rat mesenteric arteries have dense perivascular innervation (Yokomizo et al. 2015) and this inhibitory effect of nifedipine indicates that under our experimental conditions, stimulation of sympathetic nerves is sufficient to elicit electromechanical coupling (Mishima et al. 1984). However, at higher frequencies of nerve stimulation, responses were mediated by both VOCCs and $InsP_3$, and modulated by the myoendothelial pathway. This finding is in line with a previous report that stimulation of sympathetic nerves at 15 Hz increased $InsP_3$-mediated $Ca^{2+}$ signaling within endothelial cells and elicited myoendothelial feedback in mouse isolated mesenteric arteries (Nausch et al. 2012). Also, voltage-independent contractions to sympathetic nerve stimulation at frequencies above 5 Hz were previously described rabbit isolated ileocolic, saphenous and ear arteries (Bulloch et al. 1991; Skärby and Högestätt 1990). The sensitivity of the lower frequency responses to nifedipine was suggested to reflect selectivity for the purinergic component of the electrically evoked response (Bulloch et al. 1991). However, in our experiments, as in rabbit ear artery (Skärby and Högestätt 1990), there was no apparent role for ATP in nerve-evoked increases in tone and so the decreased reliance of VOCC with increasing frequency of stimulation may be explained by reports that responses to high concentrations of noradrenaline are less dependent on VOCCs as reported in rabbit aorta and pulmonary artery (Casteels et al. 1977; van Breemen et al. 1982). Reliance of nerve-evoked responses on largely voltage-independent contractile mechanisms provides an effective mechanism for integration of nerve activity, the endothelium and arterial diameter in individual arterial segments within a vascular bed. Binding of noradrenaline to $\alpha_1$-adrenoceptors will cause localized vasoconstriction accompanied by

activation of endothelial $IK_{Ca}$ channels to both mediate myoendothelial feedback to limit reductions in diameter, and to dissipate the electrical signal so restricting the spread of the response to facilitate vascular control at the segmental level (**Figure 3.8**; Haug and Segal 2005; Moore et al. 2010; Behringer and Segal 2012).

In the intact perfused mesenteric bed, block of $IK_{Ca}$ channels enhanced responses to infusion of noradrenaline, indicating that the myoendothelial feedback is functional in the intact preparation, but TRAM-34 was without effect on increases in pressure evoked by U46619 or nerve stimulation. However, block of $SK_{Ca}$ channels and/or NOS did cause significant enhancement of vasoconstriction elicited by all three stimuli (noradrenaline, U46619 and nerve stimulation), indicative of a more general mechanism which unlike myoendothelial feedback, is not influenced by contractile stimuli. These experiments were conducted under conditions of constant luminal flow in which vasoconstriction leads to increases in shear stress (Kamiya and Togawa 1980; Macedo and Lautt 1996; Macedo et al. 2011). *In vivo*, endothelial sensing of shear stress, the tangential frictional force exerted by blood flowing across the cell surface, plays a dominant role in regulating tissue perfusion (Baeyens et al. 2016; Chistiakov et al. 2017; Thosar et al. 2012). For example, matching of skeletal muscle blood flow to contractile activity depends on release of NO from the endothelium of feed arteries in response to elevated shear stress caused by dilation of downstream arterioles (Sinkler and Segal 2017).

In addition to the release of NO, several lines of evidence support a functional role for $SK_{Ca}$ channels, possibly in a complex cav-1 and TRPV4 channels, in mediating the endothelial response to shear stress and release of NO. $SK_{Ca}$ channels are expressed on the luminal surface of endothelial cells of rat mesenteric arteries (Sandow et al. 2006), and co-localized with and TRPV4 channels in human microvascular and bovine coronary endothelial cells (Goedicke-Fritz et al.

2015; Lu et al. 2017). Acute exposure of bovine coronary endothelial cells to shear stress resulted in activation of both $SK_{Ca}$ and TRPV4 currents, and increased production of NO. In isolated coronary arterioles, block of $SK_{Ca}$ channels with apamin significantly inhibited shear stress-induced dilation (Lu et al. 2017) and both endothelial $SK_{Ca}$ current density and flow-mediated dilation were impaired in arteries from mice lacking either cav-1 or $SK_{Ca}$ channels (Goedicke-Fritz et al. 2015; Brähler et al. 2009). Thus, in light of these findings, we propose that in the intact mesenteric bed it is shear stress-induced activation of $SK_{Ca}$ channels and NO release that plays the dominant role in modulating vasoconstriction to both agonists and nerve stimulation. In contrast to myoendothelial feedback, which, as discussed above, can provide a localized mechanism for integration of smooth muscle contractility and endothelial function within in individual arterial segments, increases in shear stress can simultaneously stimulate large areas of the endothelium, and so have a global impact on the magnitude and distribution of blood flow across many arteries (**Figure 3.8**). Thus, I propose that together, myoendothelial feedback and endothelial sensing of increases in shear stress provide functionally distinct but complimentary mechanisms to ensure appropriate distribution of blood flow within the intact vascular bed. Previous work by our lab and others has established that in rat mesenteric arteries, myoendothelial feedback elicited by $\alpha_1$-adrenoceptor agonists is mediated by localized $InsP_3$-dependent $Ca^{2+}$ transients activating $IK_{Ca}$ channels (Tran et al. 2012) to elicit endothelial and smooth muscle hyperpolarization (Kerr et al. 2015). Such recordings are not possible in the intact, perfused mesenteric bed due to the complexity of the preparation and thus I have utilized block of $IK_{Ca}$ channels to functionally assess the role of myoendothelial feedback in modulating arterial tone (Kerr et al. 2015; Nausch et al. 2012; Tran et al. 2012). An alternative approach would have been to use tissues from mice lacking $IK_{Ca}$ channels but to date, the majority of functional (Kansui et al. 2008; Kerr et al. 2015;

Lamboley et al. 2005; Tran et al. 2012) and immunofluorescence (Sandow et al. 2006) studies on $InsP_3/IK_{Ca}$ channel-mediated myoendothelial feedback have utilized arteries from rats, with only one recent study describing this pathway in arteries from mice (Looft-Wilson et al. 2017).

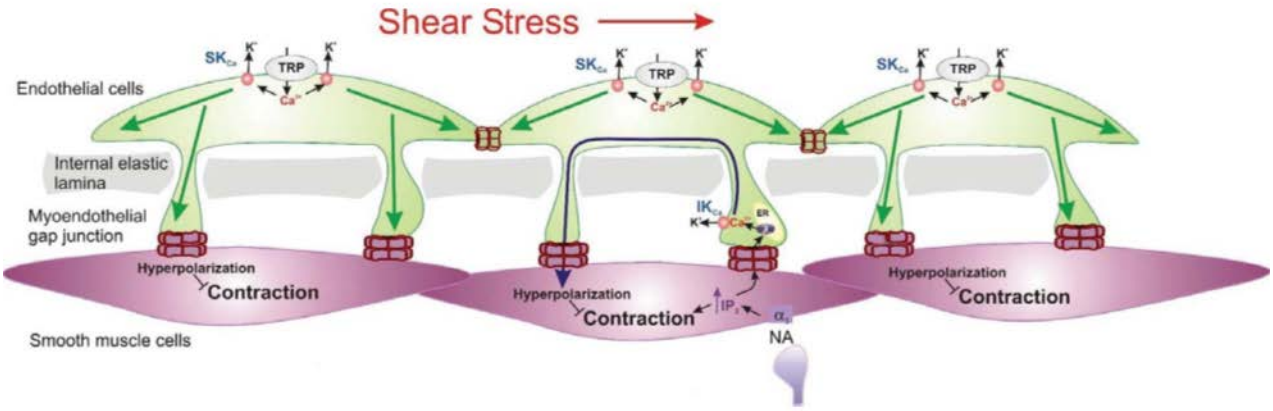



**Figure 3.8: Schematic illustrating proposed role of myoendothelial feedback in modulating nerve-evoked reductions in arterial diameter.** Noradrenaline released from perivascular sympathetic nerves binds to $\alpha_1$-adrenoceptors to cause vasoconstriction accompanied by $InsP_3$-mediated activation of endothelial $IK_{Ca}$ channels to both mediate myoendothelial feedback to limit reductions in diameter. Opening of $IK_{Ca}$ channels also dissipates the electrical signal so restricting the spread of the response and so facilitating vascular control at the segmental level. However, in the intact mesenteric bed, activation of $SK_{Ca}$ channels in response to shear stress will evoke hyperpolarization across large areas of endothelial cells that will spread through myoendothelial gap junctions to surrounding smooth muscle cells to inhibit contraction. NO does contribute to both myoendothelial feedback and responses to shear stress but for clarity has been omitted from this schematic.

As in previous studies, the dependence of myoendothelial feedback on generation $InsP_3$ was illustrated in our experiments with isolated arteries by the use of xestospongin C and U73122. We did not conduct the same experiments in the perfused bed as PLC, $InsP_3$ and DAG have all been implicated in the effects of shear stress on endothelial cells (Nollert et al. 1990; Prasad et al. 1993) so potentially confounding any conclusions as to the role of myoendothelial feedback.

In the mesenteric bed perfused at a constant flow rate, decreases in arterial diameter augment shear stress because of its inverse relationship to the third power of the internal vessel diameter (Kamiya and Togawa 1980). An alternative method to enhance shear stress is to increase the flow rate or viscosity of fluid through isolated vessels mounted on cannulae. This has the advantage of allowing quantification of changes in shear stress but interpretation of data obtained from this approach could be confounded by the previously described interaction between myogenic reactivity and shear stress (Kuo et al. 1990). It must be acknowledged that although evoking vasoconstriction under conditions of constant flow and increasing flow through vessels both augment shear stress, they may do so via different mechanism. Such methodological differences, together with species and vessel variation, could contribute to the wide range of signaling molecules and ion channels which have been implicated in the endothelial response. For example, deletion of $K_{ir}2.1$ channels results in loss of flow-induced release of NO in isolated mouse mesenteric arteries whereas $K_{Ca}$ channels appear to mediate NO-independent responses to flow in the same vessels (Ahn et al. 2017). In contrast, assessment of blood flow in mouse gluteus muscle using intra-vital microscopy demonstrated that flow-induced of feed arteries is mediated by NO with no apparent contribution of endothelial $K_{Ca}$ channels (Sinkler and Segal 2017).

To summarize, in the present study I have used functional approaches to demonstrate that the ability of myoendothelial feedback is not a universal mechanism to limit agonist-evoked

responses in isolated arteries, rather its contribution is determined by the nature the underlying contractile mechanisms. Furthermore, although myoendothelial feedback can limit reductions in diameter within discrete arterial segments, it is shear stress-induced activation of $SK_{Ca}$ channels that is responsible for endothelial engagement to limit vasoconstriction to both agonists and nerve stimulation at the level of the intact mesenteric bed.

These findings add to previous work indicating that within a vascular bed, arterial vasoconstriction may be controlled at the segmental level, whereas increases in blood flow are coordinated over large distances (reviewed by Behringer and Segal 2012). In the setting of this model, we propose that engagement of $InsP_3/IK_{Ca}$ channel by nerve-evoked responses in arterial segments will not only limit reductions in arterial diameter, but opening of $IK_{Ca}$ channels will dissipate the electrical signal and so spatially restrict the response (Behringer and Segal 2012). In contrast, activation of $SK_{Ca}$ channels and release of NO in response to increases in shear stress will provide a global endothelial response to vasoconstriction to ensure appropriate distribution of blood flow within the intact vascular bed (Behringer and Segal 2012).

# Chapter 4: Modulation of myogenic reactivity by $K_{Ca}$ channel activators: role of NO and $K_{ir}$ channels

## 4.1 Introduction

As discussed in **Chapter 1**, NO and opening of endothelial $SK_{Ca}$ and $IK_{Ca}$ channels leading to EDH, are both crucial to the ability of endothelial cells to regulate smooth muscle contractility and therefore resistance artery diameter. Inhibition by apamin and either charybdotoxin or TRAM-34, blockers of $SK_{Ca}$ and $IK_{Ca}$ channels, has been considered a unique characteristic of EDH-mediated vasorelaxation (Bryan et al. 2005; Doughty et al. 1999; Garland et al. 1995; Marrelli et al. 2003; McNeish et al. 2006; Yamamoto et al. 1999). However, as shown in **Chapter 3,** elucidation of the myoendothelial feedback pathway by our lab (Kerr et al. 2015; Wei et al. 2018) and others (Nausch et al. 2012; Tran et al. 2012) indicates that rather than being independent, these vasorelaxant pathways may intersect.

In support of a link between NO and EDH, numerous studies have shown that agonist-induced release of endothelium-derived NO occurs concurrently with $K_{Ca}$ channel-mediated hyperpolarization (reviewed by Busse et al. 2002) and in rabbit superior mesenteric artery, ACh-evoked hyperpolarization is linked to release of NO (Stankevicius et al. 2006). Moreover, in the same study, 1-ethyl-2-benzimidazolinone (1-EBIO; Syme et al. 2000), an opener of $SK_{Ca}$ and $IK_{Ca}$ channels, induced oxyhaemoglobin-sensitive increases in NO release. A direct role for $K_{Ca}$ channels in NO production has been demonstrated in cultured human vascular endothelial cells (Sheng and Braun 2007; Sheng et al. 2009), and $K_{Ca}$ channel activators 6,7-Dichloro-1H-indole-2,3-dione 3-oxime (NS309) and 5,6-Dichloro-1-ethyl-1,3-dihydro-2H-benzimidazol-2-one (DCEBIO) enhanced ATP-evoked membrane hyperpolarization and increases in $[Ca^{2+}]_i$, along with acute NO synthesis in the same system. In porcine retinal arterioles, NS309 and CyPPA enhanced NO-mediated bradykinin relaxations (Dalsgaard et al. 2010) and in rat isolated skeletal muscle arteries, NS309 and DCEBIO augmented ACh-induced dilation (Brähler et al. 2009), in

part through enhancement of the NO-dependent component of the response (Sheng et al. 2009). Doxycycline-induced suppression of the SK3 channel impaired NO-mediated dilation to ACh and to shear stress stimulation in mouse isolated carotid arteries and in skeletal muscle arterioles *in vivo* (Brähler et al. 2009). Also, as shown in **Chapter 3,** in the perfused mesenteric bed, activation of $SK_{Ca}$ channels appears to be responsible for shear stress-induced NO release.

As described in **Chapter 1**, the myogenic reactivity, the ability of small resistance arteries to constrict in response to increases in intravascular pressure, is a crucial autoregulatory mechanism to maintain constant blood flow within tissues and organs in the face of changes in blood pressure( Davis and Hill 1999; El-Yazbi and Abd-Elrahman 2017; Hill et al. 2009; Ashley Izzard and Heagerty 2014). The ability to contract in response to increases in pressure is an intrinsic property of smooth muscle cells in small arteries (Davis and Hill 1999). Although the endothelium is not required for development of myogenic reactivity (Falcone et al. 1991, 1993; Hill et al. 2006; Kuo et al. 1990), it may be able to exert a modulatory influence in some resistance arteries (Meininger and Davis 1992; Palomares and Cipolla 2014).

In **Chapter 3**, I showed that in isolated rat mesenteric arteries, myogenic reactivity is not modulated by the $InsP_3/IK_{Ca}$ channel-mediated myoendothelial feedback pathway(Wei et al. 2018), but that the $IK_{Ca}$ channel activator SKA-31 can dilate endothelium-intact myogenically active mesenteric vessels (**Figure 3.4**).

My goal in conducting the experiments described in this chapter was to further explore the functional role of $SK_{Ca}$ and $IK_{Ca}$ channels in regulating resistance artery diameter, and in particular, to investigate potential interactions between these channels and NO in mediating vasodilation in myogenically active resistance arteries. Thus, I hypothesized that *small molecule*

*activators of endothelial $K_{Ca}$ channels modulate myogenic reactivity at least in part through endothelium-derived NO.*

To test this hypothesis, I have investigated the functional effects of small molecule activators of endothelial $K_{Ca}$ channels, CyPPA and SKA-31 on myogenic reactivity in isolated mesenteric arteries. Strong inward rectifier channels of the $K_{ir}2$ series are activated by membrane potential hyperpolarization and have been suggested to boost endothelium-dependent vasodilatations by $SK_{Ca}$ and $IK_{Ca}$ channel activation (Jackson 2005; Longden and Nelson 2015; Sonkusare et al. 2012). Thus, I also investigated the functional role of $K_{ir}$ channels in the actions of the endothelium-dependent dilator ACh and the activators of $SK_{Ca}$ and $IK_{Ca}$ channel. I chose to use rat mesenteric arteries for this study as they develop myogenic reactivity and both NO and $K_{Ca}$ channel-mediated EDH contribute to endothelium-dependent relaxations to ACh (Waldron and Garland 1994; **Figure 4.23**).

## 4.2 Methods

A full descriptions of methods are provided in Chapter 2.

## 4.3 Results

*4.3.1 Rat mesenteric arteries express $SK_{Ca}$ and $IK_{Ca}$ channels:* Expression of mRNA for $SK_{Ca}$ (SK1-3) and $IK_{Ca}$ (IK) channel subunits was detected in rat mesenteric arteries via qPCR and expression levels were compared to ß-actin mRNA (**Figure 4.1**). As shown in previous studies, mRNA for both SK3 and IK were present (Hilgers and Webb 2007; Burnham et al. 2006).

Endothelial expression of $SK_{Ca}$ (SK3) and $IK_{Ca}$ (IK) channel proteins were identified in whole artery mounts of third order rat mesenteric arteries by the use of confocal immunofluorescence (**Figure 4.2**). As our lab has shown previously (Kerr et al. 2015), and in line with their role in myoendothelial feedback, $IK_{Ca}$ channels were localized at internal elastic lamina (IEL) holes, communication sites between endothelial and smooth muscle cells. In contrast, $SK_{Ca}$

channels were located at adjacent endothelial cell borders, as had previously been observed in porcine coronary artery (Burnham et al. 2002).

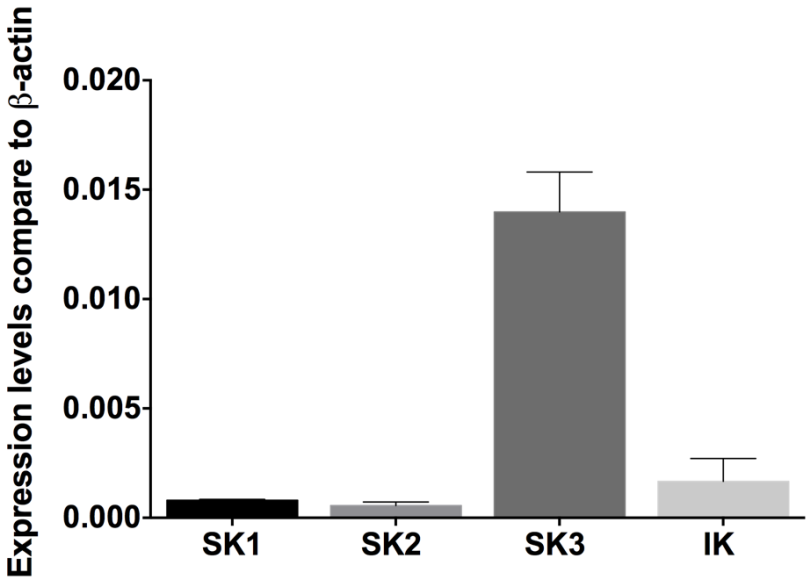



**Figure 4.1: mRNA expression of $K_{Ca}$ channel subunits in rat mesenteric arteries.** Expression levels of $SK_{Ca}$ 2.1, $SK_{Ca}$ 2.2, $SK_{Ca}$ 2.3, and $IK_{Ca}$ mRNA was determined in rat mesenteric arteries using qPCR analysis and compared to ß-actin mRNA. Relative expression was expressed as $2^{-\Delta C_t}$, $\Delta C_t = C_{t\,(gene)} - C_{t\,(ß\text{-}actin)}$. Data were obtained from mesenteric arteries isolated from 4 different rats performed in triplicates.

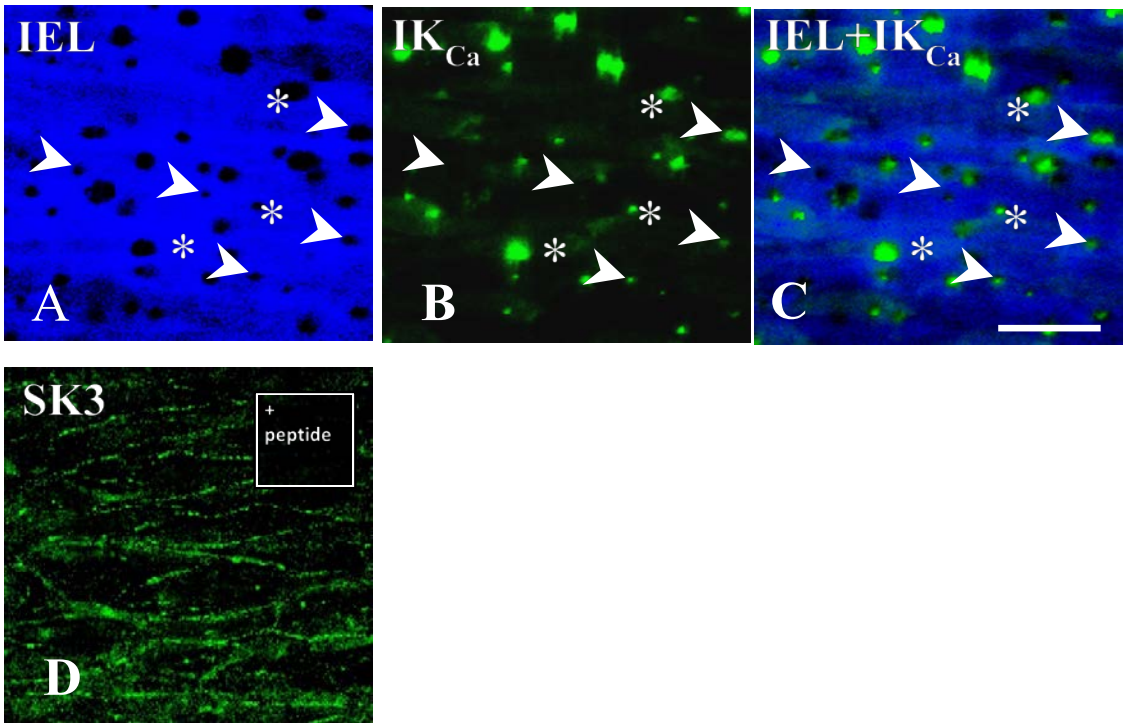



**Figure 4.2: Confocal immunohistochemistry of $K_{Ca}$ channels in third order rat mesenteric arteries.** Confocal stacks at the endothelial to smooth muscle interface (a site of mesenteric artery EC-SM contact) demonstrate discrete punctate $IK_{Ca}$ protein distribution (B). Holes in the internal elastic lamina (IEL) occur as dark spots (examples with arrowheads, A), with overlay of IEL autofluorescence and single confocal sections at the EC-SM interface showing $IK_{Ca}$ channel localization at a portion of hole sites (C). Panel D indicates diffuse expression of $SK_{Ca}$ protein on endothelial cell surface. Peptide block and incubation in secondary antibody alone resulted in labelling (data not shown), Antibody characterization, as per previous studies (Chadha et al. 2010; Sandow et al. 2009). Longitudinal vessel axis left to right, with n=3, each from a different animal. Scale bar denotes 25 µm. Confocal imaging carried out by Dr. Shaun Sandow, University of the Sunshine Coast.

*4.3.2 Characterization of myogenic reactivity in rat mesenteric arteries:* Myogenic reactivity was examined in 3$^{rd}$ and 4$^{th}$ order mesenteric resistance arteries mounted in a pressure myograph and subjected to step-wise increases in intraluminal pressure (20 to 120 mmHg). Arteries relatively maintained their diameter across the whole range whereas in $Ca^{2+}$-free buffer, passive dilation was observed at each step (**Figure 4.3a** and **b**). Active and passive vessel diameters were not significantly different between endothelium intact and denuded arteries (**Figure 4.3c**). At 80 mmHg, the mean diameters for endothelium-intact and denuded arteries were 230.3 ± 10.3 µm and

233.5 ± 10.3 µm (P > 0.05, t-test), respectively, and in $Ca^{2+}$ free solution these values were 310.7 ± 8.0 µm (n=6) and 322.9 ± 12.7 µm (n=11; P>0.05, t-test), respectively.

Time controls were performed to confirm that myogenic reactivity did not vary significantly over the time course of an experiment. In these, three pressure ramps were constructed in the same tissue with a period of 20 minutes between ramps. As shown in **Figure 4.4a**, no statistical difference was observed between the three ramps; at 80 mmHg the diameters of vessels in the three successive pressure ramps were 225.3 ± 19.2 µm, 226.21 ± 18.4 µm and 202.3 ± 18.1 µm (n=5-6; P>0.05), respectively. Also, bath application of DMSO (1:350 dilution), an organic solvent used to dissolve a number of the drugs used in experiments described in this thesis, did not affect myogenic reactivity (**Figure 4.4b**); at 80 mmHg, the mean arterial diameters were 216.5 ± 20.1 µm and 191.4 ± 24.8 µm (n=6; P>0.05), in the absence and presence of DMSO, respectively. From this point onwards, data of myogenic tone will be expressed as % myogenic tone (see **Methods Section 2.4.1**), as this is a standard method of reporting myogenic tone in the literature.

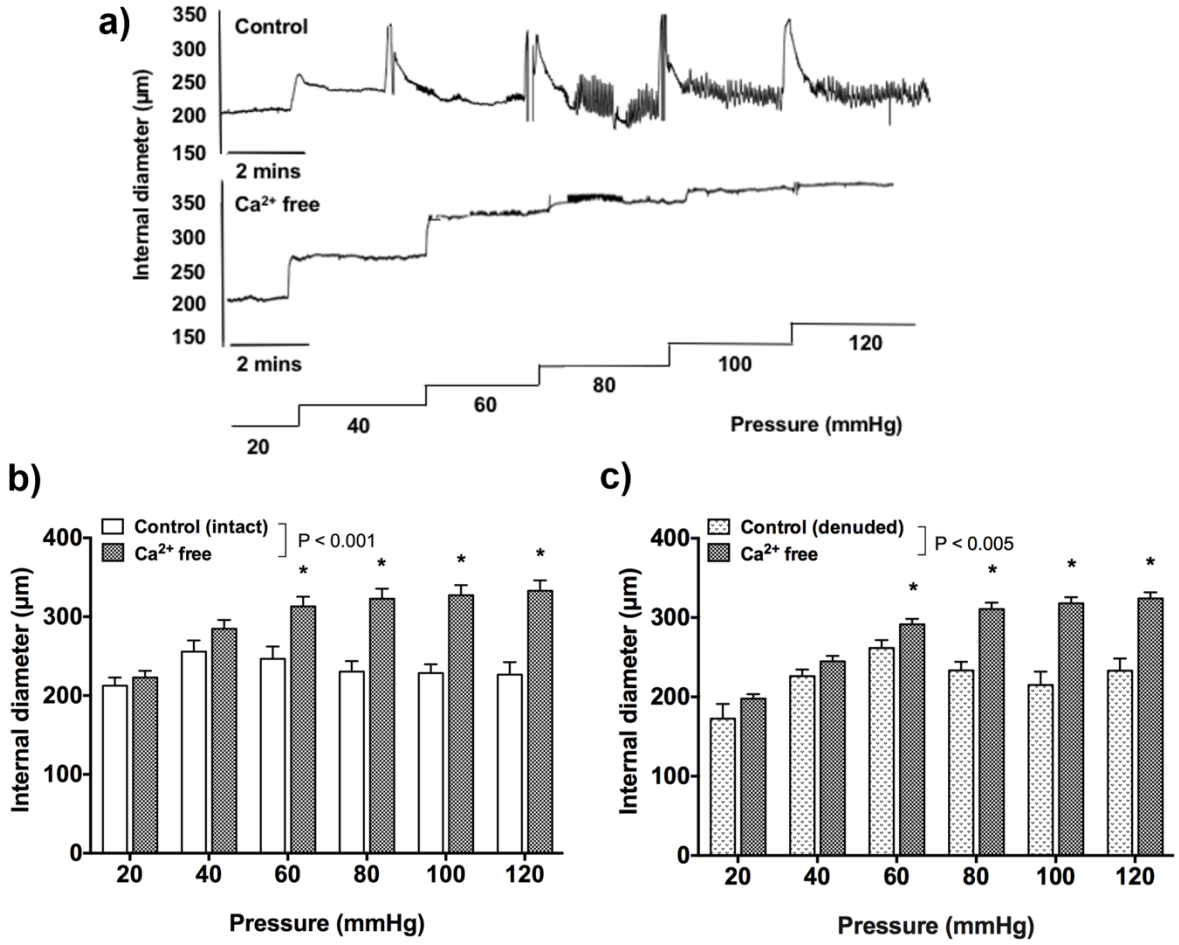



**Figure 4.3: Myogenic reactivity in rat mesenteric arteries is not influenced by the endothelium.** Segments of rat mesenteric resistance artery were mounted in a pressure myograph and subjected to increases in pressure from 20-120 mmHg in 20 mmHg increments. **a)** Representative traces of pressure-diameter relationship for an isolated segment of an obtained in the presence and absence of extracellular $Ca^{2+}$. **b)** Mean pressure-diameter relationships in the presence and absence of extracellular $Ca^{2+}$. **c)** Mean pressure-diameter relationships for endothelium-denuded arteries in the presence and absence of extracellular $Ca^{2+}$. Values are presented as mean ± SEM (n=5-6) of actual diameter recordings. Two-way ANOVA was performed, * denotes statistical significant compared to control values ($P<0.05$).

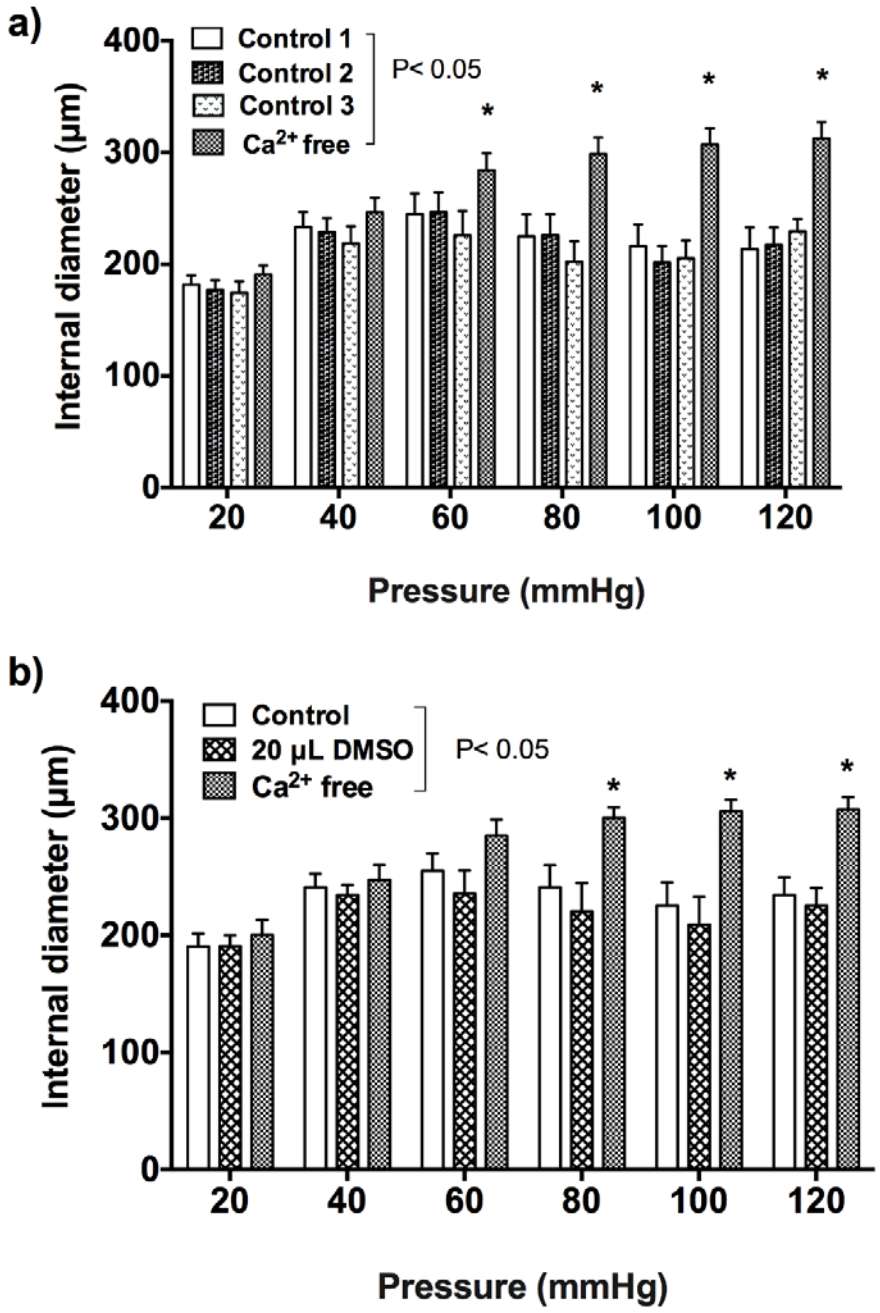



**Figure 4.4: Myogenic reactivity in rat mesenteric resistance arteries is reproducible over time and not affected by dimethyl sulfoxide (DMSO).** Segments of rat mesenteric resistance artery were mounted in a pressure myograph and subjected to increases in pressure from 20-120 mmHg in 20 mmHg increments. **a)** Mean pressure-diameter relationships in the presence and absence of extracellular $Ca^{2}$, pressure ramp performed three times. **b)** Mean pressure-diameter relationships for endothelium-denuded arteries in the presence and absence of 20 µl DMSO or extracellular $Ca^{2+}$. Values are presented as mean ± SEM (n=5-6). Two-way ANOVA was performed, * denotes statistical significant compared to control values ($P<0.05$).

*4.3.3 Myogenic reactivity is enhanced by combined inhibition of NO, $SK_{Ca}$ and $IK_{Ca}$ channels:* I demonstrated in Chapter 3 that myogenic reactivity in rat mesenteric arteries is not affected by block of NOS by L-NAME (100 μM) nor by the $IK_{Ca}$ channel inhibitor TRAM-34 (5 μM; **Figures 3.4c** and **d**).

I also used another inhibitor of $IK_{Ca}$ channels, NS 6180 (1 μM), which is structurally unrelated to TRAM-34, which blocks the channel by binding to a different site than TRAM-34 (Strøbaek et al. 2013; Nguyen et al. 2017), but is equipotent TRAM-34 in inhibiting human, mouse and rat $IK_{Ca}$ channels ($IC_{50}$ 10-20 nM). As with TRAM-34, NS 6180 had no effect on myogenic reactivity (**Figure 4.5**). Similarly, block of $SK_{Ca}$ channels with apamin (250 nM), alone or in combination with TRAM-34, was also without effect on myogenic reactivity (**Figure 4.6**). However, application of L-NAME, apamin and TRAM-34 together did significantly enhance myogenic reactivity in rat mesenteric arteries at pressures from 20-100 mmHg (**Figure 4.7**).

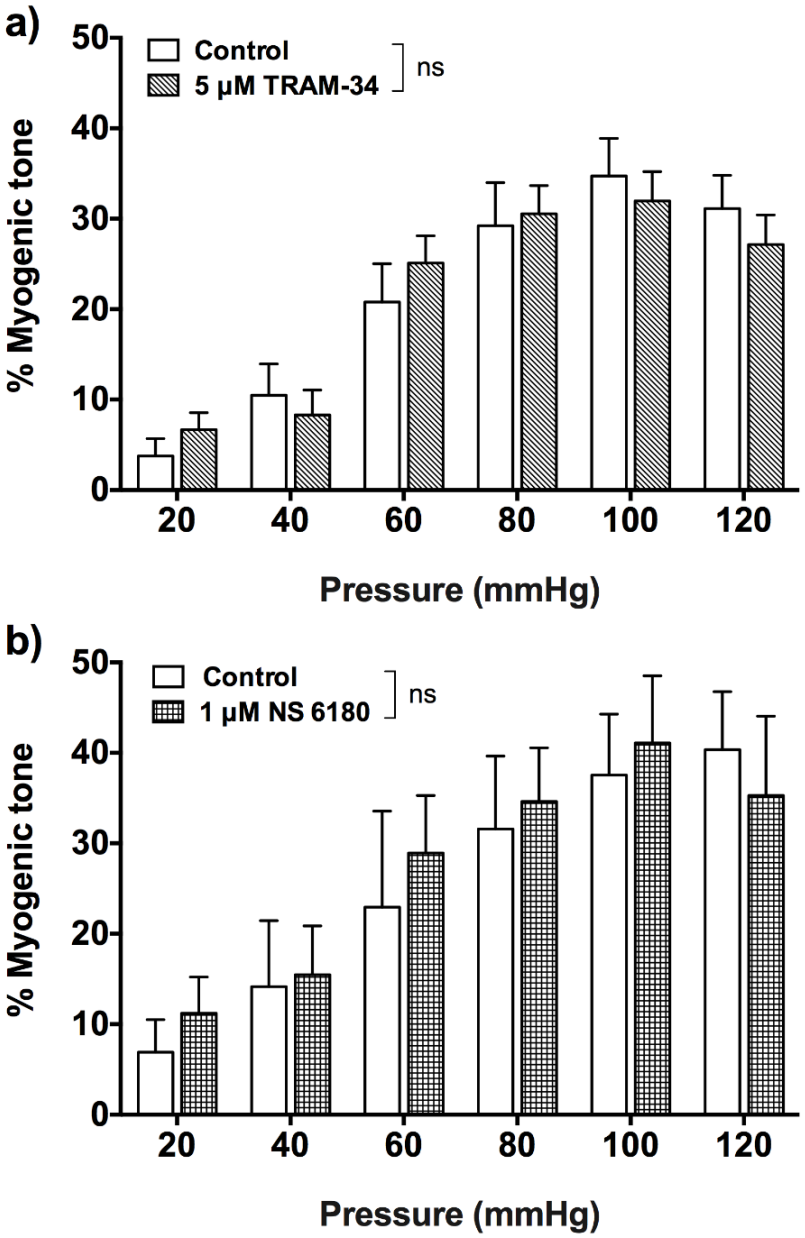



**Figure 4.5: Inhibition of $IK_{Ca}$ channels by TRAM-34 or NS 6180 does not affect myogenic reactivity.** Segments of rat mesenteric resistance artery were mounted in a pressure myograph and subjected to increases in pressure from 20-120 mmHg in 20 mmHg increments. Myogenic tone is expressed as the percentage difference in active diameter versus passive diameter of small mesenteric arteries. Mean % myogenic tone in endothelium-intact arteries in the absence and presence of **a)** 5µM TRAM-34 (n=6) **b)** 1 µM NS 6180 (n=5). Two-way ANOVA was performed ($P>0.05$).

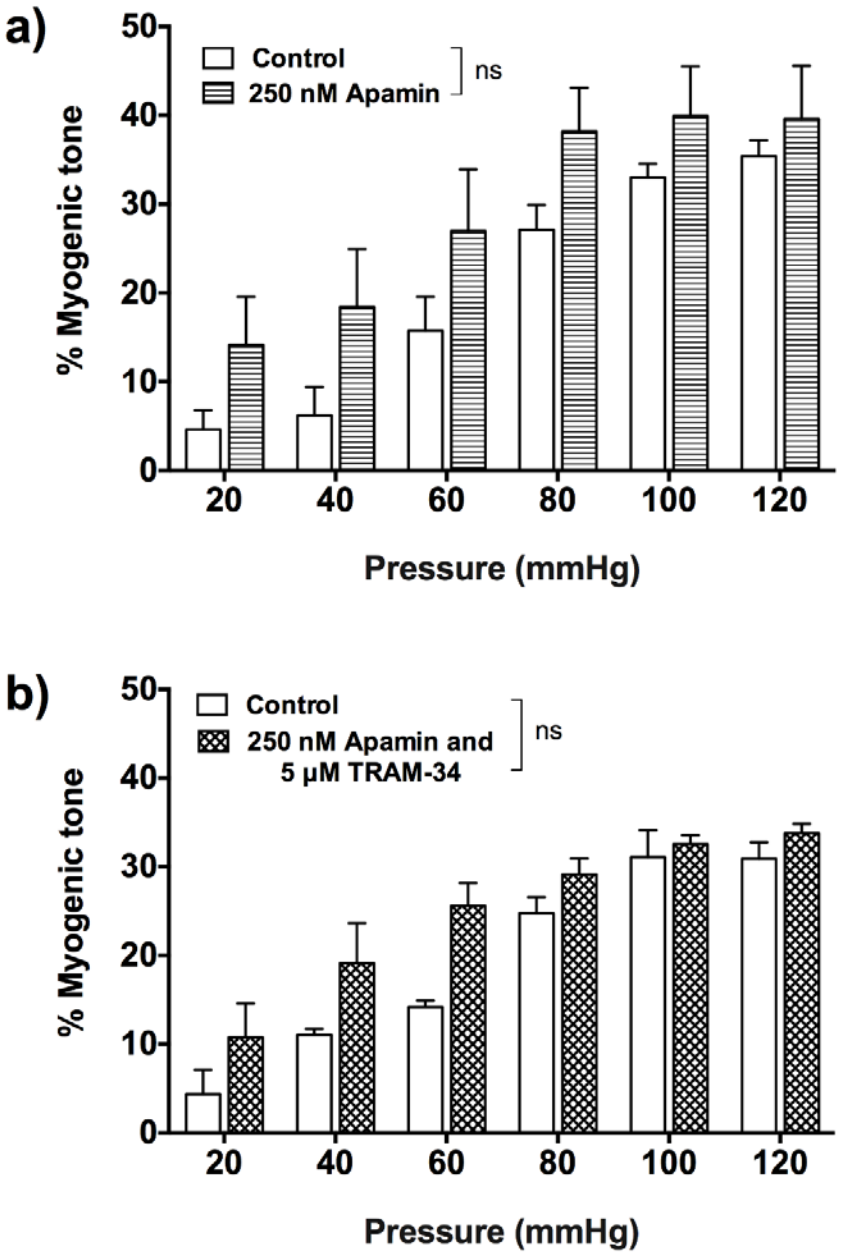



**Figure 4.6: Inhibition of endothelial $SK_{Ca}$ channels by apamin alone and inhibition of both $SK_{Ca}$ and $IK_{Ca}$ channels does not affect myogenic reactivity.** Segments of rat mesenteric resistance artery were mounted in a pressure myograph and subjected to increases in pressure from 20-120 mmHg in 20 mmHg increments. Myogenic tone is expressed as the percentage difference in active diameter versus passive diameter of small mesenteric arteries. Mean % myogenic tone in endothelium-intact arteries in the absence and presence of **a)** 250 nM apamin (n=5). **b)** 250 nM apamin and 5 µM TRAM-34 (n=4). Two-way ANOVA was performed (P>0.05).

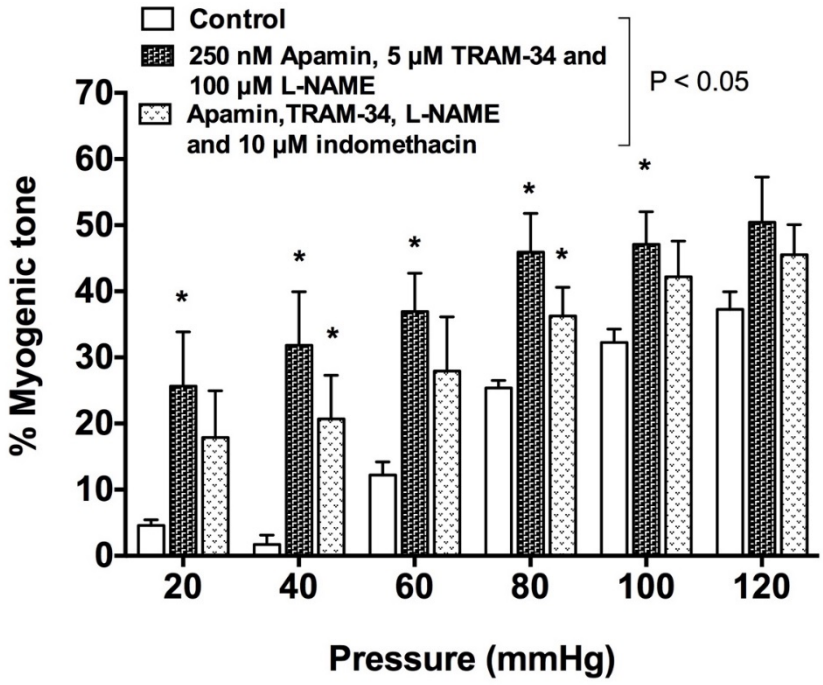



**Figure 4.7: Combined inhibition of NOS, $SK_{Ca}$ and $IK_{Ca}$ channels enhances myogenic reactivity.** Segments of rat mesenteric resistance artery were mounted in a pressure myograph and subjected to increases in pressure from 20-120 mmHg in 20 mmHg increments. Myogenic tone is expressed as the percentage difference in active diameter versus passive diameter of small mesenteric arteries. Mean % myogenic tone in endothelium-intact arteries in the absence and presence of 250nM apamin, 5 µM TRAM-34 and 100 µM L-NAME or 250nM apamin, 5 µM TRAM-34 100 µM L-NAME and 10 µM indomethacin (n=5). Two-way ANOVA was performed, *denotes statistically significant compared to control values ($P<0.05$).

This potentiation of myogenic tone seen with block of both NOS and endothelial $K_{Ca}$ channel activity was in contrast to the lack of effect of endothelial removal on the pressure-induced constriction (**Figure 4.3**). A similar pattern was observed in isolated rat adipose tissue (Undavia et al. 2003) and cerebral arterioles (Bai et al. 2004), and in mesenteric arteries from diabetic mice (Lagaud et al. 2001), leading to the suggestion that the endothelium releases a combination of constricting and dilating factors, such that there is no net effect of endothelium removal. The endothelial COX pathway is a potential source of vasoconstrictor mediators (Butcher et al. 2013; Ellinsworth et al. 2014; Lot et al. 1993) but, the COX inhibitor, indomethacin (10 µM) did not

significantly affect myogenic reactivity (**Figure 4.8**) and did not prevent the enhancement of myogenic tone seen in the presence of inhibitors of NOS and $K_{Ca}$ channels.

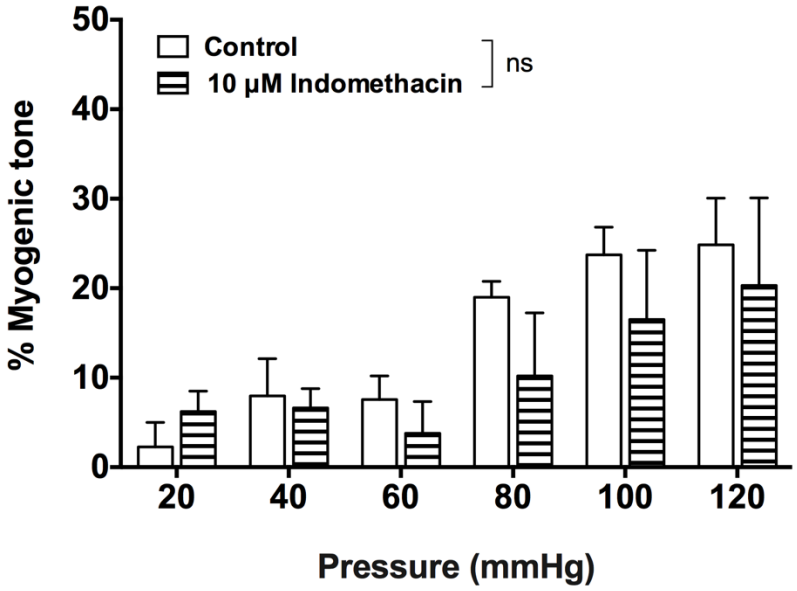



**Figure 4.8: Inhibition of COX does not significantly affect myogenic reactivity.** Segments of rat mesenteric resistance artery were mounted in a pressure myograph and subjected to increases in pressure from 20-120 mmHg in 20 mmHg increments. Myogenic tone is expressed as the percentage difference in active diameter versus passive diameter of small mesenteric arteries. Mean % myogenic tone in endothelium-intact arteries in the absence and presence of 10 µM indomethacin (n=5). Two-way ANOVA was performed (P>0.05).

Having characterized the myogenic reactivity in rat isolated mesenteric arteries, I moved on to determine whether small molecule activators of endothelial $SK_{Ca}$ and $IK_{Ca}$ channels, CyPPA and SKA-31, can modulate myogenic reactivity in these vessels.

*4.3.4 CyPPA and SKA-31 evoke smooth muscle hyperpolarization in mesenteric arteries*: Bolus doses of CyPPA (50 µmoles) and SKA-31 (50 µmoles) each evoked hyperpolarization of the smooth muscle resting membrane potential in endothelium-intact second order mesenteric arteries (**Figure 4.10**; representative traces are included in **Figure 4.9)**. The mean resting membrane potential of smooth muscle cells was -44.6 ± 1.7 mV (n=10). Resting membrane potential was not altered by the block of $SK_{Ca}$ and $IK_{Ca}$ channels inhibitors apamin -40 ± 1.6 mV (n=5) and TRAM-

34 -40.8 ± 1.32 mV (n=5), respectively (P>0.05). CyPPA evoked a hyperpolarization of 10.8 ± 1.2 mV (n=5) which was abolished by apamin (50 nM). SKA-31 caused a hyperpolarization of 9.4 ± 1.8 mV (n=5) which was abolished by TRAM-34 (1 μM).

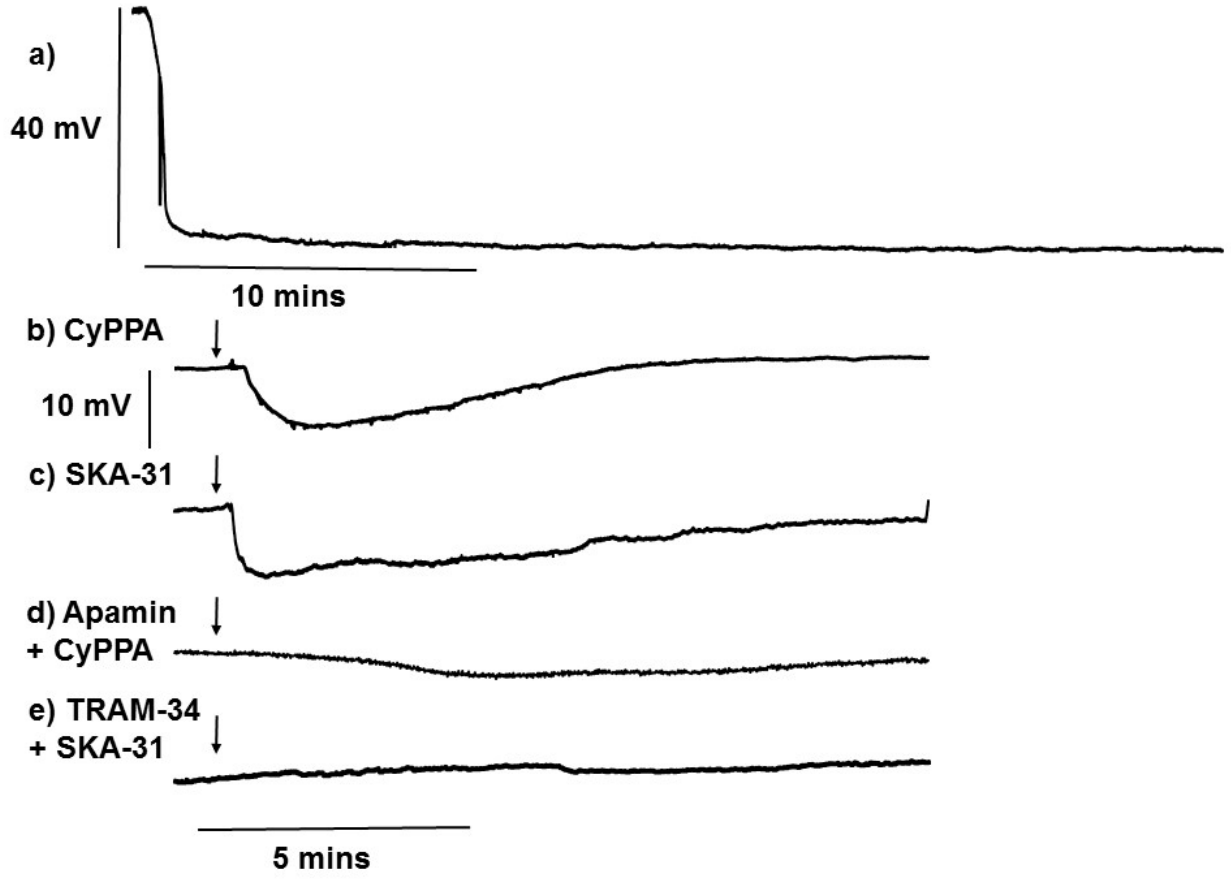



**Figure 4.9: Representative traces of CyPPA and SKA-31 evoked smooth muscle hyperpolarization in rat mesenteric arteries.** Segments of mesenteric artery were pinned out in a superfusion chamber to record smooth muscle membrane potential using sharp glass microelectrodes. Representative traces showing **a)** resting membrane potential; hyperpolarization to **b)** CyPPA (50 μmole) **c)** SKA-31 (50 μmole); hyperpolarization was blocked in presence of **d)** Apamin (50 nM) and CyPPA (50 μmole) **e)** TRAM-34 (1 μM) and SKA-31 (1μM).

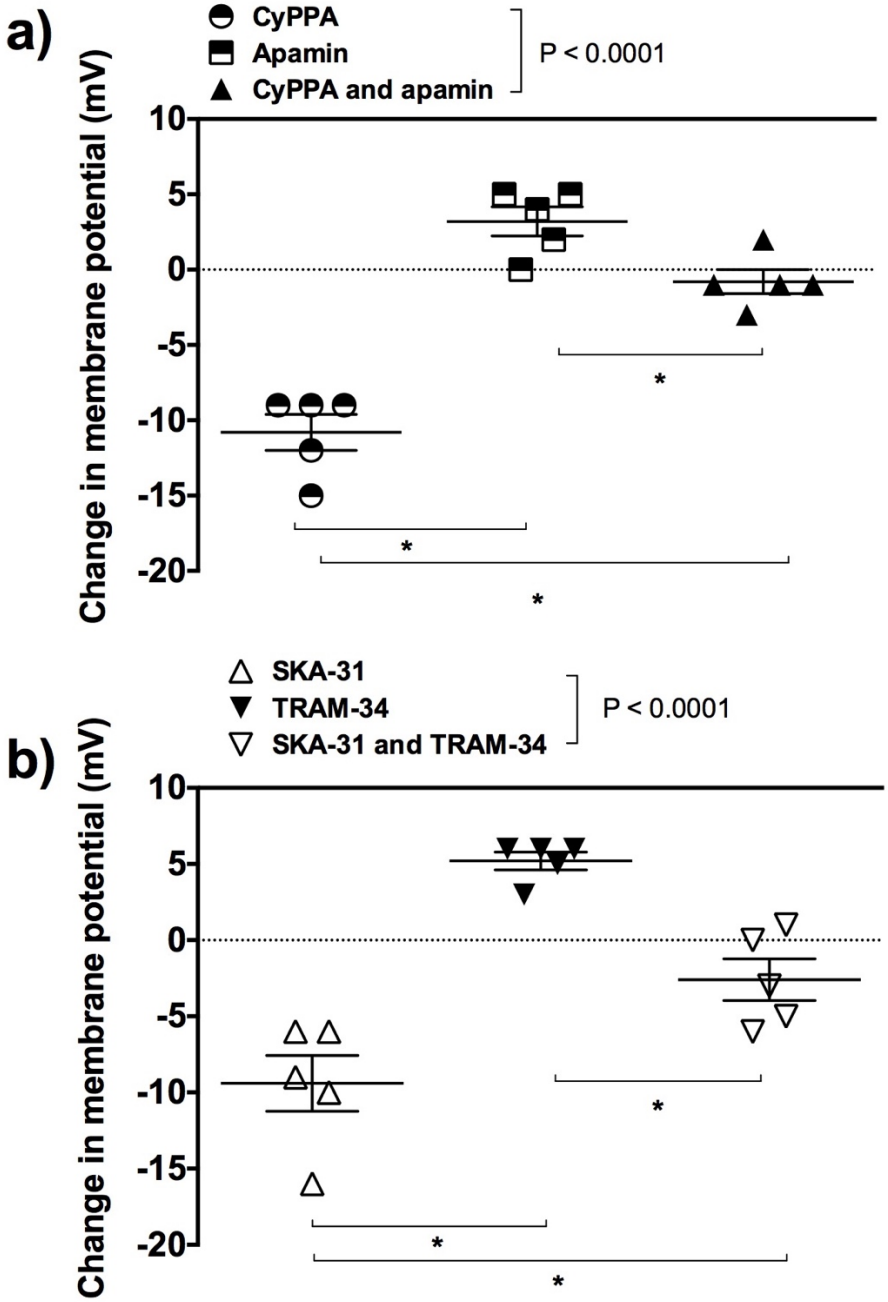



**Figure 4.10: CyPPA and SKA-31 evoked smooth muscle hyperpolarization in rat mesenteric arteries.** Segments of mesenteric artery were pinned out in a superfusion chamber to record smooth muscle membrane potential using sharp glass microelectrodes. Mean data showing membrane potential recorded in the absence and presence of **a)** CyPPA (50 μmoles), apamin (50 nM), and CyPPA and apamin (n=5). **b)** SKA-31 (50 μmoles), TRAM-34 (1 μM) and SKA-31 and TRAM-34 (n=5). One-way ANOVA was performed. *denotes statistically significant P<0.05.

*4.3.5 CyPPA and SKA-31 evoke endothelium-dependent relaxation of pressurized arteries pre-constricted with PE:* The ability of the $K_{Ca}$ channel activators CyPPA and SKA-31 to evoke dilation of PE-induced vasoconstriction was assessed in arteries mounted on a pressure myograph, held at a constant intravascular pressure of 60 mmHg and constricted with PE (3 μM); at this concentration, arterial diameter was reduced from 249.6 ± 11.79 μm to 52.26 ± 12.73 μm (n=13, P<0.05, t-test). In endothelium-intact arteries, CyPPA (10 nM-10 μM) evoked concentration-dependent dilation of PE-induced constriction which was significantly inhibited by either apamin (50 nM) or L-NAME (100 μM; **Figure 4.11a**). In contrast, CyPPA was without effect on arterial diameter in endothelium-denuded arteries (**Figure 4.11b**). Thus, these data show that in rat mesenteric arteries, CyPPA (up to a concentration of 10 μM) elicits endothelium-dependent relaxation which is dependent on both $SK_{Ca}$ channels and NO. For the remaining studies, I used 5 μM CyPPA as at this concentration the response was blocked by apamin.

Experiments following the same format were conducted with SKA-31 (10 nM - 30 μM). SKA-31 evoked concentration-dependent dilation of PE-constricted arteries which were unaffected by L-NAME (**Figure 4.12a)**. Removal of the endothelium (**Figure 4.12b)** or addition of TRAM-34 (5 μM) abolished dilation to SKA-31 but a modest dilation was still observed with 10 μM (**Figure 4.12c**). Apamin was without effect on responses to SKA-31 (**Figure 4.12c**). Thus, these data show that in rat mesenteric arteries, SKA-31 (up to a concentration of 10 μM) elicits endothelium-dependent relaxation of PE-constricted arteries which is dependent on $IK_{Ca}$ channels. For the remaining studies, I used 5 μM SKA-31 was used to examine the effect of $IK_{Ca}$ channels activation on myogenic reactivity in small rat mesenteric arteries.

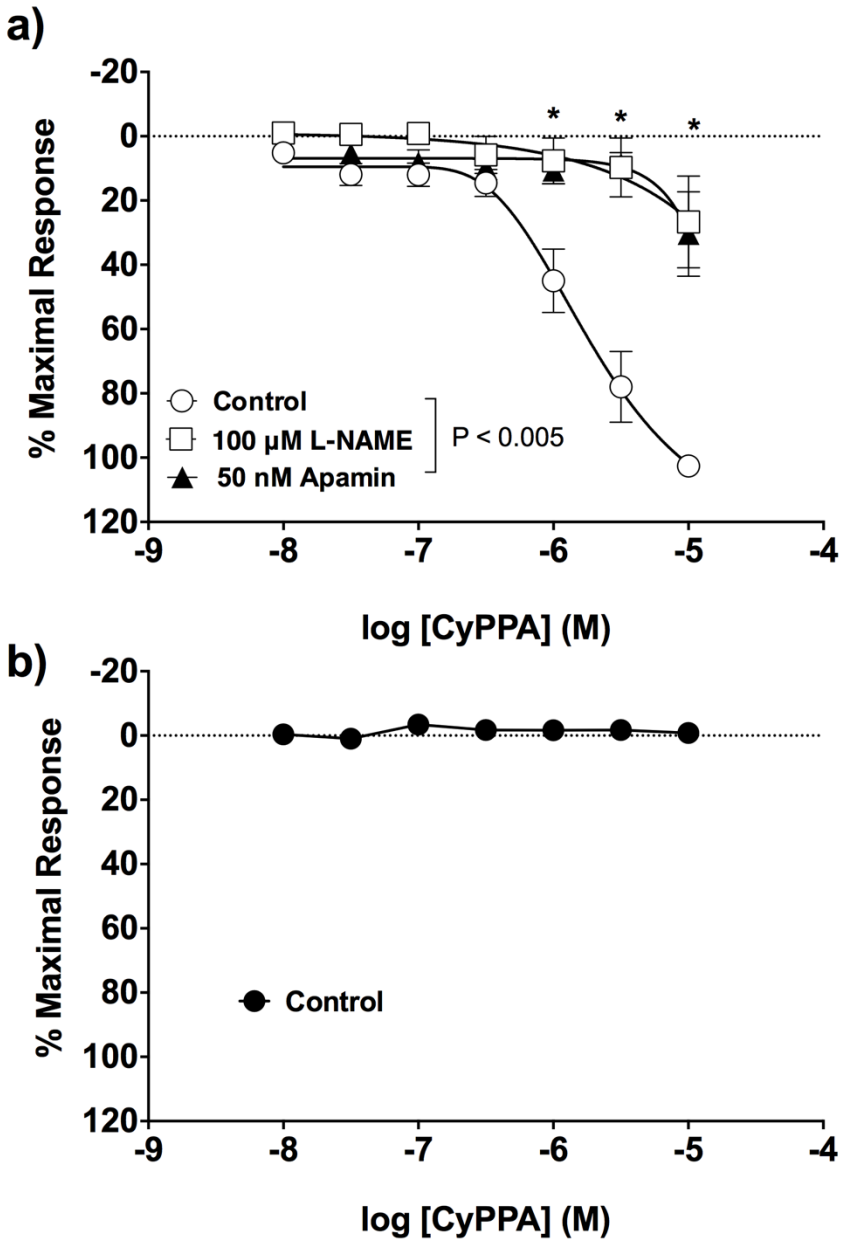



**Figure 4.11: CyPPA evokes endothelium-dependent dilation of arteries pre-constricted with PE, a response dependent on NO and $SK_{Ca}$ channels.** Small resistance mesenteric arteries were mounted on a pressure myograph and pressurized at 60 mmHg. PE (3µM) was used to constrict the vessels. **a)** Mean concentration-response curves for CyPPA-induced reversal of PE-induced tone in endothelium-intact arteries in the presence and absence of 100 µM L-NAME or 50 nM apamin (n=4-7). **b)** Mean concentration-response curves for CyPPA-induced reversal of PE-induced tone in endothelium-denuded arteries (n=5). Values shown as mean ± SEM. Two-way ANOVA was performed, *denotes statistically significant compared to control values ($P<0.05$).

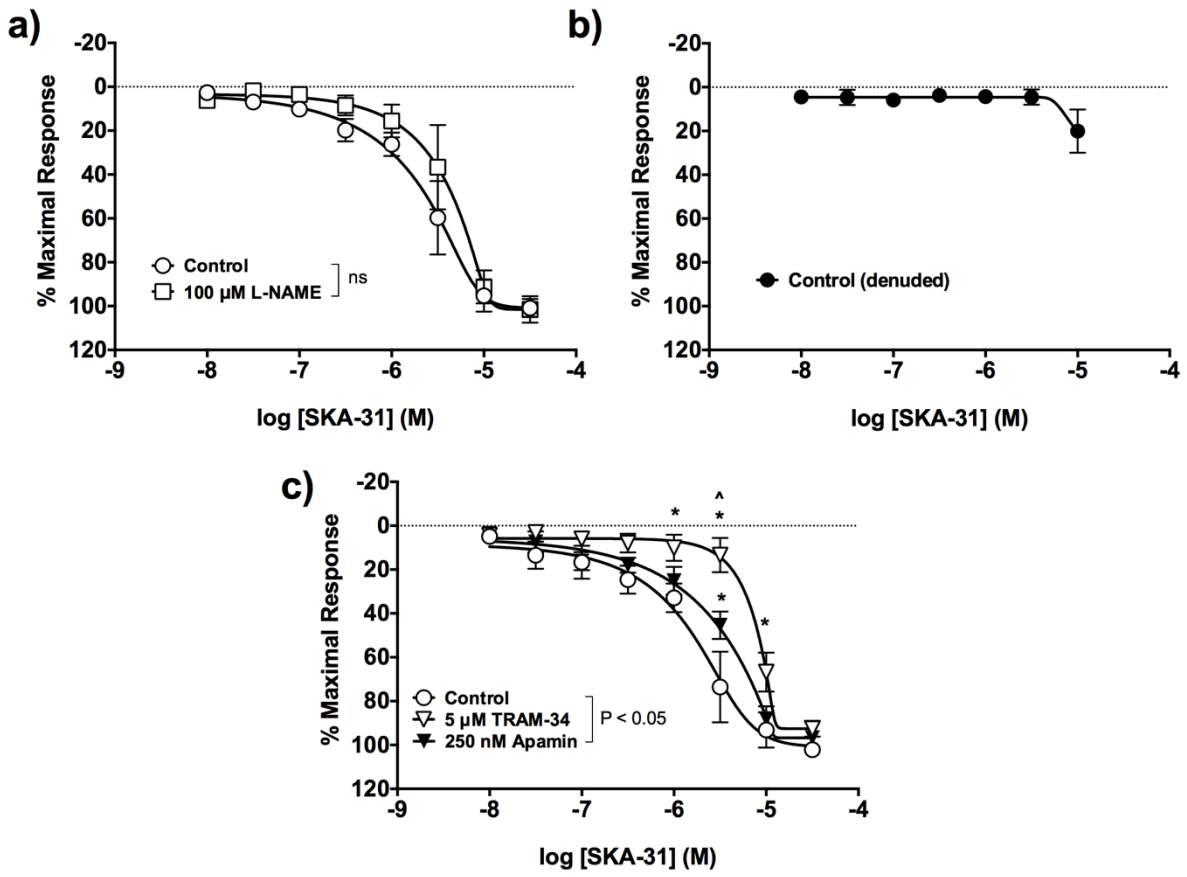



**Figure 4.12: SKA-31 evokes dilation of arteries pre-constricted with PE, a response that is dependent on the endothelium and $IK_{Ca}$ channels but not dependent on NO nor $SK_{Ca}$.** Small resistance mesenteric arteries were mounted on a pressure myograph and pressurized at 60 mmHg. PE was used to constrict the vessels. Mean concentration-response curves for SKA-31-induced reversal of PE-induced tone in the **a)** presence and absence of 100 µM L-NAME (n=5) **b)** in endothelium-denuded arteries (n=5) **c)** presence and absence of 5 µM TRAM-34 or 250 nM apamin (n=5). Values shown as mean ± SEM (n=5), two-way ANOVA was performed. *denotes statistically significant compared to control values ($P<0.05$). ^denotes statistically significant compared to apamin treated vessels ($P<0.05$).

*4.3.6 CyPPA inhibits myogenic reactivity in an endothelium- and NO-dependent manner:* In the presence of CyPPA (5 μM), myogenic reactivity was abolished at intravascular pressures of 40 to 120 mmHg (**Figure 4.13b**). The diameter values at 80 mmHg for control, 5μM CyPPA and $Ca^{2+}$ free condition were 245.166 ± 13.67 μm, 340.01 ± 12.45 μm and 345.54 ± 10.676 μm (n=5), respectively. This effect was lost in endothelium-denuded arteries in which diameters in the presence of CyPPA were not significantly different from controls (P>0.05) (**Figure 4.13c**).

Apamin, a selective inhibitor of $SK_{Ca}$ channels, blocked the effect of CyPPA in endothelium-intact arteries and thus restored myogenic reactivity at intravascular pressures 60-120 mmHg (**Figure 4.14a**). The effect of CyPPA in endothelium-intact arteries was also abolished by L-NAME (100 μM) which restored the myogenic reactivity at intravascular pressures 60-120 mmHg, such that the % myogenic tone in presence of CyPPA and L-NAME was not significantly different compared to the percent of myogenic tone obtained under control condition (**Figure 4.14b**). Thus, CyPPA inhibits myogenic reactivity through endothelial $SK_{Ca}$ channels and is NO-dependent.

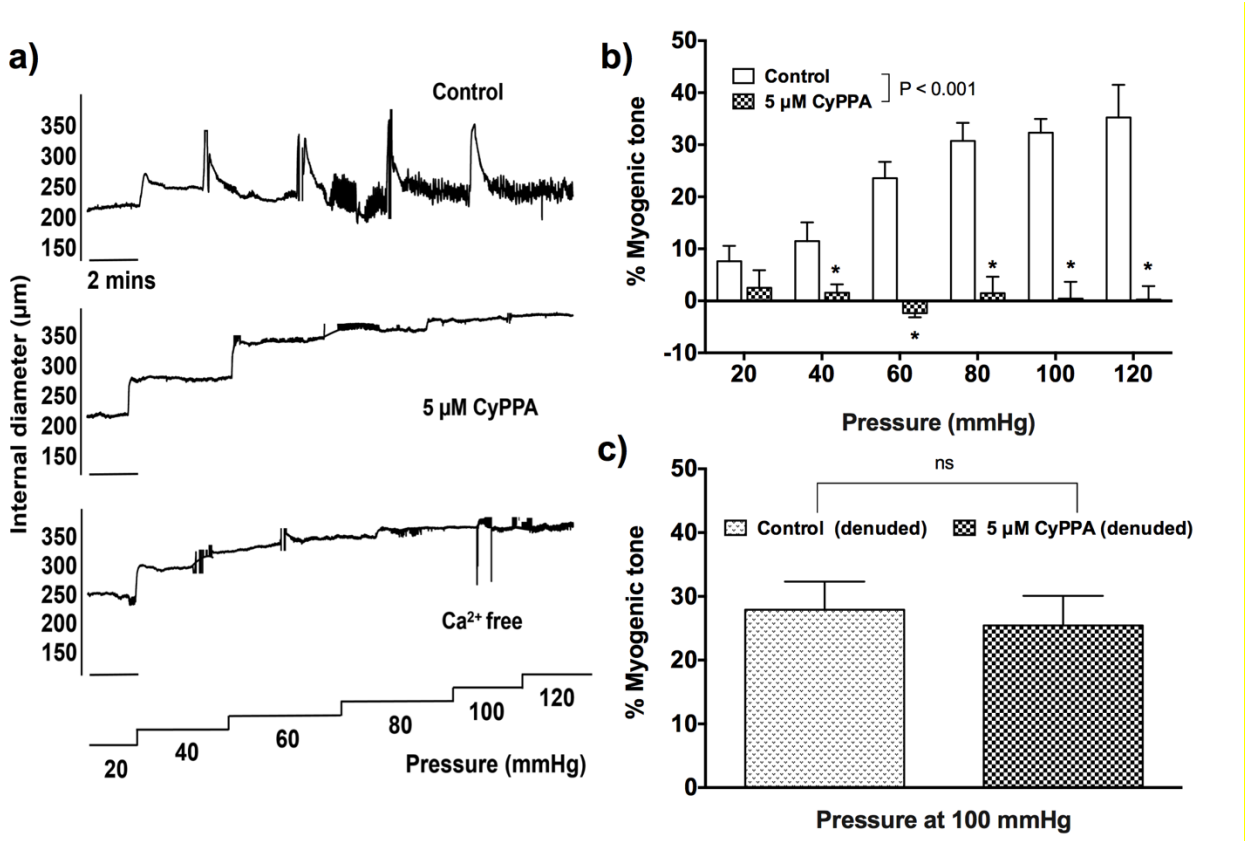



**Figure 4.13: CyPPA inhibits myogenic reactivity in an endothelium-dependent manner.** Segments of rat mesenteric resistance artery were mounted in a pressure myograph and subjected to increases in pressure from 20-120 mmHg in 20 mmHg increments. Myogenic tone is expressed as the percentage difference in active diameter versus passive diameter of small mesenteric arteries. **a)** Representative traces of pressure-diameter relationships in an endothelium-intact artery in the absence and presence of 5 µM CyPPA and extracellular $Ca^{2+}$. Mean % myogenic tone in **b)** endothelium-intact arteries in the absence and presence of 5 µM CyPPA (n=6) **c)** endothelium-denuded arteries at 100 mmHg in the absence and presence of 5 µM CyPPA (n=5). Two-way ANOVA and paired t-test was performed *denotes statistically significant compared to control values ($P<0.05$).

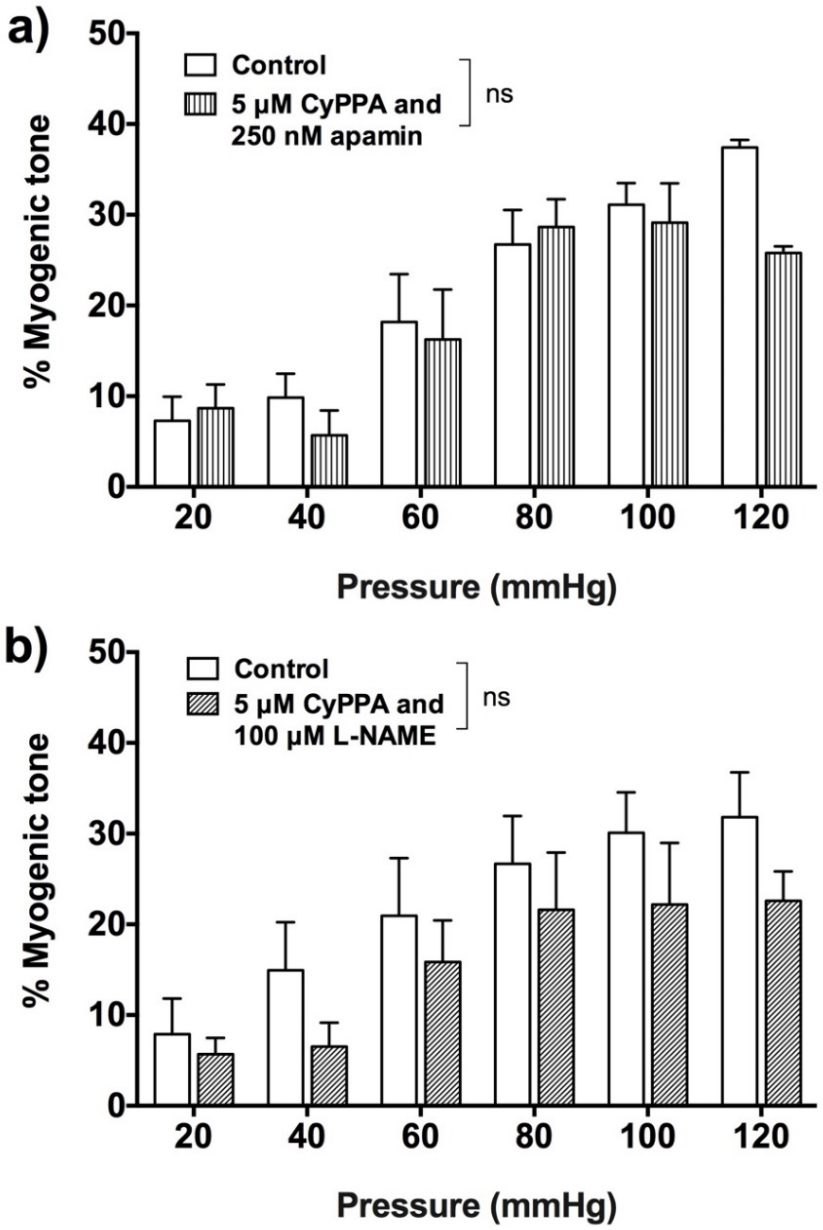



**Figure 4.14: The effect of CyPPA is blocked by apamin and L-NAME.** Segments of rat mesenteric resistance artery were mounted in a pressure myograph and subjected to increases in pressure from 20-120 mmHg in 20 mmHg increments. Myogenic tone is expressed as the percentage difference in active diameter versus passive diameter of small mesenteric arteries. Mean % myogenic tone in endothelium-intact arteries in the absence and presence of **a)** 5 µM CyPPA and 250 apamin (n=5) **b)** 5 µM CyPPA and 100 µM L-NAME (n=5). Two-way ANOVA was performed (P>0.05).

*4.3.7 SKA-31 inhibits myogenic reactivity in an endothelium-independent manner:* In the presence of SKA-31 (5 μM), myogenic reactivity was abolished at intravascular pressures of 40 to 120 mmHg (**Figure 4.15a, b**). The diameter values at 80 mmHg for control, 5μM SKA-31 and $Ca^{2+}$ free condition were 215.59 ± 20.92 μm, 309.16 ± 15.07 μm and 304.11 ± 18.93 μm (n=6), respectively. In contrast to CyPPA, the inhibition of myogenic reactivity caused by SKA-31 was not affected by removal of the endothelium (**Figure 4.15c**). In endothelium-denuded arteries, the diameter values at 80 mmHg for control and SKA-31 and $Ca^{2+}$ free condition were 218.51 ± 18.13 μm, 296.52 ± 16.03 μm and 316.32 ± 15.79 μm (n=5), respectively. This contrasts with the effect of SKA-31 on PE-evoked tone (**Figure 4.12b**) that showed under those conditions, the effect was endothelium-dependent. In the presence of the selective $IK_{Ca}$ channels inhibitor TRAM-34 (5 μM; **Figure 4.16**), the attenuation of the myogenic reactivity by SKA-31 was not statistically different to control.

To investigate the potential contribution of $K_{ir}$ channels to the actions of CyPPA and SKA-31, I first examined the expression of $K_{ir}$ channels in these arteries and the ability of increases in extracellular KCl to evoke $K_{ir}$-dependent hyperpolarization and relaxation to demonstrate the $K_{ir}$ channels are functional in rat mesenteric arteries. I used $Ba^{2+}$ (50 -100 μM) to examine the role of $K_{ir}$ channels in rat mesenteric arteries as this concentration range has been shown to selectively block $K_{ir}$ channels (Nelson and Quayle 1995).

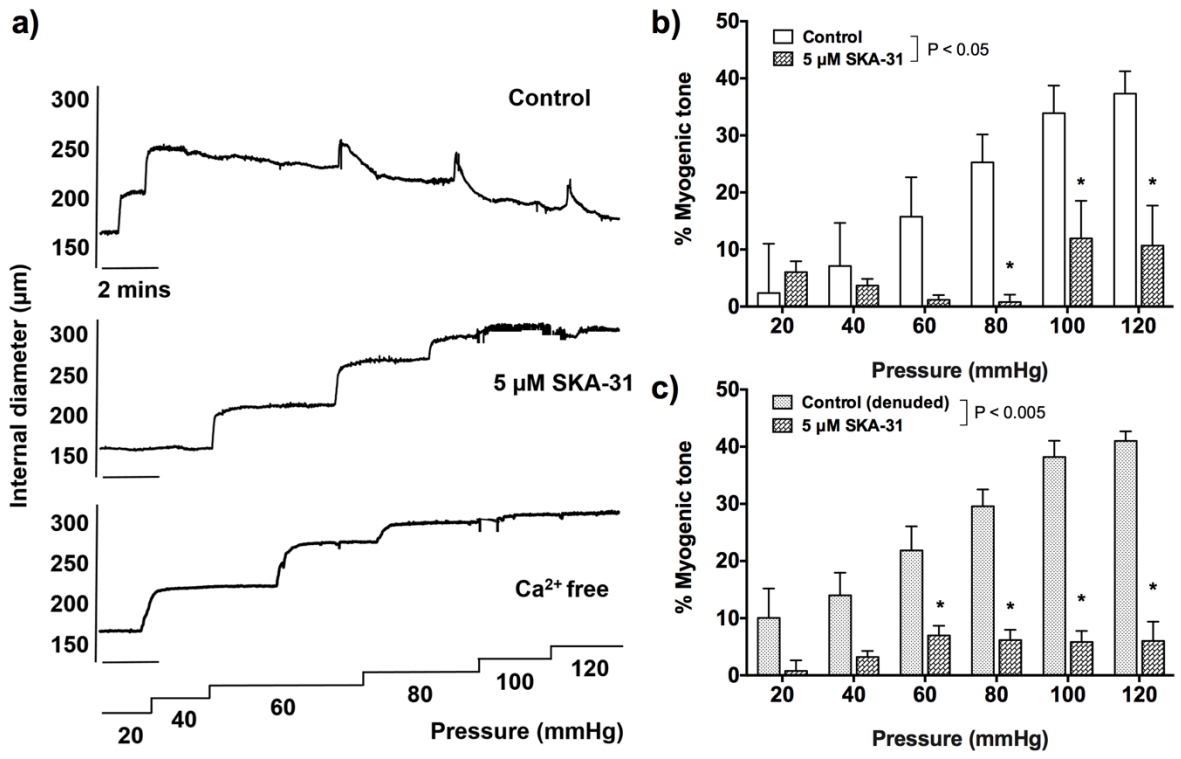



**Figure 4.15: Activation of $IK_{Ca}$ channels inhibits myogenic reactivity in an endothelium-independent manner.** Segments of rat mesenteric resistance artery were mounted in a pressure myograph and subjected to increases in pressure from 20-120 mmHg in 20 mmHg increments. Myogenic tone is expressed as the percentage difference in active diameter versus passive diameter of small mesenteric arteries. **a)** Representative trace of pressure-diameter relationships in endothelium-intact artery in the absence and presence of 5 µM SKA-31 and extracellular $Ca^{2+}$. Mean % myogenic tone in **b)** endothelium-intact arteries in the absence and presence of 5 µM SKA-31 (n=5) **c)** in endothelium-denuded arteries in the absence and presence of 5 µM SKA-31 (n=4). Two-way ANOVA was performed. *denotes statistically significant compared to control values ($P<0.05$).

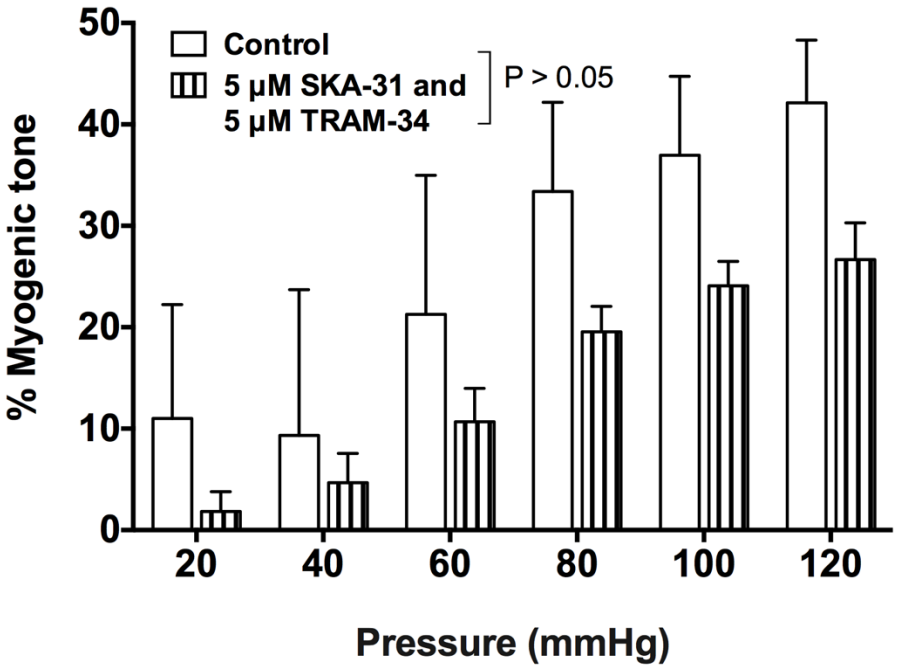



**Figure 4.16: The effect of SKA-31 is blocked by TRAM-34.** Segments of rat mesenteric resistance artery were mounted in a pressure myograph and subjected to increases in pressure from 20-120 mmHg in 20 mmHg increments. Myogenic tone is expressed as the percentage difference in active diameter versus passive diameter of small mesenteric arteries. Mean % myogenic tone in endothelium-intact arteries in the absence and presence of 5 µM SKA-31 and 5 µM TRAM-34 (n=5). Two-way ANOVA was performed (P>0.05).

*4.3.8 Expression of $K_{ir}$ channels in rat mesenteric arteries:* The expression of mRNA for $K_{ir}$ channel subtypes in rat mesenteric arteries was investigated using qPCR with expression levels compared to ß-actin expression. The expression of mRNA for $K_{ir}$2.1, 2.2, 2.4 was identified along with lower levels of expression of the non-classical subtypes $K_{ir}$ 7.1 and 3.4 (n=4). $K_{ir}$1.1 and 3.1 were not detected (**Figure 4.17**). Heart, brain and kidney were used as positive controls. The expression of $K_{ir}$2.1 and 2.2 channel proteins were identified in whole mount preparations of third order rat mesenteric arteries using immunohistochemistry (**Figure 4.18**).

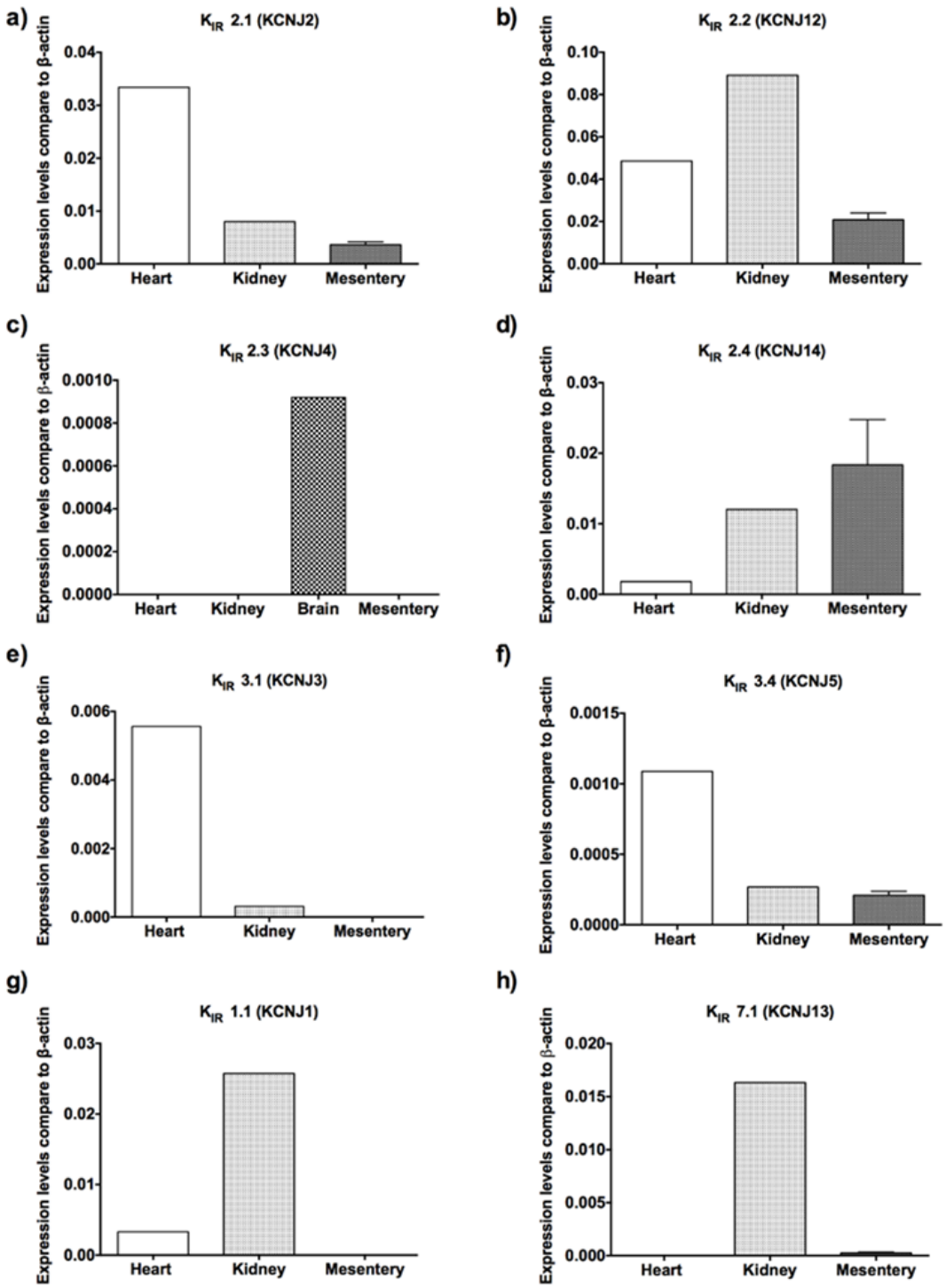
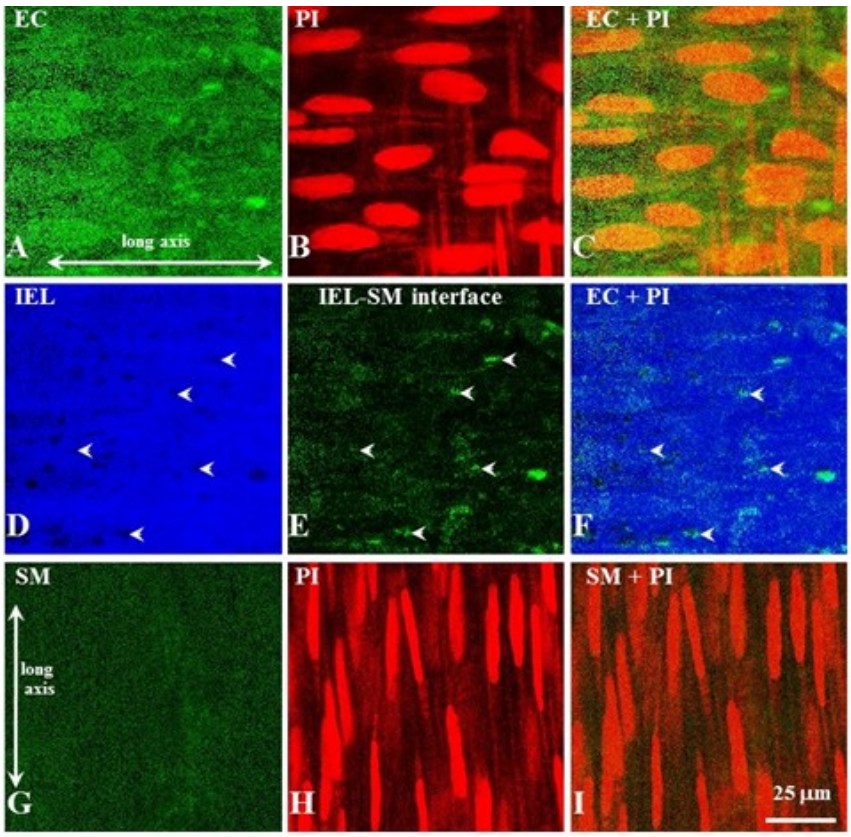



**Figure 4.17: mRNA expression of $K_{ir}$ channels in mesenteric arteries.** Expression levels of $K_{ir}$2.1, 2.2, 2.3, 2.4, 3.1, 3.4, 1.1 and 7.1 mRNA determined in rat mesenteric arteries using qPCR analysis. Relative expression levels are compared to the level of ß-actin and expressed as $2^{-\Delta C_t}$, $\Delta C_t = C_{t\,(gene)} - C_{t\,(ß\text{-}actin)}$. Data were obtained from mesenteric arteries isolated from 4 different rats. Tissue from heart, kidney and brain was used as a positive control.

a)


EC
PI
EC + PI
A
long axis
B
C
IEL
IEL-SM interface
EC + PI
D
E
F
SM
PI
SM + PI
long axis
G
H
I
25 µm

b)

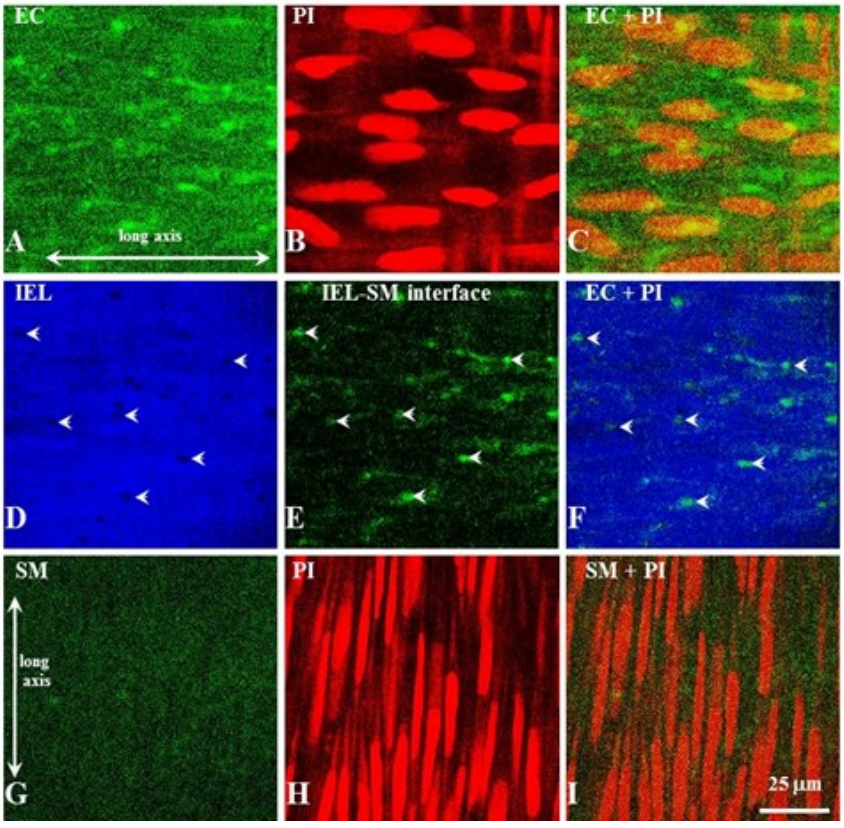

EC
PI
EC + PI
A
long axis
B
C
IEL
IEL-SM interface
EC + PI
D
E
F
SM
PI
SM + PI
long axis
G
H
I
25 µm

**Figure 4.18: Localization of $K_{ir}$2.1 and 2.2 channel proteins in third order rat mesenteric arteries using confocal immunohistochemistry.** The expression of $K_{ir}$2.1 protein (**aA**; green) and $K_{ir}$2.2 protein (**bA**; green) are found on endothelial cells surface. **aB** and **bB** indicate endothelial cell (EC) nuclei (red). Expression of $K_{ir}$2.1 (**aC**) and $K_{ir}$2.2 (**bC**) both overlap with where EC nuclei are found. Panels **aD** and **bD** indicate autofluorences of internal elastic lamina (IEL; blue), black dots indicate holes in the IEL. Expression of both $K_{ir}$2.1 (**aE**) and $K_{ir}$2.2 (**bE**) are also found in the interface between IEL and smooth muscle (SM) overlapping with holes in the IEL (**aF** and **bF**). Expression of $K_{ir}$2.1 (**aG, aI**) and $K_{ir}$2.2 (**bG, bI**) are also identified on the surface of SM (green). **aH** and **bH** indicated smooth muscle (SM) nuclei stained in red. $K_{ir}$2.1 and $K_{ir}$2.2 antibodies are from Mark Chen, GlaxoSmithKline, Stevenage, U.K., and Craig Neylon, University of Melbourne, respectively. Peptide block and incubation in secondary antibody alone resulted in no labelling (data not shown), Antibody characterization, as per previous studies (Chadha et al. 2010; Sandow et al. 2009). Propidum iodide stains for nuclei. Longitudinal vessel axis left to right, with n=3, each from a different animal. Confocal imaging carried out by Dr. Shaun Sandow, University of the Sunshine Coast, Australia.

*4.3.9 Increases in extracellular KCl evoked smooth muscle cell hyperpolarization in mesenteric arteries:* Segments of rat mesenteric arteries were cut open longitudinally and pinned to the bottom of a Sylgard chamber. Sharp glass electrode was used to record smooth muscle membrane potential in endothelium-intact and denuded arteries (**Figure 4.19**). The resting membrane potential was not significantly different between endothelium-intact and denuded arteries (-49.75 ± 1.38 vs -44.5 ± 2.63; n=4; P>0.05). Application of $Ba^{2+}$ (50 μM), a selective blocker of $K_{ir}$ channels, caused a significant depolarization of smooth muscle cells in all arteries suggesting that $K_{ir}$ channels are involved in regulation of the resting membrane potential of mesenteric arteries. At a concentration of 7 mM, KCl evoked smooth muscle hyperpolarization which was not different between endothelium-intact and denuded arteries and were both inhibited by $Ba^{2+}$ (50 μM). However, at a concentration of 15 mM, KCl hyperpolarized endothelium-intact arteries but not endothelium-denuded arteries, an effect blocked by $Ba^{2+}$ (50 μM; **Figure 4.19b**).

*4.3.10 Investigation of the ability of $K_{ir}$ channels to modulate tone in mesenteric arteries:* The ability of $K_{ir}$ channels to mediate vasorelaxation was determined by application of KCl to arteries

mounted in a wire myograph. At concentrations between 5 and 13 mM, KCl evoked relaxation of PE-induced tone in both endothelium-intact and denuded mesenteric arteries; further addition of KCl reduced evoked relaxations in a concentration dependent manner. The effect of KCl was abolished by $Ba^{2+}$ (50 μM; **Figure 4.20**). The pattern of KCl-induced relaxation in endothelium-denuded arteries is statistically different compare to the pattern in endothelium intact arteries ($P<0.05$).

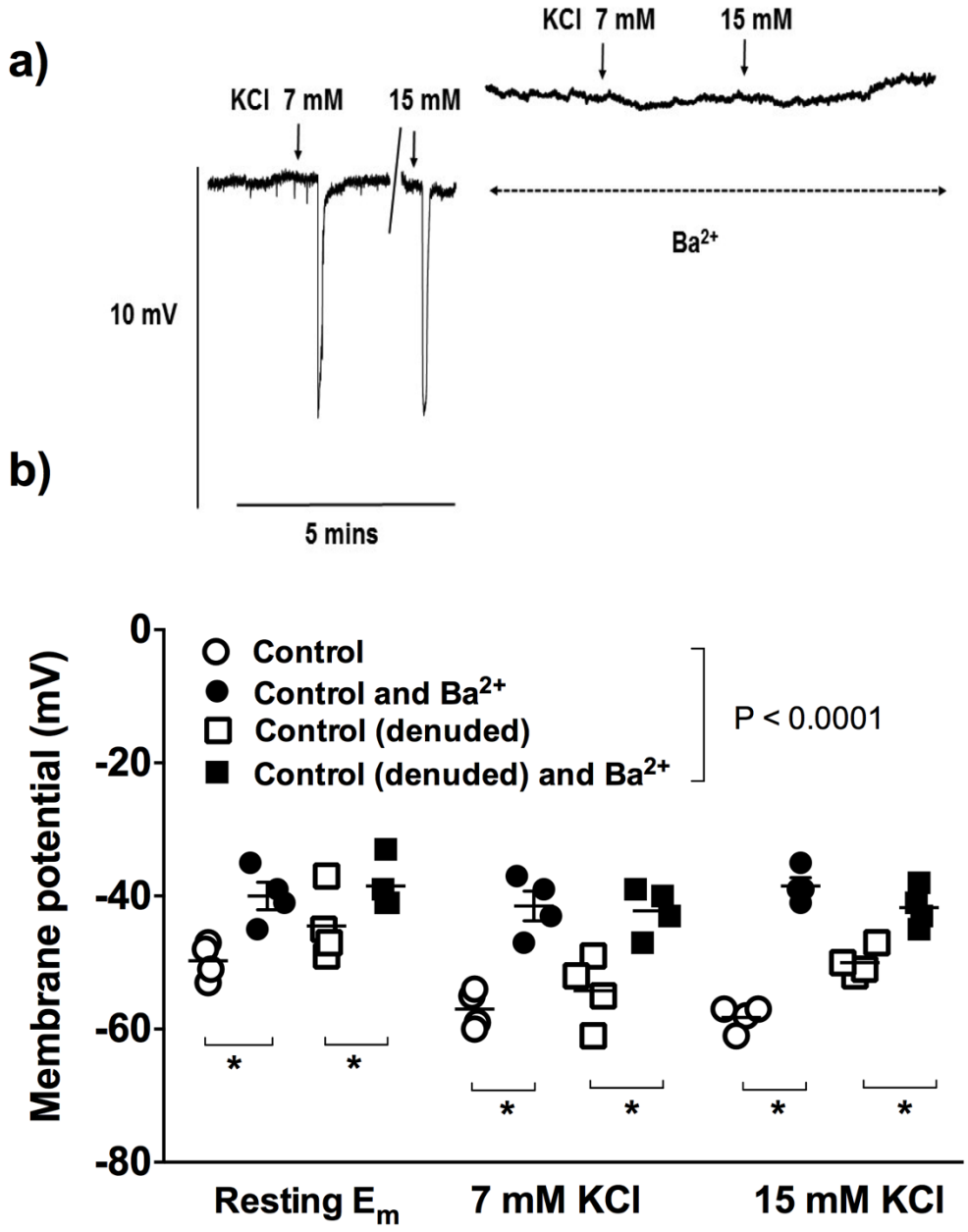



**Figure 4.19: KCl-evoked smooth muscle hyperpolarization which was inhibited by $Ba^{2+}$.** Segments of mesenteric artery were pinned out in a superfusion chamber to record smooth muscle membrane potential using sharp glass microelectrodes. **a)** Representative trace showing hyperpolarization to KCl (7 mM and 15 mM) in the absence and presence of $Ba^{2+}$ (50 μM). **b)** Mean data showing membrane potential recorded in the absence and presence of 7 mM and 15 mM KCl and/or $Ba^{2+}$ (50 μM). Values shown as mean ± SEM (n=4). Two-way ANOVA was performed. *denotes statistically significant $P<0.05$.

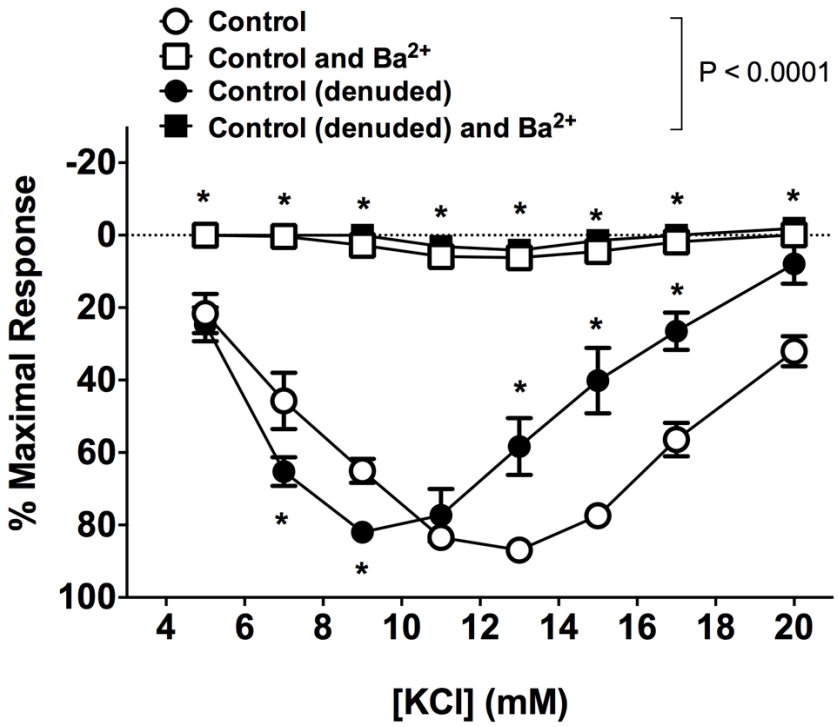



**Figure 4.20: $K_{ir}$ channels mediate relaxations to raised extracellular KCl in rat mesenteric arteries.** Segments of rat mesenteric resistance artery were mounted in a wire myograph and tone induced with PE (3 μM). Mean concentration-response curves to KCl endothelium-intact and denuded arteries in the presence and absence of 50 μM $Ba^{2+}$ (n=4). Values shown as mean ± SEM. Two-way ANOVA was performed. *denotes statistical significance compared to endothelium-intact values (P<0.05).

The ability of $K_{ir}$ channels to mediate vasodilation of myogenically active vessels was determined in arteries mounted in a pressure myograph and maintained at an intravascular pressure of 80 mmHg. KCl-induced dilation was observed up to a concentration of 13 mM in endothelium-intact arteries and the response was abolished with the presence of 50 μM $Ba^{2+}$ (**Figure 4.21b**). In endothelium-denuded arteries, KCl-induced relaxation was observed up to a concentration of 9 mM and was abolished by $Ba^{2+}$ (50 μM; **Figure 4.21c**).

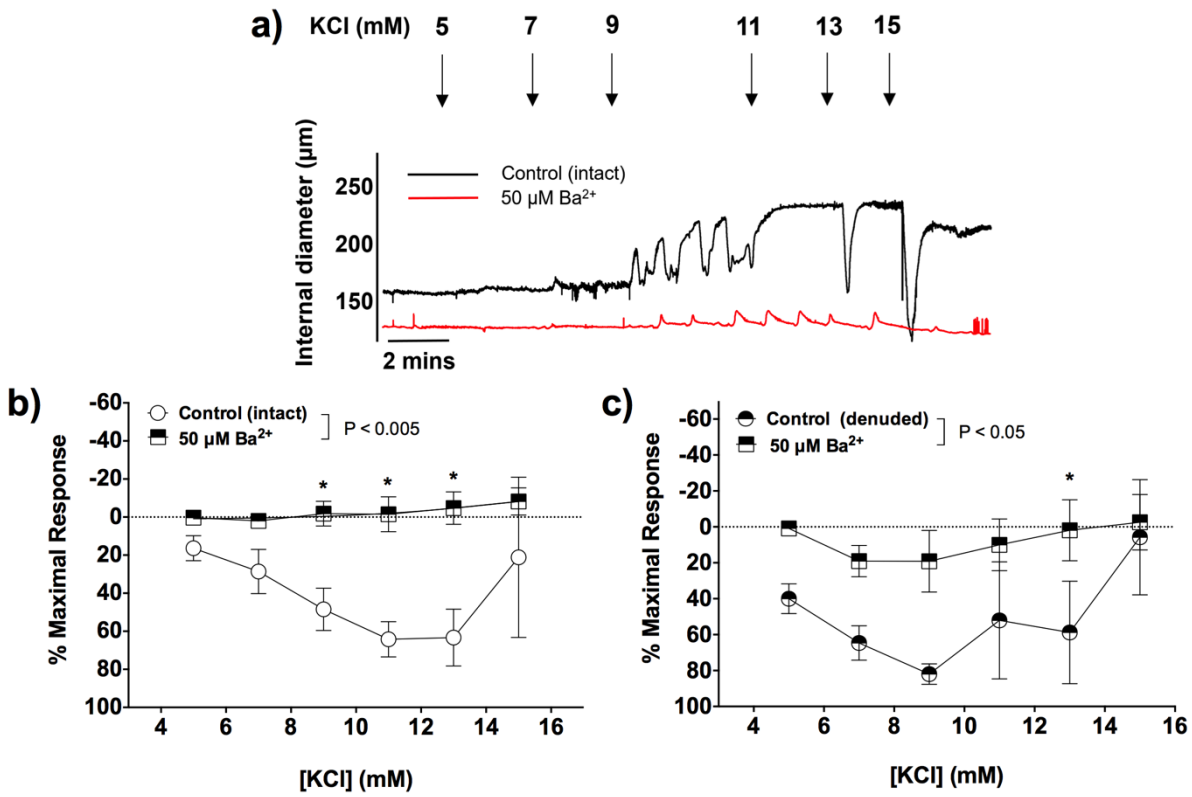



**Figure 4.21: $K^+$-induced dilation is inhibited by $Ba^{2+}$.** Small resistance mesenteric arteries were mounted on a pressure myograph and pressurized at 80 mmHg. **a)** Representative trace of KCl-induced vasodilation in endothelium-intact artery in absence and presence of 50 µM $Ba^{2+}$. KCl concentration-response curves performed in **b)** endothelium-intact arteries in the presence and absences of 50 µM $Ba^{2+}$ (n=5). **c)** endothelium-denuded arteries in the presence and absence of 50 µM $Ba^{2+}$ (n=6). Values shown as mean ± SEM. Two-way ANOVA was performed. *denotes statistically significant compared to control values (P<0.05).

$Ba^{2+}$ blocks all $K_{ir}$ channels and so to investigate the contribution of $K_{ir}2$ and 3 series channels in KCl-induced dilation, the effects of ML-133 (Wu et al. 2010), an inhibitor of $K_{ir}2$ series channels, and tertiapin (Jin and Lu 1999) an inhibitor of $K_{ir}3.1/3.4$ on KCl-evoked responses in endothelium-intact myogenically active arteries was determined. ML-133 (20 µM) significantly inhibited $K^+$-induced dilation (**Figure 4.22a**) but tertiapin (300 nM) was without effect (**Figure 4.22b**) suggesting that $K_{ir}2$ series but not $K_{ir}3.1/3.4$ may mediate the functional response of these vessels to increases in extracellular $K^+$.

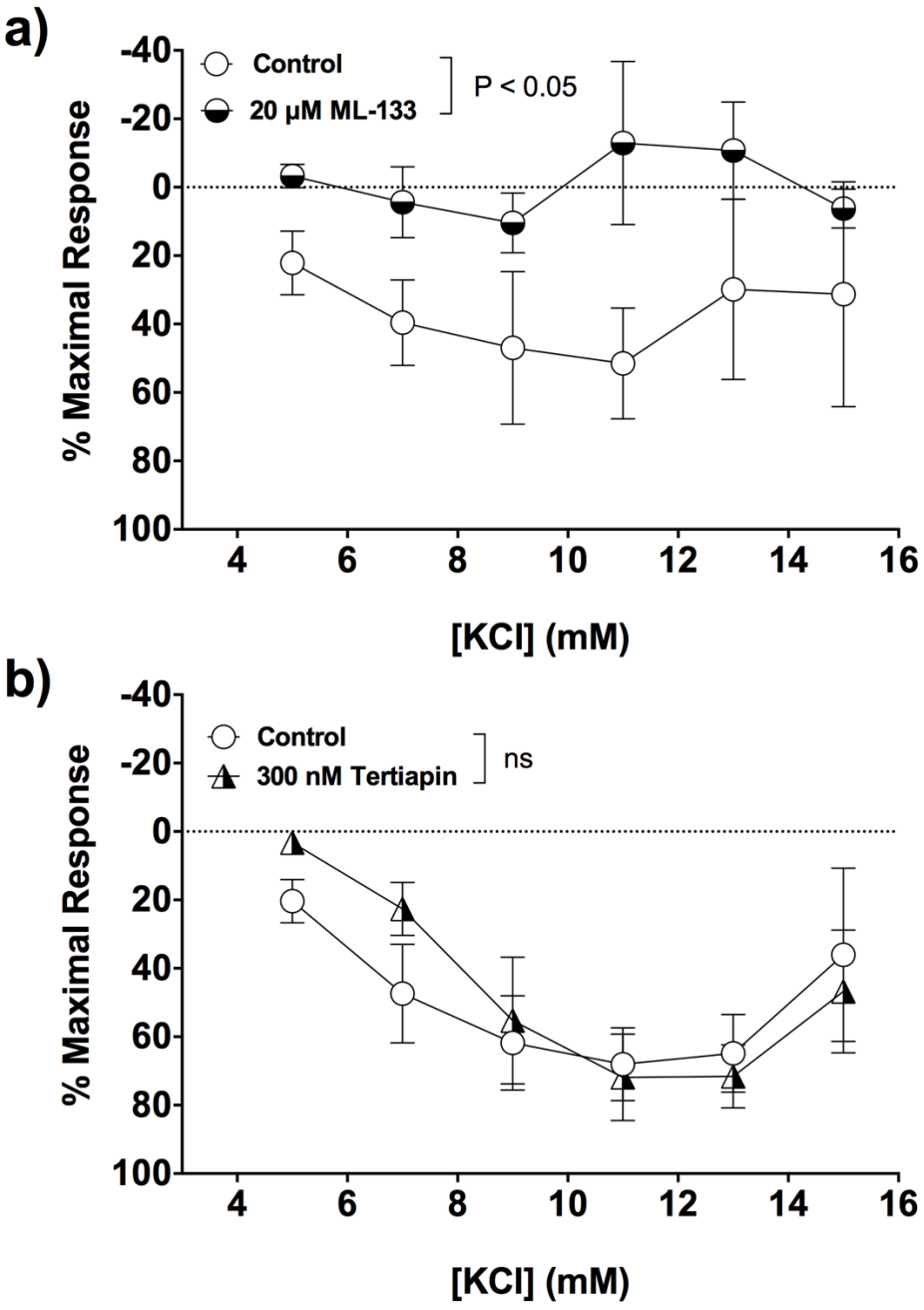



**Figure 4.22: ML-133, a blocker of $K_{ir}2$ series channels, inhibits $K^+$-induced dilation.** Small resistance mesenteric arteries were mounted on a pressure myograph and pressurized at 80 mmHg. KCl concentration-response curves were conducted in the presence and absence of **a)** 20 µM ML-133 and **b)** 300 nM tertiapin, (both n=5). Values shown as mean ± SEM, two-way ANOVA. * denotes statistically significant compared to control values.

*4.3.11 Investigation of the functional contribution of $K_{ir}$ channels to ACh-evoked relaxation in rat mesenteric arteries:* The contribution of $K_{ir}$ channels to ACh-evoked relaxation of PE-induced tone was examined in 3rd order mesenteric resistance arteries mounted in a wire myograph using $Ba^{2+}$ to block $K_{ir}$ channels. ACh-evoked concentration-dependent relaxation that was attenuated by $Ba^{2+}$ (100 μM) $pEC_{50}$ value decreased from 7.12 ± 0.13 to 6.94 ± 0.12 (n=5, P<0.05; **Figure 4.23e**). However, it was not inhibited by either NOS inhibitor L-NAME (100 μM) nor L-NAME and $Ba^{2+}$ (**Figure 4.23e**).

Block of endothelial $SK_{Ca}$ and $IK_{Ca}$ channels with apamin and TRAM-34, caused a rightward shift in the concentration-response curve to ACh and significantly decreased the $pEC_{50}$ value from 7.47 ± 0.17 to 6.22 ± 0.42 (n=5; **Figure 4.23c**). In the presence of these blockers, $Ba^{2+}$ (100 μM) caused further inhibition of ACh-evoked relaxation reducing the maximum relaxation from 97.1 ± 1.56 to 56.42 ± 10.35 % (n=5; **Figure 4.23c**). Inhibition of both endothelial $K_{Ca}$ channels and NOS caused a significant attenuation of relaxation to all concentrations of ACh and reduced the maximum relaxation from 97.64 ± 0.66 to 47.34 ± 14.78 % (n=5). Under these conditions, addition of $Ba^{2+}$ abolished relaxation to ACh (**Figure 4.23d**).

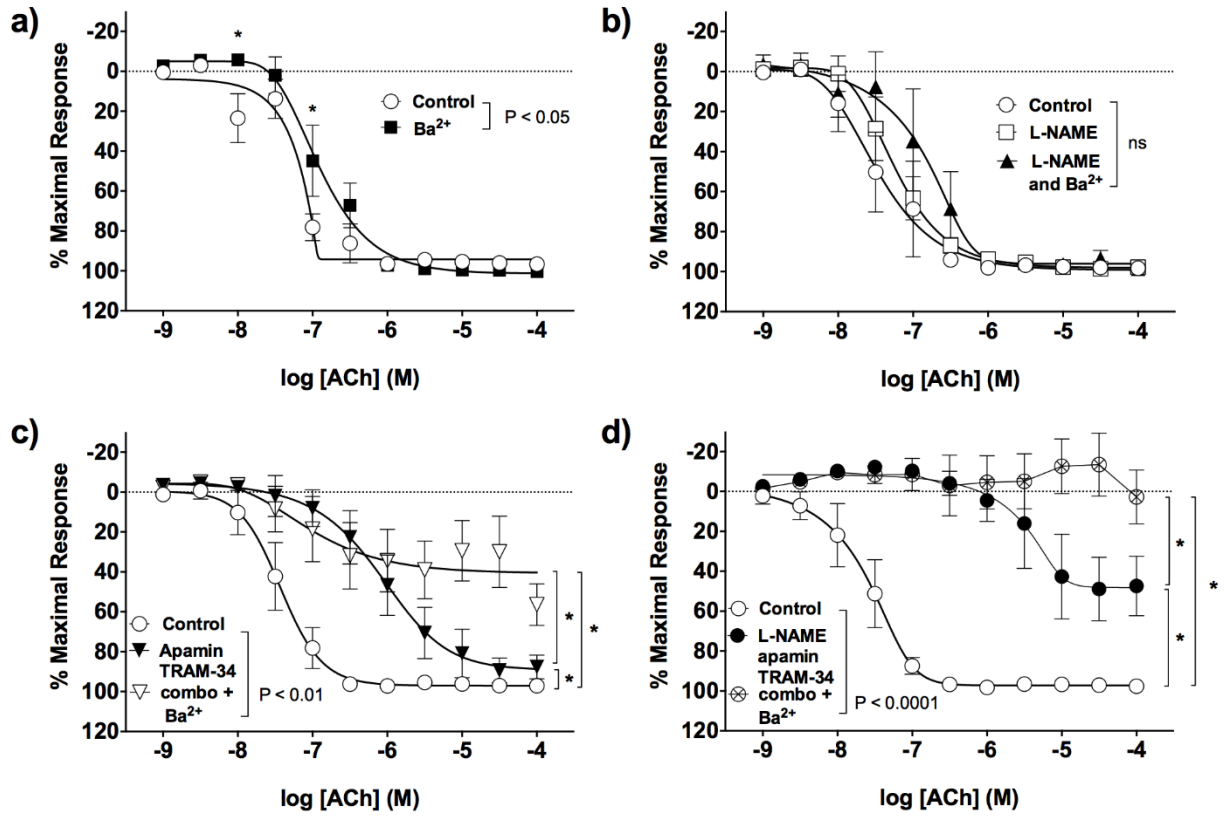



**Figure 4.23: $K_{ir}$ channels contribute to ACh-induced vasorelaxation in rat mesenteric arteries mounted in wire myograph.** Mean concentration-response curves to ACh were constructed in arteries mounted on a wire myograph and tone was raised with PE in the presence and absence of **a)** $Ba^{2+}$ (100 μM; n=5) * denotes statistically significance compared to control values. **b)** L-NAME (100 μM) or L-NAME and $Ba^{2+}$ ((100 μM and 100 μM $Ba^{2+}$; n=4) * denotes statistical significance compared to control values. ^ denotes statistical significance compared to 100 μM L-NAME **c)** 50 nM apamin and 5 μM TRAM-34 and 50 nM apamin, 5 μM TRAM-34 and 100 μM $Ba^{2+}$(n=5). *denotes statistically significance. **d)** TRAM-34 (5 μM), apamin (50 nM) and L-NAME (100 μM) and TRAM-34 (5 μM), apamin (50 nM), L-NAME (100 μM) and $Ba^{2+}$ (100 μM) (n=5). Values shown as mean ± SEM. Two-way ANOVA was performed. * denotes statistical significant compared to control values (P<0.05).

*4.3.12 Investigation of the functional role of $K_{ir}$ channels in modulating myogenic reactivity in rat mesenteric arteries:* In the presence of $Ba^{2+}$ (50 μM), the myogenic reactivity of endothelium-intact mesenteric arteries was not different from control (**Figure 4.24**). This is in contrast to a published study showing that $Ba^{2+}$ (100 μM) does enhance myogenic tone in mouse mesenteric

arteries (Sonkusare et al. 2012). As shown above, using qPCR I found that mRNA in addition to the classical $K_{ir}2.1$ and 2.2, mRNA for $K_{ir}3.4$ and 7.1 was also present in mesenteric arteries. Therefore, selective blockers of these channels were used to determine if they play a functional role in modulating pressure-induced vasoconstriction: tertiapin (300 nM; Jin and Lu 1999) is a selective inhibitor for $K_{ir}3.1$ and 3.4 channels, VU 590 (5 µM; Lewis et al. 2009) is a selective inhibitor for $K_{ir}1.1$ and 7.1 subtypes and ML-133 (20 µM; Wu et al. 2010) is a selective inhibitor of $K_{ir}2$ series channels. However, the application of the three inhibitors individually or in combination did not alter the myogenic reactivity in rat mesenteric arteries or the level of myogenic tone at each pressure was not significantly different from control values (**Figure 4.25**).

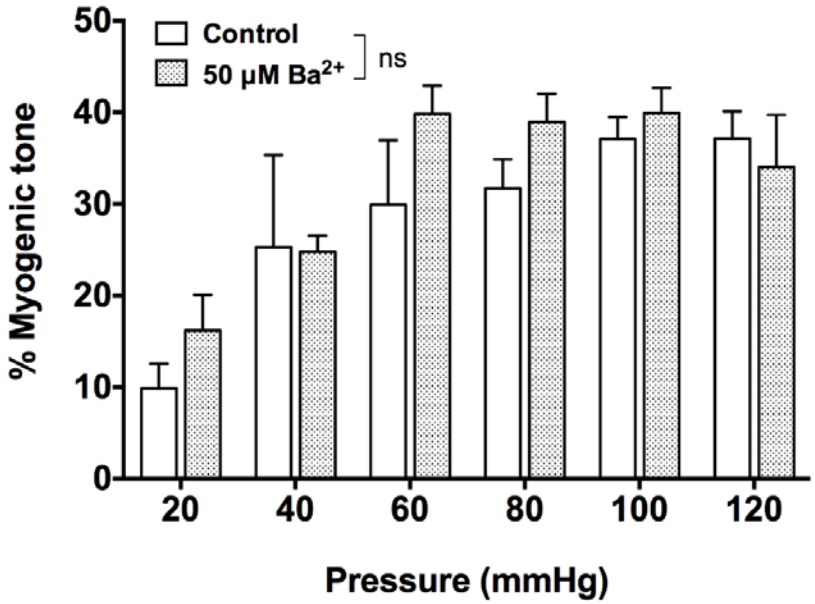



**Figure 4.24: Block of $K_{ir}$ channels by $Ba^{2+}$ does not enhance myogenic reactivity in rat mesenteric arteries.** Segments of rat mesenteric resistance artery were mounted in a pressure myograph and subjected to increases in pressure from 20-120 mmHg in 20 mmHg increments. Myogenic tone is expressed as the percentage difference in active diameter versus passive diameter of small mesenteric arteries. Mean % myogenic tone in endothelium-intact arteries in the absence and presence of 50 µM $Ba^{2+}$ (n=5). Two-way ANOVA was performed (P> 0.05).

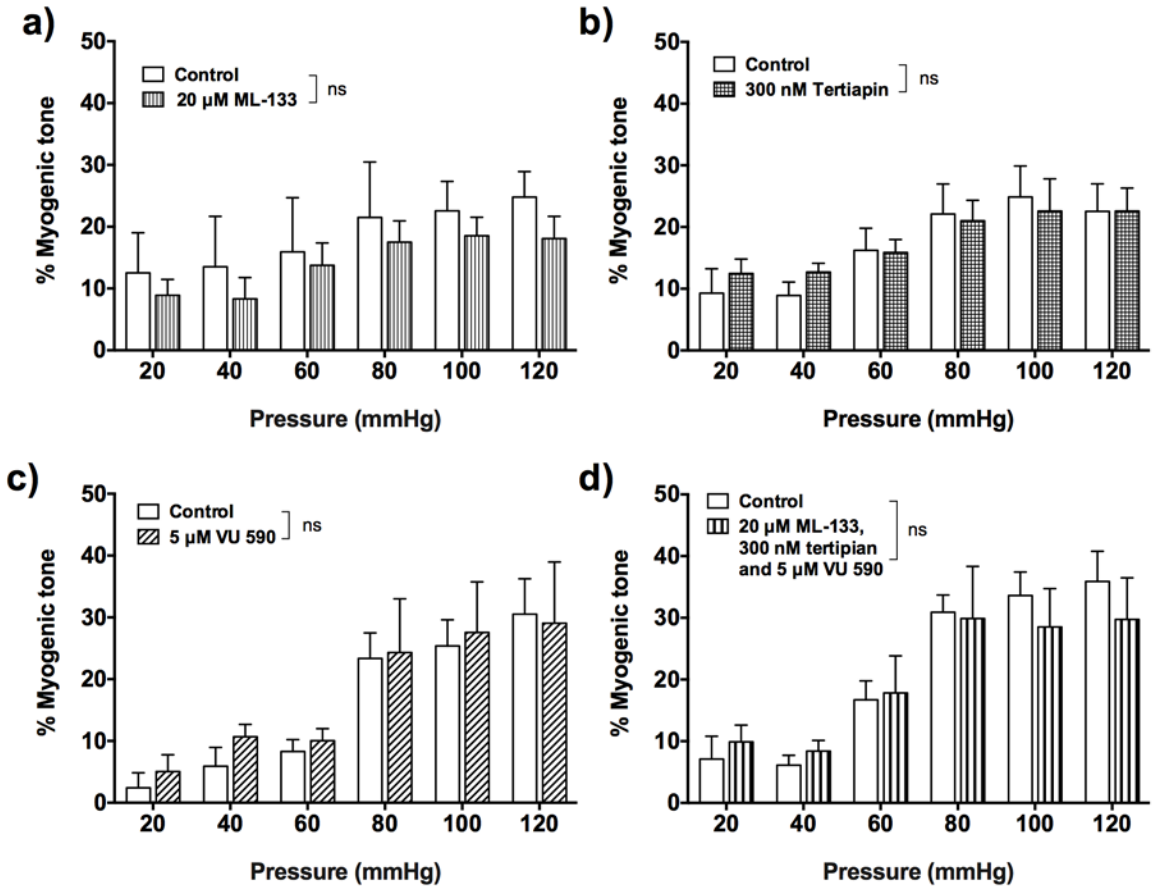



**Figure 4.25: Pharmacological inhibition of $K_{ir}$2 series, 3.1/3.4, 7.1 and 1.1 channels did not alter myogenic reactivity.** Segments of rat mesenteric resistance artery were mounted in a pressure myograph and subjected to increases in pressure from 20-120 mmHg in 20 mmHg increments. Myogenic tone is expressed as the percentage difference in active diameter versus passive diameter of small mesenteric arteries. Mean % myogenic tone in endothelium-intact arteries in the absence and presence of **a)** 20 μM ML-133 (n=5) **b)** 300 nM tertiapin (n=5). **c)** 5 μM VU 590 (n=4) **d)** 20 μM ML-133, 300 nM tertiapin, and 5 μM VU 590 (n=5). Two-way ANOVA was performed (P>0.05).

Previous work has linked $K_{ir}$ channels to shear stress-induced dilation in mouse mesenteric arteries (Ahn et al. 2017). I show here that in the perfused rat mesenteric bed, block of $K_{ir}$ channels with $Ba^{2+}$ (50 and 100 μM) resulted in an enhancement of nerve-evoked constriction in endothelium-intact (**Figure 4.26a)** but not in endothelium-denuded preparations (**Figure 4.26b)**. Although $K_{ir}$ channels do not modulate myogenic tone in isolated rat mesenteric arteries, endothelial $K_{ir}$ channels can participate in activation of the endothelium by increases in shear stress

in the intact mesenteric bed.

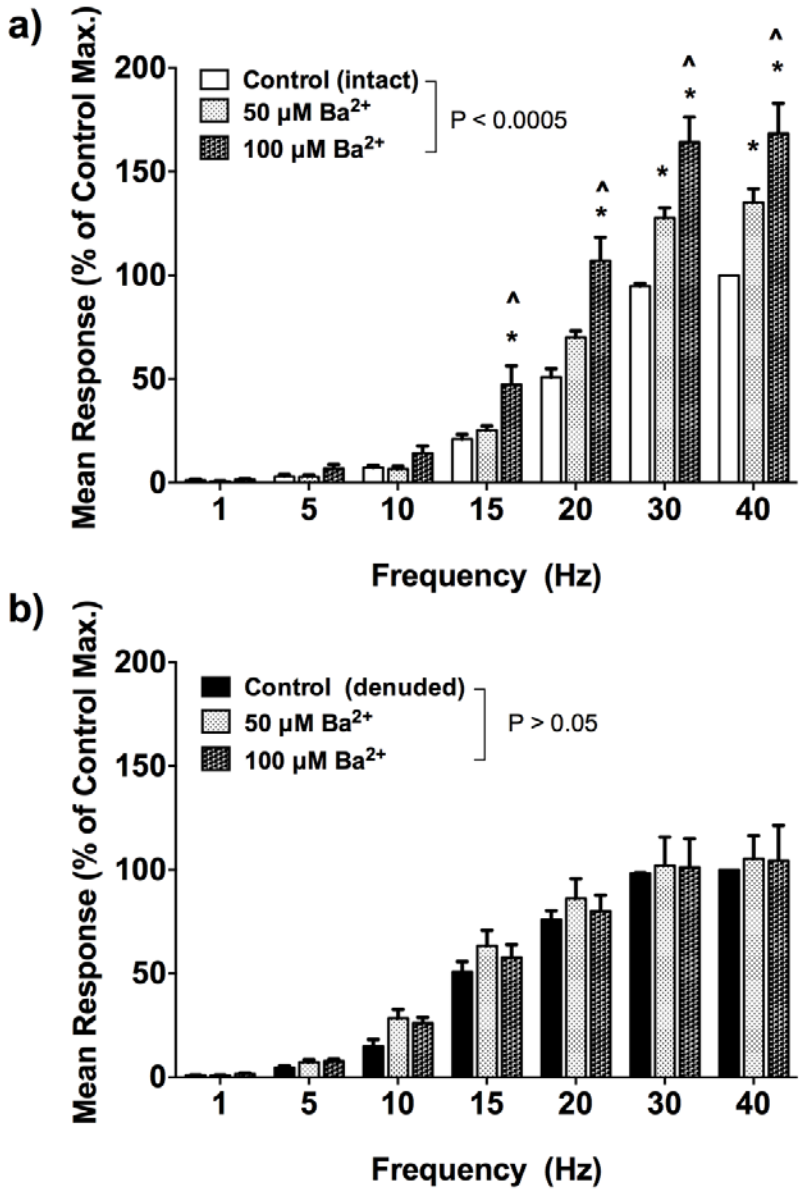



**Figure 4.26: $Ba^{2+}$ caused endothelium-dependent enhancement of nerve-evoked constriction in rat mesenteric vascular bed.** Mesenteric vascular bed was perfused under a constant flow rate and electrical stimulations were conducted. **a)** Mean data showing frequency-response relationships in the absence and presence of $Ba^{2+}$ at concentrations of 50 µM and 100 µM alone in endothelium-intact mesenteric vascular beds. **b)** Mean data showing frequency-response relationships in the absence and presence of $Ba^{2+}$ (50 µM and 100 µM alone in endothelium-denuded mesenteric vascular beds. Values are shown as mean ± SEM (n=5), Two-way ANOVA was performed. *denotes statistical significance compared to control values (P<0.05). ^denotes statistical significance compared to 50 µM $Ba^{2+}$ (P<0.05).

*4.3.13 Investigation of the contribution of $K_{ir}$ channels to the actions of CyPPA and SKA-31 in myogenically active mesenteric arteries:* In the presence of $Ba^{2+}$ (50 μM), CyPPA was no longer able to inhibit myogenic reactivity in endothelium-intact mesenteric arteries (**Figure 4.27**). Furthermore, in the presence of CyPPA and $Ba^{2+}$ (50 μM), myogenic reactivity was significantly enhanced compare to control (**Figure 4.27b**). An interpretation of the effect of $Ba^{2+}$ on the actions of CyPPA and SKA-31 in myogenically active arteries is due to direct block of $SK_{Ca}$ and $IK_{Ca}$ channels by $Ba^{2+}$. However, at this concentration $Ba^{2+}$ did not significantly alter myogenic reactivity (**Figure 4.24**), does not block $SK_{Ca}$ and $IK_{Ca}$ channels (Sonkusare et al. 2016), and did not inhibit the amplitude of smooth muscle hyperpolarization evoked by CyPPA and SKA-31. The amplitude of hyperpolarization evoked by CyPPA was comparable in the absence and presence of 50 μM $Ba^{2+}$ (-10.8 ± 2.0 vs -12 ± 1.7 mV; P>0.05, t-test). The amplitude of hyperpolarization induced by SKA-31 was also not altered in the absence and presence of $Ba^{2+}$ (50 μM) (-9.4 ± 1.8 vs -9.3 ± 2.1 mV; P>0.05, t-test).

At a concentration of 50 μM, $Ba^{2+}$ did not significantly affect the ability of SKA-31 to inhibit myogenic reactivity (**Figure 4.28b**). However, at a concentration of 100 μM, $Ba^{2+}$ prevented the actions of SKA-31 (**Figure 4.28c**).

Inhibition of $K_{ir}2$ channels with ML-133 (20 μM) did not significantly alter myogenic reactivity (**Figure 4.25a**) but in the presence of ML-133, the effects of CyPPA and SKA-31 were attenuated (**Figure 4.29**). Tertiapin (300 nM), an inhibitor of $K_{ir}3.1$ and 3.4 channels, which did not affect myogenic reactivity by itself (**Figure 4.25b**), also reduced the effects of CyPPA and SKA-31. In the presence of tertiapin plus either CyPPA or SKA-31, myogenic reactivity was not significantly different from controls (**Figure 4.30**).

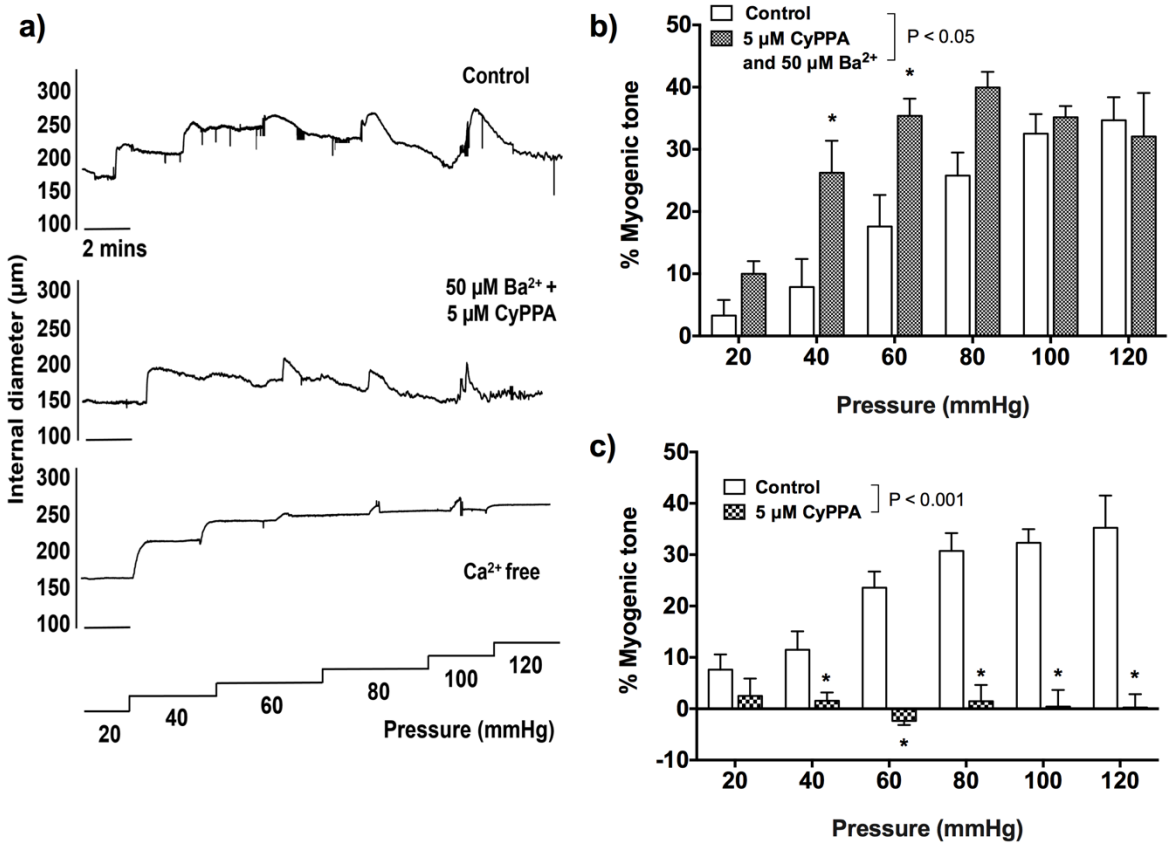



**Figure 4.27: The effect of CyPPA on myogenic tone is dependent on $K_{ir}$ channels.** Segments of rat mesenteric resistance artery were mounted in a pressure myograph and subjected to increases in pressure from 20-120 mmHg in 20 mmHg increments. **a)** Representative trace of pressure-diameter relationships in the absence and presence of 50 μM $Ba^{2+}$ and 5 μM CyPPA, and extracellular $Ca^{2+}$. Myogenic tone is expressed as the percentage difference in active diameter versus passive diameter of small mesenteric arteries. Mean % myogenic tone in endothelium-intact arteries in the absence and presence of **b)** 5 μM CyPPA and 50 μM $Ba^{2+}$ (n=5). **c)** 5 μM CyPPA alone (n=6). Two-way ANOVA was performed. *denotes statistically significant compared to control values (P<0.05). Panel **c)** comes from **Figure 4.13**.

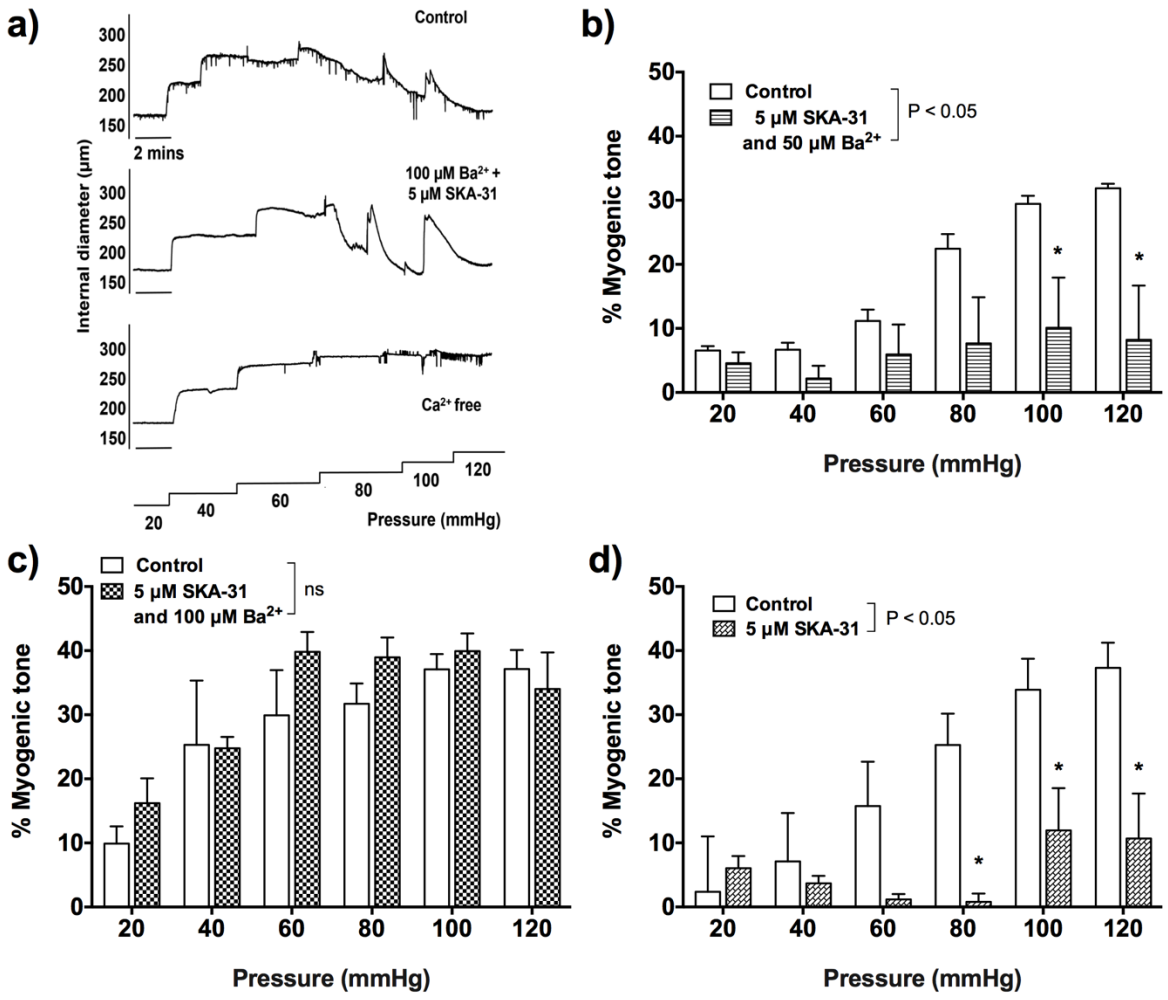



**Figure 4.28: The effect of SKA-31 on myogenic tone is dependent on $K_{ir}$ channels.** Segments of rat mesenteric resistance artery were mounted in a pressure myograph and subjected to increases in pressure from 20-120 mmHg in 20 mmHg increments. **a)** Representative trace of pressure-diameter relationships in the absence and presence of 50 µM $Ba^{2+}$ and 5 µM SKA-31 and extracellular $Ca^{2+}$. Myogenic tone is expressed as the percentage difference in active diameter versus passive diameter of small mesenteric arteries. Mean % myogenic tone in endothelium-intact arteries in the absence and presence of **b)** 5 µM SKA-31 and 50 µM $Ba^{2+}$ (n=4) **c)** 5 µM SKA-31 and 100 µM $Ba^{2+}$ (n=5). **d)** 5 µM SKA-31 alone (n=5). Two-way ANOVA was performed. *denotes statistically significant compared to control values (P<0.05). Panel **d)** comes from **Figure 4.15**.

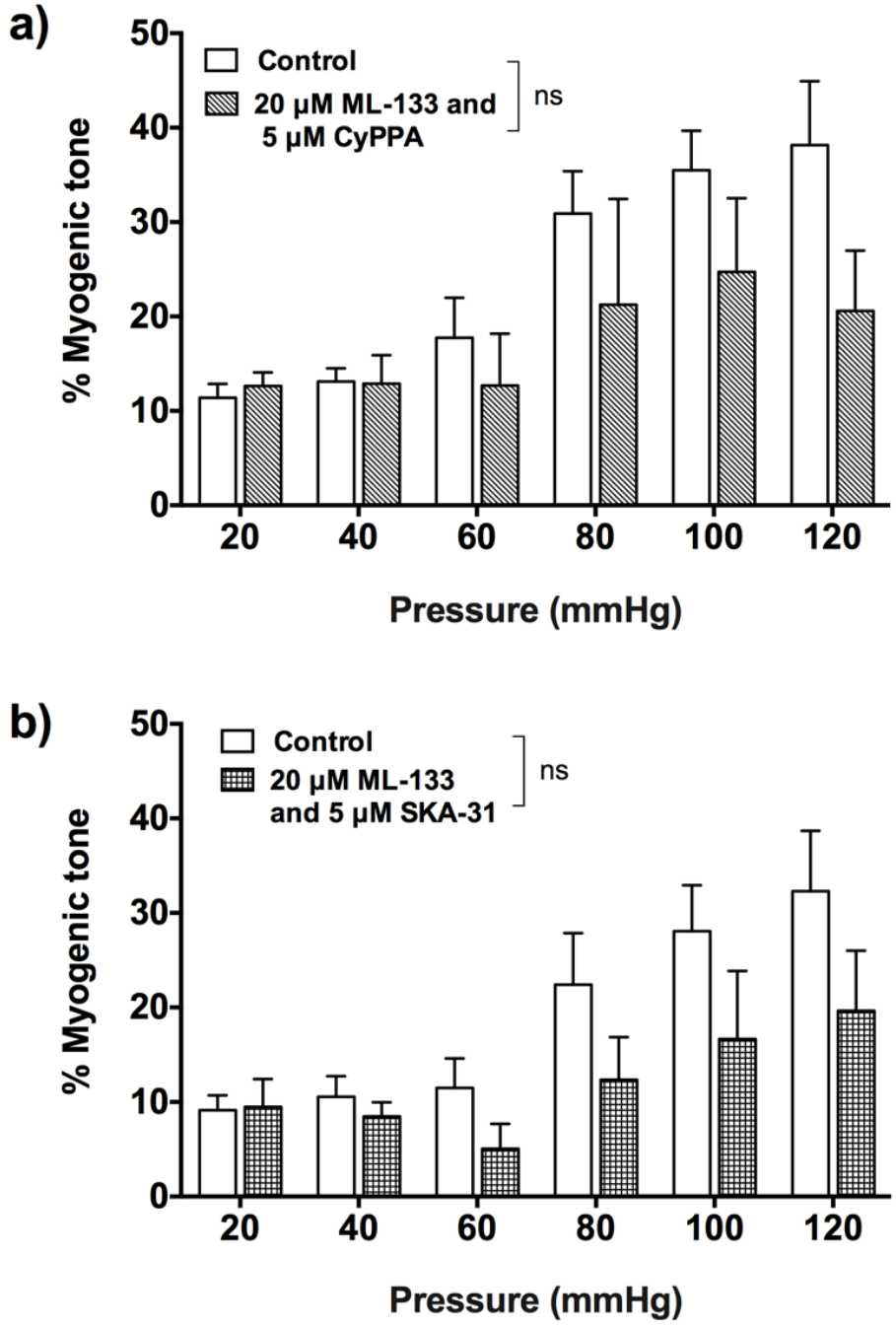



**Figure 4.29: Inhibition of $K_{ir}2$ channels reduces the effects of CyPPA and SKA-31 on myogenic reactivity.** Segments of rat mesenteric resistance artery were mounted in a pressure myograph and subjected to increases in pressure from 20-120 mmHg in 20 mmHg increments. Myogenic tone is expressed as the percentage difference in active diameter versus passive diameter of small mesenteric arteries. Mean % myogenic tone in endothelium-intact arteries in the absence and presence of **a)** 20 µM ML-133 and 5 µM CyPPA (n=5). **b)** 20 µM ML-133 and 5 µM SKA-31 (n=6). Two-way ANOVA was performed (P>0.05).

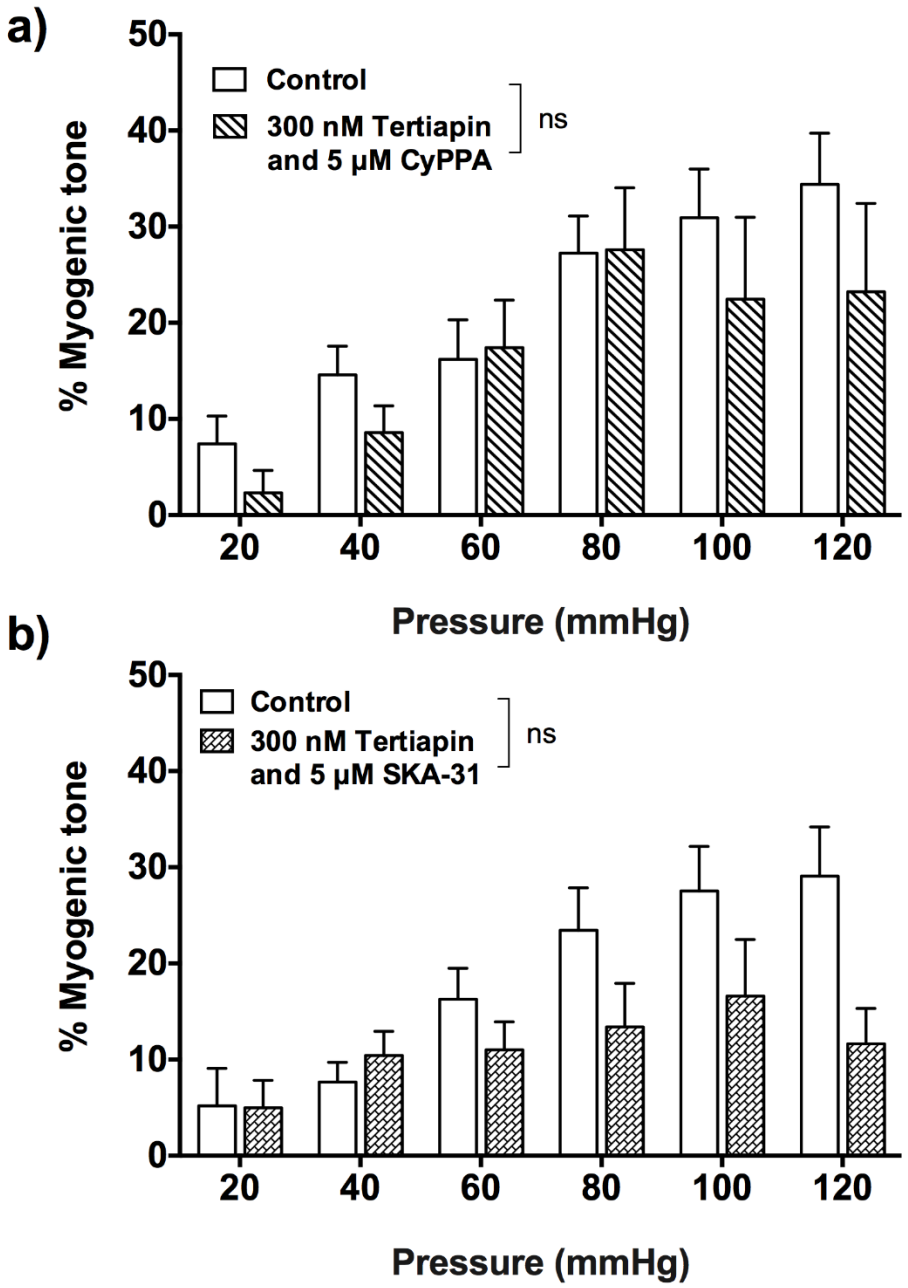



**Figure 4.30: Inhibition of $K_{ir}3.1/3.4$ channels reduces the effect of CyPPA and SKA-31.** Segments of rat mesenteric resistance artery were mounted in a pressure myograph and subjected to increases in pressure from 20-120 mmHg in 20 mmHg increments. Myogenic tone is expressed as the percentage difference in active diameter versus passive diameter of small mesenteric arteries. Mean % myogenic tone in endothelium-intact arteries in the absence and presence of **a)** 300 nM tertiapin and 5 µM CyPPA (n=5). **b)** 300 nM tertiapin and 5 µM SKA-31 (n=5). Two-way ANOVA was performed. * denotes statistically significant compared to control values ($P<0.05$).

## 4.4 Discussion

The data presented in this chapter show that small molecule activators of $SK_{Ca}$ and $IK_{Ca}$ channels can inhibit the development of myogenic reactivity and that this effect is also dependent on the activity of $K_{ir}$ channels. CyPPA, a putative activator of $SK_{Ca}$ channels, elicited smooth muscle hyperpolarization and inhibited myogenic reactivity that was endothelium-dependent, inhibited by the selective $SK_{Ca}$ channel blocker apamin, and mediated, at least in part, by NO. In contrast, the putative $IK_{Ca}$ channel activator SKA-31 elicited smooth muscle hyperpolarization, but with respect to modulating myogenic reactivity, acted independently of the endothelium. As $IK_{Ca}$ channels have not been identified in vascular smooth muscle cells of mesenteric arteries, this finding suggests that SKA-31 may have previously unreported, off-target effects which also lead to smooth muscle relaxation. Together, these data support my hypothesis of a link between NO and vasorelaxation elicited by agents that activate endothelial $SK_{Ca}$ channels. My work also reveals a role for $K_{ir}$ channels in amplifying vasodilator responses driven by changes in membrane potential in mesenteric resistance arteries.

$SK_{Ca}$ (SK3) and $IK_{Ca}$ (IK1) channels are functionally expressed on most endothelial cells but not normal vascular smooth muscle cells (Ledoux et al. 2006). In this study, $IK_{Ca}$ channels were localized to endothelial cells in a proportion of the holes in the IEL, whereas $SK_{Ca}$ channels were located at adjacent endothelial cell borders, as has previously been observed in porcine coronary, rat mesenteric, skeletal muscle and cerebral resistance arteries (Kerr et al. 2015; Sandow et al. 2006; Senadheera et al. 2012; Tran et al. 2012). This localization facilitates activation of different channel populations by localized increase in $[Ca^{2+}]_i$ and is in line with differential roles of these channels in endothelium-dependent modulation of resistance artery diameter: $IK_{Ca}$ channels mediate myoendothelial feedback (see **Chapter 3**) whereas $SK_{Ca}$ channels can be activated by increases in shear stress (Kerr et al. 2015; Tran et al. 2012; Wei et al. 2018). $IK_{Ca}$ and

$SK_{Ca}$ channels have not been reported to be expressed in smooth muscle cells of rat mesenteric arteries (Kerr et al. 2015; Sandow et al. 2006), but recently $IK_{Ca}$ channels have been identified in smooth muscle cells of radial arteries from non-pregnant and pregnant rats (Murphy et al. 2016), and in rat middle cerebral arteries ( McNeish et al. 2006).

I chose to investigate endothelial modulation of myogenic reactivity as the ability to constrict in response to increase in intravascular pressure is a key autoregulatory mechanism in resistance arteries, and contributes to total peripheral vascular resistance and thus blood pressure. Characterization of the myogenic reactivity of third and fourth order resistance arteries was undertaken as there is very limited data in the literature on the nature of the response to pressure in these vessels with most studies of myogenic reactivity involving cerebral (Knot and Nelson 1998), skeletal muscle (Kotecha and Hill 2005) and renal (Loutzenhiser et al. 2002) arteries (for review see Izzard and Heagerty 2014). In a number of previous studies, rat mesenteric arteries have in fact been shown to develop little or no myogenic tone, which from my own experience I suggest may be due to tissue handling (see Cockell and Poston 1996; Dunn and Das 2017 for examples). I found that mesenteric resistance arteries developed myogenic reactivity at intravascular pressures of 60 mmHg and above, and that this response was not modulated by removal of the endothelium or block of either eNOS, COX or $SK_{Ca}$ and $IK_{Ca}$ channels. This is in line with the findings of **Chapter 3** that under normal conditions, myogenic reactivity is not dependent on the endothelium (Meininger and Davis 1992; Palomares and Cipolla 2014) and, in contrast to some other vessels such as rat cremaster and cerebral artery (Marrelli 2002; Mishra et al. 2015) and female gerbil spiral artery (Reimann et al. 2013), is not normally modulated by it. However, inhibition of eNOS, $SK_{Ca}$ and $IK_{Ca}$ channels together, potentiated myogenic reactivity, indicating that the endothelium may release a combination of constricting and dilating factors,

such that there is no net effect of endothelium removal.

In the current study of rat mesenteric arteries, CyPPA evoked apamin-sensitive hyperpolarization, and at concentrations of 10 nM to 10 μM, elicited endothelium-dependent dilation which was sensitive to either apamin or L-NAME. CyPPA also abolished myogenic reactivity in an endothelium- and NO-dependent manner that was prevented by block of $SK_{Ca}$ channels with apamin. Several studies have investigated the effects of CyPPA (Haddock et al. 2011; Hougaard et al. 2007) on vascular function. For example, in porcine retinal arteries ( Dalsgaard et al. 2010) mounted in a wire myograph, CyPPA (1–100 μM) induced concentration-dependent relaxations of induced tone; these responses were blocked by apamin, endothelial removal and inhibition of NO signaling only at concentrations up to 10 μM. However, only a few reports have described the effects of CyPPA on smooth muscle membrane potential. A higher concentration of CyPPA (30 μM) evoked smooth muscle hyperpolarization in mesenteric arteries from healthy and obese rats, a response which was abolished by apamin, but for which the endothelium-dependence was not demonstrated (Haddock et al. 2011). The same concentration of CyPPA was shown to cause EDH of smooth muscle in uterine arteries from healthy and diabetic pregnant rats (Gokina et al. 2015); the effect of apamin on the hyperpolarization was not determined, but it was shown to inhibit CyPPA-induced dilation and CyPPA-activated currents in isolated endothelial cells. Thus, my findings are in line with previous work and support the proposal that CyPPA activates endothelial $SK_{Ca}$ channels to elicit EDH and is associated with NO mediated smooth muscle relaxation (Dalsgaard et al. 2010).

The mechanism linking activation of $SK_{Ca}$ channels to NO is unclear. There is now a significant body of evidence supporting a structural link between TRPV4 and $SK_{Ca}$ channels in endothelial cells (Saliez et al. 2008) and demonstrating that activation of $SK_{Ca}$ channels in

response to a number of stimuli, including agonists and increases in shear stress, is mediated by TRPV4-mediated $Ca^{2+}$ influx (Sonkusare et al. 2012). Thus, the dependence of the effects of CyPPA on NO could be related to the localization of $SK_{Ca}$ channels to the luminal surface (Sandow et al. 2006) of endothelial cells in signaling microdomains with cav-1, eNOS and mechanosensitive TRPV4 channels. SK3 and cav-1 were co-immunoprecipitated from endothelial cells of rat mesenteric and porcine coronary arteries (Absi et al. 2009). eNOS, TRPV4 and $SK_{Ca}$ channels were shown to be targeted to caveolae in freshly isolated bovine coronary endothelial cells, and TRPV4 and SK3 could be co-immunoprecipitated by cav-1 antibodies from the same cells (Lu et al. 2017). In addition, shear stress led to the activation of $SK_{Ca}$ currents by $Ca^{2+}$ influx through TRPV4 channels in cultured endothelial cells. The increase in $[Ca^{2+}]_i$, and activation of $SK_{Ca}$ channels were blocked by the TRPV4 inhibitor HC067047 indicating that TRPV4-mediated $Ca^{2+}$ entry evoked by shear stress was responsible for activation of $SK_{Ca}$ channels. However, in mouse small pulmonary and mesenteric, and rat carotid arteries (Köhler et al. 2006), TRPV4 activity (Marziano et al. 2017; Mendoza et al. 2010) is linked to NO production rather than activation of $SK_{Ca}$ channels, whereas in rat pulmonary arteries vasodilation to the TRPV4 agonist GSK1016790A was mediated by NO, $SK_{Ca}$ and $IK_{Ca}$ channels (Addison et al. 2016), supporting the idea of differential $Ca^{2+}$ signaling networks. Endothelial cells in pressurized resistance arteries/arterioles show spontaneous $Ca^{2+}$ events (Duza and Sarelius 2004; McSherry et al. 2005) but how they are modulated by increases in pressure has received little attention. Activation of TRPV4 was shown to increase endothelial $Ca^{2+}$events leading to EDH-mediated dilation to low pressures (Bagher et al. 2012) (<50 mmHg) in rat skeletal muscle arteries but how changes in pressure impact the TRPV4-NOS axis has not been explored.

One possibility to explain the NO-dependence of CyPPA-mediated inhibition of myogenic reactivity in the current study could reflect CyPPA causing $SK_{Ca}$-mediated hyperpolarization to further enhance TRPV4-mediated $Ca^{2+}$ influx resulting in production of NO **(Figure 4.31)**. However, it would then be expected that in the presence of L-NAME to block NOS, EDH would still be able to mediate relaxation when in fact L-NAME abolished the actions of CyPPA. In rat cerebral and pulmonary arteries L-NAME inhibited EDH-mediated relaxation via a COX-$TXA_2$ pathway leading to inhibition of $SK_{Ca}$ channels (Addison et al. 2016; McNeish and Garland 2007). Also, in other cell types there is evidence of crosstalk between eNOS and COX (see reviews Clancy et al. 2000; Silva et al. 2016). For example, in rat hepatic endothelial cells, NO inhibits the production of $TXA_2$ by reducing the activity of COX (Rosado et al. 2012). This mechanism could contribute to L-NAME sensitivity of the actions of CyPPA seen in my studies although the fact that L-NAME alone did not potentiate myogenic reactivity suggests it is not a major factor. It is also possible that NO plays a facilitatory role in CyPPA-mediated hyperpolarization and relaxation, thus, removal of endogenous NO with L-NAME inhibited the effect of $SK_{Ca}$ channel activation. The hypothesis that NO facilitates the spread of hyperpolarization through gap junctions will be examined in **Chapter 5**.

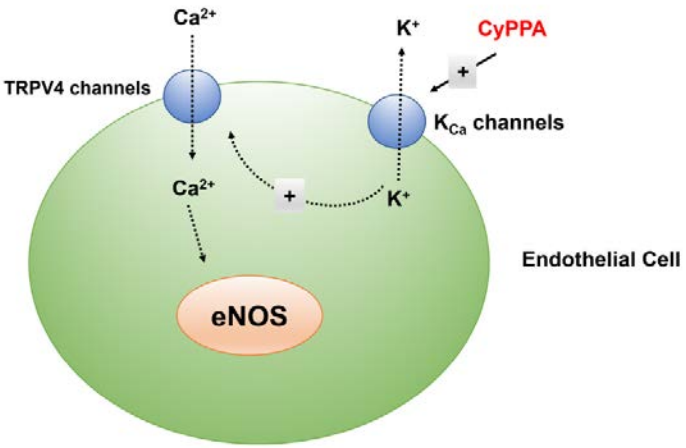



**Figure 4.31: Schematic illustrating proposed effect of CyPPA in modulating myogenic reactivity.** See text for details.

SKA-31 evoked TRAM-34-sensitive smooth muscle hyperpolarization, and at concentrations of 10 nM to 30 μM elicited concentration-dependent dilation in PE-stimulated vessels which was unaffected by L-NAME. Removal of the endothelium or addition of TRAM-34 inhibited dilation to SKA-31 at lower concentrations but vasodilation was still observed with 10 μM SKA-31. SKA-31 (5 μM) abolished myogenic reactivity at intravascular pressures of 60 to 120 mmHg (**Figure 4.15c**), an effect that was not dependent on the endothelium but was only partial prevented by blockers of $IK_{Ca}$ channels. Thus, in contrast to CyPPA, NO does not appear play a role in the effects of SKA-31 on smooth muscle contractility and it can have actions independent of the endothelium. The reason for the difference in the endothelium-dependence of the effects of SKA-31 in arteries constricted with PE as compared to myogenically active arteries is not clear. A possibility could be that the different mechanisms of contraction (PE vs myogenic) reveal an "off-target" effect of SKA-31 in myogenic arteries. Recent evidence has shown that the development of myogenic tone is associated with $Ca^{2+}$ influx through TRPM4 channels, with inhibition of TRPM4 with 9-phenanthrol attenuating the myogenic response in rat cerebral arteries (Gonzales et al. 2010) as described in **Section 1.3.2**. Interestingly, 9-phenanthrol has also been found to activate endothelial $IK_{Ca}$ channels in rat mesenteric arteries (Garland et al. 2015). Due to similarities in the chemical structures of SKA-31 and 9-phenanthrol, and the fact that both of these chemicals can activate $IK_{Ca}$ channels, it is reasonable to speculate that SKA-31 may block smooth muscle TRPM4 channels as an "off-target" effect to inhibit the development myogenic tone. However, the exact mechanism is unknown and requires further investigation.

Previous reports have also shown that NO is not involved in the actions of SKA-31. At a concentration of 10 μM, SKA-31 dilated myogenically active, rat cremaster and middle cerebral arteries, an effect that was largely, but not completely, inhibited by the $SK_{Ca}$ channel blocker

UCL1684 and TRAM-34, abolished by endothelial denudation, but unaffected by block of eNOS (Mishra et al. 2015). In mouse skeletal muscle arterioles, SKA-31 (1-30 μM) induced concentration-dependent dilation and enhanced the effects of ACh, effects that were independent of eNOS and were lost by deletion of $K_{Ca}3.1$ channels (Radtke et al. 2013). At a low concentration, SKA-31 (0.5 μM) enhanced endothelium-dependent vasodilation to ACh in mice lacking eNOS (Hasenau et al. 2011).

Alternatively, a lack of effect of L-NAME could be due to the ability of SKA-31 to act directly on smooth muscle cells; as discussed above, SKA-31 may be able to block TRPM4 channels to directly inhibit smooth muscle contraction[232]. An endothelium-independent action of SKA-31 has not previously been described in the literature, but i.p. injection of SKA-31 reduced mean arterial blood pressure in mice lacking connexin 40 (Radtke et al. 2013). This connexin connects endothelial cells and is reportedly an essential component in MEGJs (Mather et al. 2005; Sandow et al. 2002) in a number of vessels, and so the ability of SKA-31 to affect the vasculature in mice lacking this protein suggests that the response is independent of myoendothelial coupling, i.e. not dependent on the transfer of an electrical signal from endothelial to smooth muscle cells.

Strong inward rectifier channels of the $K_{ir}2$ series are activated by membrane potential hyperpolarization and have been suggested to boost vasodilatation elicited by activation of endothelial or smooth muscle potassium channels (Jackson 2005; Longden and Nelson 2015; Smith et al. 2008). For example, in rat isolated coronary and cerebral arteries, $Ba^{2+}$ attenuated dilation to activators of $K_{ATP}$ channels, and to UTP and ACh, all known to activate endothelial $K_{Ca}$ channels (Marrelli et al. 2003; Smith et al. 2008). In the same study (Smith et al. 2008), expression of mRNA for $K_{ir}2.1$ and 2.2 was shown to be ~2- to 6-fold higher in samples of isolated smooth muscle cells from cerebral and coronary arteries as compared to mesenteric vessels. Also,

a $Ba^{2+}$-sensitive current was recorded only in smooth muscle cells from cerebral and coronary arteries, and $Ba^{2+}$-sensitive dilation to elevated $[K^+]_o$ was seen in endothelium-denuded coronary and cerebral but not mesenteric arteries. These data indicate that functional $K_{ir}$ channels are present in smooth muscle cells of coronary and cerebral but not mesenteric arteries. An earlier study (Crane et al. 2003) also provided evidence for the presence of $K_{ir}$ channels in endothelial but not vascular smooth muscle cells of rat mesenteric arteries, and demonstrated that dilation of these vessels to increases in $[K^+]_o$ was insensitive to $Ba^{2+}$ but inhibited by ouabain, a blocker of $Na^+/K^+$ATPase. However, there appears to be significant variation in channel expression as in another study, $K_{ir}2.1$ but not $K_{ir}2.2$, accounted for the strong inwardly rectifying currents in isolated smooth muscle cells of rat coronary and basilar arteries (Bradley et al. 1999).

In the present study, mRNA for both $K_{ir}2.1$ and 2.2 was detected in intact mesenteric arteries, and $K_{ir}2.1$ and 2.2 channel proteins were identified in both endothelial and smooth muscle cells of whole mount preparations by the use of confocal immunohistochemistry. mRNA for $K_{ir}3.4$ was also detected but lack of suitable antibodies precluded protein detection by immunohistochemistry. $K_{ir}$ channels are involved in regulation of membrane potential as application of $Ba^{2+}$ depolarized the smooth muscle membrane potential of isolated mesenteric arteries and supporting previous studies in rat renal, coronary, cerebral and mesenteric arteries (Weston et al. 2002). Furthermore, increases in $[K^+]_o$ elicited smooth muscle hyperpolarization and relaxation in both endothelium-intact and denuded arteries which, in all cases was blocked by $Ba^{2+}$ indicating that in contrast to previous studies, functional $K_{ir}$ channels are present in the smooth muscle cells in the rat mesenteric arteries. KCl evoked U-shaped concentration-response curves are consistent with the biophysical properties of strong inward rectifier $K_{ir}$ channels that display voltage-dependent block of channel conductances (details are discussed in **section 1.2.4c**).

Interestingly, my experiments showed that there were differences in the sensitivity of endothelium-intact and denuded arteries to increases in $[K^+]_o$. Under both isometric and isobaric conditions, denuded arteries maximally dilated at a concentration of 9 mM $[K^+]_o$ whereas endothelium-intact arteries maximally dilated a concentration of 13mM. The reason for this is unclear but could indicate a spatially distinct distribution of $K_{ir}2.1$ and $K_{ir}2.2$ channels between endothelial and smooth muscle cells and/or difference in their biophysical properties. In endothelium-intact arteries, the spread of EDH amplifies the effect to limit the depolarization elicited by higher $[K^+]_o$ acting on smooth muscle cells. In support of this proposal, while smooth muscle hyperpolarization to 7 mM $[K^+]_o$ was the same in intact and denuded arteries, at a concentration of 15 mM, the membrane potential of smooth muscle cells in endothelium-denuded arteries was significantly more depolarized indicating the hyperpolarizing influence of the endothelium had been lost. Vasodilatation to $[K^+]_o$ in rat mesenteric arteries pre-constricted with PE has previously been attributed to activation of $Na^+,K^+$-ATPase (Crane et al. 2003; Smith et al. 2008) and in rat femoral arteries both $K_{ir}$ channels and $Na^+,K^+$-ATPase were responsible for the response (Savage et al. 2003). My data demonstrating that $Ba^{2+}$, at a concentration that does not affect other ion channels such as Kv, $K_{Ca}$ and $K_{ATP}$ (reviewed by Nelson and Quayle 1995), inhibits both smooth muscle hyperpolarization and relaxation to $[K^+]_o$, and that ML-133 inhibits the relaxation, suggests that as in mouse mesenteric arteries, this response can be fully accounted for by activation of $K_{ir}$ channels (Sonkusare et al. 2016). A study has reported TWIK-2 channels, a subtype of twin pore $K^+$ potassium channels, were sensitive to $Ba^{2+}$ ($IC_{50}$=80 μM; Lloyd et al. 2009). While TWIK-2 channel mRNA expression has been identified in mesenteric arteries, block of these channels failed to generate an increase in artery tone compared to that observed in pulmonary arteries (Gardener et al. 2004). In my study, myogenic reactivity was unaffected by 50

μM $Ba^{2+}$, but to rule out a potential role of TWIK-2 channels a higher concentration of $Ba^{2+}$ is required.

Endothelium-dependent relaxations to ACh in isolated mesenteric arteries were also inhibited by $Ba^{2+}$, an effect that was enhanced when $SK_{Ca}$ and $IK_{Ca}$ channel activity was also blocked. Indeed, in the presence of L-NAME, apamin and TRAM-34, $Ba^{2+}$ abolished the remaining response. This finding supports the concept that endothelium-dependent vasodilators that elevate $[Ca^{2+}]_i$ and hyperpolarize the endothelium through the activation of $SK_{Ca}$ and $IK_{Ca}$ channels will engage $K_{ir}$ channels to boost their signal strength (Sonkusare et al. 2016). It has also been proposed that activation of $K_{ir}$ channels may also be enhanced by efflux of $K^+$ through $SK_{Ca}$ and $IK_{Ca}$ channels leading to localized increases in $[K^+]_o$ in the restricted space between endothelial and smooth muscle membranes (Edwards et al. 1998), although supporting evidence for this idea is limited.

$Ba^{2+}$ does not enhance myogenic reactivity suggesting $K_{ir}$ channels may not act to provide a "brake" on myogenic vasoconstriction as has been shown for other $K^+$ channels such as Kv channels (Hirst et al. 1986; Nelson and Quayle 1995; Smith et al. 2008). However, $Ba^{2+}$ did cause endothelium-dependent enhancement of nerve-evoked vasoconstriction in the rat perfused mesenteric bed showing that in addition to ACh-evoked relaxation, endothelial $K_{ir}$ channels in mesenteric arteries can be engaged by physical stimuli.

As discussed in **Chapter 1**, the negative slope conductance of $K_{ir}$ channels means that their activity is enhanced by hyperpolarization (Nelson and Quayle 1995; Nelson et al. 1990) and so engagement of these channels can "boost" responses to other stimuli that elicit opening of $K^+$ channels. Therefore, I investigated the possibility that inhibition of myogenic reactivity caused by CyPPA and SKA-31 in rat mesenteric resistance arteries involves recruitment of $K_{ir}$ channels: the

effects of CyPPA were blocked by 50 μM $Ba^{2+}$ but 100 μM $Ba^{2+}$ was required to inhibit the effects of SKA-31. $Ba^{2+}$ does not block $SK_{Ca}$ or $IK_{Ca}$ channels (Sonkusare et al. 2016) and the reason for this difference in concentration dependence is unclear. Interestingly, block of $K_{ir}$ channels not only abolished the effect of CyPPA, in the presence of $Ba^{2+}$ and CyPPA myogenic reactivity was enhanced compared to control values suggesting that activation of $SK_{Ca}$ channels led to recruitments of more $K_{ir}$ channels.

As CyPPA acts in an endothelium-dependent manner, these experiments were conducted in endothelium-intact mesenteric arteries. $K_{ir}$ channels are expressed on both endothelial and smooth muscle cells in these arteries as shown by immunohistochemistry and by the relaxations to raised $[K^+]_o$. Thus, the effects of SKA-31 may be less sensitive to $Ba^{2+}$ as by acting on the smooth muscle, it may be directly recruiting a different population of $K_{ir}$ channels that have subtle differences in $Ba^{2+}$ sensitivity. Here, SKA-31 may inhibit smooth muscle TRPM4 channels, channels important for the development of myogenic tone, causing hyperpolarization, and so activate smooth muscle $K_{ir}$ channels (see above and **Section 1.3.2** for details). CyPPA, in contrast, will only target the endothelial $SK_{Ca}$ channels. The effects of both CyPPA and SKA-31 on myogenic reactivity were reduced by ML-133 and tertiapin suggesting that these agents recruit both $K_{ir}2$ series and $K_{ir}$ 3.4 (GIRK) channels (supported by the identification of mRNA for these channels in the arteries). To date, the role of GIRK channels has mainly been studied in the central nervous system and heart where they are activated by agents such as adenosine and GABA (Lüscher and Slesinger 2010). However, stretch-activation of these channels via membrane bound $PIP_2$ has also been described which could enable them to be activated by increases in intravascular pressure (Ji et al. 1998; Zhang et al. 2004; Sui et al. 1998).

Evidence suggests that at high concentrations $Ba^{2+}$ is able to block other $K^+$ channels (e.g. $K_{ATP}$, $K_V$ and $BK_{Ca}$) but at concentrations less than 100 μM, $Ba^{2+}$ selectively block $K_{ir}$ channels (McCarron and Halpern 1990; Nelson and Quayle 1995; Nelson et al. 1995). At a concentration less than 100 μM TWIK-2 channel activity was reduced (Lloyd et al. 2009), however, in mesenteric arteries the effect of $Ba^{2+}$ on TWIK-2 channels was absent (Gardener et al. 2004) suggesting it is not involved in the regulation of rat mesenteric arterial tone. In my experiments, I have shown $Ba^{2+}$ does not inhibit the peak change in smooth muscle membrane potential elicited by SKA-31 and CyPPA suggesting $Ba^{2+}$ does not inhibit the activities of $SK_{Ca}$ and $IK_{Ca}$ channels. This is supported by data obtained in patch-clamp experiments using freshly isolated endothelial cells which $Ba^{2+}$ does not affect $K_{Ca}$ channel currents (Sonkusare et al. 2016). In addition, inhibition of $BK_{Ca}$ and Kv channels have both been shown to potentiate myogenic reactivity in mesenteric (Plane et al. 2005), uterine (Hu et al. 2012), and cerebral arteries (Ledoux et al. 2006; Nelson et al. 1995), an effect which is not seen with $Ba^{2+}$.

A limitation of this study is that recordings of membrane potential and arterial diameter were made under different experimental conditions; arteries pinned to the bottom of a dish and arteries mounted in a pressure myograph. Simultaneous recording of smooth muscle membrane potential and vessel diameter is technically challenging as vessel movements greatly reduce the chances of successful, long duration impalements. Therefore, we measured the membrane potential in pinned out arteries as has been widely used in published studies (Edwards et al. 1988; Edwards, Dora, et al. 1998; Quayle et al. 1993; Wei et al. 2018).

To summarize, in the present study I have used functional approaches (wire and pressure myography) and recordings of intracellular membrane potential, supported by qPCR and immunohistochemistry, to demonstrate that small molecule activators of $SK_{Ca}$ and $IK_{Ca}$ channels

can modulate myogenic reactivity through a $K_{ir}$ channel-dependent mechanism. The actions of CyPPA are blocked by apamin, a selective inhibitor of $SK_{Ca}$ channels and are blocked by the NOS inhibitor L-NAME. This indicates that either CyPPA enhances NO release or that block of NOS leads to inhibition of $SK_{Ca}$-mediated EDH. In contrast, SKA-31 can act via an NO-independent pathway in arteries pre-constricted with PE, and in myogenically active arteries, can act independently of the endothelium. However, inhibition of myogenic reactivity by both CyPPA (via activation of endothelial $SK_{Ca}$ channels) and SKA-31 (via activation of endothelial $IK_{Ca}$ channels and possibly inhibition of smooth muscle TRPM4 channels) involves recruitment of $K_{ir}$ channels. In addition, I propose SKA-31 could be a novel pharmacological tool to examine the function of TRPM4 channels which there is only one inhibitor (9-phenanthrol) currently available.

The traditional view has been that $K_{ir}$ channels provide a resting current whose passive properties set basal membrane potential and enable blood flow to be increased in response to increases in $[K^+]_o$. However, my findings, together with recent published studies, indicate that these channels may also fulfill an important physiological role in amplifying arterial diameter changes elicited by stimuli which hyperpolarize the endothelial or smooth muscle cell membrane potential including agonists and physiological stimuli such as increases in shear stress. My data also support the findings of **Chapter 3** that the mechanisms underlying control of resistance artery diameter are context- and stimulus specific, and that the endothelium can play a central role in co-ordinating changes in arterial diameter in response to different stimuli.

# Chapter 5: NO facilitates endothelium-dependent smooth muscle hyperpolarization in rat basilar artery

## 5.1 Introduction

Release of endothelium-derived NO in response to agonists and flow occurs concurrently with hyperpolarization of the endothelial cell membrane potential caused by opening of $SK_{Ca}$ and $IK_{Ca}$ channels. As previously discussed, release of NO and transfer of endothelial hyperpolarization to surrounding smooth muscle cells (EDH) have generally been regarded as distinct pathways for regulation of vascular tone. This idea came from experiments in which agonist-evoked relaxation of isolated arteries was found not to be blocked by inhibitors of NOS or COX, and the "residual" relaxation was inhibited by blockers of endothelial $SK_{Ca}$ and $IK_{Ca}$ channels, apamin and charybdotoxin (for review see Garland et al. 1995; Félétou and Vanhoutte 1999). In fact, in very few studies were changes in membrane potential measured and the defining characteristic of an EDH responses became the ability of $K_{Ca}$ channel inhibitors to block NOS inhibitor-resistant relaxations in isolated arteries.

However, recent data indicate endothelial $K_{Ca}$ channels may modulate the release and/or actions of NO (Mishra et al. 2013; Sheng and Braun 2007; Sheng et al. 2009; Stankevicius et al. 2006), and studies showing inhibition of EDH of smooth muscle by NOS inhibitors suggest that NO could also contribute to EDH. For example, in rabbit carotid artery EDH and relaxation to ACh was closely correlated with the release of NO, and the NO donor, linsidomine, caused both smooth muscle hyperpolarization and relaxation (Cohen et al. 1997). In rat basilar arteries, both endothelium-dependent relaxations and EDH of smooth muscle cell membrane potential evoked by ACh were blocked by the NOS inhibitor L-NAME suggesting that NO may mediate both effects (Allen et al. 2002). But, in the same vessels, NO produced only a very small hyperpolarization of

smooth muscle membrane potential at very high concentrations which were associated with maximal relaxation (Rand and Garland 1992).

In large conduit arteries such as rabbit aorta (Moore et al. 1990), agonist-evoked endothelium-dependent relaxation can be fully accounted for by NO. In smaller arteries, the persistence of relaxations to agonists in the presence of NOS inhibition which were then abolished by inhibitors of $K_{Ca}$ channels, led to the conclusion that the importance of EDH increases with decreasing artery diameter. For example, whereas ACh-evoked relaxation was abolished by L-NAME in rat and rabbit aorta (Rees et al. 1990; Moore et al. 1990), in rat cremaster arterioles, L-NAME alone had only a modest effect on ACh-evoked relaxations but in the presence of L-NAME, apamin and charybdotoxin, relaxations were blocked, the signature of an EDH-mediated response (Potocnik et al. 2009). The rationale for the apparent increased role of EDH in mediating endothelium-dependent relaxation in smaller versus large vessels, is the prevalence of electrical coupling between endothelial and smooth muscle cells via MEGJs (Sandow et al. 2006). This type of cell-cell connection is extremely limited in conduit arteries and so a diffusible mediator is required for effective communication between the two cell types. However, in small vessels, the density of MEGJs increases, and so electrical communication is more effective and plays a greater role in mediating vasorelaxation to stimuli acting on the endothelium (Senadheera et al. 2012; Straub et al. 2014).

Based on these findings, the prevailing view has been that NO is unimportant in endothelial to smooth muscle communication in small arteries. But, NOS inhibitors do have a significant effect on agonist-evoked vasorelaxation in some resistance arteries indicating that although it may not be the major mediator, NO certainly plays a contributory role (Parsons et al. 1994). In isolated rat mesenteric arteries, L-NAME caused a rightward shift in the concentration-response curves for

both ACh-evoked relaxation and membrane hyperpolarization indicating that, although exogenous NO does not elicit smooth muscle hyperpolarization in these vessels, it may play a facilitatory role in the EDH response (Waldron and Garland 1994). Building on these findings, I have tested the hypothesis that *NO can facilitate $K_{Ca}$ channel mediated, endothelium-dependent smooth muscle hyperpolarization.*

For these experiments I chose to use 1-EBIO and NS309, as like receptor agonists such as ACh, these agents stimulate EDH by activating both $SK_{Ca}$ and $IK_{Ca}$ channels (Strøbæk et al. 2004). All experiments were conducted in the rat basilar artery as previous work in our lab had shown that in this vessel, ACh-evokes endothelium-dependent relaxation and EDH, both of which are inhibited by the NOS inhibitor L-NAME (Allen et al. 2002).

## 5.2 Methods

Please see Chapter 2 for a complete description of methods.

## 5.3 Results

*5.3.1 Endothelium-dependent vasorelaxation and EDH elicited by ACh were abolished by block of NO signaling and endothelial $K_{Ca}$ channels:* In rat basilar arteries mounted under isometric conditions in the wire myograph, ACh (1 nM to 10 μM) evoked endothelium-dependent relaxation of 5-HT (3 μM)-evoked tone (**Figures 5.1a and c**). In separate experiments, bolus doses of ACh (10 μmoles) elicited hyperpolarization of basilar artery smooth muscle cell membrane potential in endothelium-intact (**Figure 5.1b**) but not denuded arteries (n=4).

Both ACh-evoked relaxation and EDH were inhibited by the NOS inhibitor L-NAME (100 μM) or the soluble guanylyl cyclase inhibitor 1H-[1,2,4]oxadiazolo[4,3,-a]quinoxalin-1-one (ODQ; 10 μM), suggesting that as previously proposed (Allen et al. 2002), ACh-evoked changes in tension and membrane potential in this artery can be accounted for by the release of NO which activates soluble guanylyl cyclase and PKG (Waldron and Garland 1994; **Figure 5.1**). However,

both ACh-evoked relaxation and EDH were also significantly inhibited by the combination of apamin (50 nM) and TRAM-34 (1 μM), blockers of $SK_{Ca}$ and $IK_{Ca}$ channels, respectively, (**Figure 5.1**) indicating that these channels also contribute to the actions of ACh. Concentration-relaxation curves for ACh in the presence of L-NAME and/or apamin and TRAM-34 are shown in **Figure 5.1c**. For clarity the data showing the effect of ODQ is not included on the graph; in the presence of ODQ (10 μM) the maximal relaxation to ACh was reduced from 93.4 ± 1.6 % to 20 ± 3.1 % (n=5; $P<0.05$). L-NAME, ODQ, and the combination of apamin and TRAM-34 were without effect on smooth muscle cell resting membrane potential; mean resting smooth muscle membrane potential under control conditions and in the presence of L-NAME, ODQ or apamin and TRAM-34 were -42.1 ± 2.6 mV, -39.6 ± 1.9 mV, 37.5 ± 2.9 mV and 38.5 ± 4 mV (n=6; $P>0.05$), respectively.

*5.3.2 Endothelium-dependent vasorelaxation and EDH evoked by 1-EBIO and NS309 were abolished by block of NO signaling and endothelial $K_{Ca}$ channels:* 1-EBIO (1 μM to 1 mM) and NS309 (1 nM to 10 μM), activators of both $SK_{Ca}$ and $IK_{Ca}$ channels, each caused endothelium-dependent relaxation of isolated segments of basilar artery mounted in the wire myograph (**Figure 5.2a**). Bolus doses of 1-EBIO (100 μmoles) and NS309 (1 μmole) elicited hyperpolarization of the smooth muscle membrane potential in endothelium-intact segments of rat basilar artery **(Figure 5.2b and e**) but were without effect on the tone and smooth muscle membrane potential in endothelium-denuded arteries (n=4 each). Vasorelaxation and EDH to both agents were significantly inhibited by L-NAME, ODQ or apamin and TRAM-34 **(Figure 5.2c-e**). The difference in potency between 1-EBIO and NS309 seen in these experiments is in line with that reported in the literature (Strøbæk et al. 2004).

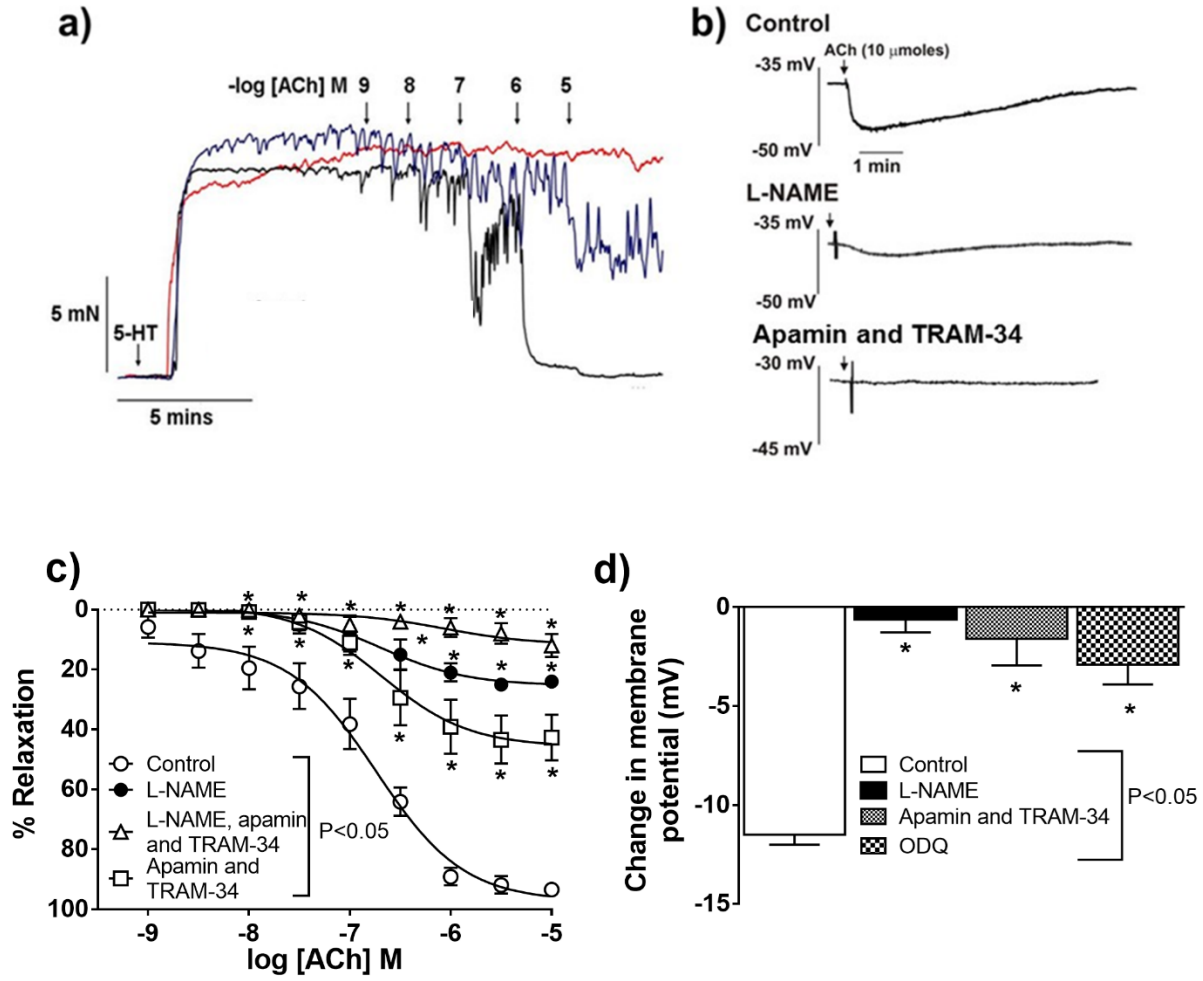



**Figure 5.1: Relaxation and hyperpolarization to ACh are inhibited by block of NOS signaling and/or endothelial $K_{Ca}$ channels.** Representative traces showing ACh-evoked **a)** relaxation and **b)** hyperpolarization in endothelium intact-arteries in the absence and presence of L-NAME (100 μM) and/or apamin (50 nM) and TRAM-34 (1 μM). Arrows indicate addition of ACh (10 μmoles). In **a)** the black line is control relaxations, the blue line is in the presence of apamin and TRAM-34, and the red line is in the presence of L-NAME, apamin and TRAM-34. Mean data showing **c)** relaxation (n=6) and **d)** hyperpolarization (n=5) to ACh in the absence and presence of blockers of NOS signalling and endothelial $K_{Ca}$ channels (*data contributed by Frances Plane)*. Two-way ANOVA was performed. *denotes statistically significant compared to control values ($P<0.05$).

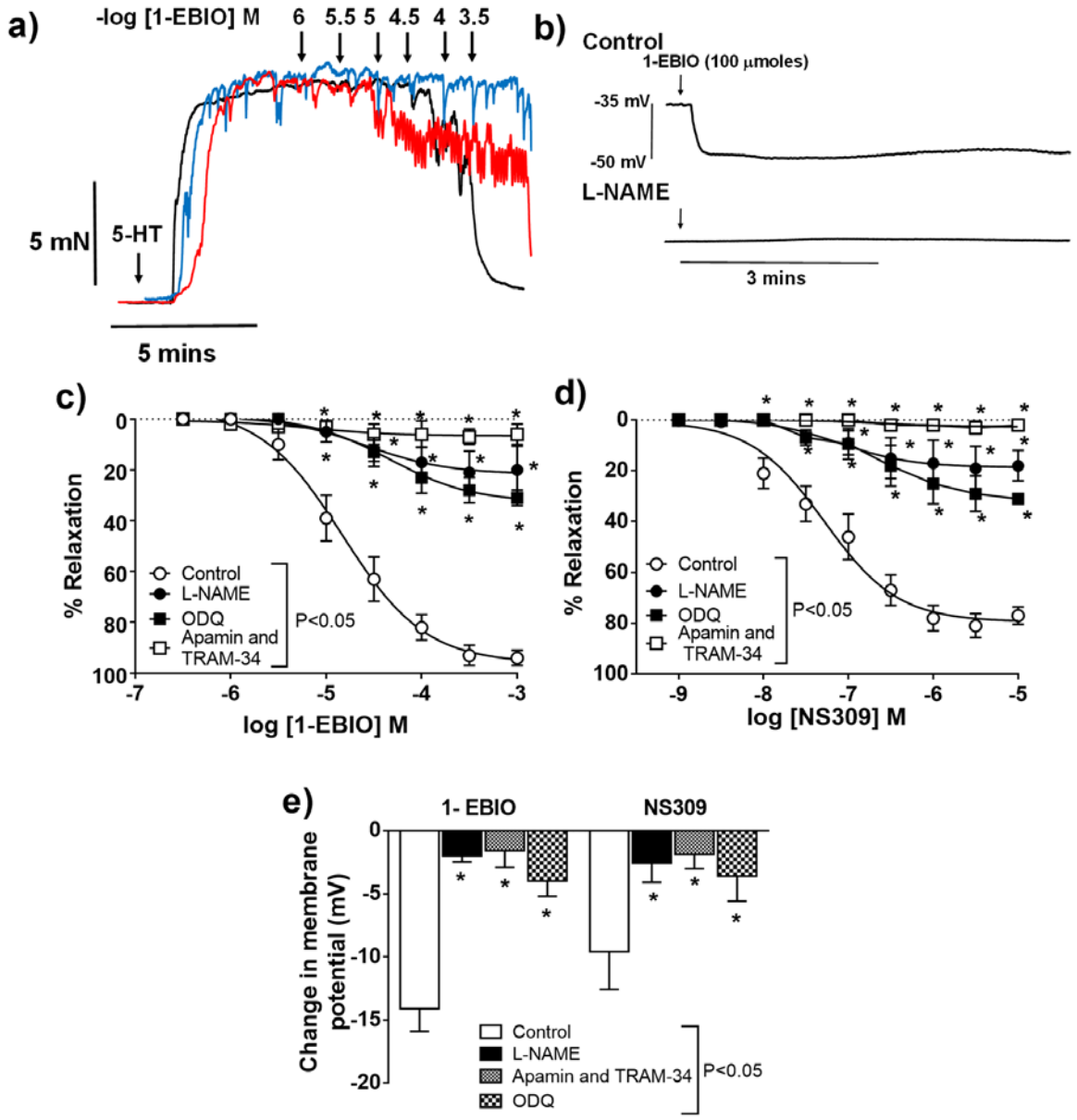



**Figure 5.2: Relaxation and hyperpolarization to 1-EBIO and NS309 are inhibited by block of NOS and/or endothelial $K_{Ca}$ channels.** Representative traces showing EBIO-evoked **a)** relaxation and **b)** hyperpolarization in endothelium intact-arteries in the absence and presence of L-NAME (100 µM). Arrows indicate addition of 1-EBIO (100 µmoles). In **a)** the black line is control relaxations, the red line is in the presence of L-NAME and the blue line is in the presence of apamin and TRAM-34. Mean data showing relaxations to **c)** 1-EBIO and **d)** NS309 in the absence and presence of L-NAME, ODQ (10 µM), or apamin and TRAM-34 (n=5). **e)** Mean data showing hyperpolarization to 1-EBIO and NS309 in the absence and presence of L-NAME, ODQ, or apamin and TRAM-34 (n=5). Two-way ANOVA was performed. *denotes statistically significant compared to control values (P<0.05).

*5.3.3 DEA NONOate evoked endothelium-independent relaxation in rat basilar artery but had no effect on smooth muscle membrane potential:* Based on these findings I then investigated whether NO can evoke smooth muscle membrane hyperpolarization and relaxation in isolated endothelium-denuded segments of rat basilar artery using the NO donor DEA NONOate, which at 37ºC and pH 7.4 spontaneously liberates NO (Keefer et al. 1996). DEA NONOate (100 μmoles) did not alter resting membrane potential of smooth muscle cells (n=6; **Figure 5.3a**). However, DEA NONOate (1 nM to 10 μM) evoked concentration dependent relaxations which were significantly inhibited by ODQ (10 μM) but unaffected by block of $BK_{Ca}$ channels by iberiotoxin (IbTX; 100 nM; **Figure 5.3b**). In these experiments, IbTX significantly enhanced 5-HT-evoked tone and so the concentration of 5-HT was titrated to give a comparable level of tone in the absence and presence of IbTX.

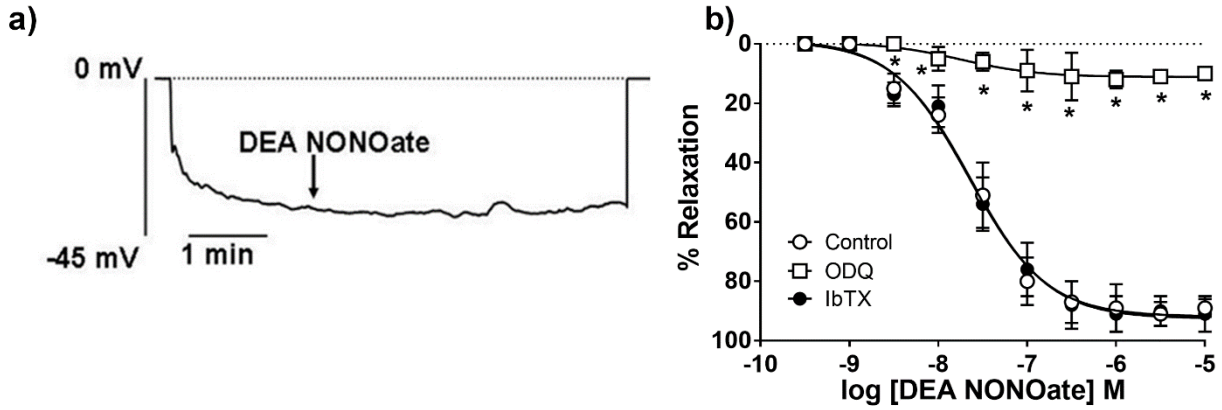



**Figure 5.3: DEA NONOate evokes vasorelaxation without altering smooth muscle membrane potential. a)** Representative trace showing DEA NONOate (100 μmoles) does not alter smooth muscle membrane potential (n=6). **b)** Mean data showing relaxations to DEA NONOate in the absence and presence of ODQ (10 μM) or IbTX (100 nM; n=6). Two-way ANOVA was performed. *denotes statistically significant compared to control values ($P<0.05$).

*5.3.4. Exogenous NO overcomes the effects of L-NAME on smooth muscle hyperpolarization and relaxation to ACh, 1-EBIO and NS309.* Although DEA NONOate had no effect on smooth muscle

membrane potential, addition of a low concentration of DEA NONOate (20 nM) to the perfusate partially overcame the inhibition of endothelium-dependent relaxations and hyperpolarizations to ACh, 1-EBIO and NS309 seen in the presence of L-NAME (**Figure 5.4**). In the presence of DEA NONOate and L-NAME relaxations to lower concentrations of these agents were enhanced relative to L-NAME alone and were not significantly different to control values (**Figure 5.4 c-e**). Smooth muscle hyperpolarizations elicited by ACh, 1-EBIO and NS309 were also enhanced and not different from control values (**Figure 5.4a, b**). In the presence of L-NAME and DEA NONOate, addition of ODQ (10 μM) or $K_{Ca}$ channel blockers apamin and TRAM-34 abolished hyperpolarizations (**Figure 5.4b**) and relaxations to ACh, 1-EBIO and NS309 (**Table 5.1**). Thus, the potentiating effect of DEA NONOate appears to be mediated by soluble guanylyl cyclase and endothelial $SK_{Ca}$ and $IK_{Ca}$ channels. At the concentration used in these experiments, DEA NONOate evokes around 20% relaxation of 5-HT-induced tone. Thus, control experiments were conducted in which the concentration used to evoke tone was titrated to a comparable level but this was without effect on the inhibition of ACh-evoked responses caused by L-NAME.

| | **Control** | **L-NAME** | **DEA NONOate and L-NAME** | **DEA NONOate, L-NAME and ODQ** | **DEA NONOate, L-NAME, apamin and TRAM-34** |
|---|---|---|---|---|---|
| **ACh** | 93.4 ± 1.6 | 24.0 ± 2.1* | 58.5 ± 4.9* | 5.1 ± 3.7* | 1.4 ± 1.3* |
| **1-EBIO** | 94.0 ± 3.2 | 20.0 ± 9.3* | 52.1 ± 6.0* | 6.7 ± 4.7* | 2.8 ± 1.9* |
| **NS309** | 81.0 ± 4.7 | 19.7 ± 8.6* | 54.0 ± 2.5* | 2.0 ± 1.8* | 4.5 ± 3.0* |

**Table 5.1. Effect of DEA NONOate on relaxations to ACh, 1-EBIO and NS309 is prevented by block of soluble guanylyl cyclse or endothelial $K_{Ca}$ channels.** Maximal relaxations (%) to ACh, 1-EBIO and NS309 in the absence and presence of DEA NONOate (20 nM) and/or L-NAME (100 mM), ODQ (10 mM), apamin (50 nM) and TRAM-34 (1 μM). n=5-6. Two-way ANOVA was performed. *denotes statistically significant compared to control values ($p<0.05$).

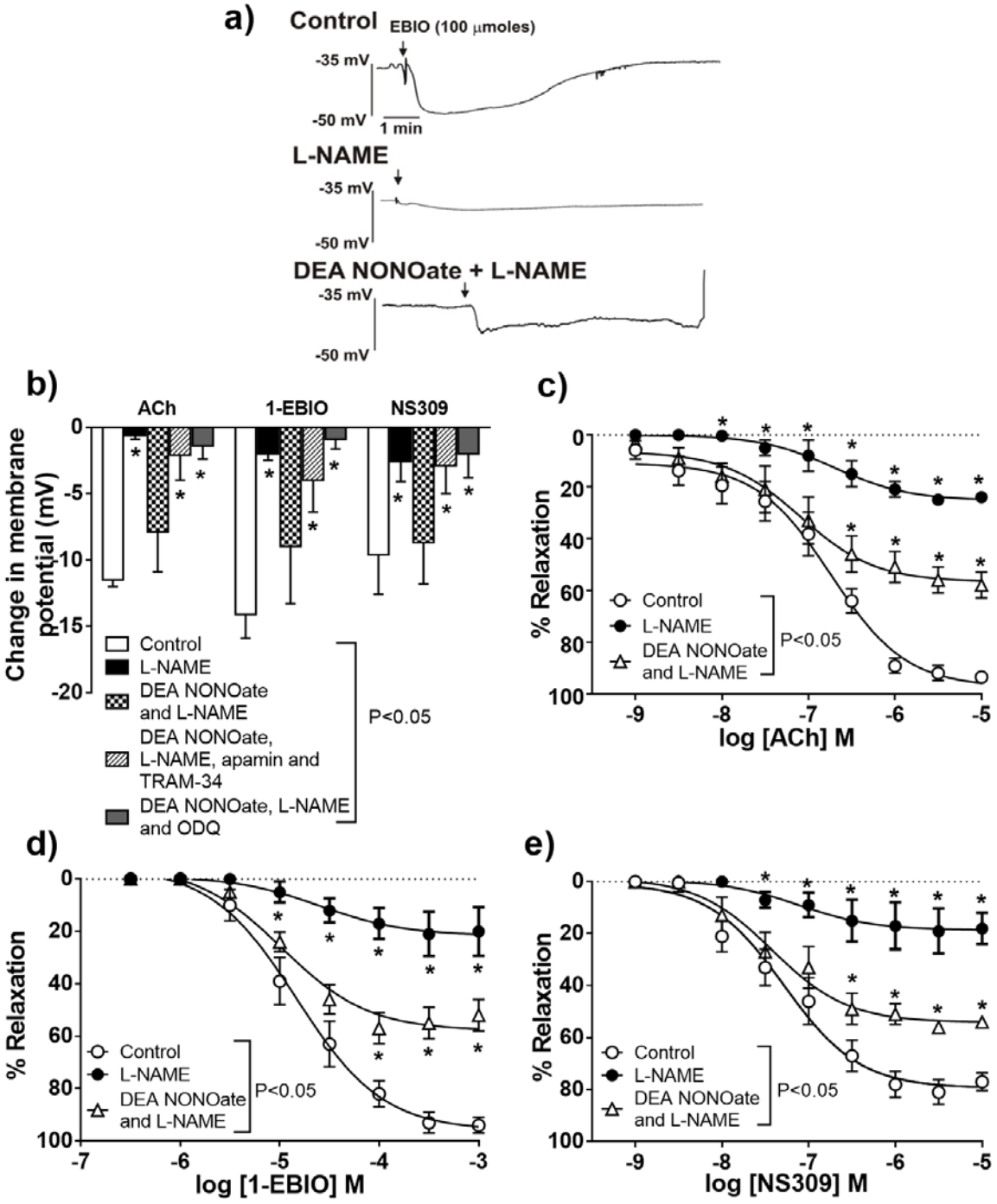



**Figure 5.4: Prior addition of DEA NONOate overcomes inhibition of EDH caused by block of NOS. a)** Representative traces showing hyperpolarization to 1-EBIO (100 μmoles) in the absence presence of L-NAME (100 μM) alone and L-NAME plus DEA NONOate (20 nM). Arrows indicate addition of 1-EBIO (100 μM)]. Mean data showing the effect of DEA NONOate on **b)** hyperpolarization and relaxation to **c)** ACh (*data contributed by Frances Plane)*, **d)** 1-EBIO and **e)** NS309 in the presence of L-NAME (n=5). Two-way ANOVA was performed. * denotes P<0.01 compared to control.

*5.3.5 Endothelium-independent vasorelaxation and smooth muscle hyperpolarization to the $K_{ATP}$ channel opener pinacidil were unaffected by DEA NONOate:* Pinacidil (0.01-10 μM) evoked relaxation of 5-HT (3 μM)-evoked tone in endothelium-denuded segments of rat basilar artery mounted in the wire myograph (**Figure 5.5a**). In separate experiments, bolus doses of pinacidil (100 μmoles) elicited hyperpolarization of basilar artery smooth muscle cell membrane potential in endothelium-denuded arteries. Both relaxation and hyperpolarization to pinacidil were unaffected by L-NAME (100 μM), ODQ (10 μM), or apamin (50 nM) and TRAM-34 (1 μM: **Figure 5.5**). Furthermore, in the presence of L-NAME, DEA NONOate (20 nM) did not enhance relaxation to pinacidil; the maximal relaxations to pinacidil (10 μM) in the presence of L-NAME alone and with DEA NONOate were 94.3 ± 2.8 % and 95.1 ± 4.7 % (n=4; P>0.05), respectively.

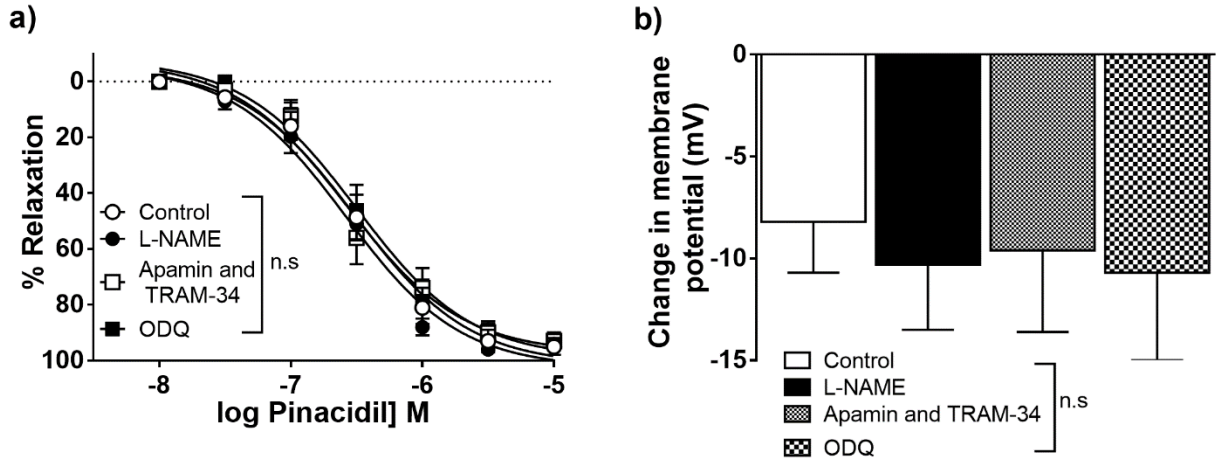



**Figure 5.5: Relaxation and hyperpolarization to pinacidil are not affected by block of NOS signaling or endothelial $K_{Ca}$ channels. a)** Mean data showing relaxations to pinacidil in the absence and presence of L-NAME (100 μM), ODQ (10 μM) or apamin (50 nM) and TRAM-34 (1 μM; n=4). **b)** Mean data showing hyperpolarization to pinacidil (100 μmoles) in the absence and presence of L-NAME, ODQ or apamin and TRAM-34 (n=4). Two-way ANOVA was performed (P>0.05).

## 5.4 Discussion

In this chapter, the role of NO in both smooth muscle relaxation and hyperpolarization elicited by activation of endothelial $SK_{Ca}$ and $IK_{Ca}$ channels in rat basilar arteries was investigated. As in previous studies, block of both relaxation and EDH to ACh by L-NAME was at first interpreted as indicating that NO mediates both endothelium-dependent relaxation and hyperpolarization in this artery. However, further investigation revealed that exogenous NO did not elicit hyperpolarization in this vessel. Furthermore, when endogenous NO production was blocked, ACh-evoked relaxation and EDH could be restored by a low concentration of an NO donor, DEA NONOate. Thus, it appears that in addition to acting as an independent vasorelaxant, NO may play a facilitatory role in EDH-mediated responses in rat basilar arteries.

NO is the primary mediator of endothelium-dependent vasorelaxation in large conduit arteries such as rat aorta (Nagao et al. 1992). However, in small resistance arteries in which endothelial and smooth muscle cells have tight electrical coupling through gap junctions, EDH (Garland et al. 1995) appears to be the dominant mediator of endothelium-dependent relaxation and a role of NO has not been described. Identification of a facilitatory role for NO in enhancing EDH-mediated changes in smooth muscle tone may demonstrate that NO plays a more important role in small arteries than previously recognized. This is consistent with the permissive role of NO described in earlier studies (Bryan et al. 1995; Golding et al. 2001) in which basal release of endogenous NO and sufficient levels of cGMP are required to elicit $\alpha_2$-adrenoceptor- and $K^+$-mediated vasodilation.

As in the current study in rat basilar arteries, inhibition of NOS has been shown to inhibit both endothelium-dependent relaxation and EDH in arteries such as rabbit carotid artery (Cohen et al. 1997) and guinea-pig uterine and spiral arteries (Jiang et al. 2004; Tare et al. 1990). These findings led to the proposal that NO is an endothelium-derived hyperpolarizing factor, and that

activation of $K^+$ channels contributes to NO-mediated vasodilation (Edwards et al. 1998; Kerr et al. 2012; McNeish et al. 2005; Potocnik et al. 2009). In support of this proposal, exogenous NO can cause smooth muscle cell hyperpolarization in rabbit mesenteric (Murphy and Brayden 1995) and carotid arteries (Cohen et al. 1997), and guinea-pig uterine (Tare et al. 1990) and coronary arteries (Parkington et al. 1995) although there is variation in the identity of the $K^+$ channel involved. For example, in rabbit mesenteric arteries, NO-mediated hyperpolarization appears to be due to activation of smooth muscle $K_{ATP}$ channels (Murphy and Brayden 1995), whereas in rat aorta a role for these channels was discounted (Vanheel and Van de Voorde 1997), and in horse penile arteries NO appears to activate $BK_{Ca}$ channels (Simonsen et al. 1995). NO can directly activate $BK_{Ca}$ channels in isolated smooth muscle cells from rat mesenteric resistance arteries and rabbit aorta (Bolotina et al. 1994; Mistry and Garland 1998), and relaxations of rat mesenteric and rabbit carotid arteries to NO are inhibited by blockers of $BK_{Ca}$ channels(Plane et al. 2001; Plane et al. 1998). However, in the present study, the lack of effect of DEA NONOate on membrane potential and the inability of IbTX to modulate relaxations to DEA NONOate suggests that activation of $K^+$ channels does not contribute to the vasorelaxant actions of NO in rat basilar arteries. This finding is similar to published reports in which NO either had no effect on smooth muscle membrane potential or evoked hyperpolarization only at very high concentrations (Garland and McPherson 1992; Tare et al. 1990).

The lack of effect of NO on membrane potential raises the question of how the NO donor DEA NONOate can potentiate endothelium-dependent relaxation and EDH elicited by ACh and $K_{Ca}$ channels activators when endogenous NO production is blocked. In endothelium-denuded arteries, relaxation and smooth muscle hyperpolarization to the $K_{ATP}$ channel opener pinacidil were unaffected by DEA NONOate demonstrating that the observed potentiating effects are not

due to direct actions on the smooth muscle. EDH elicited by ACh, and $K_{Ca}$ channel openers is mediated by electrotonic spread of charge from the endothelial to smooth muscle cells via MEGJs (Sandow et al. 2006), low resistance pathways that allow the flux of ions and small signaling molecules between the two cell layers. Thus, one possibility is that NO can regulate the permeability of MEGJs to facilitate EDH (Straub et al. 2011).

The potential physiological importance of NO in regulating gap junctional communication has been recently recognized and is illustrated by the description of hemoglobin-α being enriched at MEGJs where it regulates the effects of NO on vascular reactivity (Straub et al. 2012). Data obtained from experiments using co-cultured endothelial and smooth muscle cells, and isolated arteries have demonstrated that both endogenous (Hoffmann et al. 2003; Kameritsch et al. 2005; Lidington et al. 2003; McKinnon et al. 2009; Rodenwaldt et al. 2007) and exogenous NO (Straub et al. 2011) can alter the expression and function of MEGJs (Haefliger et al. 1999; Hoffmann et al. 2003; reviewed by Looft-Wilson et al. 2012). MEGJs are composed of connexin proteins which form pores between endothelial and smooth muscle cells. Each gap junction is composed of two opposing hemichannels each consisting of six connexin subunits, which join to form a pore between adjacent cells (Ellinsworth et al. 2016; de Wit and Griffith 2010). Individual gap junctions aggregate into plaques, which can be visualized by electron microscopy at points of cell-cell contact (Sandow and Hill 2000). Expression and distribution of different connexin proteins varies between vessels but the major ones associated with MEGJs in both rat mesenteric and basilar arteries are connexin43 and 37 (Haddock et al. 2006; Sandow et al. 2006). In co-cultures of endothelial and smooth muscle cells, NO can directly enhance the permeability of MEGJs through S-nitrosylation of connexin43 at cysteine 271 (Straub et al. 2011). In the same study, abundant amounts of eNOS and connexin43 were co-localized to MEGJs in both co-cultured cells and intact

mesenteric, coronary, cremaster and thoracodorsal arteries. Interestingly, the connexin43 isolated from these tissues was constitutively S-nitrosylated on cysteine 271 and de-nitrosylation of connexin43 by S-nitrosoglutathione reductase led to a reduction in heterocellular communication between endothelial and smooth muscle cells (Straub et al. 2011). Thus, it was proposed that this post-translational modification of connexin43 by NO may be important for maintaining the open state and permeability of MEGJs.

In addition to directly nitrosylating connexins, NO can increase levels of second messengers to activate kinase enzymes which will phosphorylate connexin proteins to enhance electrical communication (Sáez et al. 1997; Weng et al. 2002), assembly (Paulson et al. 2000), and trafficking to cell membrane (Hoffmann et al. 2003). For example, in cultured human umbilical vein endothelial cells, increases in gap junction number and function caused by exogenous NO is dependent on both cGMP-PKG and cAMP-PKA pathways (Hoffmann et al. 2003); in addition to activating soluble guanylyl cyclase to increase levels of cGMP, NO can indirectly increase the activity of PKA by inhibiting phosphodiesterase activity and so elevate cAMP levels (Francis et al. 2010; Patel et al. 2006). Phosphorylation of connexins by PKC and PKA to increase opening of gap junctions has also been demonstrated in freshly isolated neonatal rat and pig cardiomyocytes (Sáez et al. 1997; Weng et al. 2002), and in rats, chronic treatment with NOS inhibitor L-NAME *in vivo* can cause arterial hypertension associated with attenuated expression of connexin 43 (Haefliger et al. 1999). However, in the short duration of my experiments it is unlikely that there would be sufficient time for changes in protein expression, and thus any acute effects of NO on MEGJ function are more likely mediated through S-nitrosylation and/or PKG-mediated phosphorylation of connexin proteins. This proposal is supported by the fact that the

potentiating effect of DEA NONOate on endothelium-dependent relaxation and hyperpolarization was prevented by the soluble guanylyl cyclase inhibiter ODQ.

Another possible explanation for effects of NO on EDH is that NO may suppress $Cl^-$ efflux in smooth muscle cells which would increase membrane resistance and so enhance the effect of subsequent $K^+$ channel opening on smooth muscle membrane potential (Zhang, Vogalis, and Goyal 1998; Blouquit-Laye et al. 2012; Sakagami et al. 2001). Under physiological conditions, inhibition of $Cl^-$ channels has been reported to have a minimal effect on resting membrane potential as the equilibrium potential of $Cl^-$ is close to the resting membrane potential of smooth muscle cells (Despopoulos and Silbernagl 2003; Guyton and Hall 2006). This could explain why exogenous NO did not affect the resting membrane potential of smooth muscle cells in my experiments, however, the lack of effect of DEA NONOate on responses to pinacidil which acts to directly hyperpolarize the smooth muscle does not support this proposal.

In the present study on rat basilar arteries, block of NOS had a profound effect on EDH whereas in rat mesenteric arteries, block of NOS causes a rightward shift in the concentration-response curve for ACh-evoked EDH (Waldron and Garland 1994). A potential reason for this difference is that the density of MEGJs is higher in rat mesenteric versus basilar arteries (Sandow et al. 2002, 2009). A higher density of connections will provide greater coupling efficiency between endothelial and smooth muscle cells so may reduce the influence of NO on endothelial to smooth muscle communication.

To summarize, in this chapter I have found that relaxation and hyperpolarization to ACh, and the $K_{Ca}$ channel activators 1-EBIO and NS309 were inhibited by block of NOS and/or endothelial $K_{Ca}$ channels in rat basilar arteries. This could indicate a role for NO in relaxation and smooth muscle hyperpolarization to these agents. However, exogenous NO did not elicit smooth

muscle cell membrane hyperpolarization, and application of an NO donor could overcome the inhibition of EDH caused by block of NOS suggesting that NO plays a facilitatory role in EDH. How NO enhances EDH of smooth muscle is unclear but, based on recent published studies, NO may modulate MEGJs to facilitate spread of charge from endothelial to smooth muscle cells. These findings have significant implications for previous work in which the sensitivity of contractile and electrical responses to NOS inhibition has been interpreted as showing a causal role for NO. Also, these data may contribute to shifting our perception of the role of NO from being just a vasodilator to also being a facilitator of EDH, and further support the proposal that rather than two separate pathways, NO and EDH are intrinsically linked.

# Chapter 6: General Discussion

Maintenance of appropriate blood supply to tissues and organs requires endothelial and smooth muscle cells to act in a co-ordinated manner in response to mechanical and chemical stimuli. Stimulation of the endothelium by changes in shear stress, and circulating mediators leads to release of diffusible relaxing and contracting factors, and also to electrical coupling to alter the contractile state of surrounding smooth muscle cells. Conversely, stimulation of smooth muscle cells by neurotransmitters and increases in intravascular pressure can lead to flux of second messengers into endothelial cells to activate both electrical and chemical pathways which will feedback to limit smooth muscle contraction.

Activation of endothelial pathways for modulation of smooth muscle contractility is mediated by a rise in $[Ca^{2+}]_i$ leading to recruitment of downstream effectors, two of the most important of which are NOS and $K_{Ca}$ channels. NOS converts L-arginine to NO which elicits relaxation of smooth muscle cells via stimulation of soluble guanylyl cyclase to increase cGMP and activate PKG-mediated phosphorylation of numerous target proteins. Activation of $K_{Ca}$ channels hyperpolarizes the endothelial cell membrane potential which in turn spreads to surrounding smooth muscle cells (EDH) to limit contraction by reducing the open probability VOCCs. NO and EDH have long being regarded as separate, parallel pathways for endothelium-dependent vasodilation and the gold-standard test for EDH-mediated responses is the demonstration that application of $SK_{Ca}$ and $IK_{Ca}$ channel blockers apamin and charybdotoxin or TRAM-34, inhibits the relaxation remaining in the presence of an NOS inhibitor such as L-NAME. However, physiologically, the idea of two completely independent pathways for relaxation is counter to the sort of signal integration required to fine tune changes in arterial diameter in response to simultaneous stimuli such as release of neurotransmitter from sympathetic nerves and

increases in shear stress due to vasoconstriction. Thus, in my thesis I have investigated how the NO and EDH pathways may be integrated to regulate smooth muscle cell contractility.

One aspect of this has been to identify how contractile stimulation of smooth muscle cells leads to recruitment of inhibitory endothelial mechanism to limit further contraction, a process termed myoendothelial feedback. Our lab and others have shown that in resistance arteries, stimulation of smooth muscle cells by $\alpha_1$-adrenoceptor agonists leads to flux of $InsP_3$ from smooth muscle to endothelial cells to elicit localized increases in $Ca^{2+}$, activation of $IK_{Ca}$ channels located at MEGJs and also production of NO.

Building on this work, in **Chapter 3**, I demonstrated that in individual arteries, the contribution of myoendothelial feedback is determined by the nature of the underlying contractile mechanisms. In the case of agonists and sympathetic vasoconstriction, engagement of the endothelium appears to be dependent on the ability of the stimulus to generate $InsP_3$. Similarly, for sympathetic vasoconstriction the ability of the endothelium to limit increases in tone is mirrored by the frequency-dependence of contribution nifedipine-resistant mechanisms to increases in tone. These experiments revealed that at lower frequencies, nerve-evoked increases in tone can be fully accounted for by depolarization leading to increased $Ca^{2+}$ influx though L-type VOCCs, but at higher frequencies there is an increasing contribution of $InsP_3$. Sympathetic nerves play a major role in regulating arterial diameter *in vivo* and reliance on largely voltage-independent contractile mechanisms provides an effective mechanism for integration of nerve activity with the endothelium within individual arterial segments. Also, voltage-dependent contractions are limited by opening of Kv and $BK_{Ca}$ channels and so an increasing dependence on voltage-independent mechanisms with high frequencies of stimulation may provide a mechanism to escape these control mechanisms and achieve a higher degree of contraction.

Moving on to the intact mesenteric bed, I found that instead of myoendothelial feedback, it is shear stress-induced activation of $SK_{Ca}$ channels and release of NO that provides a global endothelial response to vasoconstriction to ensure appropriate distribution of blood flow within the intact vascular bed. Thus, the findings from this work highlight the influence of contractile stimulus on the ability of the endothelium to modulate smooth muscle function, and also the role of the endothelium as a site of information exchange, able to integrate responses to direct mechanical stimuli and flux of mediators from smooth muscle cells to regulate arterial diameter.

The second aspect of my work has been to investigate interactions between the NO and EDH pathways, research which was stimulated by studies suggesting a link between $K_{Ca}$ channel-mediated hyperpolarization and NO production. In **Chapter 4,** I explored the mechanisms underlying the ability of small molecule $K_{Ca}$ channel activators to inhibit myogenic reactivity and in particular whether endothelium-derived NO plays a role in the actions of these agents. I chose to examine the effects of these agents on myogenic reactivity as this is a crucial autoregulatory process and the underlying contractile processes are different to those mediating agonist-evoked contractions of isolated arteries. I found that CyPPA, an activator of $SK_{Ca}$ channels, elicited endothelium- and NO-dependent inhibition of myogenic reactivity, as hypothesized but that the actions of SKA-31, a putative activator of $IK_{Ca}$ channels, were not exclusively endothelium-dependent which has not previously been reported. The fact that SKA-31 appeared to evoke endothelium-dependent dilation in arteries stimulated to constrict with the $\alpha_1$-adrenoceptor agonist PE but acted in an endothelium-independent manner in myogenically active arteries further highlights the necessity to consider context when examining how vasodilator stimuli are working. It is already established that the relative contribution of NO and EDH to agonist-evoked endothelium-dependent relaxation can be influenced by the nature of the contractile stimuli (Plane

and Garland 1996). However, this may be the first example in which the mechanism of action of a chemical agent in terms of whether it acts as an endothelium-dependent or –independent relaxant is determined by the background contractile pathways. An implication of this may be that drugs should not be described as endothelium-dependent or –independent vasodilators without reference to the context.

The data from this chapter also reveal a role for $K_{ir}$ channels in amplifying the vasodilator responses driven by changes in membrane potential in mesenteric resistance arteries. Together with recently published work, these findings indicate that $K_{ir}$ channels may play an important physiological role in amplifying vasodilator responses to hyperpolarizing stimuli and as such may have the potential to be drug targets for new approaches to enhance endothelium-dependent dilator responses in disease states associated with endothelial dysfunction.

Following on with the same theme of a link, in **Chapter 5,** I investigated whether there could be a facilitatory relationship between NO and EDH in mediating vasodilation to ACh. I found that relaxation and hyperpolarization to ACh, and the $K_{Ca}$ channel activators 1-EBIO and NS309 were inhibited by block of NOS and/or endothelial $K_{Ca}$ channels and, as in published studies, I initially interpreted these findings as indicating that NO could be an endothelium-derived hyperpolarizing factor and $K_{Ca}$ channel activators can evoke NO-mediated relaxation and smooth muscle hyperpolarization. However, I then found that exogenous NO does not elicit membrane hyperpolarization in the basilar artery and that an NO donor can partially overcome the inhibition of EDH caused by block of NOS. How NO enhances EDH of smooth muscle is unclear. NO can suppresses $Cl^-$ efflux which would increase membrane resistance and so enhance the effect of subsequent $K^+$ channel mediated hyperpolarization (Zhang et al. 1998; Blouquit-Laye et al. 2012; Sakagami et al. 2001). However, a more likely possibility is that NO modulates MEGJ

communication, a proposal supported by recently published data showing that NOS is localized with MEGJs.

Whatever the underlying mechanism, the findings from **Chapter 5** have implications for the data obtained in my other two studies. Facilitation of EDH by NO could play a role in myoendothelial feedback (**Chapter 3**) and inhibition of the EDH pathway by removal of NO could contribute to the observed L-NAME sensitivity of responses to CyPPA (**Chapter 4**). These data also have implications for previously published studies in which loss of the response to an endothelium-dependent vasodilator in the presence of NOS inhibition has also been interpreted as showing a causal relaxant role for NO, as it could shift our view of NO from being an independent vasodilator to a facilitator of hyperpolarization.

In conclusion, traditionally, NO and $K_{Ca}$ channels have been regarded as two distinct endothelium-dependent pathways for modulation of resistance artery diameter. This thesis presents several lines of evidence to support the proposal that endothelial membrane potential, and the integrated activity of $SK_{Ca}/IK_{Ca}$ channels and NO regulate the contractile state of smooth muscle in a manner which is influenced by the nature of vasoconstrictor stimulus.

Over all, the mechanisms governing regulation of blood flow operate as a finely-tuned stimulus- and context-dependent network with the endothelium playing a central role in signal integration.

*6.1 Future Directions:* The data presented in this thesis open up many potential avenues for future research such as:

1. Further investigation of the role of endothelial cells in integrating responses to multiple stimuli to regulate tissue perfusion and blood pressure using approaches such as arteries from

animals lacking key components of the myoendothelial feedback pathway such as $IK_{Ca}$ channels, connexin proteins, and TRPC3 channels.

2. Further investigation of interactions between NO and EDH in terms of determining if NO does contribute to setting smooth muscle membrane resistance, and how it modulates MEGJs in intact arteries. The use of animals lacking connexin proteins, key components of MEGJs may be useful in this pursuit as will imaging and biochemical approaches to investigate NO-mediated modifications of gap-junction proteins such as S-nitrosylation and PKG-mediated protein phosporylation and how these influence electrical communication.

3. Exploration of the role of $K_{ir}$ as amplifiers of vasodilation to physiological stimuli such as increases in shear stress and in myoendothelial feedback using $K_{ir}2.1$ and 2.2 channels KO animals.

4. Endothelial membrane potential as a determinant of vascular health: Evaluation of the impact of risk factors for cardiovascular disease such as elevated glucose on endothelial membrane potential regulation, and interactions between $K_{Ca}$ and $K_{ir}$ channel activity, and the NO and COX pathways to determine if activating $K_{Ca}$ or $K_{ir}$ channels could provide a new therapeutic strategy to overcome endothelial dysfunction seen in animal models of diabetes mellitus.

# References


Abd El-Rahman, Rasha R, Osama F Harraz, Suzanne E Brett, Yana Anfinogenova, Rania E Mufti, Daniel Goldman, and Donald G Welsh. 2013. "Identification of L- and T-Type Ca2+ Channels in Rat Cerebral Arteries: Role in Myogenic Tone Development." *American journal of physiology. Heart and circulatory physiology* 304(1): H58-71.

Abdullaev, Iskandar F, Jonathan M Bisaillon, Marie Potier, Jose C Gonzalez, Rajender K Motiani, and Mohamed Trebak. 2008. "Stim1 and Orai1 Mediate CRAC Currents and Store-Operated Calcium Entry Important for Endothelial Cell Proliferation." *Circulation research* 103(11): 1289–99.

Absi, M, M P Burnham, A H Weston, E Harno, M Rogers, and G Edwards. 2009. "Effects of Methyl β-Cyclodextrin on EDHF Responses in Pig and Rat Arteries; Association between SKCa Channels and Caveolin-Rich Domains." *British Journal of Pharmacology* 151(3): 332–40.

Adams, D J, J Barakeh, R Laskey, and C Van Breemen. 1989. "Ion Channels and Regulation of Intracellular Calcium in Vascular Endothelial Cells." *FASEB journal : official publication of the Federation of American Societies for Experimental Biology* 3(12): 2389–2400.

Addison, M. Pule, Thakur Uttam Singh, Subhashree Parida, Soumen Choudhury, Jaya Kiran Kasa, Susanth V. Sukumaran, Sajad Ahmad Darzi, Kannan Kandasamy, Vishakha Singh, Dinesh Kumar, and Santosh Kumar Mishra. 2016. "NO Synthase Inhibition Attenuates EDHF-Mediated Relaxation Induced by TRPV4 Channel Agonist GSK1016790A in the Rat Pulmonary Artery: Role of TxA2." *Pharmacological Reports* 68(3): 620–26.

Adelman, John P., James Maylie, and Pankaj Sah. 2012. "Small-Conductance Ca $^{2+}$ -Activated K $^{+}$ Channels: Form and Function." *Annual Review of Physiology* 74(1): 245–69.

Ahn, Sang Joon, Ibra S. Fancher, Jing-Tan Bian, Chong Xu Zhang, Sarah Schwab, Robert Gaffin, Shane A. Phillips, and Irena Levitan. 2017. "Inwardly Rectifying K $^{+}$ Channels Are Major Contributors to Flow-Induced Vasodilatation in Resistance Arteries." *The Journal of Physiology* 595(7): 2339–64.

Aiello, E A, Odile Clement-Chomienne, D P Sontag, Michael P Walsh, and William C Cole. 1996. "Protein Kinase C Inhibits Delayed Rectifier K+ Current in Rabbit Vascular Smooth Muscle Cells." *Am J Physiol* 271(1 Pt 2): H109–19.

Akaike, N, H Kanaide, T Kuga, M Nakamura, J Sadoshima, and H Tomoike. 1989. "Low-Voltage-Activated Calcium Current in Rat Aorta Smooth Muscle Cells in Primary Culture." *The Journal of Physiology* 416(1): 141–60.

Albert, A P, and W A Large. 2002. "A Ca2+-Permeable Non-Selective Cation Channel Activated by Depletion of Internal Ca2+ Stores in Single Rabbit Portal Vein Myocytes." *The Journal of physiology* 538(Pt 3): 717–28.

Alejandro-Aiello, E, A T Malcolm, M P Walsh, and William C Cole. 1998. "SS-Adrenoceptor Activiation of PKA Regulate Delayed Rectifier K + Channels of Vascular Smooth Muscle Cell." *Am.J.Physiol.* 275: H448–59.

Alioua, A., J. P. Huggins, and E. Rousseau. 1995. "PKG-I Alpha Phosphorylates the Alpha-Subunit and Upregulates Reconstituted GKCa Channels from Tracheal Smooth Muscle." *American Journal of Physiology-Lung Cellular and Molecular Physiology* 268(6): L1057–63.

Allen, Bruce G., and Michael P. Walsh. 1994. "The Biochemical Basis of the Regulation of Smooth-Muscle Contraction." *Trends in Biochemical Sciences* 19(9): 362–68.

Allen, Tracy, Mircea Iftinca, William C Cole, and Frances Plane. 2002. “Smooth Muscle Membrane Potential Modulates Endothelium-Dependent Relaxation of Rat Basilar Artery via Myo-Endothelial Gap Junctions.” *The Journal of physiology* 545(Pt 3): 975–86.

Alonso-Carbajo, Lucía, Miklos Kecskes, Griet Jacobs, Andy Pironet, Ninda Syam, Karel Talavera, and Rudi Vennekens. 2017. “Muscling in on TRP Channels in Vascular Smooth Muscle Cells and Cardiomyocytes.” *Cell Calcium* 66: 48–61.

Amberg, Gregory C., and Manuel F. Navedo. 2013. “Calcium Dynamics in Vascular Smooth Muscle.” *Microcirculation* 20(4): 281–89.

Amberg, Gregory C., Manuel F. Navedo, Madeline Nieves-cintrón, Jeffery D. Molkentin, and Luis F. Santana. 2007. “Calcium Sparklets Regulate Local and Global Calcium in Murine Arterial Smooth Muscle.” *Journal of Physiology* 579(1): 187–201.

Amberg, Gregory C, Adrian D Bonev, Charles F Rossow, Mark T Nelson, and Luis F Santana. 2003. “Modulation of the Molecular Composition of Large Conductance, Ca(2+) Activated K(+) Channels in Vascular Smooth Muscle during Hypertension.” *The Journal of clinical investigation* 112(5): 717–24.

Ando, Joji, Teruhiko Komatsuda, and Akira Kamiya. 1988. “Cytoplasmic Calcium Response to Fluid Shear Stress in Cultured Vascular Endothelial Cells.” *In Vitro Cellular & Developmental Biology* 24(9): 871–77.

von Anrep, G. 1912. “On Local Vascular Reactions and Their Interpretation.” *The Journal of Physiology* 45(5): 318–27.

Armstrong, Ehrin J, Caitlin T Harskamp, and April W Armstrong. 2013. “Psoriasis and Major Adverse Cardiovascular Events: A Systematic Review and Meta-Analysis of Observational Studies.” *J Am Heart Assoc* 2(2): e000062.

Azar, S, MA Johnson, J Scheinman, L Bruno, and L Tobian. 1979. “Regulation of Glomerular Capillary Pressure and Filtration Rate in Young Kyoto Hypertensive Rats.” *Clinical Science* 56: 203–9.

Baeyens, Nicolas, Chirosree Bandyopadhyay, Brian G Coon, Sanguk Yun, and Martin A Schwartz. 2016. “Endothelial Fluid Shear Stress Sensing in Vascular Health and Disease.” *The Journal of Clinical Investigation* 126(3): 821–28.

Bagher, Pooneh, Timea Beleznai, Yasuo Kansui, Ray Mitchell, Christopher J Garland, and Kim A Dora. 2012. “Low Intravascular Pressure Activates Endothelial Cell TRPV4 Channels, Local Ca2+ Events, and IKCa Channels, Reducing Arteriolar Tone.” *Proceedings of the National Academy of Sciences of the United States of America* 109(44): 18174–79.

Bai, Ni, Farzad Moien-Afshari, Hiroshi Washio, Anie Min, and Ismail Laher. 2004. “Pharmacology of the Mouse-Isolated Cerebral Artery.” *Vascular pharmacology* 41(3): 97–106.

Bayliss, W. M. 1902. “On the Local Reactions of the Arterial Wall to Changes of Internal Pressure.” *The Journal of physiology* 28(3): 220–31.

Beech, D. J. 2012. “Integration of Transient Receptor Potential Canonical Channels with Lipids.” *Acta Physiologica* 204(2): 227–37.

Behringer, Erik J. 2017. “Calcium and Electrical Signaling in Arterial Endothelial Tubes: New Insights into Cellular Physiology and Cardiovascular Function.” *Microcirculation* 24(3): e12328.

Behringer, Erik J., and Steven S. Segal. 2015. “Membrane Potential Governs Calcium Influx into Microvascular Endothelium: Integral Role for Muscarinic Receptor Activation.” *Journal of Physiology* 593(20): 4531–48.

Behringer, Erik J, and Steven S Segal. 2012. “Spreading the Signal for Vasodilatation: Implications for Skeletal Muscle Blood Flow Control and the Effects of Ageing.” *The Journal of physiology* 590(24): 6277–84.
Belik, Jaques. 1995. “The Myogenic Response of Arterial Vessels Is Increased in Fetal Pulmonary Hypertension.” *Pediatric Research* 37(2): 196–201.
Bell, Tracy D, Gerald F DiBona, Ying Wang, and Michael W Brands. 2006. “Mechanisms for Renal Blood Flow Control Early in Diabetes as Revealed by Chronic Flow Measurement and Transfer Function Analysis.” *Journal of the American Society of Nephrology : JASN* 17(8): 2184–92.
Bény, J. 1997. “Electrical Coupling between Smooth Muscle Cells and Endothelial Cells in Pig Coronary Arteries.” *Pflugers Archiv : European journal of physiology* 433(3): 364–67.
Berger, Miles, John A. Gray, and Bryan L. Roth. 2009. “The Expanded Biology of Serotonin.” *Annual Review of Medicine* 60(1): 355–66.
Berridge, Michael J. 2008. “Smooth Muscle Cell Calcium Activation Mechanisms.” *The Journal of Physiology* 586(21): 5047–61.
Bevan, S., S. Hothi, G. Hughes, I. F. James, H. P. Rang, K. Shah, C. S.J. Walpole, and J. C. Yeats. 1992. “Capsazepine: A Competitive Antagonist of the Sensory Neurone Excitant Capsaicin.” *British Journal of Pharmacology* 107(2): 544–52.
Bezanilla, Francisco. 2008. “How Membrane Proteins Sense Voltage.” *Nature Reviews Molecular Cell Biology* 9(4): 323–32.
Bezprozavanny, I, James Watras, and B Ehrlich. 1991. “Bell-Shaped Calcium-Response Curves of Ins(1,4,5)P3-and Calcium-Gated Channels from Endoplasmic Reticulum of Cerebellum.” *Nature* 351: 751–54.
Bichet, Delphine, Friederike A. Haass, and Lily Yeh Jan. 2003. “Merging Functional Studies with Structures of Inward-Rectifier K+ Channels.” *Nature Reviews Neuroscience* 4(12): 957–67.
Billaud, M., A. W. Lohman, S. R. Johnstone, L. A. Biwer, S. Mutchler, and B. E. Isakson. 2014. “Regulation of Cellular Communication by Signaling Microdomains in the Blood Vessel Wall.” *Pharmacological Reviews* 66(2): 513–69.
Blouquit-Laye, Sabine, Luc Dannhoffer, Camille Braun, A.-T. Dinh-Xuan, Edouard Sage, and Thierry Chinet. 2012. “Effect of Nitric Oxide on Epithelial Ion Transports in Noncystic Fibrosis and Cystic Fibrosis Human Proximal and Distal Airways.” *AJP: Lung Cellular and Molecular Physiology* 303(7): L617–25.
Blum, M., C. Pils, U. A. Müller, and J. Strobel. 2006. “Messung Der Myogenen Antwort Retinaler Arteriolen Bei Diabetischer Retinopathie.” *Der Ophthalmologe* 103(3): 209–13.
Bogle, R G, A R Baydoun, J D Pearson, and G E Mann. 1996. “Regulation of L-Arginine Transport and Nitric Oxide Release in Superfused Porcine Aortic Endothelial Cells.” *The Journal of physiology* 490 ( Pt 1: 229–41.
Bohlen, H. Glenn, Robert W. Gore, and Phillip M. Hutchins. 1977. “Comparison of Microvascular Pressures in Normal and Spontaneously Hypertensive Rats.” *Microvascular Research* 13(1): 125–30.
Boittin, F X, N Macrez, G Halet, and J Mironneau. 1999. “Norepinephrine-Induced Ca(2+) Waves Depend on InsP(3) and Ryanodine Receptor Activation in Vascular Myocytes.” *The American journal of physiology* 277(1 Pt 1): C139-51.
Bolla, Manlio, Khalid Matrougui, Laurent Loufrani, Jacques Maclouf, Bernard I. Levy, Sylviane Levy-Toledano, Aïda Habib, and Daniel Henrion. 2002. “p38 Mitogen-Activated Protein

Kinase Activation Is Required for Thromboxane- Induced Contraction in Perfused and Pressurized Rat Mesenteric Resistance Arteries." *Journal of Vascular Research* 39(4): 353–60.

Bolotina, Victoria M., Soheil Najibi, James J. Palacino, Patrick J. Pagano, and Richard A. Cohen. 1994. "Nitric Oxide Directly Activates Calcium-Dependent Potassium Channels in Vascular Smooth Muscle." *Nature* 368(6474): 850–53.

Bond, P A. 1967. "Metabolism of Propranolol ('Inderal'), a Potent, Specific Beta-Adrenergic Receptor Blocking Agent." *Nature* 213(5077): 721.

Bonev, A D, J H Jaggar, M Rubart, and M T Nelson. 1997. "Activators of Protein Kinase C Decrease Ca2+ Spark Frequency in Smooth Muscle Cells from Cerebral Arteries." *The American journal of physiology* 273(6 Pt 1): C2090-5.

Bradley, A. B., and K. G. Morgan. 1987. "Alterations in Cytoplasmic Calcium Sensitivity during Porcine Coronary Artery Contractions as Detected by Aequorin." *The Journal of Physiology* 385(1): 437–48.

Bradley, K K, J H Jaggar, A D Bonev, T J Heppner, E R Flynn, M T Nelson, and B Horowitz. 1999. "Kir2.1 Encodes the Inward Rectifier Potassium Channel in Rat Arterial Smooth Muscle Cells." *J Physiol* 515 ( Pt 3(1999): 639–51.

Brähler, Sebastian, Anuradha Kaistha, Volker J Schmidt, Stephanie E Wölfle, Christoph Busch, Brajesh P Kaistha, Michael Kacik, Anna-Lena Hasenau, Ivica Grgic, Han Si, Chris T Bond, John P Adelman, Heike Wulff, Cor de Wit, Joachim Hoyer, and Ralf Köhler. 2009. "Genetic Deficit of SK3 and IK1 Channels Disrupts the Endothelium-Derived Hyperpolarizing Factor Vasodilator Pathway and Causes Hypertension." *Circulation* 119(17): 2323–32.

Brain, Susan D, and Helen M Cox. 2009. "Neuropeptides and Their Receptors: Innovative Science Providing Novel Therapeutic Targets." *British Journal of Pharmacology* 147(S1): S202–11.

Brakemeier, S, Anne Kersten, Ines Eichler, Ivica Grgic, Andreas Zakrzewicz, Hartmut Hopp, Ralf Köhler, and Joachim Hoyer. 2003. "Shear Stress-Induced up-Regulation of the Intermediate-Conductance Ca2+-Activated K+ Channel in Human Endothelium." *Cardiovascular Research* 60(3): 488–96.

Brawley, L, A M Shaw, and A MacDonald. 2000. "$\beta_1$ -, $\beta_2$ - and Atypical $\beta$ -Adrenoceptor-Mediated Relaxation in Rat Isolated Aorta." *British Journal of Pharmacology* 129(4): 637–44.

Brayden, Joseph E, Yao Li, and Matthew J Tavares. 2013. "Purinergic Receptors Regulate Myogenic Tone in Cerebral Parenchymal Arterioles." *Journal of Cerebral Blood Flow and Metabolism* 33(2): 293–99.

van Breemen, Cornelis, Allen Mangel, Mohammad Fahim, and Kaushik Meisheri. 1982. "Selectivity of Calcium Antagonistic Action in Vascular Smooth Muscle." *The American Journal of Cardiology* 49(3): 507–10.

Brenner, Robert, Guillermo J. Peréz, Adrian D. Bonev, Delrae M. Eckman, Jon C. Kosek, Steven W. Wiler, Andrew J. Patterson, Mark T. Nelson, and Richard W. Aldrich. 2000. "Vasoregulation by the β1 Subunit of the Calcium-Activated Potassium Channel." *Nature* 407(6806): 870–76.

Briones, Ana M, Craig J Daly, Francesc Jimenez-Altayo, Sonia Martinez-Revelles, Jose M Gonzalez, John C McGrath, and Elisabet Vila. 2005. "Direct Demonstration of β1- and Evidence against β2- and β3-Adrenoceptors, in Smooth Muscle Cells of Rat Small

Mesenteric Arteries." *British Journal of Pharmacology* 146(5): 679–91.
Brozovich, F V, C J Nicholson, C V Degen, Yuan Z Gao, M Aggarwal, and K G Morgan. 2016. "Mechanisms of Vascular Smooth Muscle Contraction and the Basis for Pharmacologic Treatment of Smooth Muscle Disorders." *Pharmacological reviews* 68(2): 476–532.
BruceBalus. "Blausen_0055_ArteryWallStructure.png." *Wikipedia*. https://commons.wikimedia.org/wiki/File:Blausen_0055_ArteryWallStructure.png.
Bryan, Robert M, Junping You, Elke M. Golding, and Sean P. Marrelli. 2005. "Endothelium-Derived Hyperpolarizing Factor A Cousin to Nitric Oxide and Prostacyclin." *Anesthesiology: The Journal of the American Society of Anesthesiologists* 102(6): 1261–77.
Bryan Jr, R M, M L Steenberg, M Y Eichler, T D Johnson, M W G Swafford, and M S Suresh. 1995. "Permissive Role of NO in α 2 -Adrenoceptor-Mediated Dilations in Rat Cerebral Arteries." *Am J Physiol* 269: H1171–74.
Bulley, Simon, Zachary P. Neeb, Sarah K. Burris, John P. Bannister, Candice M. Thomas-Gatewood, Wanchana Jangsangthong, and Jonathan H. Jaggar. 2012. "TMEM16A/ANO1 Channels Contribute to the Myogenic Response in Cerebral Arteries." *Circulation Research* 111(8): 1027–36.
Bulloch, J. M., A. MacDonald, and J. C. McGrath. 1991. "Different Sensitivities of Rabbit Isolated Blood Vessels Exhibiting Co-Transmission to the Slow Calcium Channel Blocker, Nifedipine." *British Journal of Pharmacology* 103(3): 1685–90.
Burke, Marilyn, Malikarjuna Pabbidi, Fan Fan, Ying Ge, Ruisheng Liu, Jan Michael Williams, Allison Sarkis, Jozef Lazar, Howard J Jacob, and Richard J Roman. 2013. "Genetic Basis of the Impaired Renal Myogenic Response in FHH Rats." *American journal of physiology. Renal physiology* 304(5): F565-77.
Burnham, M P, R Bychkov, M Feâ Leâ Tou, G R Richards, P M Vanhoutte, A H Weston, and G Edwards. 2002. "Characterization of an Apamin-Sensitive Small-Conductance Ca 2+ -Activated K + Channel in Porcine Coronary Artery Endothelium: Relevance to EDHF." *British Journal of Pharmacology* 135: 1133–43.
Burnham, Matthew P, Ian T Johnson, and Arthur H Weston. 2006. "Impaired Small-Conductance Ca 2+-Activated K + Channel-Dependent EDHF Responses in Type II Diabetic ZDF Rats." *British Journal of Pharmacology* 148(4): 434–41.
Burnstock, G., and V. Ralevic. 2013. "Purinergic Signaling and Blood Vessels in Health and Disease." *Pharmacological Reviews* 66(1): 102–92.
Burnstock, Geoffrey. 2007. "Physiology and Pathophysiology of Purinergic Neurotransmission." *Physiological Reviews* 87(2): 659–797.
Burrows, M E, and P C Johnson. 1981. "Diameter, Wall Tension, and Flow in Mesenteric Arterioles during Autoregulation." *The American journal of physiology* 241: H829–37.
Busse, Rudi, Gillian Edwards, Michel Félétou, Ingrid Fleming, Paul M Vanhoutte, and Arthur H Weston. 2002. "EDHF: Bringing the Concepts Together." *Trends in pharmacological sciences* 23(8): 374–80.
Butcher, Joshua T., Adam G. Goodwill, Shyla C. Stanley, and Jefferson C. Frisbee. 2013. "Differential Impact of Dilator Stimuli on Increased Myogenic Activation of Cerebral and Skeletal Muscle Resistance Arterioles in Obese Zucker Rats." *Microcirculation* 20(7): 579–89.
Carlson, Brian E, and Daniel A Beard. 2011. "Mechanical Control of Cation Channels in the Myogenic Response." *American journal of physiology. Heart and circulatory physiology* 301(2): H331-43.

Carvajal, Jorge A., Alfredo M. Germain, Juan Pablo Huidobro-Toro, and Carl P. Weiner. 2000. "Molecular Mechanism of cGMP-Mediated Smooth Muscle Relaxation." *Journal of Cellular Physiology* 184(3): 409–20.

Casteels, R, K Kitamura, H Kuriyama, and H Suzuki. 1977. "Excitation-Contraction Coupling in the Smooth Muscle Cells of the Rabbit Main Pulmonary Artery." *The Journal of physiology* 271(1): 63–79.

Catterall, William A. 2011. "Voltage-Gated Calcium Channels." *Cold Spring Harbor Perspectives in Biology* 3(8): 1–23.

Chadha, Preet S., Rebecca E. Haddock, Lauren Howitt, Margaret J. Morris, Tim V. Murphy, T. Hilton Grayson, and Shaun L. Sandow. 2010. "Obesity Up-Regulates Intermediate Conductance Calcium-Activated Potassium Channels and Myoendothelial Gap Junctions to Maintain Endothelial Vasodilator Function." *Journal of Pharmacology and Experimental Therapeutics* 335(2): 284–93.

Chen, G, and D W Cheung. 1997. "Effect of K(+)-Channel Blockers on ACh-Induced Hyperpolarization and Relaxation in Mesenteric Arteries." *Am J Physiol* 272(5 Pt 2): H2306-12.

Chen, G, H Suzuki, and A H Weston. 1988. "Acetylcholine Releases Endothelium-Derived Hyperpolarizing Factor and EDRF from Rat Blood Vessels." *British journal of pharmacology* 95(4): 1165–74.

Chen, W T, R A Brace, J B Scott, D K Anderson, and F J Haddy. 1972. "The Mechanism of the Vasodilator Action of Potassium." *Proceedings of the Society for Experimental Biology and Medicine. Society for Experimental Biology and Medicine (New York, N.Y.)* 140(3): 820–24.

Chilian, W M, C L Eastham, and M L Marcus. 1986. "Microvascular Distribution of Coronary Vascular Resistance in Beating Left Ventricle." *The American journal of physiology* 251(4 Pt 2): H779-88.

Chilton, L., K. Loutzenhiser, E. Morales, J. Breaks, G. J. Kargacin, and R. Loutzenhiser. 2008. "Inward Rectifier K+ Currents and Kir2.1 Expression in Renal Afferent and Efferent Arterioles." *Journal of the American Society of Nephrology* 19(1): 69–76.

Chistiakov, D. A., A. N. Orekhov, and Y. V. Bobryshev. 2017. "Effects of Shear Stress on Endothelial Cells: Go with the Flow." *Acta Physiologica* 219(2): 382–408.

Chong, Hyun Lee, and Peter C. Ruben. 2008. "Interaction between Voltage-Gated Sodium Channels and the Neurotoxin, Tetrodotoxin." *Channels* 2(6): 407–12.

Christophersen, Palle, and Heike Wulff. 2015. "Pharmacological Gating Modulation of Small- and Intermediate-Conductance $Ca^{2+}$ -Activated $K^{+}$ Channels ($K_{Ca}$ 2.x and $K_{Ca}$ 3.1)." *Channels* 6950(November): 00–00.

Chu, Duc Quyen, Helen M Cox, Soraia K P Costa, Herbert Herzog, and Susan D Brain. 2003. "The Ability of Neuropeptide Y to Mediate Responses in the Murine Cutaneous Microvasculature: An Analysis of the Contribution of $Y_1$ and $Y_2$ Receptors." *British Journal of Pharmacology* 140(2): 422–30.

Cipolla, Marilyn J., Jeremiah Smith, Meghan M. Kohlmeyer, and Julie A. Godfrey. 2009. "SK Ca and IK Ca Channels, Myogenic Tone, and Vasodilator Responses in Middle Cerebral Arteries and Parenchymal Arterioles Effect of Ischemia and Reperfusion." *Stroke* 40(4): 1451–57.

Clancy, Robert, Branko Varenika, Weiqing Huang, Les Ballou, Mukundan Attur, Ashok R. Amin, and Steven B. Abramson. 2000. "Nitric Oxide Synthase/COX Cross-Talk: Nitric Oxide Activates COX-1 But Inhibits COX-2-Derived Prostaglandin Production." *Journal of*

*Immunology* 165(3): 1582–87.
Clapham, David E. 2007. "Calcium Signaling." *Cell* 131(6): 1047–58.
Clément-Chomienne, O, M P Walsh, and W C Cole. 1996. "Angiotensin II Activation of Protein Kinase C Decreases Delayed Rectifier K+ Current in Rabbit Vascular Myocytes." *The Journal of physiology* 495 ( Pt 3): 689–700.
Cockell, A P, and L Poston. 1996. "Isolated Mesenteric Arteries from Pregnant Rats Show Enhanced Flow-Mediated Relaxation but Normal Myogenic Tone." *The Journal of physiology* 495 ( Pt 2: 545–51.
Cogolludo, A., Laura Moreno, Lisardo Bosca, Juan Tamargo, and Francisco Perez-Vizcaino. 2003. "Thromboxane A2-Induced Inhibition of Voltage-Gated K+ Channels and Pulmonary Vasoconstriction: Role of Protein Kinase C." *Circulation Research* 93(7): 656–63.
Cohen, R A, F Plane, S Najibi, I Huk, T Malinski, and C J Garland. 1997. "Nitric Oxide Is the Mediator of Both Endothelium-Dependent Relaxation and Hyperpolarization of the Rabbit Carotid Artery." *Proceedings of the National Academy of Sciences of the United States of America* 94(8): 4193–98.
Coleman, R. A., P. P.A. Humphrey, I. Kennedy, G. P. Levy, and P. Lumley. 1981. "Comparison of the Actions of U-46619, a Prostaglandin H2-Analogue, With Those of Prostaglandin H2and Thromboxane a2on Some Isolated Smooth Muscle Preparations." *British Journal of Pharmacology* 73(3): 773–78.
Colinas, Olaia, Alejandro Moreno-Domínguez, Hai-Lei Zhu, Emma J. Walsh, M. Teresa Pérez-García, Michael P. Walsh, and William C. Cole. 2015. "α5-Integrin-Mediated Cellular Signaling Contributes to the Myogenic Response of Cerebral Resistance Arteries." *Biochemical Pharmacology* 97(3): 281–91.
Crane, G J, S D Walker, K A Dora, and C J Garland. 2003. "Evidence for a Differential Cellular Distribution of Inward Rectifier K Channels in the Rat Isolated Mesenteric Artery." *Journal of Vascular Research* 40(2): 159–68.
Cyrus, Tillmann, Tao Ding, and Domenico Praticò. 2010. "Expression of Thromboxane Synthase, Prostacyclin Synthase and Thromboxane Receptor in Atherosclerotic Lesions: Correlation with Plaque Composition." *Atherosclerosis* 208(2): 376–81.
Dalsgaard, T, C Kroigaard, M Misfeldt, T Bek, and U Simonsen. 2010. "Openers of Small Conductance Calcium-Activated Potassium Channels Selectively Enhance NO-Mediated Bradykinin Vasodilatation in Porcine Retinal Arterioles Abbreviations :" (January): 1496–1508.
Dalsgaard, Thomas, Christel Kroigaard, and Ulf Simonsen. 2010. "Calcium-Activated Potassium Channels - a Therapeutic Target for Modulating Nitric Oxide in Cardiovascular Disease?" *Expert opinion on therapeutic targets* 14(8): 825–37.
Dam, Vibeke Secher, Donna M. B. Boedtkjer, Jakob Nyvad, Christian Aalkjaer, and Vladimir Matchkov. 2014. "TMEM16A Knockdown Abrogates Two Different Ca2+-Activated Cl− Currents and Contractility of Smooth Muscle in Rat Mesenteric Small Arteries." *Pflügers Archiv - European Journal of Physiology* 466(7): 1391–1409.
Daneshtalab, Noriko, and John S Smeda. 2010. "Alterations in the Modulation of Cerebrovascular Tone and Blood Flow by Nitric Oxide Synthases in SHRsp with Stroke." *Cardiovascular Research* 86(1): 160–68.
Davis, M J. 1993. "Myogenic Response Gradient in an Arteriolar Network." *The American journal of physiology* 264(6 Pt 2): H2168-79.
Davis, M J, Xin Wu, Timothy R Nurkiewicz, Junya Kawasaki, George E Davis, Michael A Hill,

and Gerald A Meininger. 2001. “Integrins and Mechanotransduction of the Vascular Myogenic Response.” *American journal of physiology. Heart and circulatory physiology* 280(4): H1427–33.
Davis, Michael J. 2012. “Perspective: Physiological Role(s) of the Vascular Myogenic Response.” *Microcirculation* 19(2): 99–114.
Davis, Michael J, and Michael A Hill. 1999. “Signaling Mechanisms Underlying the Vascular Myogenic Response.” 79(2).
Denninger, John W, and Michael A Marletta. 1999. “Guanylate Cyclase and the ·NO/cGMP Signaling Pathway.” *Biochimica et Biophysica Acta (BBA) - Bioenergetics* 1411(2–3): 334–50.
Despopoulos, Agamemnon., and Stefan. Silbernagl. 2003. *Color Atlas of Physiology*. Thieme.
Devor, D C, a K Singh, R a Frizzell, and R J Bridges. 1996. “Modulation of Cl- Secretion by Benzimidazolones. I. Direct Activation of a Ca(2+)-Dependent K+ Channel.” *The American journal of physiology* 271(5 Pt 1): L775-84.
Dhaka, Ajay, Veena Viswanath, and Ardem Patapoutian. 2006. “Trp Ion Channels and Temperature Sensation.” *Annual Review of Neuroscience* 29(1): 135–61.
Dietrich, Alexander, and Thomas Gudermann. 2014. “TRPC6: Physiological Function and Pathophysiological Relevance.” *Handbook of Experimental Pharmacology* 222: 157–88.
Dietrich, Alexander, Michael Mederos Y Schnitzler, Maik Gollasch, Volkmar Gross, Ursula Storch, Galyna Dubrovska, Michael Obst, Eda Yildirim, Birgit Salanova, Hermann Kalwa, Kirill Essin, Olaf Pinkenburg, Friedrich C. Luft, Thomas Gudermann, and Lutz Birnbaumer. 2005. “Increased Vascular Smooth Muscle Contractility in TRPC6-/- Mice.” *Molecular and cellular biology* 25(16): 6980–89.
Dirksen, Robert T. 2009. “Sarcoplasmic Reticulum–mitochondrial through-Space Coupling in Skeletal Muscle.” *Applied Physiology, Nutrition, and Metabolism* 34(3): 389–95.
Dora, Kim A., and Chris J. Garland. 2013. “Linking Hyperpolarization to Endothelial Cell Calcium Events in Arterioles.” *Microcirculation* 20(3): 248–56.
Doughty, Joanne M, Frances Plane, Philip D Langton, Pietro Lampertico, James S Malter, Massimo Colombo, and Michael A Gerbert. 1999. “Charybdotoxin and Apamin Block EDHF in Rat Mesenteric Artery If Selectively Applied to the Endothelium.” *American Journal of Physiology - Heart and Circulatory Physiology* 276(3): H1107 LP-H1112.
Doyle, D A, J Morais Cabral, R A Pfuetzner, A Kuo, J M Gulbis, S L Cohen, B T Chait, and R MacKinnon. 1998. “The Structure of the Potassium Channel: Molecular Basis of K+ Conduction and Selectivity.” *Science (New York, N.Y.)* 280(5360): 69–77.
Drab, M., P Verkade, M Elger, M Kasper, M Lohn, B Lauterbach, J Menne, C Lindschau, F Mende, F C Luft, A Schedl, H Haller, and T V Kurzchalia. 2001. “Loss of Caveolae, Vascular Dysfunction, and Pulmonary Defects in Caveolin-1 Gene-Disrupted Mice.” *Science* 293(5539): 2449–52.
Du, Jianyang, Jia Xie, and Lixia Yue. 2009. “Intracellular Calcium Activates TRPM2 and Its Alternative Spliced Isoforms.” *Proceedings of the National Academy of Sciences of the United States of America* 106(17): 7239–44.
Dunn, Shannon M., and Kumuda C. Das. 2017. “Decreased EDHF-mediated Relaxation Is a Major Mechanism in Endothelial Dysfunction in Resistance Arteries in Aged Mice on Prolonged High-fat Sucrose Diet.” *Physiological Reports* 5(23): e13502.
Dunn, W. R., G. C. Wellman, and J. A. Bevan. 1994. “Enhanced Resistance Artery Sensitivity to Agonists under Isobaric Compared with Isometric Conditions.” *American Journal of*

*Physiology-Heart and Circulatory Physiology* 266(1): H147–55.
Dunn, William R., Sarah J. Wallis, and Sheila M. Gardiner. 1998. "Remodelling and Enhanced Myogenic Tone in Cerebral Resistance Arteries Isolated from Genetically Hypertensive Brattleboro Rats." *Journal of Vascular Research* 35(1): 18–26.
Duza, Tasmia, and Ingrid H Sarelius. 2004. "Increase in Endothelial Cell Ca(2+) in Response to Mouse Cremaster Muscle Contraction." *The Journal of physiology* 555(Pt 2): 459–69.
Earley, S., and J. E. Brayden. 2015. "Transient Receptor Potential Channels in the Vasculature." *Physiological Reviews* 95(2): 645–90.
Earley, Scott, Thierry Pauyo, Rebecca Drapp, Matthew J Tavares, Wolfgang Liedtke, and Joseph E Brayden. 2009. "TRPV4-Dependent Dilation of Peripheral Resistance Arteries Influences Arterial Pressure." *American journal of physiology. Heart and circulatory physiology* 297(3): H1096–1102.
Earley, Scott, Brian J. Waldron, and Joseph E. Brayden. 2004. "Critical Role for Transient Receptor Potential Channel TRPM4 in Myogenic Constriction of Cerebral Arteries." *Circulation Research* 95(9): 922–29.
Edvinsson, L, E Ekblad, R Håkanson, and C Wahlestedt. 1984. "Neuropeptide Y Potentiates the Effect of Various Vasoconstrictor Agents on Rabbit Blood Vessels." *British journal of pharmacology* 83(2): 519–25.
Edwards, F R, G.D.S. Hirst, and G.D. Silverberg. 1988. "Inward Rectification in Rat Cerebral Arterioles; Involvement of Potassium Ions N Autoregulation." *Physiology* 404: 455–66.
Edwards, G, K a Dora, M J Gardener, C J Garland, and a H Weston. 1998. "K+ Is an Endothelium-Derived Hyperpolarizing Factor in Rat Arteries." *Nature* 396(6708): 269–72.
Edwards, Gillian, Michel Félétou, and Arthur H. Weston. 2010. "Endothelium-Derived Hyperpolarising Factors and Associated Pathways: A Synopsis." *Pflügers Archiv - European Journal of Physiology* 459(6): 863–79.
Efremov, Rouslan G, Alexander Leitner, Ruedi Aebersold, and Stefan Raunser. 2015. "Architecture and Conformational Switch Mechanism of the Ryanodine Receptor." *Nature* 517(7532): 39–43.
Eichler, Ines, Judith Wibawa, Ivica Grgic, Andrea Knorr, Susanne Brakemeier, Axel R Pries, Joachim Hoyer, and Ralf Köhler. 2003. "Selective Blockade of Endothelial Ca $^{2+}$ -Activated Small- and Intermediate-Conductance K $^{+}$ -Channels Suppresses EDHF-Mediated Vasodilation." *British Journal of Pharmacology* 138(4): 594–601.
Eisner, David. 2014. "Calcium in the Heart: From Physiology to Disease." *Experimental Physiology* 99(10): 1273–82.
El-Yazbi, Ahmed F., and Khaled S. Abd-Elrahman. 2017. "ROK and Arteriolar Myogenic Tone Generation: Molecular Evidence in Health and Disease." *Frontiers in Pharmacology* 8(FEB): 1–10.
Ellinsworth, David C., Nilima Shukla, Ingrid Fleming, and Jamie Y. Jeremy. 2014. "Interactions between Thromboxane A2, Thromboxane/prostaglandin (TP) Receptors, and Endothelium-Derived Hyperpolarization." *Cardiovascular Research* 102(1): 9–16.
Ellinsworth, David C, Shaun L Sandow, Nilima Shukla, Yanping Liu, Jamie Y Jeremy, and David D Gutterman. 2016. "Endothelium-Derived Hyperpolarization and Coronary Vasodilation: Diverse and Integrated Roles of Epoxyeicosatrienoic Acids, Hydrogen Peroxide, and Gap Junctions." *Microcirculation* 23(1): 15–32.
Essin, Kirill, Andrea Welling, Franz Hofmann, Friedrich C. Luft, Maik Gollasch, and Sven Moosmang. 2007. "Indirect Coupling between Ca v 1.2 Channels and Ryanodine Receptors

to Generate Ca2+ Sparks in Murine Arterial Smooth Muscle Cells." *Journal of Physiology* 584(1): 205–19.
Evans, R J, and T C Cunnane. 1992. "Relative Contributions of ATP and Noradrenaline to the Nerve Evoked Contraction of the Rabbit Jejunal Artery - Dependence on Stimulation Parameters." *Naunyn-Schmiedeberg's Archives of Pharmacology* 345(4): 424–30.
Evans, R J, C Lewis, C Virginio, K Lundstrom, G Buell, A Surprenant, and R A North. 1996. "Ionic Permeability Of, and Divalent Cation Effects On, Two ATP-Gated Cation Channels (P2X Receptors) Expressed in Mammalian Cells." *Journal of Phy8iology* 497(2): 413–22.
Evanson, K. W., J. P. Bannister, M. D. Leo, and J. H. Jaggar. 2014. "LRRC26 Is a Functional BK Channel Auxiliary Subunit in Arterial Smooth Muscle Cells." *Circulation Research* 115(4): 423–31.
Falcone, J. C., H. J. Granger, and G. A. Meininger. 1993. "Enhanced Myogenic Activation in Skeletal Muscle Arterioles from Spontaneously Hypertensive Rats." *American Journal of Physiology-Heart and Circulatory Physiology* 265(6): H1847–55.
Falcone, J C, M J Davis, and G A Meininger. 1991. "Endothelial Independence of Myogenic Response in Isolated Skeletal Muscle Arterioles." *The American journal of physiology* 260(1 Pt 2): H130-5.
Falcone, J C, and G A Meininger. 1999. "Endothelin Mediates a Component of the Enhanced Myogenic Responsiveness of Arterioles from Hypertensive Rats." *MICROCIRCULATION* 6(4): 305–14.
Falloon, B. J., N. Stephens, J. R. Tulip, and A. M. Heagerty. 1995. "Comparison of Small Artery Sensitivity and Morphology in Pressurized and Wire-Mounted Preparations." *American Journal of Physiology-Heart and Circulatory Physiology* 268(2): H670–78.
Fan, Guizhen, Mariah L Baker, Zhao Wang, Mariah R Baker, Pavel A Sinyagovskiy, Wah Chiu, Steven J Ludtke, and Irina I Serysheva. 2015. "Gating Machinery of InsP 3 R Channels Revealed by Electron Cryomicroscopy." *Nature* 527: 336.
Fanger, C M, S Ghanshani, N J Logsdon, H Rauer, K Kalman, J Zhou, K Beckingham, K G Chandy, M D Cahalan, and J Aiyar. 1999. "Calmodulin Mediates Calcium-Dependent Activation of the Intermediate Conductance KCa Channel, IKCa1." *The Journal of biological chemistry* 274(9): 5746–54.
Félétou, M, and P M Vanhoutte. 1999. "The Third Pathway: Endothelium-Dependent Hyperpolarization." *Journal of physiology and pharmacology : an official journal of the Polish Physiological Society* 50(4): 525–34.
Félétou, Michel, and Paul M Vanhoutte. 2006. "Endothelium-Derived Hyperpolarizing Factor: Where Are We Now?" *Arteriosclerosis, thrombosis, and vascular biology* 26(6): 1215–25.
Fenger-Gron, J, M J Mulvany, and K L Christensen. 1995. "Mesenteric Blood Pressure Profile of Conscious, Freely Moving Rats." *The Journal of Physiology* 488(3): 753–60.
Ferreira, S. H., S. Moncada, and J. R. Vane. 1971. "Indomethacin and Aspirin Abolish Prostaglandin Release from the Spleen." *Nature New Biology* 231(25): 237–39.
Figueroa, Xavier F., and Brian R. Duling. 2009. "Gap Junctions in the Control of Vascular Function." *Antioxidants & Redox Signaling* 11(2): 251–66.
Fisslthaler, B, S Dimmeler, C Hermann, R Busse, and I Fleming. 2000. "Phosphorylation and Activation of the Endothelial Nitric Oxide Synthase by Fluid Shear Stress." *Acta Physiologica Scandinavica* 168(1): 81–88.
Fitzpatrick, F A. 2004. "Cyclooxygenase Enzymes: Regulation and Function." *Current pharmaceutical design* 10(6): 577–88.

Förstermann, Ulrich, and William C Sessa. 2012. “Nitric Oxide Synthases: Regulation and Function.” *European Heart Journal* 33(7): 829–37, 837a–837d.
Foskett, J. Kevin, Carl White, King-ho Cheung, and Don-On Daniel Mak. 2007. “Inositol Trisphosphate Receptor Ca 2+ Release Channels.” *Physiological Reviews* 87(2): 593–658.
Francis, Sharron H, Jennifer L Busch, Jackie D Corbin, and David Sibley. 2010. “cGMP-Dependent Protein Kinases and cGMP Phosphodiesterases in Nitric Oxide and cGMP Action.” *Pharmacological reviews* 62(3): 525–63.
Freichel, M, S H Suh, a Pfeifer, U Schweig, C Trost, P Weissgerber, M Biel, S Philipp, D Freise, G Droogmans, F Hofmann, V Flockerzi, and B Nilius. 2001. “Lack of an Endothelial Store-Operated Ca2+ Current Impairs Agonist-Dependent Vasorelaxation in TRP4-/- Mice.” *Nature cell biology* 3(2): 121–27.
Frischauf, Irene, Martin Muik, Isabella Derler, Judith Bergsmann, Marc Fahrner, Rainer Schindl, Klaus Groschner, and Christoph Romanin. 2009. “Molecular Determinants of the Coupling between STIM1 and Orai Channels: Differential Activation of Orai1-3 Channels by a STIM1 Coiled-Coil Mutant.” *The Journal of biological chemistry* 284(32): 21696–706.
Funk, C D, and G A FitzGerald. 2007. “COX-2 Inhibitors and Cardiovascular Risk.” *J Cardiovasc Pharmacol* 50(5): 470–79.
Furness, J B, and J M Marshall. 1974. “Correlation of the Directly Observed Responses of Mesenteric Vessles of the Rat to Nerve Stimulation and Noradrenaline with the Distribution of Adrenergic Nerves.” *The Journal of physiology* 239(1): 75–88.
Gafni, Juliette, Julia A. Munsch, Tien H. Lam, Michelle C. Catlin, Lucio G. Costa, Tadeusz F. Molinski, and Isaac N. Pessah. 1997. “Xestospongins: Potent Membrane Permeable Blockers of the Inositol 1,4,5- Trisphosphate Receptor.” *Neuron* 19(3): 723–33.
García-Cardeña, Guillermo, Roger Fan, Vijay Shah, Raffaella Sorrentino, Giuseppe Cirino, Andreas Papapetropoulos, and William C. Sessa. 1998. “Dynamic Activation of Endothelial Nitric Oxide Synthase by Hsp90.” *Nature* 392(6678): 821–24.
Garcia, Susana R., Ashley S. Izzard, Anthony M. Heagerty, and Stuart J. Bund. 1997. “Myogenic Tone in Coronary Arteries from Spontaneously Hypertensive Rats.” *Journal of Vascular Research* 34(2): 109–16.
Garcia, Susana R, and Stuart J Bund. 1998. “Nitric Oxide Modulation of Coronary Artery Myogenic Tone in Spontaneously Hypertensive and Wistar-Kyoto Rats.” *Clin Sci (Lond)* 94(3): 225–29.
Gardener, M. J., I. T. Johnson, M. P. Burnham, G. Edwards, A. M. Heagerty, and A. H. Weston. 2004. “Functional Evidence of a Role for Two-Pore Domain Potassium Channels in Rat Mesenteric and Pulmonary Arteries.” *British Journal of Pharmacology* 142(1): 192–202.
Gardiner, S M, A M Compton, T Bennett, R M Palmer, and S Moncada. 1990. “Regional Haemodynamic Changes during Oral Ingestion of NG-Monomethyl-L-Arginine or NG-Nitro-L-Arginine Methyl Ester in Conscious Brattleboro Rats.” *British journal of pharmacology* 101(1): 10–12.
Gardiner, S M, P A Kemp, T Bennett, R M Palmer, and S Moncada. 1992. “Nitric Oxide Synthase Inhibitors Cause Sustained, but Reversible, Hypertension and Hindquarters Vasoconstriction in Brattleboro Rats.” *European journal of pharmacology* 213(3): 449–51.
Garland, C J, and K A Dora. 2016. “EDH: Endothelium-Dependent Hyperpolarization and Microvascular Signalling.” *Acta Physiologica*: 1–10.
Garland, C J, and G A McPherson. 1992. “Evidence That Nitric Oxide Does Not Mediate the Hyperpolarization and Relaxation to Acetylcholine in the Rat Small Mesenteric Artery.”

*British journal of pharmacology* 105(2): 429–35.
Garland, Christopher J., Frances Plane, Barbara K. Kemp, and Thomas M. Cocks. 1995. "Endothelium-Dependent Hyperpolarization: A Role in the Control of Vascular Tone." *Trends in Pharmacological Sciences* 16(1): 23–30.
Garland, Christopher J, S V Smirnov, P Bagher, C S Lim, C Y Huang, R Mitchell, C Stanley, A Pinkney, and K A Dora. 2015. "TRPM4 Inhibitor 9-Phenanthrol Activates Endothelial Cell Intermediate Conductance Calcium-Activated Potassium Channels in Rat Isolated Mesenteric Artery." *British Journal of Pharmacology* 172(4): 1114–23.
Garland, CJ, PL Yarova, F Jiménez-Altayó, and KA Dora. 2011. "Vascular Hyperpolarization to β-Adrenoceptor Agonists Evokes Spreading Dilatation in Rat Isolated Mesenteric Arteries." *British Journal of Pharmacology* 164(3): 913–21.
Garthwaite, J, E Southam, C L Boulton, E B Nielsen, K Schmidt, and B Mayer. 1995. "Potent and Selective Inhibition of Nitric Oxide-Sensitive Guanylyl Cyclase by 1H-[1,2,4]oxadiazolo[4,3-A]quinoxalin-1-One." *Molecular pharmacology* 48(2): 184–88.
Gericke, Adrian, Jan J. Sniatecki, Veronique G. A. Mayer, Evgeny Goloborodko, Andreas Patzak, Jürgen Wess, and Norbert Pfeiffer. 2011. "Role of $M_1$, $M_3$, and $M_5$ Muscarinic Acetylcholine Receptors in Cholinergic Dilation of Small Arteries Studied with Gene-Targeted Mice." *American Journal of Physiology-Heart and Circulatory Physiology* 300(5): H1602–8.
Ghosh, D., A.U. Syed, M.P. Prada, M.A. Nystoriak, L.F. Santana, M. Nieves-Cintrón, and M.F. Navedo. 2017. "Calcium Channels in Vascular Smooth Muscle." *Advances in Pharmacology* 78: 49–87.
Giachini, F. R. C., C.-W. Chiao, F. S. Carneiro, V. V. Lima, Z. N. Carneiro, A. M. Dorrance, R. C. Tostes, and R. C. Webb. 2009. "Increased Activation of Stromal Interaction Molecule-1/Orai-1 in Aorta From Hypertensive Rats: A Novel Insight Into Vascular Dysfunction." *Hypertension* 53(2): 409–16.
Goedicke-Fritz, Sybelle, Anuradha Kaistha, Michael Kacik, Sabrina Markert, Andreas Hofmeister, Christoph Busch, Sebastian Bänfer, Ralf Jacob, Ivica Grgic, and Joachim Hoyer. 2015. "Evidence for Functional and Dynamic Microcompartmentation of Cav-1/TRPV4/KCain Caveolae of Endothelial Cells." *European Journal of Cell Biology* 94(7–9): 391–400.
Gokina, Natalia I., Adrian D. Bonev, Julie Phillips, Alexander P. Gokin, Kelsey Veilleux, Karen Oppenheimer, and Gabriela Goloman. 2015. "Impairment of $IK_{Ca}$ Channels Contributes to Uteroplacental Endothelial Dysfunction in Rat Diabetic Pregnancy." *American Journal of Physiology - Heart and Circulatory Physiology* 309(4): H592–604.
Gokina, Natalia I., Kristen M. Park, Keara McElroy-Yaggy, and George Osol. 2005. "Effects of Rho Kinase Inhibition on Cerebral Artery Myogenic Tone and Reactivity." *J Appl Physiol* 98(5): 1940–48.
Golding, E. M., M. L. Steenberg, T. D. Johnson, and R. M. Bryan. 2001. "Nitric Oxide in the Potassium-Induced Response of the Rat Middle Cerebral Artery: A Possible Permissive Role." *Brain Research* 889(1–2): 98–104.
Gonzales, A. L., Z. I. Garcia, G. C. Amberg, and S. Earley. 2010. "Pharmacological Inhibition of TRPM4 Hyperpolarizes Vascular Smooth Muscle." *AJP: Cell Physiology* 299(5): C1195–1202.
Gonzales, Albert L., Ying Yang, Michelle N. Sullivan, Lindsey Sanders, Fabrice Dabertrand, David C. Hill-Eubanks, Mark T. Nelson, and Scott Earley. 2014. "A PLCγ1-Dependent,

Force-Sensitive Signaling Network in the Myogenic Constriction of Cerebral Arteries." *Science Signaling* 7(327): ra49-ra49.
Gonzales, Albert L, and Scott Earley. 2012. "Endogenous Cytosolic Ca(2+) Buffering Is Necessary for TRPM4 Activity in Cerebral Artery Smooth Muscle Cells." *Cell calcium* 51(1): 82–93.
Goto, Kenichi, Nicole M Rummery, T Hilton Grayson, and Caryl E Hill. 2004. "Attenuation of Conducted Vasodilatation in Rat Mesenteric Arteries during Hypertension: Role of Inwardly Rectifying Potassium Channels." *The Journal of physiology* 561(Pt 1): 215–31.
Goulopoulou, Styliani, and R Clinton Webb. 2014. "Symphony of Vascular Contraction: How Smooth Muscle Cells Lose Harmony to Signal Increased Vascular Resistance in Hypertension." *Hypertension (Dallas, Tex. : 1979)* 63(3): e33-9.
Gratton, Jean-Philippe, Jason Fontana, Daniel S. O'Connor, Guillermo García-Cardeña, Timothy J. McCabe, and William C. Sessa. 2000. "Reconstitution of an Endothelial Nitric-Oxide Synthase (eNOS), hsp90, and Caveolin-1 Complex *in Vitro*." *Journal of Biological Chemistry* 275(29): 22268–72.
Gray, David W., and Ian Marshall. 1992. "Novel Signal Transduction Pathway Mediating Endothelium-Dependent β-Adrenoceptor Vasorelaxation in Rat Thoracic Aorta." *British Journal of Pharmacology* 107(3): 684–90.
Grayson, T. Hilton, Timothy V. Murphy, and Shaun L. Sandow. 2017. "Transient Receptor Potential Canonical Type 3 Channels: Interactions, Role and Relevance - A Vascular Focus." *Pharmacology and Therapeutics* 174: 79–96.
Grayson, T.Hilton, Rebecca E. Haddock, Tracy P. Murray, Richard J.H. Wojcikiewicz, and Caryl E. Hill. 2004. "Inositol 1,4,5-Trisphosphate Receptor Subtypes Are Differentially Distributed between Smooth Muscle and Endothelial Layers of Rat Arteries." *Cell Calcium* 36(6): 447–58.
Greenwood, Iain A, and Susumu Ohya. 2009. "REVIEW New Tricks for Old Dogs: KCNQ Expression and Role in Smooth Muscle." *British Journal of Pharmacology* 156: 1196–1203.
Grimm, Christian, Robert Kraft, Sophie Sauerbruch, Günter Schultz, and Christian Harteneck. 2003. "Molecular and Functional Characterization of the Melastatin-Related Cation Channel TRPM3." *Journal of Biological Chemistry* 278(24): 21493–501.
Groschner, Klaus, Wolfgang F. Graier, and Walter R. Kukovetz. 1992. "Activation of a Small-Conductance Ca2+-Dependent K+ Channel Contributes to Bradykinin-Induced Stimulation of Nitric Oxide Synthesis in Pig Aortic Endothelial Cells." *BBA - Molecular Cell Research* 1137(2): 162–70.
Grundy, David. 2015. "Principles and Standards for Reporting Animal Experiments in *The Journal of Physiology* and *Experimental Physiology*." *The Journal of Physiology* 593(12): 2547–49.
Gruszczynska-Biegala, Joanna, Pawel Pomorski, Marta B. Wisniewska, and Jacek Kuznicki. 2011. "Differential Roles for STIM1 and STIM2 in Store-Operated Calcium Entry in Rat Neurons" ed. David Holowka. *PLoS ONE* 6(4): e19285.
Guidotti, Guido G, and Gian C Gazzola. 1987. "Dependence of L-Arginine Accumulation Potential in Cultured Human Fibroblasts." *American Journal of Physiology* 253(3): C391-7.
Gutman, G. A., K George Chandy, Stephan Grissmer, Michel Lazdunski, David McKinnon, Luis A Pardo, Gail A Robertson, Bernardo Rudy, Michael C Sanguinetti, Walter Stühmer, and Xiaoliang Wang. 2005. "International Union of Pharmacology. LIII. Nomenclature and

Molecular Relationships of Voltage-Gated Potassium Channels." *Pharmacological Reviews* 57(4): 473–508.
Guyton, Arthur C., and John E. Hall. 2006. Elsevier Inc *Textbook of Medical Physiology*.
Haddock, Rebecca E., T. Hilton Grayson, Therese D. Brackenbury, Kate R. Meaney, Craig B. Neylon, Shaun L. Sandow, and Caryl E. Hill. 2006. "Endothelial Coordination of Cerebral Vasomotion via Myoendothelial Gap Junctions Containing Connexins 37 and 40." *American Journal of Physiology-Heart and Circulatory Physiology* 291(5): H2047–56.
Haddock, Rebecca E., T. Hilton Grayson, Margaret J. Morris, Lauren Howitt, Preet S. Chadha, and Shaun L. Sandow. 2011. "Diet-Induced Obesity Impairs Endothelium-Derived Hyperpolarization via Altered Potassium Channel Signaling Mechanisms." *PLoS ONE* 6(1).
Haddy, F J. 1983. "Potassium Effects on Contraction in Arterial Smooth Muscle Mediated by Na+, K+-ATPase." *Federation proceedings* 42(2): 239–45.
Haddy, F J, and J B Scott. 1968. "Metabolically Linked Vasoactive Chemicals in Local Regulation of Blood Flow." *Physiological reviews* 48(4): 688–707.
Haefliger, Jacques-antoine, Paolo Meda, Andrea Formenton, Philippe Wiesel, Hans R Brunner, Pascal Nicod, Daniel Hayoz, Jacques-antoine Haefliger, Paolo Meda, Andrea Formenton, Philippe Wiesel, Anne Zanchi, Hans R Brunner, Pascal Nicod, and Daniel Hayoz. 1999. "Aortic Connexin43 Is Decreased During Hypertension Induced by Inhibition of Nitric Oxide Synthase." 43.
Harder, D R. 1984. "Pressure-Dependent Membrane Depolarization in Cat Middle Cerebral Artery." *Circulation Research* 55(2): 197–202.
Harder, David R, John Smeda, and Julian Lombard. 1985. "BRIEF COMMUNICATIONS Enhanced Myogenic Depolarization in Hypertensive Cerebral Arterial Muscle." *Circ Res* 57: 319–22.
Harraz, Osama F et al. 2014. "Ca<inf>v</inf>3.2 Channels and the Induction of Negative Feedback in Cerebral Arteries." *Circulation Research* 115(7): 650–61.
Harraz, Osama F, Suzanne E Brett, Anil Zechariah, Monica Romero, Jose L Puglisi, Sean M Wilson, and Donald G Welsh. 2015. "Genetic Ablation of CaV3.2 Channels Enhances the Arterial Myogenic Response by Modulating the RyR-BKCa Axis." *Arteriosclerosis, thrombosis, and vascular biology* 35(8): 1843–51.
Harraz, Osama F, Frank Visser, Suzanne E Brett, Daniel Goldman, Anil Zechariah, Ahmed M Hashad, Bijoy K Menon, Tim Watson, Yves Starreveld, and Donald G Welsh. 2015. "CaV1.2/CaV3.x Channels Mediate Divergent Vasomotor Responses in Human Cerebral Arteries." *The Journal of general physiology* 145(5): 405–18.
Harvey, Lodish, Arnold Berk, S Lawrence Zipursky, Paul Matsudaira, David Baltimore, and James Darnell. 2000. *Molecular Cell Biology*. W.H. Freeman.
Hasenau, A-L, G Nielsen, C Morisseau, B D Hammock, H Wulff, and R Köhler. 2011. "Improvement of Endothelium-Dependent Vasodilations by SKA-31 and SKA-20, Activators of Small- and Intermediate-Conductance Ca2+ -Activated K+ -Channels." *Acta physiologica (Oxford, England)* 203(1): 117–26.
Hashad, Ahmed M., Neil Mazumdar, Monica Romero, Anders Nygren, Kamran Bigdely-Shamloo, Osama F. Harraz, Jose L. Puglisi, Edward J. Vigmond, Sean M. Wilson, and Donald G. Welsh. 2016. "Interplay among Distinct Ca 2+ Conductances Drives Ca 2+ Sparks/spontaneous Transient Outward Currents in Rat Cerebral Arteries." *The Journal of Physiology* 4(4): 1111–26.
Haug, Sara J., and Steven S. Segal. 2005. "Sympathetic Neural Inhibition of Conducted

Vasodilatation along Hamster Feed Arteries: Complementary Effects of $\alpha_1$ - and $\alpha_2$ - Adrenoreceptor Activation." *The Journal of Physiology* 563(2): 541–55.
Hayashi, Koichi, Murray Epstein, and Rodger Loutzenhiser. 1992. "Enhanced Myogenic Responsiveness of Renal Interlobular Arteries in Spontaneously Hypertensive Rats." *Hypertension* 19(2): 153–60.
Hayashi, Koichi, Murray Epstein, Rodger Loutzenhiser, Hayley K Forster Hayashi M Epstein, and H Forster. 1992. "Impaired Myogenic Responsiveness of the Afferent Arteriole in Streptozotocin-Induced Diabetic Rats: Role of Eicosanoid Derangements1." *J. Am. Soc. Nephrol* 2(2): 1578–86.
Haynes, J, J Robinson, L Saunders, A E Taylor, and S J Strada. 1992. "Role of cAMP-Dependent Protein Kinase in cAMP-Mediated Vasodilation." *The American journal of physiology* 262(2 Pt 2): H511-6.
Hemmens, B, and B Mayer. 1998. "Enzymology of Nitric Oxide Synthases." *Methods in molecular biology (Clifton, N.J.)* 100: 1–32.
Hemmings, Denise G, Sukrutha Veerareddy, Philip N Baker, and Sandra T Davidge. 2005. "Increased Myogenic Responses in Uterine but Not Mesenteric Arteries from Pregnant Offspring of Diet-Restricted Rat Dams." *Biology of reproduction* 72(4): 997–1003.
Hideaki, Karaki. 1989. "Ca2+ Localization and Sensitivity in Vascular Smooth Muscle." *Trends in Pharmacological Sciences* 10(8): 320–25.
Hilgers, Rob H. P., and R. Clinton Webb. 2007. "Reduced Expression of $SK_{Ca}$ and $IK_{Ca}$ Channel Proteins in Rat Small Mesenteric Arteries during Angiotensin II-Induced Hypertension." *American Journal of Physiology-Heart and Circulatory Physiology* 292(5): H2275–84.
Hill-Eubanks, D. C., A. L. Gonzales, S. K. Sonkusare, and M. T. Nelson. 2014. "Vascular TRP Channels: Performing Under Pressure and Going with the Flow." *Physiology* 29(5): 343–60.
Hill, M A, M J Davis, G A Meininger, S J Potocnik, and T V Murphy. 2006. "Arteriolar Myogenic Signalling Mechanisms: Implications for Local Vascular Function." *Clin Hemorheol Microcirc* 34(1–2): 67–79.
Hill, M A, and G A Meininger. 1994. "Calcium Entry and Myogenic Phenomena in Skeletal Muscle Arterioles." *The American journal of physiology* 267(3 Pt 2): H1085-92.
Hill, Michael A., and Gerald A. Meininger. 2012. "Arteriolar Vascular Smooth Muscle Cells: Mechanotransducers in a Complex Environment." *International Journal of Biochemistry and Cell Biology* 44(9): 1505–10.
Hill, Michael A., Gerald A. Meininger, Michael J. Davis, and Ismail Laher. 2009. "Therapeutic Potential of Pharmacologically Targeting Arteriolar Myogenic Tone." *Trends in Pharmacological Sciences* 30(7): 363–74.
Hill, Michael A, Hui Zou, Simon J Potocnik, Gerald A Meininger, and Michael J Davis. 2001. "Invited Review: Arteriolar Smooth Muscle Mechanotransduction: Ca(2+) Signaling Pathways Underlying Myogenic Reactivity." *Journal of applied physiology (Bethesda, Md. : 1985)* 91(2): 973–83.
Himmel, H M, A R Whorton, and H C Strauss. 1993. "Intracellular Calcium, Currents, and Stimulus-Response Coupling in Endothelial Cells." *Hypertension (Dallas, Tex. : 1979)* 21(1): 112–27.
Himpens, B, and R Casteels. 1990. "Different Effects of Depolarization and Muscarinic Stimulation on the Ca2+/force Relationship during the Contraction-Relaxation Cycle in the

Guinea Pig Ileum." *Pflugers Archiv : European journal of physiology* 416(1–2): 28–35.
Hirano, Katsuya, Mayumi Hirano, and Akiko Hanada. 2009. "Involvement of STIM1 in the Proteinase-Activated Receptor 1-Mediated Ca $^{2+}$ Influx in Vascular Endothelial Cells." *Journal of Cellular Biochemistry* 108(2): 499–507.
Hirata, Masakazu, Yasunori Hayashi, Fumitaka Ushikubi, Yoshifumi Yokota, Ryoichiro Kageyama, Shigetada Nakanishi, and Shuh Narumiya. 1991. "Cloning and Expression of cDNA for a Human Thromboxane A2 Receptor." *Nature* 349(6310): 617–20.
Hirst, G.D.S., G.D. Silverberg, and D.F. Van Helden. 1986. "The Action Potential and the Underlying Ionic Currents." *J. Physiol.* 371: 289–304.
Hirst, G D, and F R Edwards. 1989. "Sympathetic Neuroeffector Transmission in Arteries and Arterioles." *Physiol Rev.* 69(2): 546–604.
Hoffmann, Anke, Torsten Gloe, Ulrich Pohl, and Stefan Zahler. 2003. "Nitric Oxide Enhances de Novo Formation of Endothelial Gap Junctions." *Cardiovascular Research* 60(2): 421–30.
Hofmann, Thomas, Alexander G. Obukhov, Michael Schaefer, Christian Harteneck, Thomas Gudermann, and Günter Schultz. 1999. "Direct Activation of Human TRPC6 and TRPC3 Channels by Diacylglycerol." *Nature* 397(6716): 259–63.
Hooper, Robert, Xuexin Zhang, Marie Webster, Christina Go, Joseph Kedra, Katie Marchbank, Donald L Gill, Ashani T Weeraratna, Mohamed Trebak, and Jonathan Soboloff. 2015. "Novel Protein Kinase C-Mediated Control of Orai1 Function in Invasive Melanoma." *Molecular and cellular biology* 35(16): 2790–98.
Horiuchi, Tetsuyoshi, Hans H. Dietrich, Kazuhiro Hongo, and Ralph G. Dacey. 2002. "Mechanism of Extracellular K+-Induced Local and Conducted Responses in Cerebral Penetrating Arterioles." *Stroke* 33(11): 2692–99.
Hoshi, T., A. Pantazis, and R. Olcese. 2013. "Transduction of Voltage and Ca $^{2+}$ Signals by Slo1 BK Channels." *Physiology* 28(3): 172–89.
Hou, X., L. Pedi, M. M. Diver, and S. B. Long. 2012. "Crystal Structure of the Calcium Release-Activated Calcium Channel Orai." *Science* 338(6112): 1308–13.
Hougaard, C, B L Eriksen, S Jørgensen, T H Johansen, T Dyhring, L S Madsen, D Strøbaek, and P Christophersen. 2007. "Selective Positive Modulation of the SK3 and SK2 Subtypes of Small Conductance Ca2+-Activated K+ Channels." *British journal of pharmacology* 151(5): 655–65.
Van Hove, Ce, C Van der Donckt, Ag Herman, H Bult, and P Fransen. 2009. "Vasodilator Efficacy of Nitric Oxide Depends on Mechanisms of Intracellular Calcium Mobilization in Mouse Aortic Smooth Muscle Cellsb ph_396 920..930." *British Journal of Pharmacology* 158: 920–30.
Hu, X-Q, D Xiao, R Zhu, X Huang, S Yang, SM Wilson, and L Zhang. 2012. "Chronic Hypoxia Suppresses Pregnancy-Induced Upregulation of Large-Conductance Ca2+-Activated K+ Channel Activity in Uterine Arteries." *Hypertension* 60: 214–22.
Huang, An, and A. Koller. 1996. "Both Nitric Oxide and Prostaglandin-Mediated Responses Are Impaired in Skeletal Muscle Aterioles of Hypertensive Rats." *Journal of Hypertension* 4: 887–95.
Huang, An, and Akos Koller. 1997. "Endothelin and Prostaglandin H2enhance Arteriolar Myogenic Tone in Hypertension." *Hypertension* 30(5): 1210–15.
Huang, An, Dong Sun, and Akos Koller. 1993. "Endothelial Dysfunction Augments Myogenic Arteriolar Constriction in Hypertension." *Hypertension* 22(6): 913–21.

Huang, Paul L., Zhihong Huang, Hiroshi Mashimo, Kenneth Bloch, D., Michael A. Moskowitz, John Bevan, A., and Mark C. Fishman. 1995. "Hypertension in Mice Lacking the Gene for Endothelial Nitric Oxide Synthase." *Nature* 377(6546): 239–42.

Hussain, Monira B., and Ian Marshall. 1997. "Characterization of $\alpha_1$ -Adrenoceptor Subtypes Mediating Contractions to Phenylephrine in Rat Thoracic Aorta, Mesenteric Artery and Pulmonary Artery." *British Journal of Pharmacology* 122(5): 849–58.

Hwa, Joyce J, and John A Bevan. 1986. "Stretch-Dependent (Myogenic) Tone in Rabbit Ear Resistance Arteries." *The American journal of physiology* 250(1 Pt 2): H87-95.

Inoue, R, T Okada, H Onoue, Y Hara, S Shimizu, S Naitoh, Y Ito, and Y Mori. 2001. "The Transient Receptor Potential Protein Homologue TRP6 Is the Essential Component of Vascular alpha(1)-Adrenoceptor-Activated Ca(2+)-Permeable Cation Channel." *Circulation research* 88(3): 325–32.

Inoue, Ryuji, Zhong Jian, and Yasuhiro Kawarabayashi. 2009. "Mechanosensitive TRP Channels in Cardiovascular Pathophysiology." *Pharmacology and Therapeutics* 123(3): 371–85.

Ito, Isamu, Yagna P R Jarajapu, Dennis L Guberski, Maria B Grant, and Harm J Knot. 2006. "Myogenic Tone and Reactivity of Rat Ophthalmic Artery in Acute Exposure to High Glucose and in a Type II Diabetic Model." *Invest Ophthalmol Vis Sci* 47: 683–92.

Izzard, A S, S J Bund, and A M Heagerty. 1996. "Myogenic Tone in Mesenteric Arteries from Spontaneously Hypertensive Rats." *Am J Physiol* 270(1 Pt 2): H1-6.

Izzard, Ashley S, and Anthony M Heagerty. 2014. "Myogenic Properties of Brain and Cardiac Vessels and Their Relation to Disease." *Current vascular pharmacology* 12(6): 829–35.

Jackson, W.F. F. 2016. 78 Advances in Pharmacology *Potassium Channels in Regulation of Vascular Smooth Muscle Contraction and Growth*. 1st ed. Elsevier Inc.

Jackson, W F. 2000. "Ion Channels and Vascular Tone." *Hypertension (Dallas, Tex. : 1979)* 35(1 Pt 2): 173–78.

Jackson, William F. 2005. "Potassium Channels in the Peripheral Microcirculation." *Microcirculation (New York, N.Y. : 1994)* 12(1): 113–27.

Jackson, William F. 2018. "KV Channels and the Regulation of Vascular Smooth Muscle Tone." *Mircroculation* (October 2017): 1–13.

Jaggar, J. H., G. C. Wellman, T. J. Heppner, V. A. Porter, G. J. Perez, M. Gollasch, T. Kleppisch, M. Rubart, A. S. Stevenson, W. J. Lederer, H. J. Knot, A. D. Bonev, and Mark T. Nelson. 1998. "Ca2+ Channels, Ryanodine Receptors and Ca2+-Activated K+ Channels: A Functional Unit for Regulating Arterial Tone." *Acta Physiologica Scandinavica* 164(4): 577–87.

Jaggar, J H, V A Porter, W J Lederer, and M T Nelson. 2000. "Calcium Sparks in Smooth Muscle." *American journal of physiology. Cell physiology* 278(2): C235–56.

Jaggar, Jonathan H. 2001. "Intravascular Pressure Regulates Local and Global Ca(2+) Signaling in Cerebral Artery Smooth Muscle Cells." *American journal of physiology. Cell physiology* 281(2): C439–48.

Jalil, Jorge E. 2008. "Blood Pressure Reduction, Potassium Channels, and the Endothelium Insights From L-Serine." *Hypertension* 51: 626–28.

Jan, L Y, and Y N Jan. 1992. "Structural Elements Involved in Specific K+ Channel Functions." *Annual Review of Physiology* 54(1): 537–55.

Jarajapu, Yagna P. R., and Harm J. Knot. 2002. "Role of Phospholipase C in Development of Myogenic Tone in Rat Posterior Cerebral Arteries." *American Journal of Physiology Heart and Circulatory Physiology* 283(6): H2234–38.

Jarajapu, Yagna P R, Paul Coats, John C McGrath, Chris Hillier, and Allan MacDonald. 2001. "Functional Characterization of $\alpha_1$ -Adrenoceptor Subtypes in Human Skeletal Muscle Resistance Arteries." *British Journal of Pharmacology* 133(5): 679–86.

Jennings, Lisa K. 2009. "Mechanisms of Platelet Activation: Need for New Strategies to Protect against Platelet-Mediated Atherothrombosis." *Thrombosis and Haemostasis* 102(2): 248–57.

Jewell, Ryan P., Christine M. Saundry, Adrian D. Bonev, Bruce I. Tranmer, and George C. Wellman. 2004. "Inhibition of $Ca^{++}$ Sparks by Oxyhemoglobin in Rabbit Cerebral Arteries." *Journal of Neurosurgery* 100(2): 295–302.

Ji, Guangju, Morris E Feldman, Kai Su Greene, Vincenzo Sorrentino, Hong-Bo Xin, and Michael I Kotlikoff. 2004. "RYR2 Proteins Contribute to the Formation of Ca 2+ Sparks in Smooth Muscle." *The Journal of General Physiology* 123(4): 377–86.

Ji, S, S A John, Y Lu, and J N Weiss. 1998. "Mechanosensitivity of the Cardiac Muscarinic Potassium Channel. A Novel Property Conferred by Kir3.4 Subunit." *The Journal of biological chemistry* 273(3): 1324–28.

Jiang, Zhi-Gen, Xiaorui Shi, Hui Zhao, Jun-Qiang Si, and Alfred L. Nuttall. 2004. "Basal Nitric Oxide Production Contributes to Membrane Potential and Vasotone Regulation of Guinea Pig in Vitro Spiral Modiolar Artery." *Hearing Research* 189(1–2): 92–100.

Jin, Weili, and Zhe Lu. 1999. "Synthesis of a Stable Form of Tertiapin: A High-Affinity Inhibitor for Inward-Rectifier K+ Channels." *Biochemistry* 38(43): 14286–93.

Johnson, Paul C. 1986. "Autoregulation of Blood Flow." *Circulation Research* 59(5): 483–95.

Johnson, Rosalyn P., Ahmed F. El-yazbi, Kosuke Takeya, Emma J. Walsh, Michael P. Walsh, and William C. Cole. 2009. "Ca2+ Sensitization via Phosphorylation of Myosin Phosphatase Targeting Subunit at Threonine-855 by Rho Kinase Contributes to the Arterial Myogenic Response." *Journal of Physiology* 587(11): 2537–53.

Kamata, K, N Miyata, and Y Kasuya. 1989. "Involvement of Endothelial Cells in Relaxation and Contraction Responses of the Aorta to Isoproterenol in Naive and Streptozotocin-Induced Diabetic Rats." *Journal of Pharmacology and Experimental Therapeutics* 249(3).

Kameritsch, Petra, Natascha Khandoga, Wolfram Nagel, Christina Hundhausen, Darcy Lidington, and Ulrich Pohl. 2005. "Nitric Oxide Specifically Reduces the Permeability of Cx37-Containing Gap Junctions to Small Molecules." *Journal of Cellular Physiology* 203(1): 233–42.

Kamiya, A, and T Togawa. 1980. "Adaptive Regulation of Wall Shear Stress to Flow Change in the Canine Carotid Artery." *The American journal of physiology* 239(1): H14–21.

Kamm, K E, and J T Stull. 2001. "Dedicated Myosin Light Chain Kinases with Diverse Cellular Functions." *The Journal of biological chemistry* 276(7): 4527–30.

Kansui, Yasuo, Christopher J Garland, and Kim A Dora. 2008. "Enhanced Spontaneous Ca2+events in Endothelial Cells Reflect Signalling through Myoendothelial Gap Junctions in Pressurized Mesenteric Arteries." *Cell Calcium* 44(2): 135–46.

Kargacin, Gary J. 1994. "Calcium Signaling in Restricted Diffusion Spaces." *Biophysical Journal* 67: 262–72.

Kasumu, Adebimpe W., Charlotte Hougaard, Frederik Rode, Thomas A. Jacobsen, Jean Marc Sabatier, Birgitte L. Eriksen, Dorte Strøbæk, Xia Liang, Polina Egorova, Dasha Vorontsova, Palle Christophersen, Lars Christian B. Rønn, and Ilya Bezprozvanny. 2012. "Selective Positive Modulator of Calcium-Activated Potassium Channels Exerts Beneficial Effects in a Mouse Model of Spinocerebellar Ataxia Type 2." *Chemistry & Biology* 19(10): 1340–53.

Keef, Kathleen D., Joseph R. Hume, and Juming Zhong. 2001. “Regulation of Cardiac and Smooth Muscle Ca $^{2+}$ Channels (Ca $_{V}$ 1.2a,b) by Protein Kinases.” *American Journal of Physiology-Cell Physiology* 281(6): C1743–56.

Keefer, Larry K., Raymond W. Nims, Keith M. Davies, and David A. Wink. 1996. “‘NONOates’ (1-Substituted Diazen-1-Ium-1,2-Diolates) as Nitric Oxide Donors: Convenient Nitric Oxide Dosage Forms.” *Methods in Enzymology* 268: 281–93.

Kent, K C, L J Collins, F T Schwerin, M K Raychowdhury, and J A Ware. 1993. “Identification of Functional PGH 2 /TXA 2 Receptors on Human Endothelial Cells.” *Circulation Research* 72: 958–65.

Kerr, Paul M., Raymond Tam, Deepak Narang, Kyle Potts, Dane McMillan, Kale McMillan, and Frances Plane. 2012. “Endothelial Calcium-Activated Potassium Channels as Therapeutic Targets to Enhance Availability of Nitric Oxide.” *Canadian Journal of Physiology and Pharmacology* 90(6): 739–52.

Kerr, Paul M., Ran Wei, Raymond Tam, Shaun L. Sandow, Timothy V. Murphy, Katarina Ondrusova, Stephanie E. Lunn, Cam Ha T Tran, Donald G. Welsh, and Frances Plane. 2015. “Activation of Endothelial IKCa Channels Underlies NO-Dependent Myoendothelial Feedback.” *Vascular Pharmacology* 74: 130–38.

Kim, Moon Young, Guo Hua Liang, Ji Aee Kim, Seong Hoon Park, Jong Sik Hah, and Suk Hyo Suh. 2005. “Contribution of Na $^{+}$ -K $^{+}$ Pump and K $_{IR}$ Currents to Extracellular pH-Dependent Changes of Contractility in Rat Superior Mesenteric Artery.” *American Journal of Physiology-Heart and Circulatory Physiology* 289(2): H792–800.

Kim, Sangwon F. 2014. 96 Vitamins and Hormones *The Nitric Oxide-Mediated Regulation of Prostaglandin Signaling in Medicine*. 1st ed. Elsevier Inc.

Kiyonaka, S. et al. 2009. “Selective and Direct Inhibition of TRPC3 Channels Underlies Biological Activities of a Pyrazole Compound.” *Proceedings of the National Academy of Sciences* 106(13): 5400–5405.

Knot, H J, and M T Nelson. 1998. “Regulation of Arterial Diameter and Wall [Ca2+] in Cerebral Arteries of Rat by Membrane Potential and Intravascular Pressure.” *The Journal of physiology* (Pt 1): 199–209.

Knot, H J, N B Standen, and M T Nelson. 1998. “Ryanodine Receptors Regulate Arterial Diameter and Wall [Ca2+] in Cerebral Arteries of Rat via Ca2+-Dependent K+ Channels.” *The Journal of physiology* 508 ( Pt 1: 211–21.

Kochukov, Mikhail Y., Adithya Balasubramanian, Joel Abramowitz, Lutz Birnbaumer, and Sean P. Marrelli. 2014. “Activation of Endothelial Transient Receptor Potential C3 Channel Is Required for Small Conductance Calcium-Activated Potassium Channel Activation and Sustained Endothelial Hyperpolarization and Vasodilation of Cerebral Artery.” *Journal of the American Heart Association* 3(4): 1–18.

Köhler, Ralf, Willm Thomas Heyken, Philipp Heinau, Rudolf Schubert, Han Si, Michael Kacik, Christoph Busch, Ivica Grgic, Tanja Maier, and Joachim Hoyer. 2006. “Evidence for a Functional Role of Endothelial Transient Receptor Potential V4 in Shear Stress-Induced Vasodilatation.” *Arteriosclerosis, Thrombosis, and Vascular Biology* 26(7): 1495–1502.

Köhler, Ralf, Brajesh P Kaistha, and Heike Wulff. 2010. “Vascular KCa-Channels as Therapeutic Targets in Hypertension and Restenosis Disease.” *Expert opinion on therapeutic targets* 14(2): 143–55.

Kornfeld, Mark, Max Salomonsson, Antonio Gutierrez, and A E.G. Persson. 2000. “The Influence of β-Adrenergic Activation on Noradrenergic α1activation of Rabbit Afferent

Arterioles." *Pflugers Archiv European Journal of Physiology* 441(1): 25–31.
Kotecha, Neela, and Michael A Hill. 2005. "Myogenic Contraction in Rat Skeletal Muscle Arterioles : Smooth Muscle Membrane Potential and Ca2+ Signaling." *Am. J. Physiol. Heart Circ. Physiol.* 289: 1326–34.
Krapivinsky, Grigory, Igor Medina, Lily Eng, Luba Krapivinsky, Yinhai Yang, and David E. Clapham. 1998. "A Novel Inward Rectifier K+ Channel with Unique Pore Properties." *Neuron* 20(5): 995–1005.
Kudo, Ichiro, and Makoto Murakami. 2002. "Phospholipase A2 Enzymes." *Prostaglandins & other lipid mediators* 68–69: 3–58.
von Kügelgen, I, and K Starke. 1985. "Noradrenaline and Adenosine Triphosphate as Co-Transmitters of Neurogenic Vasoconstriction in Rabbit Mesenteric Artery." *The Journal of physiology* 367: 435–55.
Kunz, Lars, Andrea Thalhammer, Frank D Berg, Ulrike Berg, Diane M Duffy, Richard L Stouffer, Gregory A Dissen, Sergio R Ojeda, and Artur Mayerhofer. 2002. "Ca 2+ - Activated, Large Conductance K Channel in the Ovary: Identification, Characterization, and Functional Involvement in Steroidogenesis." *The Journal of Clinical Endocrinology & Metabolism* 87(12): 5566–74.
Kuo, Ivana Y, Anthie Ellis, Victoria AL Seymour, Shaun L Sandow, and Caryl E Hill. 2010. "Dihydropyridine-Insensitive Calcium Currents Contribute to Function of Small Cerebral Arteries." *Journal of Cerebral Blood Flow & Metabolism* 30(6): 1226–39.
Kuo, L, W M Chilian, and M J Davis. 1990. "Coronary Arteriolar Myogenic Response Is Independent of Endothelium." *Circulation research* 66(3): 860–66.
Lagaud, G. J L, E Masih-Khan, S Kai, C. Van Breemen, and G P Dubé. 2001. "Influence of Type II Diabetes on Arterial Tone and Endothelial Function in Murine Mesenteric Resistance Arteries." *Journal of Vascular Research* 38(6): 578–89.
Lamboley, Mathieu, Philippe Pittet, Mic Ele Koenigsberger, Roger Sauser, Jean-Louis Bény, and Jean-Jacques Meister. 2005. "Evidence for Signaling via Gap Junctions from Smooth Muscle to Endothelial Cells in Rat Mesenteric Arteries: Possible Implication of a Second Messenger." *Cell Calcium* 37: 311–20.
Lamont, Christine, and W. Gil Wier. 2004. "Different Roles of Ryanodine Receptors and Inositol (1,4,5)-Trisphosphate Receptors in Adrenergically Stimulated Contractions of Small Arteries." *American Journal of Physiology-Heart and Circulatory Physiology* 287(2): H617–25.
Landmesser, Ulf, Sergey Dikalov, S. Russ Price, Louise McCann, Tohru Fukai, Steven M. Holland, William E. Mitch, and David G. Harrison. 2003. "Oxidation of Tetrahydrobiopterin Leads to Uncoupling of Endothelial Cell Nitric Oxide Synthase in Hypertension." *Journal of Clinical Investigation* 111(8): 1201–9.
Lanner, Johanna T, Dimitra K Georgiou, Aditya D Joshi, and Susan L Hamilton. 2010. "Ryanodine Receptors: Structure, Expression, Molecular Details, and Function in Calcium Release." *Cold Spring Harbor perspectives in biology* 2(11): a003996.
Laporte, Régent, Adrian Hui, and Ismail Laher. 2004. "Pharmacological Modulation of Sarcoplasmic Reticulum Function in Smooth Muscle." *Pharmacol. Rev.* 56(4): 439–513.
Launay, Pierre, Andrea Fleig, Anne Laure Perraud, Andrew M Scharenberg, Reinhold Penner, and Jean Pierre Kinet. 2002. "TRPM4 Is a Ca2+-Activated Nonselective Cation Channel Mediating Cell Membrane Depolarization." *Cell* 109(3): 397–407.
Leblanc, Normand, Jonathan Ledoux, Sohag Saleh, Amy Sanguinetti, Jeff Angermann, Kate

O'Driscoll, Fiona Britton, Brian A Perrino, and Iain A Greenwood. 2005. "Regulation of Calcium-Activated Chloride Channels in Smooth Muscle Cells: A Complex Picture Is Emerging." *Canadian Journal of Physiology and Pharmacology* 83(7): 541–56.

Ledoux, J., M E Werner, Joseph E Brayden, Mark T Nelson, Werner E Matthias, Joseph E Brayden, and Mark T Nelson. 2006. "Calcium-Activated Potassium Channels and the Regulation of Vascular Tone." *Physiology* 21(3): 69–79.

Ledoux, Jonathan, Mark S Taylor, Adrian D Bonev, Rachael M Hannah, Viktoriya Solodushko, Bo Shui, Yvonne Tallini, Michael I Kotlikoff, and Mark T Nelson. 2008. "Functional Architecture of Inositol 1,4,5-Trisphosphate Signaling in Restricted Spaces of Myoendothelial Projections." *Proceedings of the National Academy of Sciences* 105(28): 9627–32.

Leo, M. Dennis, John P. Bannister, Damodaran Narayanan, Anitha Nair, Jordan E. Grubbs, Kyle S. Gabrick, Frederick A. Boop, and Jonathan H. Jaggar. 2014. "Dynamic Regulation of 1 Subunit Trafficking Controls Vascular Contractility." *Proceedings of the National Academy of Sciences* 111(21): 7879–7879.

Leung, Yuk Ki, Juan Du, Yu Huang, and Xiaoqiang Yao. 2010. "Cyclic Nucleotide-Gated Channels Contribute to Thromboxane A2-Induced Contraction of Rat Small Mesenteric Arteries" ed. Arnold Schwartz. *PLoS ONE* 5(6): e11098.

Lewis, L. Michelle, Gautam Bhave, Brian A. Chauder, Sreedatta Banerjee, Katharina A. Lornsen, Rey Redha, Katherine Fallen, Craig W. Lindsley, C. David Weaver, and Jerod S. Denton. 2009. "High-Throughput Screening Reveals a Small-Molecule Inhibitor of the Renal Outer Medullary Potassium Channel and Kir7.1." *Molecular Pharmacology* 76(5): 1094–1103.

Li, Fan-fan, Meng-zhou He, Yin Xie, Yuan-yuan Wu, Mei-tao Yang, Yao Fan, Fu-yuan Qiao, and Dong-rui Deng. 2017. "Involvement of Dysregulated IK Ca and SK Ca Channels in Preeclampsia." *Placenta* 58: 9–16.

Li, Rong Shan, Yong Wang, Hui Shen Chen, Fang Yong Jiang, Qiang Tu, Wen Jun Li, and Rui Xing Yin. 2016. "TMEM16A Contributes to Angiotensin II-Induced Cerebral Vasoconstriction via the RhoA/ROCK Signaling Pathway." *Molecular Medicine Reports* 13(4): 3691–99.

Li, Yao, Rachael L Baylie, Matthew J Tavares, and Joseph E Brayden. 2014. "TRPM4 Channels Couple Purinergic Receptor Mechanoactivation and Myogenic Tone Development in Cerebral Parenchymal Arterioles." *Journal of Cerebral Blood Flow and Metabolism* 34(10): 1706–14.

Lidington, Darcy, Yves Ouellette, Fuyan Li, and Karel Tyml. 2003. "Conducted Vasoconstriction Is Reduced in a Mouse Model of Sepsis." *Journal of Vascular Research* 40(2): 149–58.

Lim, Hyunjung, and Sudhansu K. Dey. 2002. "Minireview: A Novel Pathway of Prostacyclin Signaling—Hanging Out with Nuclear Receptors." *Endocrinology* 143(9): 3207–10.

Lin, Qingsong, Guiling Zhao, Xi Fang, Xiaohong Peng, Huayuan Tang, Hong Wang, Ran Jing, Jie Liu, W. Jonathan Lederer, Ju Chen, and Kunfu Ouyang. 2016. "IP3 Receptors Regulate Vascular Smooth Muscle Contractility and Hypertension." *JCI Insight* 1(17): e89402.

Lin, S, K A Fagan, K X Li, P W Shaul, D M Cooper, and D M Rodman. 2000. "Sustained Endothelial Nitric-Oxide Synthase Activation Requires Capacitative Ca2+ Entry." *The Journal of biological chemistry* 275(24): 17979–85.

Lincoln, Thomas M., Nupur Dey, and Hassan Sellak. 2001. "Invited Review: cGMP-Dependent

Protein Kinase Signaling Mechanisms in Smooth Muscle: From the Regulation of Tone to Gene Expression." *Journal of applied physiology (Bethesda, Md. : 1985)* 91(3): 1421–30.
Liou, Jen, Man Lyang Kim, Won Do Heo, Joshua T. Jones, Jason W. Myers, James E. Ferrell, and Tobias Meyer. 2005. "STIM Is a Ca2+ Sensor Essential for Ca2+-Store-Depletion-Triggered Ca2+ Influx." *Current Biology* 15(13): 1235–41.
Liu, Cuiling, Ching-Yuen Ngai, Yu Huang, Wing-Hung Ko, Min Wu, Guo-Wei He, Christopher J Garland, Kim A Dora, and Xiaoqiang Yao. 2006. "Depletion of Intracellular Ca2+ Stores Enhances Flow-Induced Vascular Dilatation in Rat Small Mesenteric Artery." *British journal of pharmacology* 147(5): 506–15.
Lloyd, Eric E, Sean P Marrelli, Khodadad Namiranian, and Robert M Bryan. 2009. "Characterization of TWIK-2, a Two-Pore Domain K+ Channel, Cloned from the Rat Middle Cerebral Artery." *Experimental biology and medicine (Maywood, N.J.)* 234(12): 1493–1502.
Longden, Thomas A, Fabrice Dabertrand, Masayo Koide, Albert L Gonzales, Nathan R Tykocki, Joseph E Brayden, David Hill-Eubanks, and Mark T Nelson. 2017. "Capillary K(+)-Sensing Initiates Retrograde Hyperpolarization to Increase Local Cerebral Blood Flow." *Nature neuroscience* 20(5): 717–26.
Longden, Thomas A, and Mark T Nelson. 2015. "Vascular Inward Rectifier K+ Channels as External K+ Sensors in the Control of Cerebral Blood Flow." *Microcirculation* 22(3): 183–96.
Looft-Wilson, R C, M Billaud, S R Johnstone, A C Straub, and B E Isakson. 2012. "Interaction between Nitric Oxide Signaling and Gap Junctions: Effects on Vascular Function." *Biochimica et Biophysica Acta - Biomembranes* 1818(8): 1895–1902.
Looft-Wilson, Robin C., Cara R. Goodell, Christina A. Mutch, Stephanie M. Mutchler, Kayla L. Miller, and Monique Guraya. 2017. "Increased Myoendothelial Feedback Is Associated with Increased connexin37 and IK1 Channel Expression in Mesenteric Arteries of Diet-Induced Hyperhomocysteinemic Mice." *Microcirculation* 24(8): e12398.
Lopatin, A N, E N Makhina, and C G Nichols. 1994. "Potassium Channel Block by Cytoplasmic Polyamines as the Mechanism of Intrinsic Rectification." *Nature* 372(6504): 366–69.
Lot, T Y, G Stark, and V G Wilson. 1993. "Endothelium-Dependent Contractions to NG-Nitro-L-Arginine Methyl Ester in the Porcine Isolated Splenic Artery Are Sensitive to Cyclooxygenase and Lipoxygenase Inhibitors." *Naunyn-Schmiedeberg's archives of pharmacology* 347(1): 115–18.
Loutzenhiser, Rodger, Anil Bidani, and Lisa Chilton. 2002. "Renal Myogenic Response: Kinetic Attributes and Physiological Role." *Circulation Research* 90(12): 1316–24.
Lu, Tong, Xiao Li Wang, Qiang Chai, Xiaojing Sun, Garry C. Sieck, Zvonimir S. Katusic, and Hon Chi Lee. 2017. "Role of the Endothelial Caveolae Microdomain in Shear Stress-Mediated Coronary Vasorelaxation." *Journal of Biological Chemistry* 292(46): 19013–23.
Lu, Zhe, and Roderick MacKinnon. 1994. "Electrostatic Tuning of Mg2+ Affinity in an Inward-Rectifier K+ Channel." *Nature* 371(6494): 243–46.
Lückhoff, A., and R. Busse. 1990. "Calcium Influx into Endothelial Cells and Formation of Endothelium-Derived Relaxing Factor Is Controlled by the Membrane Potential." *Pflügers Archiv European Journal of Physiology* 416(3): 305–11.
Lückhoff, Andreas, and Rudi Busse. 1990. "Activators of Potassium Channels Enhance Calcium Influx into Endothelial Cells as a Consequence of Potassium Currents." *Naunyn-Schmiedeberg's Archives of Pharmacology* 342(1): 94–99.

Lüscher, Christian, and Paul a Slesinger. 2010. “Emerging Concepts for G Protein-Gated Inwardly Rectifying (GIRK) Channels in Health and Disease.” *Nature Reviews Neuroscience* 11(5): 301–15.
Macedo, M P, and W W Lautt. 1996. “Shear-Induced Modulation by Nitric Oxide of Sympathetic Nerves in the Superior Mesenteric Artery.” *Canadian Journal of Physiology and Pharmacology* 74: 692–700.
Macedo, M Paula, W Wayne Lautt, Kim Erlend Mortensen, Lene Nagstrup Conley, Jakob Hedegaard, Trine Kalstad, Peter Sorensen, Christian Bendixen, Arthur Revhaug, Am J Physiol, Gastrointest Liver, David W Stepp, Daphne Merkus, Yasuhiro Nishikawa, William M Chilian, Heart Circ, and Endocrinol Metab. 2011. “Shear-Induced Modulation of Vasoconstriction in the Hepatic Artery and Portal Vein by Nitric Oxide Portal Pressure Shear-Induced Modulation of Vasoconstriction in the Hepatic Artery and Portal Vein by Nitric Oxide.” *Am J Physiol* 274: 253–60.
Majed, Batoule H, and Raouf A Khalil. 2012. “Molecular Mechanisms Regulating the Vascular Prostacyclin Pathways and Their Adaptation during Pregnancy and in the Newborn.” *Pharmacological reviews* 64(3): 540–82.
Marrelli, Sean P. 2001. “Mechanisms of Endothelial P2Y1 - and P2Y2 -Mediated Vasodilatation Involve Differential [ Ca 2+]i Responses.” *Am J Physiol Heart Circ Physiol* 281: 1759–66.
Marrelli, Sean P. 2002. “Altered Endothelial Ca2+ Regulation after Ischemia/reperfusion Produces Potentiated Endothelium-Derived Hyperpolarizing Factor-Mediated Dilations.” *Stroke* 33(9): 2285–91.
Marrelli, Sean P., Maxim S. Eckmann, Michael S. Hunte, P Sean, Maxim S. Eckmann, and S Michael. 2003. “Role of Endothelial Intermediate Conductance K Ca Channels in Cerebral EDHF-Mediated Dilations.” *American Journal of Physiology - Heart and Circulatory Physiology* 285(4): H1590–99.
Martinez-Lemus, Luis A. 2012. “The Dynamic Structure of Arterioles.” *Basic and Clinical Pharmacology and Toxicology* 110(1): 5–11.
Marziano, Corina, Kwangseok Hong, Eric L Cope, Michael I Kotlikoff, Brant E Isakson, and Swapnil K Sonkusare. 2017. “Nitric Oxide-Dependent Feedback Loop Regulates Transient Receptor Potential Vanilloid 4 (TRPV4) Channel Cooperativity and Endothelial Function in Small Pulmonary Arteries.” *Journal of the American Heart Association* 6(12).
Mather, S., Kim A Dora, Shaun L Sandow, Polly Winter, and Christopher J Garland. 2005. “Rapid Endothelial Cell-Selective Loading of Connexin 40 Antibody Blocks Endothelium-Derived Hyperpolarizing Factor Dilation in Rat Small Mesenteric Arteries.” *Circulation Research* 97(4): 399–407.
Matheson, P J, M A Wilson, and R N Garrison. 2000. “Regulation of Intestinal Blood Flow.” *The Journal of surgical research* 93(1): 182–96.
Matsuda, Hiroko, Akihiro Saigusa, and Hiroshi Irisawa. 1987. “Ohmic Conductance through the Inwardly Rectifying K Channel and Blocking by Internal Mg2+.” *Nature* 325(6100): 156–59.
Mauban, Joseph R. H., Christine Lamont, C. William Balke, and W. Gil Wier. 2001. “Adrenergic Stimulation of Rat Resistance Arteries Affects Ca $^{2+}$ Sparks, Ca $^{2+}$ Waves, and Ca $^{2+}$ Oscillations.” *American Journal of Physiology-Heart and Circulatory Physiology* 280(5): H2399–2405.
Mauban, Joseph R. H., Joseph Zacharia, Seth Fairfax, and Withrow Gil Wier. 2015. “PC-PLC/sphingomyelin Synthase Activity Plays a Central Role in the Development of

Myogenic Tone in Murine Resistance Arteries." *American Journal of Physiology - Heart and Circulatory Physiology* 308(12): H1517–24.
Mayer, B, and B Hemmens. 1997. "Biosynthesis and Action of Nitric Oxide in Mammalian Cells." *Trends in biochemical sciences* 22(12): 477–81.
McCarron, J. G., and W. Halpern. 1990. "Potassium Dilates Rat Cerebral Arteries by Two Independent Mechanisms." *American Journal of Physiology-Heart and Circulatory Physiology* 259(3): H902–8.
McCarron, John G., Marnie L. Olson, Calum Wilson, Mairi E. Sandison, and Susan Chalmers. 2013. "Examining the Role of Mitochondria in Ca $^{2+}$ Signaling in Native Vascular Smooth Muscle." *Microcirculation* 20(4): 317–29.
McCarron, John G., George Osol, and William Halpern. 1989. "Myogenic Responses Are Independent of the Endothelium in Rat Pressurized Posterior Cerebral Arteries." *Journal of Vascular Research* 26(5): 315–19.
McGahon, Mary K., José A. Fernández, Durga P. Dash, Jon McKee, David A. Simpson, Alex V. Zholos, J. Graham McGeown, and Tim M. Curtis. 2016. "TRPV2 Channels Contribute to Stretch-Activated Cation Currents and Myogenic Constriction in Retinal Arterioles." *Investigative Opthalmology & Visual Science* 57(13): 5637.
McGuire, J J, H Ding, and C R Triggle. 2001. "Endothelium-Derived Relaxing Factors: A Focus on Endothelium-Derived Hyperpolarizing Factor(s)." *Canadian journal of physiology and pharmacology* 79(6): 443–70.
McKinnon, Rebecca L., Michael L. Bolon, Hong-Xing Wang, Scott Swarbreck, Gerald M. Kidder, Alexander M. Simon, and Karel Tyml. 2009. "Reduction of Electrical Coupling between Microvascular Endothelial Cells by NO Depends on connexin37." *American Journal of Physiology-Heart and Circulatory Physiology* 297(1): H93–101.
McLachlan, Elspeth M. 2003. "Transmission of Signals through Sympathetic Ganglia - Modulation, Integration or Simply Distribution?" In *Acta Physiologica Scandinavica*, Blackwell Science Ltd, 227–35.
Mcmanus, Owen B, Lisa M H Helms, Leo Pallanck, Barry Ganetzky, Richard Swanson, and Reid J Leonard. 1995. "Functional Role of the Subunit of High Conductance Calcium-Activated Potassium Channels." *Neuron* 14: 645–50.
McNeish, a J, and C J Garland. 2007. "Thromboxane A2 Inhibition of SKCa after NO Synthase Block in Rat Middle Cerebral Artery." *British journal of pharmacology* 151(4): 441–49.
McNeish, Alister J., Kim A. Dora, and Christopher J. Garland. 2005. "Possible Role for K+ in Endothelium-Derived Hyperpolarizing Factor-Linked Dilatation in Rat Middle Cerebral Artery." *Stroke* 36(7): 1526–32.
McNeish, Alister J., Shaun L. Sandow, Craig B. Neylon, Mark X. Chen, Kim A. Dora, and Christopher J. Garland. 2006. "Evidence for Involvement of Both IKCa and SKCa Channels in Hyperpolarizing Responses of the Rat Middle Cerebral Artery." *Stroke* 37(5): 1277–82.
McSherry, Iain N, Michaela M Spitaler, Hiromichi Takano, and Kim A Dora. 2005. "Endothelial Cell Ca2+ Increases Are Independent of Membrane Potential in Pressurized Rat Mesenteric Arteries." *Cell calcium* 38(1): 23–33.
Mederos y Schnitzler, Michael, Ursula Storch, and Thomas Gudermann. 2011. "AT1 Receptors as Mechanosensors." *Current opinion in pharmacology* 11(2): 112–16.
Mederos y Schnitzler, Michael, Ursula Storch, Simone Meibers, Pascal Nurwakagari, Andreas Breit, Kirill Essin, Maik Gollasch, and Thomas Gudermann. 2008. "Gq-Coupled Receptors as Mechanosensors Mediating Myogenic Vasoconstriction." *EMBO Journal* 27(23): 3092–

3103.
Meera, P, M Wallner, Z Jiang, and L Toro. 1996. “A Calcium Switch for the Functional Coupling between α ( *Hslo* ) and β Subunits (K $_{V\ ,\ Ca\ \beta}$ ) of Maxi K Channels.” *FEBS Letters* 382(1–2): 84–88.
Meininger, G A, and M J Davis. 1992. “Cellular Mechanisms Involved in the Vascular Myogenic Response.” *Am J Physiol* 263(3 Pt 2): H647-59.
Mendoza, S. A., J. Fang, D. D. Gutterman, D. A. Wilcox, A. H. Bubolz, R. Li, M. Suzuki, and D. X. Zhang. 2010. “TRPV4-Mediated Endothelial Ca2+ Influx and Vasodilation in Response to Shear Stress.” *AJP: Heart and Circulatory Physiology* 298(2): H466–76.
Minneman, K P, T L Theroux, S Hollinger, C Han, and T a Esbenshade. 1994. “Selectivity of Agonists for Cloned Alpha 1-Adrenergic Receptor Subtypes.” *Molecular pharmacology* 46(5): 929–36.
Mishima, Shuichi, Hiroko Miyahara, and Hikaru Suzuki. 1984. “Transmitter Release Modulated by Ox-Adrenoceptor Antagonists in the Rabbit Mesenteric Artery: A Comparison between Noradrenaline Outflow and Electrical Activity.” *Br. J. Pharmac* 83: 537–47.
Mishra, Ramesh C., Darrell Belke, Heike Wulff, and Andrew P. Braun. 2013. “SKA-31, a Novel Activator of SKCa and IKCa Channels, Increases Coronary Flow in Male and Female Rat Hearts.” *Cardiovascular Research* 97(2): 339–48.
Mishra, Ramesh C, Heike Wulff, Michael A Hill, and Andrew P Braun. 2015. “Inhibition of Myogenic Tone in Rat Cremaster and Cerebral Arteries by SKA-31, an Activator of Endothelial KCa2.3 and KCa3.1 Channels.” *Journal of cardiovascular pharmacology* 66(1): 118–27.
Mistry, D. K., and C. J. Garland. 1998. “Nitric Oxide (NO)-Induced Activation of Large Conductance Ca2+-Dependent K+ Channels (BK(Ca)) in Smooth Muscle Cells Isolated from the Rat Mesenteric Artery.” *Br J Pharmacol* 124(6): 1131–40.
Mitchell, Jane A., Ferhana Ali, Lucy Bailey, Laura Moreno, and Louise S. Harrington. 2008. “Role of Nitric Oxide and Prostacyclin as Vasoactive Hormones Released by the Endothelium.” *Experimental Physiology* 93(1): 141–47.
Moccia, Francesco, Roberto Berra-Romani, and Franco Tanzi. 2012. “Update on Vascular Endothelial Ca $^{2+}$ Signalling: A Tale of Ion Channels, Pumps and Transporters.” *World Journal of Biological Chemistry* 3(7): 127.
Mogford, J. E., G. E. Davis, S. H. Platts, and G. A. Meininger. 1996. “Vascular Smooth Muscle v 3 Integrin Mediates Arteriolar Vasodilation in Response to RGD Peptides.” *Circulation Research* 79(4): 821–26.
Moore, Alex W., William F. Jackson, and Steven S. Segal. 2010. “Regional Heterogeneity of α-Adrenoreceptor Subtypes in Arteriolar Networks of Mouse Skeletal Muscle.” *The Journal of Physiology* 588(21): 4261–74.
Moore, P K, O A Al-Swayeh, N W.S. Chong, R A Evans, and A Gibson. 1990. “L-N(G)-Nitro Arginine (L-NOARG), a Novel, L-Arginine-Reversible Inhibitor of Endothelium-Dependent Vasodilatation in Vitro.” *British Journal of Pharmacology* 99(2): 408–12.
Moreno-Domínguez, Alejandro, Olaia Colinas, Ahmed El-Yazbi, Emma J. Walsh, Michael A Hill, Michael P Walsh, and William C Cole. 2013. “Ca2+ Sensitization due to Myosin Light Chain Phosphatase Inhibition and Cytoskeletal Reorganization in the Myogenic Response of Skeletal Muscle Resistance Arteries.” *Journal of Physiology* 591(5): 1235–50.
Moreno-Domínguez, Alejandro, Ahmed F El-Yazbi, Hai Lei Zhu, Olaia Colinas, X Zoë Zhong, Emma J. Walsh, Dylan M. Cole, Gary J Kargacin, Michael P Walsh, and William C Cole.

2014. “Cytoskeletal Reorganization Evoked by Rho-Associated Kinase- and Protein Kinase C-Catalyzed Phosphorylation of Cofilin and Heat Shock Protein 27, Respectively, Contributes to Myogenic Constriction of Rat Cerebral Arteries.” *Journal of Biological Chemistry* 289(30): 20939–52.

Mori, Asami, Masayuki Hanada, Kenji Sakamoto, Tsutomu Nakahara, and Kunio Ishii. 2011. “Noradrenaline Contracts Rat Retinal Arterioles via Stimulation of α1A- and α1D-Adrenoceptors.” *European Journal of Pharmacology* 673(1–3): 65–69.

Mountian, I, V G Manolopoulos, H De Smedt, J B Parys, L Missiaen, and F Wuytack. 1999. “Expression Patterns of Sarco/endoplasmic Reticulum Ca(2+)-ATPase and Inositol 1,4,5-Trisphosphate Receptor Isoforms in Vascular Endothelial Cells.” *Cell calcium* 25(5): 371–80.

Mufti, Rania E., Suzanne E. Brett, Cam Ha T. Tran, Rasha Abd El-Rahman, Yana Anfinogenova, Ahmed El-Yazbi, William C. Cole, Peter P. Jones, S.R. Wayne Chen, and Donald G. Welsh. 2010a. “Intravascular Pressure Augments Cerebral Arterial Constriction by Inducing Voltage-Insensitive Ca $^{2+}$ Waves.” *The Journal of Physiology* 588(20): 3983–4005.

Mufti, Rania E, Suzanne E Brett, Cam Ha T Tran, Rasha Abd El-Rahman, Yana Anfinogenova, Ahmed El-Yazbi, William C Cole, Peter P Jones, S R Wayne Chen, and Donald G Welsh. 2010b. “Intravascular Pressure Augments Cerebral Arterial Constriction by Inducing Voltage-Insensitive Ca2+ Waves.” *The Journal of physiology* 588(Pt 20): 3983–4005.

Muik, Martin, Irene Frischauf, Isabella Derler, Marc Fahrner, Judith Bergsmann, Petra Eder, Rainer Schindl, Clemens Hesch, Bernhard Polzinger, Reinhard Fritsch, Heike Kahr, Josef Madl, Hermann Gruber, Klaus Groschner, and Christoph Romanin. 2008. “Dynamic Coupling of the Putative Coiled-Coil Domain of ORAI1 with STIM1 Mediates ORAI1 Channel Activation.” *Journal of Biological Chemistry* 283(12): 8014–22.

Mullins, F. M., C. Y. Park, R. E. Dolmetsch, and R. S. Lewis. 2009. “STIM1 and Calmodulin Interact with Orai1 to Induce Ca2+-Dependent Inactivation of CRAC Channels.” *Proceedings of the National Academy of Sciences* 106(36): 15495–500.

Mulvany, M J, and C Aalkjaer. 1990. “Structure and Function of Small Arteries.” *Physiological reviews* 70(4): 921–61.

Muraki, K., Y. Imaizumi, S. Ohya, K. Sato, T. Takii, K. Onozaki, and M. Watanabe. 1997. “Apamin-Sensitive Ca2+-Dependent K+Current and Hyperpolarization in Human Endothelial Cells.” *Biochemical and Biophysical Research Communications* 236(2): 340–43.

Muraki, Katsuhiko, Yuko Iwata, Yuki Katanosaka, Tomohiro Ito, Susumu Ohya, Munekazu Shigekawa, and Yuji Imaizumi. 2003. “TRPV2 Is a Component of Osmotically Sensitive Cation Channels in Murine Aortic Myocytes.” *Circulation Research* 93(9): 829–38.

Muraki, K, M Watanabe, and Y Imaizumi. 2000. “Nifedipine and Nisoldipine Modulate Membrane Potential of Vascular Endothelium via a Myo-Endothelial Pathway.” *Life sciences* 67(26): 3163–70.

Murányi, Andrea, Dmitry Derkach, Ferenc Erdodi, Andrea Kiss, Masaaki Ito, and David J. Hartshorne. 2005. “Phosphorylation of Thr695 and Thr850 on the Myosin Phosphatase Target Subunit: Inhibitory Effects and Occurrence in A7r5 Cells.” *FEBS Letters* 579(29): 6611–15.

Murphy, Michael E, and Joseph E Brayden. 1995. “Nitric Oxide Hyperpolarizes Rabbit Mesenteric Arteries via ATP-Sensitive Potassium Channels.” *Journal of Phy8iology* 486(1):

47–58.
Murphy, Timothy V., Arjna Kanagarajah, Sianne Toemoe, Paul P. Bertrand, T. Hilton Grayson, Fiona C. Britton, Leo Leader, Sevvandi Senadheera, and Shaun L. Sandow. 2016. "TRPV3 Expression and Vasodilator Function in Isolated Uterine Radial Arteries from Non-Pregnant and Pregnant Rats." *Vascular Pharmacology* 83: 66–77.
Muto, Y, T Nagao, and T Urushidani. 1997. "The Putative Phospholipase C Inhibitor U73122 and Its Negative Control, U73343, Elicit Unexpected Effects on the Rabbit Parietal Cell." *The Journal of pharmacology and experimental therapeutics* 282(3): 1379–88.
Nagao, T., S. Illiano, and P. M. Vanhoutte. 1992. "Heterogeneous Distribution of Endothelium-Dependent Relaxations Resistant to NG-Nitro-L-Arginine in Rats." *American Journal of Physiology-Heart and Circulatory Physiology* 263(4): H1090–94.
Nagaraja, Sridevi, Adam Kapela, Cam H Tran, Donald G Welsh, and Nikolaos M Tsoukias. 2013. "Role of Microprojections in Myoendothelial Feedback--a Theoretical Study." *The Journal of physiology* 591(Pt 11): 2795–2812.
Narang, D., P. M. Kerr, S. E. Lunn, R. Beaudry, J. Sigurdson, M. D. Lalies, A. L. Hudson, P. E. Light, A. Holt, and F. Plane. 2014. "Modulation of Resistance Artery Tone by the Trace Amine -Phenylethylamine: Dual Indirect Sympathomimetic and 1-Adrenoceptor Blocking Actions." *Journal of Pharmacology and Experimental Therapeutics* 351(1): 164–71.
Narayanan, Damodaran, Adebowale Adebiyi, and Jonathan H Jaggar. 2012. "Inositol Trisphosphate Receptors in Smooth Muscle Cells." *American journal of physiology. Heart and circulatory physiology* 302(11): H2190-210.
Nausch, L. W. M., a. D. Bonev, T. J. Heppner, Y. Tallini, M. I. Kotlikoff, and M. T. Nelson. 2012. "Sympathetic Nerve Stimulation Induces Local Endothelial Ca2+ Signals to Oppose Vasoconstriction of Mouse Mesenteric Arteries." *AJP: Heart and Circulatory Physiology* 302(3): H594–602.
Navedo, Manuel F., Edward P Cheng, Yuan Can, Scott Votaw, Jeffery D Molkentin, JD Scott, and Luis F. Santana. 2010. "Increased Coupled Gating of L-Type Ca2+ Channels During Hypertension and Timothy Syndrome." *Circulation research* 106: 748–56.
Navedo, Manuel F., M. Nieves-Cintron, Gregory C. Amberg, Can Yuan, VS. Votaw, WJ Lederer, GS McKnight, and LF Santana. 2008. "AKAP150 Is Required for Stuttering Persistent Ca2? Sparklets and Angiotensin II–Induced Hypertension." *Circulation Research* 102: e1–11.
Navedo, Manuel F, and Gregory C Amberg. 2013. "Local Regulation of L-Type Ca2+ Channel Sparklets in Arterial Smooth Muscle." *Microcirculation* 20(4): 290–98.
Navedo, Manuel F, Gregory C Amberg, Madeline Nieves, Jeffery D Molkentin, and Luis F Santana. 2006. "Mechanisms Underlying Heterogeneous Ca2+ Sparklet Activity in Arterial Smooth Muscle." *The Journal of general physiology* 127(6): 611–22.
Navedo, Manuel F, Yukari Takeda, Madeline Nieves-Cintrón, Jeffery D Molkentin, and Luis F Santana. 2010. "Elevated Ca2+ Sparklet Activity during Acute Hyperglycemia and Diabetes in Cerebral Arterial Smooth Muscle Cells." *American journal of physiology. Cell physiology* 298(2): C211-20.
Neild, Timothy O, and Neelakumari Kotecha. 1987. "Relation Between Membrane Potential and Contractile Force in Smooth Muscle of the Rat Tail Artery During Stimulation by Norepinephrine, 5-Hydroxytryptamine, and Potassium." *Circ Res* 60(5): 791–96.
Nelson, Cole S, Jennifer L Marino, and Charles N Allen. 1996. "Melatonin Receptors Activate Heteromeric G-Protein Coupled Kir3 Channels." *NeuroReport* 7(3): 717–20.

Nelson, Mark T. 1993. “Ca2+-Activated Potassium Channels and ATP-Sensitive Potassium Channels as Modulators of Vascular Tone.” *Trends in Cardiovascular Medicine* 3(2): 54–60.
Nelson, Mark T, and John M Quayle. 1995. “Physiological Roles and Properties of Potassium Channels in Arterial Smooth Muscle.” *American Physiological Society*: C799–822.
Nelson, M T. 1992. “Regulation of Arterial Tone by Potassium Channels.” *Japanese journal of pharmacology* 58 Suppl 2: 238P–242P.
Nelson, M T, H Cheng, M Rubart, L F Santana, A D Bonev, H J Knot, and W J Lederer. 1995. “Relaxation of Arterial Smooth Muscle by Calcium Sparks.” *Science* 270(5236): 633–37.
Nelson, M T, J B Patlak, J F Worley, and N B Standen. 1990. “Calcium Channels, Potassium Channels, and Voltage Dependence of Arterial Smooth Muscle Tone.” *The American journal of physiology* 259(1 Pt 1): C3-18.
Neylon, C B, R J Lang, Y Fu, A Bobik, and P H Reinhart. 1999. “Molecular Cloning and Characterization of the Intermediate-Conductance Ca(2+)-Activated K(+) Channel in Vascular Smooth Muscle: Relationship between K(Ca) Channel Diversity and Smooth Muscle Cell Function.” *Circulation research* 85(9): e33-43.
Nguyen, Hai M., Vikrant Singh, Brandon Pressly, David Paul Jenkins, Heike Wulff, and Vladimir Yarov-Yarovoy. 2017. “Structural Insights into the Atomistic Mechanisms of Action of Small Molecule Inhibitors Targeting the KCa3.1 Channel Pores.” *MOLECULAR PHARMACOLOGY Mol Pharmacol* 91(4): 392–402.
Nilius, B., and A. Szallasi. 2014. “Transient Receptor Potential Channels as Drug Targets: From the Science of Basic Research to the Art of Medicine.” *Pharmacological Reviews* 66(3): 676–814.
Nilius, B, G Droogmans, Hideaki Kaneto, Naoto Katakami, Munehide Matsuhisa, and Taka-aki Matsuoka. 2001. “Ion Channels and Their Functional Role in Vascular Endothelium.” *Physiological reviews* 81(4): 1415–59.
Nilius, Bernd. 2007. “TRP Channels in Disease.” *Biochimica et Biophysica Acta - Molecular Basis of Disease* 1772(8): 805–12.
Nilsson, Holger, Lars M. Videbæk, Catalin Toma, and Michael J Mulvany. 1998. “Role of Intracellular Calcium for Noradrenaline-Induced Depolarization in Rat Mesenteric Small Arteries.” *Journal of Vascular Research* 35(1): 36–44.
Nobe, K, and R J Paul. 2001. “Distinct Pathways of Ca(2+) Sensitization in Porcine Coronary Artery: Effects of Rho-Related Kinase and Protein Kinase C Inhibition on Force and Intracellular Ca(2+).” *Circulation research* 88(12): 1283–90.
Nollert, M U, S G Eskin, and L V McIntire. 1990. “Shear Stress Increases Inositol Trisphosphate Levels in Human Endothelial Cells.” *Biochemical and biophysical research communications* 170(1): 281–87.
Ohyanagi, M, J E Faber, and K Nishigaki. 1991. “Differential Activation of Alpha 1- and Alpha 2-Adrenoceptors on Microvascular Smooth Muscle during Sympathetic Nerve Stimulation.” *Circulation research* 68(1): 232–44.
Oike, M, M Gericke, G Droogmans, and B Nilius. 1994. “Calcium Entry Activated by Store Depletion in Human Umbilical Vein Endothelial Cells.” *Cell calcium* 16(5): 367–76.
Oliveria, Seth F, Mark L Dell’Acqua, and William A Sather. 2007. “AKAP79/150 Anchoring of Calcineurin Controls Neuronal L-Type Ca2+ Channel Activity and Nuclear Signaling.” *Neuron* 55(2): 261–75.
Osol, G. 1995. “Mechanotransduction by Vascular Smooth Muscle.” *Journal of Vascular*

*Research* 32(5): 275–92.
Osol, G, M Cipolla, and S Knutson. 1989. "A New Method for Mechanically Denuding the Endothelium of Small (50-150 Microns) Arteries with a Human Hair." *Blood vessels* 26(5): 320–24.
Osol, George, Johan Fredrik Brekke, Keara McElroy-Yaggy, and Natalia I. Gokina. 2002. "Myogenic Tone, Reactivity, and Forced Dilatation: A Three-Phase Model of in Vitro Arterial Myogenic Behavior." *American journal of physiology. Heart and circulatory physiology* 283(6): H2260-7.
Osol, George, and W Halpern. 1985. "Myogenic Properties of Cerebral Blood Vessels from Normotensive and Hypertensive Rats." *American journal of physiology. Heart and circulatory physiology* 249(5 Pt 2): H914-21.
Pakdeechote, Poungrat, Nicole M. Rummery, Vera Ralevic, and William R. Dunn. 2007. "Raised Tone Reveals Purinergic-Mediated Responses to Sympathetic Nerve Stimulation in the Rat Perfused Mesenteric Vascular Bed." *European Journal of Pharmacology* 563(1–3): 180–86.
Palomares, Sara M, and Marilyn J Cipolla. 2014. "Myogenic Tone as a Therapeutic Target for Ischemic Stroke." *Current Vascular Pharmacology* 12(6): 788–800.
Panhwar, Fouzia, Richard D Rainbow, Robert Jackson, and Noel W Davies. 2015. "Ca2+ Dependent but PKC Independent Signalling Mediates UTP Induced Contraction of Rat Mesenteric Arteries." *Journal of Smooth Muscle Research* 51(0): 58–69.
Park, K. S., Y KiM, YH Lee, Y Earm, and WK Ho. 2003. "Mechanosensitive Cation Channels in Arterial Smooth Muscle Cells Are Activated by Diacylglycerol and Inhibited by Phospholipase C Inhibitor." *Circulation Research* 93(6): 557–64.
Park, Won Sun, Jin Han, Nari Kim, Jae-Hong Ko, Sung Joon Kim, and Yung E Earm. 2005. "Activation of Inward Rectifier $K^+$ Channels by Hypoxia in Rabbit Coronary Arterial Smooth Muscle Cells." *American Journal of Physiology-Heart and Circulatory Physiology* 289(6): H2461–67.
Park, Won Sun, Jin Han, Nari Kim, Jae Boum Youn, Hyun Joo, Hyung Kyu Kim, Jae-Hong Ko, and Yung E Earm. 2005. "Endothelin-1 Inhibits Inward Rectifier K+ Channels in Rabbit Coronary Arterial Smooth Muscle Cells Through Protein Kinase C." *J Cardiovasc Pharmacol* 46: 681–89.
Parker, Ian, and Ian F. Smith. 2010. "Recording Single-Channel Activity of Inositol Trisphosphate Receptors in Intact Cells with a Microscope, Not a Patch Clamp." *The Journal of General Physiology* 136(2): 119–27.
Parkington, H C, M A Tonta, H A Coleman, and M Tare. 1995. "Role of Membrane Potential in Endothelium-Dependent Relaxation of Guinea-Pig Coronary Arterial Smooth Muscle." *The Journal of physiology* 484 ( Pt 2): 469–80.
Parkington, H, Harold A Coleman, and Marianne Tare. 2004. "Prostacyclin and Endothelium-Dependent Hyperpolarization." *Pharmacological Research* 49(6): 509–14.
Parnavelas, J G, W Kelly, and G Burnstock. 1985. "Ultrastructural Localization of Choline Acetyltransferase in Vascular Endothelial Cells in Rat Brain." *Nature* 316(6030): 724–25.
Parsons, S J, A Hill, G J Waldron, F Plane, and C J Garland. 1994. "The Relative Importance of Nitric Oxide and Nitric Oxide-Independent Mechanisms in Acetylcholine-Evoked Dilatation of the Rat Mesenteric Bed." *British journal of pharmacology* 113(4): 1275–80.
Patel, Leena S., Cheryl K. Mitchell, William P. Dubinsky, and John O'Brien. 2006. "Regulation of Gap Junction Coupling Through the Neuronal Connexin Cx35 by Nitric Oxide and cGMP." *Cell Communication & Adhesion* 13(1–2): 41–54.

Paulson, A F, P D Lampe, R A Meyer, E TenBroek, M M Atkinson, T F Walseth, and R G Johnson. 2000. “Cyclic AMP and LDL Trigger a Rapid Enhancement in Gap Junction Assembly through a Stimulation of Connexin Trafficking.” *Journal of cell science* 113 ( Pt 17): 3037–49.

Pedarzani, P, J Mosbacher, A Rivard, L A Cingolani, D Oliver, M Stocker, J P Adelman, and B Fakler. 2001. “Control of Electrical Activity in Central Neurons by Modulating the Gating of Small Conductance Ca2+-Activated K+ Channels.” *The Journal of biological chemistry* 276(13): 9762–69.

Pérez, Guillermo J., Adrian D. Bonev, and Mark T. Nelson. 2001. “Micromolar Ca $^{2+}$ from Sparks Activates Ca $^{2+}$ -Sensitive K $^{+}$ Channels in Rat Cerebral Artery Smooth Muscle.” *American Journal of Physiology-Cell Physiology* 281(6): C1769–75.

Pertusa, María, Hans Moldenhauer, Sebastián Brauchi, Ramón Latorre, Rodolfo Madrid, and Patricio Orio. 2012. “Mutagenesis and Temperature-Sensitive Little Machines.” *Mutagenesis*: 221–46.

Pfeiffer, Silvia, Eva Leopold, Kurt Schmidt, Friedrich Brunner, and Bernd Mayer. 1996. “Inhibition of Nitric Oxide Synthesis by N(G)-Nitro-L-Arginine Methyl Ester (L-NAME): Requirement for Bioactivation to the Free Acid, N(G)-Nitro-L-Arginine.” *Br J Pharmacol* 118(6): 1433–40.

Pfitzner, J. 1976. “Poiseuille and His Law.” *Anaesthesia* 31(2): 273–75.

Piascik, Michael T., Edward E. Soltis, Mary M. Piascik, and Leigh B. Macmillan. 1996. “α-Adrenoceptors and Vascular Regulation: Molecular, Pharmacologic and Clinical Correlates.” *Pharmacology & Therapeutics* 72(3): 215–41.

Plane, F., M. Zwozdesky, R. Wei, D. Godin, and P.M. Kerr. 2013. “Activation of Endothelial Calcium-Activated Potassium Channels Limits Nerve-Mediated Vasoconstriction in Resistance Arteries.” *Canadian Journal of Cardiology* 29(10): S228–29.

Plane, F, L J Sampson, J J Smith, and C J Garland. 2001. “Relaxation to Authentic Nitric Oxide and SIN-1 in Rat Isolated Mesenteric Arteries: Variable Role for Smooth Muscle Hyperpolarization.” *British Journal of Pharmacology* 133(5): 665–72.

Plane, Frances, and William Cole. 2006. “Release of Nitric Oxide Is Modulated by Endothelial Cell Membrane Potential in Rat Basilar Artery.” *Faseb J* 20(A1166).

Plane, Frances, and Christopher J Garland. 1996. “Influence of Contractile Agonists on the Mechanism of Endothelium-Dependent Relaxation in Rat Isolated Mesenteric Artery.” *British Journal of Pharmacology* 119(2): 191–93.

Plane, Frances, Rosalyn Johnson, Paul Kerr, William Wiehler, Kevin Thorneloe, Kuniaki Ishii, Tim Chen, and William Cole. 2005. “Heteromultimeric Kv1 Channels Contribute to Myogenic Control of Arterial Diameter.” *Circulation Research* 96(2): 216–24.

Plane, Frances, Katherine E. Wiley, Jamie Y. Jeremy, Richard A. Cohen, and Christopher J. Garland. 1998. “Evidence That Different Mechanisms Underlie Smooth Muscle Relaxation to Nitric Oxide and Nitric Oxide Donors in the Rabbit Isolated Carotid Artery.” *British Journal of Pharmacology* 123(7): 1351–58.

Plüger, S, J Faulhaber, M Fürstenau, M Löhn, R Waldschütz, M Gollasch, H Haller, F C Luft, H Ehmke, and O Pongs. 2000. “Mice with Disrupted BK Channel beta1 Subunit Gene Feature Abnormal Ca(2+) spark/STOC Coupling and Elevated Blood Pressure.” *Circulation research* 87(11): E53–60.

Poburko, Damon, Chiu-Hsiang Liao, Cornelis van Breemen, and Nicolas Demaurex. 2009. “Mitochondrial Regulation of Sarcoplasmic Reticulum Ca2+ Content in Vascular Smooth

Muscle Cells." *Circulation research* 104(1): 104–12.
Potocnik, Simon J., Iain McSherry, Hong Ding, Timothy V. Murphy, Neela Kotecha, Kim A. Dora, Kathryn H. Yuill, Chris R. Triggle, Michael A. Hill, Simon J. Potocnik, Iain McSherry, Hong Ding, Timothy V. Murphy, Neela Kotecha, Kim A. Dora, Kathryn H. Yuill, Chris R. Triggle, and Michael A. Hill. 2009. "Endothelium-Dependent Vasodilation in Myogenically Active Mouse Skeletal Muscle Arterioles: Role of EDH and $K^+$ Channels." *Microcirculation* 16(5): 377–90.
Prasad, A R, S A Logan, R M Nerem, C J Schwartz, and E A Sprague. 1993. "Flow-Related Responses of Intracellular Inositol Phosphate Levels in Cultured Aortic Endothelial Cells." *Circulation research* 72(4): 827–36.
Prawitt, D., M. K. Monteilh-Zoller, L. Brixel, C. Spangenberg, B. Zabel, A. Fleig, and R. Penner. 2003. "TRPM5 Is a Transient Ca2+-Activated Cation Channel Responding to Rapid Changes in [Ca2+]i." *Proceedings of the National Academy of Sciences* 100(25): 15166–71.
Pritchard, Kirkwood A., Allan W. Ackerman, Eric R. Gross, David W. Stepp, Yang Shi, Jason T. Fontana, John E. Baker, and William C. Sessa. 2001. "Heat Shock Protein 90 Mediates the Balance of Nitric Oxide and Superoxide Anion from Endothelial Nitric-Oxide Synthase." *Journal of Biological Chemistry* 276(21): 17621–24.
Qu, Lihui, L Yu, Yanli Wang, Xin Jin, Qianlong Zhang, Ping Lu, Xiufeng Yu, Weiwei Zhong, Xiaodong Zheng, Ningren Cui, Chun Jiang, and Daling Zhu. 2015. "Inward Rectifier K+ Currents Are Regulated by CaMKII in Endothelial Cells of Primarily Cultured Bovine Pulmonary Arteries" ed. Alexander G Obukhov. *PLoS One* 10(12): e0145508.
Quayle, J. M., J. G. McCarron, J. E. Brayden, and M. T. Nelson. 1993. "Inward Rectifier K+ Currents in Smooth Muscle Cells from Rat Resistance-Sized Cerebral Arteries." *American Journal of Physiology-Cell Physiology* 265(5): C1363–70.
Quayle, J. M., M. T. Nelson, and N. B. Standen. 1997. "ATP-Sensitive and Inwardly Rectifying Potassium Channels in Smooth Muscle." *Physiological Reviews* 77(4): 1165–1232.
Radtke, Josephine, Kjestine Schmidt, Heike Wulff, Ralf Köhler, and Cor de Wit. 2013. "Activation of KCa3.1 by SKA-31 Induces Arteriolar Dilatation and Lowers Blood Pressure in Normo- and Hypertensive connexin40-Deficient Mice." *British journal of pharmacology* 170(2): 293–303.
Raeymaekers, L, and F Wuytack. 1993. "Ca2+ Pumps in Smooth Muscle Cells." *Journal of muscle research and cell motility* 14(2): 141–57.
Ramsey, I. Scott, Markus Delling, and David E. Clapham. 2006. "AN INTRODUCTION TO TRP CHANNELS." *Annual Review of Physiology* 68(1): 619–47.
Rand, V E, and C J Garland. 1992. "Endothelium-Dependent Relaxation to Acetylcholine in the Rabbit Basilar Artery: Importance of Membrane Hyperpolarization." *Br J Pharmacol* 106(1): 143–50.
Randell, Amy, Killol Chokshi, Brittany Kane, Hilary Chang, Safaa Naiel, Jeffrey G Dickhout, and Noriko Daneshtalab. 2016. "Alterations to the Middle Cerebral Artery of the Hypertensive-Arthritic Rat Model Potentiates Intracerebral Hemorrhage." *PeerJ* 4: e2608.
Reading, S A, S Earley, B J Waldron, D G Welsh, and J E Brayden. 2005. "TRPC3 Mediates Pyrimidine Receptor-Induced Depolarization of Cerebral Arteries." *American journal of physiology. Heart and circulatory physiology* 288(5): H2055-61.
Reading, Stacey A, and Joseph E Brayden. 2007. "Central Role of TRPM4 Channels in Cerebral Blood Flow Regulation." *Stroke* 38: 2322–28.
Redrobe, J P, Y Dumont, J A St-Pierre, and R Quirion. 1999. "Multiple Receptors for

Neuropeptide Y in the Hippocampus: Putative Roles in Seizures and Cognition." *Brain research* 848(1–2): 153–66.
Rees, D D, R M Palmer, R Schulz, H F Hodson, and S Moncada. 1990. "Characterization of Three Inhibitors of Endothelial Nitric Oxide Synthase in Vitro and in Vivo." *British journal of pharmacology* 101(3): 746–52.
Reimann, Katrin, Gayathri Krishnamoorthy, and Philine Wangemann. 2013. "NOS Inhibition Enhances Myogenic Tone by Increasing Rho-Kinase Mediated Ca2+ Sensitivity in the Male but Not the Female Gerbil Spiral Modiolar Artery." *PLoS ONE* 8(1).
Ricciotti, Emanuela, and Garret A. Fitzgerald. 2011. "Prostaglandins and Inflammation." *Arteriosclerosis, Thrombosis, and Vascular Biology* 31(5): 986–1000.
Robertson, B E, A D Bonev, and M T Nelson. 1996. "Inward Rectifier K+ Currents in Smooth Muscle Cells from Rat Coronary Arteries: Block by Mg2+, Ca2+, and Ba2+." *Am J Physiol* 271(2 Pt 2): 696–705.
Rodenwaldt, Barbara, Ulrich Pohl, and Cor de Wit. 2007. "Endogenous and Exogenous NO Attenuates Conduction of Vasoconstrictions along Arterioles in the Microcirculation." *American Journal of Physiology-Heart and Circulatory Physiology* 292(5): H2341–48.
Roos, Jack, Paul J. DiGregorio, Andriy V. Yeromin, Kari Ohlsen, Maria Lioudyno, Shenyuan Zhang, Olga Safrina, J. Ashot Kozak, Steven L. Wagner, Michael D. Cahalan, Gönül Veliçelebi, and Kenneth A. Stauderman. 2005. "STIM1, an Essential and Conserved Component of Store-Operated Ca $^{2+}$ Channel Function." *The Journal of Cell Biology* 169(3): 435–45.
Rosado, Eugenio, Aina Rodríguez-Vilarrupla, Jorge Gracia-Sancho, Montserrat Monclús, Jaume Bosch, and Joan-Carles Carles García-Pagán. 2012. "Interaction between NO and COX Pathways Modulating Hepatic Endothelial Cells from Control and Cirrhotic Rats." *Journal of Cellular and Molecular Medicine* 16(10): 2461–70.
Ross, Tyler D, Brian G Coon, Sanguk Yun, Nicolas Baeyens, Keiichiro Tanaka, Mingxing Ouyang, and Martin A Schwartz. 2013. "Integrins in Mechanotransduction." *Current Opinion in Cell Biology* 25(5): 613–18.
Rossitch, E, E Alexander, P M Black, and J P Cooke. 1991. "L-Arginine Normalizes Endothelial Function in Cerebral Vessels from Hypercholesterolemic Rabbits." *Journal of Clinical Investigation* 87(4): 1295–99.
Roszer, Tamyás. 2012. The Biology of Subcellular Nitric Oxide *The Biology of Subcellular Nitric Oxide*.
Ruhle, Brian, and Mohamed Trebak. 2013. "Emerging Roles for Native Orai Ca2+ Channels in Cardiovascular Disease." *Current topics in membranes* 71: 209–35.
Rummery, Nicole M., and Caryl E. Hill. 2004. "Vascular Gap Junctions and Implications for Hypertension." *Clinical and Experimental Pharmacology and Physiology* 31(10): 659–67.
Rummery, Nicole M, James a Brock, Poungrat Pakdeechote, Vera Ralevic, and William R Dunn. 2007. "ATP Is the Predominant Sympathetic Neurotransmitter in Rat Mesenteric Arteries at High Pressure." *The Journal of physiology* 582(Pt 2): 745–54.
Saddouk, F. Z., R. Ginnan, and H. A. Singer. 2017. "Ca2 +/Calmodulin-Dependent Protein Kinase II in Vascular Smooth Muscle." In *Advances in Pharmacology*, , 171–202.
Sáez, Juan C., Angus C. Nairn, Andrew J. Czernik, Glenn I. Fishman, David C. Spray, and Elliot L. Hertzberg. 1997. "Phosphorylation of Connexin43 and the Regulation of Neonatal Rat Cardiac Myocyte Gap Junctions." *Journal of Molecular and Cellular Cardiology* 29(8): 2131–45.

Sakagami, Kenji, Hajime Kawamura, David M. Wu, and Donald G. Puro. 2001. "Nitric oxide/cGMP-Induced Inhibition of Calcium and Chloride Currents in Retinal Pericytes." *Microvascular Research* 62(2): 196–203.

Sakai, T. 1990. "Acetylcholine Induces Ca-Dependent K Currents in Rabbit Endothelial Cells." *Japanese journal of pharmacology* 53(2): 235–46.

Saleh, S. N., Albert P. Albert, C. M. Peppiatt, and W. A. Large. 2006. "Angiotensin II Activates Two Cation Conductances with Distinct TRPC1 and TRPC6 Channel Properties in Rabbit Mesenteric Artery Myocytes." *Journal of Physiology* 577(2): 479–95.

Saleh, Sohag N., and Iain A. Greenwood. 2005. "Activation of Chloride Currents in Murine Portal Vein Smooth Muscle Cells by Membrane Depolarization Involves Intracellular Calcium Release." *American Journal of Physiology-Cell Physiology* 288(1): C122–31.

Saliez, J., C. Bouzin, G. Rath, P. Ghisdal, F. Desjardins, R. Rezzani, L. F. Rodella, J. Vriens, B. Nilius, O. Feron, J. L. Balligand, and C. Dessy. 2008. "Role of Caveolar Compartmentation in Endothelium-Derived Hyperpolarizing Factor-Mediated Relaxation-ca2+ Signals and Gap Junction Function Are Regulated by Caveolin in Endothelial Cells." *Circulation* 117(8): 1065–74.

Salvemini, Daniela, Sangwon F Kim, and Vincenzo Mollace. 2013. "Reciprocal Regulation of the Nitric Oxide and Cyclooxygenase Pathway in Pathophysiology: Relevance and Clinical Implications." *American journal of physiology. Regulatory, integrative and comparative physiology* 304(7): R473-87.

Sancho, Maria, Nina C. Samson, Bjorn O. Hald, Ahmed M. Hashad, Sean P. Marrelli, Suzanne E. Brett, and Donald G. Welsh. 2017. "KIRchannels Tune Electrical Communication in Cerebral Arteries." *Journal of Cerebral Blood Flow and Metabolism* 37(6): 2171–84.

Sandow, Shaun L., Craig B. Neylon, Mao X. Chen, and Christopher J. Garland. 2006. "Spatial Separation of Endothelial Small- and Intermediate-Conductance Calcium-Activated Potassium Channels (KCa) and Connexins: Possible Relationship to Vasodilator Function?" *Journal of Anatomy* 209(5): 689–98.

Sandow, Shaun L, Rebecca E Haddock, Caryl E Hill, Preet S Chadha, Paul M Kerr, Donald G Welsh, and Frances Plane. 2009. "What's Where and Why at a Vascular Myoendothelial Microdomain Signalling Complex." *Clinical and experimental pharmacology & physiology* 36(1): 67–76.

Sandow, Shaun L, and Caryl E Hill. 2000. "Incidence of Myoendothelial Gap Junctions in the Proximal and Distal Mesenteric Arteries of the Rat Is Suggestive of a Role in Endothelium-Derived Hyperpolarizing Factor–Mediated Responses." *Circulation Research* 86(3): 341 LP-346.

Sandow, Shaun L, Marianne Tare, Harold A Coleman, Caryl E Hill, and Helena C Parkington. 2002. "Involvement of Myoendothelial Gap Junctions in the Actions of Endothelium-Derived Hyperpolarizing Factor." *Circulation research* 90(10): 1108–13.

Sankaranarayanan, Ananthakrishnan, Girija Raman, Christoph Busch, Tim Schultz, Pavel I Zimin, Joachim Hoyer, Ralf Köhler, and Heike Wulff. 2009. "Naphtho[1,2-D]thiazol-2-Ylamine (SKA-31), a New Activator of KCa2 and KCa3.1 Potassium Channels, Potentiates the Endothelium-Derived Hyperpolarizing Factor Response and Lowers Blood Pressure." *Molecular pharmacology* 75(2): 281–95.

Santana, Luis F, and Manuel F Navedo. 2009. "Molecular and Biophysical Mechanisms of Ca2+ Sparklets in Smooth Muscle." *Journal of Molecular and Cellular Cardiology* 47(4): 436–44.

Sauvé, R, M Chahine, J Tremblay, and P Hamet. 1990. “Single-Channel Analysis of the Electrical Response of Bovine Aortic Endothelial Cells to Bradykinin Stimulation: Contribution of a Ca2(+)-Dependent K+ Channel.” *Journal of hypertension. Supplement : official journal of the International Society of Hypertension* 8(7): S193-201.

Savage, Declan, Joanne Perkins, Chin Hong Lim, and Stuart J Bund. 2003. “Functional Evidence That K+ Is the Non-Nitric Oxide, Non-Prostanoid Endothelium-Derived Relaxing Factor in Rat Femoral Arteries.” *Vascular pharmacology* 40(1): 23–28.

Sawada, Naoki, Hiroshi Itoh, Jun Yamashita, Kentaro Doi, Mayumi Inoue, Ken Masatsugu, Yasutomo Fukunaga, Satsuki Sakaguchi, Masakatsu Sone, Ken-ichi Yamahara, Takami Yurugi, and Kazuwa Nakao. 2001. “cGMP-Dependent Protein Kinase Phosphorylates and Inactivates RhoA.” *Biochemical and Biophysical Research Communications* 280(3): 798–805.

Schleifenbaum, J., M. Kassmann, I. A. Szijarto, H. C. Hercule, J.-Y. Tano, S. Weinert, M. Heidenreich, A. R. Pathan, Y.-M. Anistan, N. Alenina, N. J. Rusch, M. Bader, T. J. Jentsch, and M. Gollasch. 2014. “Stretch-Activation of Angiotensin II Type 1a Receptors Contributes to the Myogenic Response of Mouse Mesenteric and Renal Arteries.” *Circulation Research* 115(2): 263–72.

Schleifer, H, B Doleschal, M Lichtenegger, R Oppenrieder, I Derler, I Frischauf, T N Glasnov, C O Kappe, C Romanin, and K Groschner. 2012. “Modulation of Cellular Ca2+ Handling by Pyrazoles.” *British Journal of Pharmacology* 167(8): 1712–22.

Schlossmann, Jens, Robert Feil, and Franz Hofmann. 2003. “Signaling through NO and cGMP-dependent Protein Kinases.” *Annals of Medicine* 35(1): 21–27.

Scholey, J W, and T W Meyer. 1989. “Control of Glomerular Hypertension by Insulin Administration in Diabetic Rats.” *The Journal of clinical investigation* 83(4): 1384–89.

Schubert, Rudolf, Ulrike Krien, Iris Wulfsen, Dorrit Schiemann, Gernot Lehmann, Norbert Ulfig, Ruediger W. Veh, Jürgen R. Schwarz, and Hristo Gagov. 2004. “Nitric Oxide Donor Sodium Nitroprusside Dilates Rat Small Arteries by Activation of Inward Rectifier Potassium Channels.” *Hypertension* 43(4): 891–96.

Schubert, Rudolf, Darcy Lidington, and Steffen Sebastian Bolz. 2008. “The Emerging Role of Ca2+sensitivity Regulation in Promoting Myogenic Vasoconstriction.” *Cardiovascular Research* 77(1): 8–18.

Schumacher, Maria A., Andre F. Rivard, Hans Peter Bächinger, and John P. Adelman. 2001. “Structure of the Gating Domain of a Ca2+-Activated K+ Channel Complexed with Ca2+/calmodulin.” *Nature* 410(6832): 1120–24.

Schumacher, Maria A, Matthew Crum, and Marshall C Miller. 2004. “Crystal Structures of Apocalmodulin and an Apocalmodulin/SK Potassium Channel Gating Domain Complex.” *Structure* 12(5): 849–60.

Schwartz, Martin Alexander. 2010. “Integrins and Extracellular Matrix in Mechanotransduction.” *Cold Spring Harbor perspectives in biology* 2(12): a005066–a005066.

Scotland, Ramona S., Sharmila Chauhan, Patrick J.T. Vallance, and Amrita Ahluwalia. 2001. “An Endothelium-Derived Hyperpolarizing Factor-like Factor Moderates Myogenic Constriction of Mesenteric Resistance Arteries in the Absence of Endothelial Nitric Oxide Synthase-Derived Nitric Oxide.” *Hypertension* 38(4): 833–39.

Seager, Joanna M., Timothy V. Murphy, and Christopher J. Garland. 1994. “Importance of Inositol (1,4,5)-Trisphosphate, Intracellular Ca2+ Release and Myofilament Ca2+

Sensitization in 5-Hydroxytryptamine-Evoked Contraction of Rabbit Mesenteric Artery." *British Journal of Pharmacology* 111(2): 525–32.
Segal, Steven S. 2015. "Integration and Modulation of Intercellular Signaling Underlying Blood Flow Control HHS Public Access." *J Vasc Res. J Vasc Res* 52(2): 136–57.
Senadheera, Sevvandi, Youngsoo Kim, T. Hilton Grayson, Sianne Toemoe, Mikhail Y. Kochukov, Joel Abramowitz, Gary D. Housley, Rebecca L. Bertrand, Preet S. Chadha, Paul P. Bertrand, Timothy V. Murphy, Marianne Tare, Lutz Birnbaumer, Sean P. Marrelli, and Shaun L. Sandow. 2012. "Transient Receptor Potential Canonical Type 3 Channels Facilitate Endothelium-Derived Hyperpolarization-Mediated Resistance Artery Vasodilator Activity." *Cardiovascular Research* 95(4): 439–47.
Sepulveda, F. V., L. Pablo Cid, Jacques Teulon, and María Isabel Niemeyer. 2015. "Molecular Aspects of Structure, Gating, and Physiology of pH-Sensitive Background K2P and Kir K+-Transport Channels." *Physiological Reviews* 95(1): 179–217.
Sessa, William C. 2005. "Regulation of Endothelial Derived Nitric Oxide in Health and Disease." *Memórias do Instituto Oswaldo Cruz* 100 Suppl(Suppl 1): 15–18.
Sharif-Naeini, Reza, Alexandra Dedman, Joost H. A. Folgering, Fabrice Duprat, Amanda Patel, Bernd Nilius, and Eric Honoré. 2008. "TRP Channels and Mechanosensory Transduction: Insights into the Arterial Myogenic Response." *Pflügers Archiv - European Journal of Physiology* 456(3): 529–40.
Sharif-Naeini, Reza, Joost H.A. Folgering, Delphine Bichet, Fabrice Duprat, Inger Lauritzen, Malika Arhatte, Martine Jodar, Alexandra Dedman, Franck C. Chatelain, Uwe Schulte, Kevin Retailleau, Laurent Loufrani, Amanda Patel, Frederick Sachs, Patrick Delmas, Dorien J.M. Peters, and Eric Honoré. 2009. "Polycystin-1 and -2 Dosage Regulates Pressure Sensing." *Cell* 139(3): 587–96.
Shatarat, Amjad, William R. Dunn, and Vera Ralevic. 2014. "Raised Tone Reveals ATP as a Sympathetic Neurotransmitter in the Porcine Mesenteric Arterial Bed." *Purinergic Signalling* 10(4): 639–49.
Shaw, Linda, Stephen O'Neill, Carolyn J P Jones, Clare Austin, and Michael J Taggart. 2004. "Comparison of U46619-, Endothelin-1- or Phenylephrine-Induced Changes in Cellular Ca2+ Profiles and Ca2+ Sensitisation of Constriction of Pressurised Rat Resistance Arteries." *British journal of pharmacology* 141(4): 678–88.
Shen, J.-B., B Jiang, and A J Pappano. 2000. "Comparison of L-Type Calcium Channel Blockade by Nifedipine And/or Cadmium in Guinea Pig Ventricular Myocytes." *Journal of Pharmacology and Experimental Therapeutics* 294(2): 562–70.
Sheng, J Z, S Ella, M J Davis, M A Hill, and A P Braun. 2009. "Openers of SKCa and IKCa Channels Enhance Agonist-Evoked Endothelial Nitric Oxide Synthesis and Arteriolar Vasodilation." *Faseb J* 23(4): 1138–45.
Sheng, Jian-zhong, and Andrew P Braun. 2007. "Small- and Intermediate-Conductance Ca2+-Activated K+ Channels Directly Control Agonist-Evoked Nitric Oxide Synthesis in Human Vascular Endothelial Cells." *American journal of physiology. Cell physiology* 293(1): C458–67.
Shimokawa, H. 2014. "2014 Williams Harvey Lecture: Importance of Coronary Vasomotion Abnormalities--from Bench to Bedside." *European Heart Journal* 35(45): 3180–93.
Si, H. 2006. "Impaired Endothelium-Derived Hyperpolarizing Factor-Mediated Dilations and Increased Blood Pressure in Mice Deficient of the Intermediate-Conductance Ca2+-Activated K+ Channel." *Circulation Research* 99(5): 537–44.

Sieber, F E, D A Wilson, D F Hanley, and R J Traystman. 1993. “Extracellular Potassium Activity and Cerebral Blood Flow during Moderate Hypoglycemia in Anesthetized Dogs.” *The American journal of physiology* 264(6 Pt 2): H1774-80.

Silva, Bruno R, Tiago D Paula, Michele Paulo, and Lusiane M Bendhack. 2016. “Nitric Oxide Signaling and the Cross Talk with Prostanoids Pathways in Vascular System.” *Medicinal chemistry (Shariqah (United Arab Emirates))*.

Simonsen, U, D Prieto, I Sánez de Tejada, and A García-Sacristán. 1995. “Involvement of Nitric Oxide in the Non-Adrenergic Non-Cholinergic Neurotransmission of Horse Deep Penile Arteries: Role of Charybdotoxin-Sensitive K(+)-Channels.” *British journal of pharmacology* 116(6): 2582–90.

Sinkler, Shenghua Y., and Steven S. Segal. 2017. “Rapid *versus* Slow Ascending Vasodilatation: Intercellular Conduction *versus* Flow-Mediated Signalling with Tetanic *versus* Rhythmic Muscle Contractions.” *The Journal of Physiology* 595(23): 7149–65.

Skärby, T V, and E D Högestätt. 1990. “Differential Effects of Calcium Antagonists and Bay K 8644 on Contractile Responses to Exogenous Noradrenaline and Adrenergic Nerve Stimulation in the Rabbit Ear Artery.” *British journal of pharmacology* 101(4): 961–67.

Skomedal, T., J.B. Osnes, and I. Oye. 1980. “Competitive Blockade of a-Adrenergic Receptors in Rat Heart by Prazosin.” *Acta Pharmacol. Toxicol.* 47: 217–22.

Smaje, Laurence, B. W. Zweifach, and M. Intaglietta. 1970. “Micropressures and Capillary Filtration Coefficients in Single Vessels of the Cremaster Muscle of the Rat.” *Microvascular Research* 2(1): 96–110.

Smallridge, Robert C, Richard J Galloway, and D Gist. 1992. “U-73122, an Aminosteroid Phospholipase C Antagonist, Noncompetitively Inhibits Thyrotropin-Releasing Hormone Effects in GH3 Rat Pituitary Cells.” *Journal of Endocrinology* 131(4).

Smeda, J S, and S King. 2000. “Electromechanical Alterations in the Cerebrovasculature of Stroke-Prone Rats.” *Stroke; a journal of cerebral circulation* 31(3): 751–59.

Smeda, John S, and Noriko Daneshtalab. 2011. “The Effects of Poststroke Captopril and Losartan Treatment on Cerebral Blood Flow Autoregulation in SHRsp with Hemorrhagic Stroke.” *Journal of Cerebral Blood Flow and Metabolism* 31(2): 476–85.

Smith, J B. 1996. “Calcium Homeostasis in Smooth Muscle Cells.” *New horizons (Baltimore, Md.)* 4(1): 2–18.

Smith, Pamela D, Suzanne E Brett, Kevin D Luykenaar, Shaun L Sandow, Sean P Marrelli, Edward J Vigmond, and Donald G Welsh. 2008. “KIR Channels Function as Electrical Amplifiers in Rat Vascular Smooth Muscle.” *The Journal of Physiology* 586(4): 1147–60.

Smith, William L., and Lawrence J. Marnett. 1991. “Prostaglandin Endoperoxide Synthase: Structure and Catalysis.” *Biochimica et Biophysica Acta (BBA)/Lipids and Lipid Metabolism* 1083(1): 1–17.

Smyth, Emer M. 2010. “Thromboxane and the Thromboxane Receptor in Cardiovascular Disease.” *Clinical lipidology* 5(2): 209–19.

Smyth, Emer M., Tilo Grosser, Miao Wang, Ying Yu, and Garret A. FitzGerald. 2009. “Prostanoids in Health and Disease: Fig. 1.” *Journal of Lipid Research* 50(Supplement): S423–28.

Sneddon, P., and G. Burnstock. 1984. “ATP as a Co-Transmitter in Rat Tail Artery.” *European Journal of Pharmacology* 106(1): 149–52.

Somjen, G G. 1979. “Extracellular Potassium in the Mammalian Central Nervous System.” *Annual Review of Physiology* 41(1): 159–77.

Somlyo, A. P., and A. V. Somlyo. 2003. “Ca2+ Sensitivity of Smooth Muscle and Nonmuscle Myosin II: Modulated by G Proteins, Kinases, and Myosin Phosphatase.” *Physiological Reviews* 83(4): 1325–58.

Sonkusare, SK, T Dalsgaard, AD Bonev, and MT Nelson. 2016. “Inward Rectifier Potassium (Kir2.1) Channels as End-Stage Boosters of Endothelium- Dependent Vasodilators.” *The Journal of Physiology* 12: 1–15.

Sonkusare, Swapnil K, Adrian D Bonev, Jonathan Ledoux, Wolfgang Liedtke, Michael I Kotlikoff, Thomas J Heppner, David C Hill-eubanks, and Mark T Nelson. 2012. “Elementary Ca2+ Signals Through Endothelial TRPV4 Channels Regulate Vascular Function.” *Science* 524(May): 597–602.

Spassova, Maria A, Thamara Hewavitharana, Wen Xu, Jonathan Soboloff, and Donald L Gill. 2006. “A Common Mechanism Underlies Stretch Activation and Receptor Activation of TRPC6 Channels.” *Proceedings of the National Academy of Sciences* 103(44): 16586–91.

Spradling, A., G. Rubin, E Hafen, and G Rubin. 1982. “Transposition of Cloned P Elements into Drosophila Germ Line Chromosomes.” *Science* 218(4570): 341–47.

van der Staay, F J, R J Fanelli, A Blokland, and B H Schmidt. 1999. “Behavioral Effects of Apamin, a Selective Inhibitor of the SK(Ca)-Channel, in Mice and Rats.” *Neuroscience and biobehavioral reviews* 23(8): 1087–1110.

Stanfield, P R, N W Davies, P A Shelton, M J Sutcliffe, I A Khan, W J Brammar, and E C Conley. 1994. “A Single Aspartate Residue Is Involved in Both Intrinsic Gating and Blockage by Mg2+ of the Inward Rectifier, IRK1.” *The Journal of physiology* 478 ( Pt 1: 1–6.

Stankevicius, E, V Lopez-Valverde, L Rivera, AD Hughes, M J Mulvany, and Ulf Simonsen. 2006. “Combination of Ca2+ -Activated K+ Channel Blockers Inhibits Acetylcholine-Evoked Nitric Oxide Release in Rat Superior Mesenteric Artery.” *British journal of pharmacology* 149(5): 560–72.

Stankevicus, E, T Dalsgaard, C Kroigaard, L Beck, E Boedtkjer, M Misfeldt, G Nielsen, O Schjorring, and A Hughes. 2011. “Opening of Small and Intermediate Calcium-Activated Potassium Channels Induces Relaxation Mainly Mediated by Nitric-Oxide Release in Large Arteries and Endothelium- Derived Hyperpolarizing Factor in Small Arteries from Rat.” *J Pharmacol Exp Ther* 339(3): 842–50.

Stewart, Michael, Maurice Needham, Peter Bankhead, Tom A Gardiner, C. Norman Scholfield, Tim M Curtis, and J. Graham McGeown. 2012. “Feedback via Ca2+-Activated Ion Channels Modulates Endothelin 1 Signaling in Retinal Arteriolar Smooth Muscle.” *Investigative ophthalmology & visual science* 53(6): 3059–66.

Stocker, Martin. 2004. “Ca2+-Activated K+ Channels: Molecular Determinants and Function of the SK Family.” *Nature Reviews Neuroscience* 5(10): 758–70.

Straub, A. C., M. Billaud, S. R. Johnstone, A. K. Best, S. Yemen, S. T. Dwyer, R. Looft-Wilson, J. J. Lysiak, B. Gaston, L. Palmer, and B. E. Isakson. 2011. “Compartmentalized Connexin 43 S-Nitrosylation/Denitrosylation Regulates Heterocellular Communication in the Vessel Wall.” *Arteriosclerosis, Thrombosis, and Vascular Biology* 31(2): 399–407.

Straub, Adam C., Alexander W. Lohman, Marie Billaud, Scott R. Johnstone, Scott T. Dwyer, Monica Y. Lee, Pamela Schoppee Bortz, Angela K. Best, Linda Columbus, Benjamin Gaston, and Brant E. Isakson. 2012. “Endothelial Cell Expression of Haemoglobin α Regulates Nitric Oxide Signalling.” *Nature* 491(7424): 473–77.

Straub, Adam C., Angela C. Zeigler, and Brant E. Isakson. 2014. “The Myoendothelial Junction:

Connections That Deliver the Message." *Physiology* 29(4): 242–49.
Strøbaek, D, DT Brown, DP Jenkins, Y-J Chen, N Coleman, Y Ando, P Chiu, S Jørgensen, J Demnitz, H Wulff, and P Christophersen. 2013. "NS6180, a New KCa 3.1 Channel Inhibitor Prevents T-Cell Activation and Inflammation in a Rat Model of Inflammatory Bowel Disease." *British Journal of Pharmacology* 168(2): 432–44.
Strøbæk, Dorte, Lene Teuber, Tino D. Jørgensen, Philip K. Ahring, Katrine Kjær, Rie S. Hansen, Søren Peter Olesen, Palle Christophersen, and Bo Skaaning-Jensen. 2004. "Activation of Human IK and SK Ca 2+-Activated K + Channels by NS309 (6,7-Dichloro-1H-Indole-2,3-Dione 3-Oxime)." *Biochimica et Biophysica Acta - Biomembranes* 1665(1–2): 1–5.
Suchyna, Thomas M, Janice H Johnson, Katherine Hamer, Joseph F Leykam, Douglas A Gage, Henry F Clemo, Clive M Baumgarten, and Frederick Sachs. 2000. "Identification of a Peptide Toxin from Grammostola Spatulata Spider Venom That Blocks Cation-Selective Stretch-Activated Channels." *J. Gen. Physiol* 115: 583–98.
Suh, Byung-Chang, and Bertil Hille. 2008. "PIP2 Is a Necessary Cofactor for Ion Channel Function: How and Why?" *Annual review of biophysics* 37: 175–95.
Sui, J L, Jérme Petit-Jacques, and Diomedes E Logothetis. 1998. "Activation of the Atrial KACh Channel by the Betagamma Subunits of G Proteins or Intracellular Na+ Ions Depends on the Presence of Phosphatidylinositol Phosphates." *Proceedings of the National Academy of Sciences of the United States of America* 95(3): 1307–12.
Sun, Dong, An Huang, Akos Koller, and Gabor Kaley. 2001. "Endothelial KCa Channels Mediate Flow-Dependent Dilation of Arterioles of Skeletal Muscle and Mesentery." *Microvascular Research* 61(2): 179–86.
Suzuki, Kimiaki, Shin-ya Saito, and Tomohisa Ishikawa. 2012. "Involvement of Phosphatidylcholine-Specific Phospholipase C in Thromboxane A2 Receptor-Mediated Extracellular Ca2+ Influx in Rat Aorta." *European Journal of Pharmacology* 677(1–3): 123–30.
Swärd, Karl, Mitsuo Mita, David P Wilson, Jing Ti Deng, Marija Susnjar, and Michael P Walsh. 2003. "The Role of RhoA and Rho-Associated Kinase in Vascular Smooth Muscle Contraction." *Current hypertension reports* 5(1): 66–72.
Sybertz, E J, G Vander Vliet, and T Baum. 1983. "Analysis of the Vasoconstrictor Responses to Potassium Depolarization and Norepinephrine and Their Antagonism by Differing Classes of Vasodilators in the Perfused Rat Hindquarters." *The Journal of pharmacology and experimental therapeutics* 227(3): 621–26.
Syme, Colin A, Aaron C Gerlach, Ashvani K Singh, Daniel C Devor, and A Colin. 2000. "Pharmacological Activation of Cloned Intermediate- and Small-Conductance Ca2+ -Activated K + Channels Pharmacological Activation of Cloned Intermediate- and Small-Conductance Ca 2+ -Activated KCa Channels." *Am J Physiol Cell Physiol* (21): 570–81.
Szasz, T., and R.C. Webb. 2017. "Rho-Mancing to Sensitize Calcium Signaling for Contraction in the Vasculature." In *Advances in Pharmacology (San Diego, Calif.)*, , 303–22.
Tajada, Sendoa, Pilar Cidad, Alejandro Moreno-Domínguez, M. Teresa Pérez-García, and José R. López-López. 2012. "High Blood Pressure Associates with the Remodelling of Inward Rectifier $K^+$ Channels in Mice Mesenteric Vascular Smooth Muscle Cells." *The Journal of Physiology* 590(23): 6075–91.
Takaki, Aya, Keiko Morikawa, Masato Tsutsui, Yoshinori Murayama, Ender Tekes, Hiroto Yamagishi, Junko Ohashi, Toyotaka Yada, Nobuyuki Yanagihara, and Hiroaki Shimokawa. 2008. "Crucial Role of Nitric Oxide Synthases System in Endothelium-Dependent

Hyperpolarization in Mice." *The Journal of experimental medicine* 205(9): 2053–63.
Takano, Hiromichi, Kim A. Dora, Michaela M. Spitaler, and Chris J. Garland. 2004. "Spreading Dilatation in Rat Mesenteric Arteries Associated with Calcium-Independent Endothelial Cell Hyperpolarization." *Journal of Physiology* 556(3): 887–903.
Tanemoto, Masayuki, Nobuyoshi Kittaka, Atsushi Inanobe, and Yoshihisa Kurachi. 2000. "In Vivo Formation of a Proton-Sensitive K+ Channel by Heterometric Subunit Assemble of Kir5.1 with Kir4.1." *J Physiol* 524(3): 587–92.
Tare, M, H C Parkington, H a Coleman, T O Neild, and G J Dusting. 1990. "Hyperpolarization and Relaxation of Arterial Smooth Muscle Caused by Nitric Oxide Derived from the Endothelium." *Nature* 346(6279): 69–71.
Taylor, Mark S., Adrian D. Bonev, Tobias P. Gross, Delrae M. Eckman, Joseph E. Brayden, Chris T. Bond, John P. Adelman, and Mark T. Nelson. 2003. "Altered Expression of Small-Conductance Ca2+-Activated K+ (SK3) Channels Modulates Arterial Tone and Blood Pressure." *Circulation Research* 93(2): 124–31.
Thorneloe, Kevin S., and Mark T. Nelson. 2005. "Ion Channels in Smooth Muscle: Regulators of Intracellular Calcium and Contractility." *Canadian Journal of Physiology and Pharmacology* 83(3): 215–42.
Thosar, Saurabh S, Blair D Johnson, Jeanne D Johnston, and Janet P Wallace. 2012. "Sitting and Endothelial Dysfunction: The Role of Shear Stress." *Medical science monitor : international medical journal of experimental and clinical research* 18(12): RA173-80.
Tosun, M, R J Paul, and R M Rapoport. 1998. "Role of Extracellular Ca++ Influx via L-Type and Non-L-Type Ca++ Channels in Thromboxane A2 Receptor-Mediated Contraction in Rat Aorta." *The Journal of pharmacology and experimental therapeutics* 284(3): 921–28.
Toussaint, Fanny, Chimène Charbel, Alexandre Blanchette, and Jonathan Ledoux. 2015. "CaMKII Regulates Intracellular Ca2+ Dynamics in Native Endothelial Cells." *Cell Calcium* 58(3): 275–85.
Tran, C. H. T., M. S. Taylor, F. Plane, S. Nagaraja, N. M. Tsoukias, V. Solodushko, E. J. Vigmond, T. Furstenhaupt, M. Brigdan, and D. G. Welsh. 2012. "Endothelial Ca2+ Wavelets and the Induction of Myoendothelial Feedback." *AJP: Cell Physiology* 302(8): C1226–42.
Tran, Cam Ha T, Edward J Vigmond, Frances Plane, and Donald G Welsh. 2009. "Mechanistic Basis of Differential Conduction in Skeletal Muscle Arteries." *The Journal of physiology* 587: 1301–18.
Trebak, Mohamed. 2012. "STIM/Orai Signalling Complexes in Vascular Smooth Muscle." *The Journal of physiology* 590(17): 4201–8.
Trebak, Mohamed, Gary St J Bird, Richard R McKay, Lutz Birnbaumer, and James W Putney. 2003. "Signaling Mechanism for Receptor-Activated Canonical Transient Receptor Potential 3 (TRPC3) Channels." *The Journal of biological chemistry* 278(18): 16244–52.
Triggle, Chris R., Samson Mathews Samuel, Shalini Ravishankar, Isra Marei, Gnanapragasam Arunachalam, and Hong Ding. 2012. "The Endothelium: Influencing Vascular Smooth Muscle in Many Ways." *Canadian Journal of Physiology and Pharmacology* 90(6): 713–38.
Tsai, Emily J, and David A Kass. 2009. "Cyclic GMP Signaling in Cardiovascular Pathophysiology and Therapeutics." *Pharmacology and Therapeutics* 122(3): 216–38.
Tsien, R W, D Lipscombe, D V Madison, K R Bley, and A P Fox. 1988. "Multiple Types of Neuronal Calcium Channels and Their Selective Modulation." *Trends in neurosciences*

11(10): 431–38.
Tykocki, Nathan R, Adrian D Bonev, Thomas Andrew Longden, Thomas J Heppner, and Mark T Nelson. 2017. “Inhibition of Vascular Smooth Muscle Inward-Rectifier K+ Channels Restores Myogenic Tone in Mouse Urinary Bladder Arterioles.” *American journal of physiology. Renal physiology* (25): ajprenal.00682.2016.
Uchida, H, Y Tanaka, K Ishii, and K Nakayama. 1999. “L-Type Ca2+ Channels Are Not Involved in Coronary Endothelial Ca2+ Influx Mechanism Responsible for Endothelium-Dependent Relaxation.” *Research communications in molecular pathology and pharmacology* 104(2): 127–44.
Undavia, Samir S, Valentina Berger, Gabor Kaley, and Edward J Messina. 2003. “Myogenic Responses of Isolated Adipose Tissue Arterioles.” *Microvascular Research* 66(2): 140–46.
VanBavel, E, E. T. Van der Meulen, and J A.E. Spaan. 2001. “Role of Rho-Associated Protein Kinase in Tone and Calcium Sensitivity of Cannulated Rat Mesentric Small Arteries.” *Experimental Physiology* 86(5): 585–92.
Vanbavel, Ed, Jos P M Wesselman, and Jos A E Spaan. 1998. “Myogenic Activation and Calcium Sensitivity of Cannulated Rat Mesenteric Small Arteries.” *Circ Res* 82(2): 210–20.
Vanheel, B, and J Van de Voorde. 1997. “Nitric Oxide Induced Membrane Hyperpolarization in the Rat Aorta Is Not Mediated by Glibenclamide-Sensitive Potassium Channels.” *Canadian journal of physiology and pharmacology* 75(12): 1387–92.
Vanhoutte, P. M., H. Shimokawa, M. Feletou, and E. H.C. Tang. 2017. “Endothelial Dysfunction and Vascular Disease – a 30th Anniversary Update.” *Acta Physiologica* 219(1): 22–96.
Vanhoutte, P M, T J Verbeuren, and R C Webb. 1981. “Local Modulation of Adrenergic Neuroeffector Interaction in the Blood Vessel Well.” *Physiological Reviews* 61(1): 151–247.
Vanhoutte, Paul M. 2003. “Endothelial Control of Vasomotor Function: From Health to Coronary Disease.” *Circulation journal : official journal of the Japanese Circulation Society* 67(7): 572–75.
Vanhoutte, Paul M., Yingzi Zhao, Aimin Xu, and Susan W.S. Leung. 2016. “Thirty Years of Saying NO.” *Circulation Research* 119(2): 375–96.
VanWijk, Marja J, Kees Boer, Esther T van der Meulen, Otto P Bleker, Jos A E Spaan, and Ed VanBavel. 2002. “Resistance Artery Smooth Muscle Function in Pregnancy and Preeclampsia.” *American journal of obstetrics and gynecology* 186(1): 148–54.
Vásquez-Vivar, J, B Kalyanaraman, P Martásek, N Hogg, B S Masters, H Karoui, P Tordo, and K A Pritchard. 1998. “Superoxide Generation by Endothelial Nitric Oxide Synthase: The Influence of Cofactors.” *Proceedings of the National Academy of Sciences of the United States of America* 95(16): 9220–25.
Venkatachalam, Kartik, Fei Zheng, and Donald L. Gill. 2003. “Regulation of Canonical Transient Receptor Potential (TRPC) Channel Function by Diacylglycerol and Protein Kinase C.” *Journal of Biological Chemistry* 278(31): 29031–40.
Vettoretti, Simone, Peter Ochodnicky, Hendrik Buikema, Rob H Henning, C Alex Kluppel, Dick de Zeeuw, and Richard PE van Dokkum. 2006. “Altered Myogenic Constriction and Endothelium-Derived Hyperpolarizing Factor-Mediated Relaxation in Small Mesenteric Arteries of Hypertensive Subtotally Nephrectomized Rats.” *Journal of Hypertension* 24(11): 2215–23.
Villalba, Nuria, Edgaras Stankevicius, Albino Garcia-Sacristán, Ulf Simonsen, and Dolores Prieto. 2007. “Contribution of Both $Ca^{2+}$ Entry and $Ca^{2+}$ Sensitization to the $\alpha_1$ -

Adrenergic Vasoconstriction of Rat Penile Small Arteries." *American Journal of Physiology-Heart and Circulatory Physiology* 292(2): H1157–69.
Vriens, Joris, Grzegorz Owsianik, Thomas Hofmann, Stephan E. Philipp, Julia Stab, Xiaodi Chen, Melissa Benoit, Fenqin Xue, Annelies Janssens, Sara Kerselaers, Johannes Oberwinkler, Rudi Vennekens, Thomas Gudermann, Bernd Nilius, and Thomas Voets. 2011. "TRPM3 Is a Nociceptor Channel Involved in the Detection of Noxious Heat." *Neuron* 70(3): 482–94.
Vuori, K. 1998. "Integrin Signaling: Tyrosine Phosphorylation Events in Focal Adhesions." *Journal of Membrane Biology* 165(3): 191–99.
Waldron, G J, and C J Garland. 1994. "Contribution of Both Nitric Oxide and a Change in Membrane Potential to Acetylcholine-Induced Relaxation in the Rat Small Mesenteric Artery." *British journal of pharmacology* 112(3): 831–36.
Walsh, M P. 1991. "The Ayerst Award Lecture 1990. Calcium-Dependent Mechanisms of Regulation of Smooth Muscle Contraction." *Biochemistry and cell biology = Biochimie et biologie cellulaire* 69(12): 771–800.
Walsh, Michael P., Marija Susnjar, Jingti Deng, Cindy Sutherland, Eniko Kiss, and David P. Wilson. 2007. "Phosphorylation of the Protein Phosphatase Type 1 Inhibitor Protein CPI-17 by Protein Kinase C." In *Protein Phosphatase Protocols*, New Jersey: Humana Press, 209–24.
Wang, Xiaodong, and Van Breemen. 1999. "Depolarization-Mediated Inhibition of Ca2+ Entry in Endothelial Cells." *Am J Physiol* 277(4 Pt 2): H1498-504.
Weatherall, K. L., V. Seutin, J.-F. Liegeois, and N. V. Marrion. 2011. "Crucial Role of a Shared Extracellular Loop in Apamin Sensitivity and Maintenance of Pore Shape of Small-Conductance Calcium-Activated Potassium (SK) Channels." *Proceedings of the National Academy of Sciences* 108(45): 18494–99.
Weekley, L Bruce. 1991. "Melatonin-induced Relaxation of Rat Aorta: Interaction with Adrenergic Agonists." *Journal of Pineal Research* 11(1): 28–34.
Wei, R., S. E. Lunn, R. Tam, S. L. Gust, B. Classen, P. M. Kerr, and F. Plane. 2018. "Vasoconstrictor Stimulus Determines the Functional Contribution of Myoendothelial Feedback to Mesenteric Arterial Tone." *The Journal of Physiology*.
Weiss, Sharon, and Nathan Dascal. 2015. "Molecular Aspects of Modulation of L-Type Calcium Channels by Protein Kinase C." *Current molecular pharmacology* 8(1): 43–53.
Welsh, Donald G., Anthony D. Morielli, Mark T. Nelson, and Joseph E. Brayden. 2002. "Transient Receptor Potential Channels Regulate Myogenic Tone of Resistance Arteries." *Circulation Research* 90(3): 248–50.
Weng, S, M Lauven, T Schaefer, L Polontchouk, R Grover, and S Dhein. 2002. "Pharmacological Modification of Gap Junction Coupling by an Antiarrhythmic Peptide via Protein Kinase C Activation." *The FASEB journal : official publication of the Federation of American Societies for Experimental Biology* 16(9): 1114–16.
Werner-Felmayer, G., E.R. Werner, D. Fuchs, A. Hausen, G. Reibnegger, Kurt Schmidt, G. Weiss, and H. Wachter. 1993. "Pteridine Biosynthesis in Human Endothelial Cells." *Journal of Biological Chemistry* 268(3): 1842–46.
Wessler, I., C. J. Kirkpatrick, and K. Racké. 1999. "The Cholinergic 'Pitfall': Acetylcholine, a Universal Cell Molecule in Biological Systems, Including Humans." *Clinical and Experimental Pharmacology and Physiology* 26(3): 198–205.
Westcott, Erika B., Erica L. Goodwin, Steven S. Segal, and William F. Jackson. 2012. "Function

and Expression of Ryanodine Receptors and Inositol 1,4,5-Trisphosphate Receptors in Smooth Muscle Cells of Murine Feed Arteries and Arterioles." *The Journal of Physiology* 590(8): 1849–69.
Westcott, Erika B., and Steven S. Segal. 2013. "Perivascular Innervation: A Multiplicity of Roles in Vasomotor Control and Myoendothelial Signaling." *Microcirculation* 20(3): 217–38.
Westcott, Erika B, and William F Jackson. 2011. "Heterogeneous Function of Ryanodine Receptors, but Not IP3 Receptors, in Hamster Cremaster Muscle Feed Arteries and Arterioles." *American journal of physiology. Heart and circulatory physiology* 300(5): H1616-30.
Weston, A H, G R Richards, M P Burnham, M Félétou, P M Vanhoutte, and G Edwards. 2002. "K+-Induced Hyperpolarization in Rat Mesenteric Artery: Identification, Localization and Role of Na+/K+-ATPases." *British Journal of Pharmacology* 136(6): 918–26.
Wible, Barbara A., Maurizio Taglialatela, Eckhard Ficker, and Arthur M. Brown. 1994. "Gating of Inwardly Rectifying K+ Channels Localized to a Single Negatively Charged Residue." *Nature* 371(6494): 246–49.
Williams, George S B, Liron Boyman, Aristide C Chikando, Ramzi J Khairallah, and W J Lederer. 2013. "Mitochondrial Calcium Uptake." *Proceedings of the National Academy of Sciences* 110(26): 10479–86.
Wilson, David P., Marija Susnjar, Enikő Kiss, Cindy Sutherland, and Michael P. Walsh. 2005. "Thromboxane A 2 -Induced Contraction of Rat Caudal Arterial Smooth Muscle Involves Activation of Ca 2+ Entry and Ca 2+ Sensitization: Rho-Associated Kinase-Mediated Phosphorylation of MYPT1 at Thr-855, but Not Thr-697." *Biochemical Journal* 389(3): 763–74.
de Wit, Cor, Markus Boettcher, and Volker J Schmidt. 2008. "Signaling across Myoendothelial Gap Junctions--Fact or Fiction?" *Cell communication & adhesion* 15(3): 231–45.
de Wit, Cor, and Tudor M. Griffith. 2010. "Connexins and Gap Junctions in the EDHF Phenomenon and Conducted Vasomotor Responses." *Pflügers Archiv - European Journal of Physiology* 459(6): 897–914.
Wit, Cor De, Bernhard Jahrbeck, Christian Scha, Steffen-sebastian Bolz, and Ulrich Pohl. 1998. "Nitric Oxide Opposes Myogenic Pressure Response Predominantly in Large Arterioles In Vivo." *Hypertension* 31: 787–94.
Worzfeld, Thomas, Nina Wettschureck, and Stefan Offermanns. 2008. "G(12)/G(13)-Mediated Signalling in Mammalian Physiology and Disease." *Trends in pharmacological sciences* 29(11): 582–89.
Wray, Susan, and Theodor Burdyga. 2010. "Sarcoplasmic Reticulum Function in Smooth Muscle." *Physiological Reviews* 90(1): 113–78.
Wu, BN, KD Luykenaar, JE Brayden, WR Giles, RL Corteling, WB Wiehler, and DG Welsh. 2007. "Hyposmotic Challenge Inhibits Inward Rectifying K+ Channels in Cerebral Arterial Smooth Muscle Cells." *American journal of physiology Heart and circulatory physiology* 292(2): H1085-94.
Wu, Meng, Hao-ran Wang, Haibo Yu, Elena Makhina, Jia Xu, Eric S Dawson, Corey R Hopkins, Craig W Lindsley, Owen B McManus, and Min Li. 2010. Probe Reports from the NIH Molecular Libraries Program *A Potent and Selective Small Molecule Kir2.1 Inhibitor*. National Center for Biotechnology Information (US).
Wu, Ming-Ming, Jie Lou, Bin-Lin Song, Yuan-Feng Gong, Yan-Chao Li, Chang-Jiang Yu, Qiu-Shi Wang, Tian-Xing Ma, Ke Ma, H Criss Hartzell, Dayue Darrel Duan, Dan Zhao, and

Zhi-Ren Zhang. 2014. “Hypoxia Augments the Calcium-Activated Chloride Current Carried by Anoctamin-1 in Cardiac Vascular Endothelial Cells of Neonatal Mice.” *British Journal of Pharmacology* 171(15): 3680–92.

Wulff, H, M J Miller, W Hansel, S Grissmer, M D Cahalan, and K G Chandy. 2000. “Design of a Potent and Selective Inhibitor of the Intermediate-Conductance Ca2+-Activated K+ Channel, IKCa1: A Potential Immunosuppressant.” *Proceedings of the National Academy of Sciences of the United States of America* 97(14): 8151–56.

Wulff, Heike, Aaron Kolski-Andreaco, Ananthakrishnan Sankaranarayanan, Jean-Marc Sabatier, and Vikram Shakkottai. 2007. “Modulators of Small- and Intermediate-Conductance Calcium-Activated Potassium Channels and Their Therapeutic Indications.” *Current medicinal chemistry* 14(13): 1437–57.

Xia, X.-M. M et al. 1998. “Mechanism of Calcium Gating in Small-Conductance Calcium-Activated Potassium Channels.” *Nature* 395(6701): 503–7.

Xia, Y, A L Tsai, V Berka, and J L Zweier. 1998. “Superoxide Generation from Endothelial Nitric-Oxide Synthase. A Ca2+/calmodulin-Dependent and Tetrahydrobiopterin Regulatory Process.” *The Journal of biological chemistry* 273(40): 25804–8.

Xu, S.-Z., and David J Beech. 2001. “TrpC1 Is a Membrane-Spanning Subunit of Store-Operated Ca2+ Channels in Native Vascular Smooth Muscle Cells.” *Circulation Research* 88(1): 84–87.

Yamagishi, Toshio, Teruyuki Yanagisawa, and Norio Taira. 1992. “Activation of Phospholipase C by the Agonist U46619 Is Inhibited by Cromakalim-Induced Hyperpolarization in Porcine Coronary Artery.” *Biochemical and Biophysical Research Communications* 187(3): 1517–22.

Yamamoto, Kimiko, Takaaki Sokabe, Takahiro Matsumoto, Kimihiro Yoshimura, Masahiro Shibata, Norihiko Ohura, Toru Fukuda, Takashi Sato, Keisuke Sekine, Shigeaki Kato, Masashi Isshiki, Toshiro Fujita, Mikio Kobayashi, Koichi Kawamura, Hirotake Masuda, Akira Kamiya, and Joji Ando. 2006. “Impaired Flow-Dependent Control of Vascular Tone and Remodeling in P2X4-Deficient Mice.” *Nature Medicine* 12(1): 133–37.

Yamamoto, Yoshimichi, Kenro Imaeda, and Hikaru Suzuki. 1999. “Endothelium-Dependent Hyperpolarization and Intercellular Electrical Coupling in Guinea-Pig Mesenteric Arterioles.” *Journal of Physiology* 514(2): 505–13.

Yang, Hui, Su Juan Kuang, Fang Rao, Yu Mei Xue, Xiao Ying Liu, Zhi Xin Shan, Xiao Hong Li, Jie Ning Zhu, Zhi Ling Zhou, Xiao juan Zhang, Qiu Xiong Lin, Xi Yong Yu, and Chun Yu Deng. “Species-Specific Differences in the Role of L-Type Ca2+ Channels in the Regulation of Coronary Arterial Smooth Muscle Contraction.” *Naunyn-Schmiedeberg’s Archives of Pharmacology* 389(2): 151–57.

Yang, Jian, Yuh Nung Jan, and Lily Y Jan. 1995. “Control of Rectification and Permeation by Residues in Two Distinct Domains in an Inward Rectifier K+ Channel.” *Neuron* 14(5): 1047–54.

Yang, X P, and S Chiba. 2002. “Neuropeptide Y Inhibits Double Peaked Vasoconstrictor Responses to Periarterial Nerve Stimulation Primarily through Prejunctional Y2 Receptor Subtype in Canine Splenic Arteries.” *Autonomic & autacoid pharmacology* 22(2): 119–26.

Yasuda, Noritaka et al. 2008. “Conformational Switch of Angiotensin II Type 1 Receptor Underlying Mechanical Stress-Induced Activation.” *EMBO reports* 9(2): 179–86.

Yokomizo, Ayako, Shingo Takatori, Narumi Hashikawa-Hobara, Mitsuhiro Goda, and Hiromu Kawasaki. 2015. “Characterization of Perivascular Nerve Distribution in Rat Mesenteric

Small Arteries." *Biological & Pharmaceutical Bulletin* 38(11): 1757–64.
Yoo, Sungjae, Ji Yeon Lim, and Sun Wook Hwang. 2014. "Sensory TRP Channel Interactions with Endogenous Lipids and Their Biological Outcomes." *Molecules* 19(4): 4708–44.
Yuan, Qi, Jingyi Yang, Gaetano Santulli, Steven R. Reiken, Anetta Wronska, Mindy M. Kim, Brent W. Osborne, Alain Lacampagne, Yuxin Yin, and Andrew R. Marks. 2016. "Maintenance of Normal Blood Pressure Is Dependent on IP3R1-Mediated Regulation of eNOS." *Proceedings of the National Academy of Sciences* 113(30): 8532–37.
Yue, Zhichao, Jia Xie, Albert S. Yu, Jonathan Stock, Jianyang Du, and Lixia Yue. 2015. "Role of TRP Channels in the Cardiovascular System." *American Journal of Physiology-Heart and Circulatory Physiology* 308(3): H157–82.
Yuhki, K, H Kashiwagi, F Kojima, J Kawabe, and F Ushikubi. 2010. "Roles of Prostanoids in the Pathogenesis of Cardiovascular Diseases." *International angiology : a journal of the International Union of Angiology* 29(2 Suppl): 19–27.
Zacharia, Joseph, Chris Hillier, and Allan MacDonald. 2004. "$\alpha_1$ -Adrenoceptor Subtypes Involved in Vasoconstrictor Responses to Exogenous and Neurally Released Noradrenaline in Rat Femoral Resistance Arteries." *British Journal of Pharmacology* 141(6): 915–24.
Zacharia, Joseph, Jin Zhang, and W. Gil Wier. 2007. "Ca2+ Signaling in Mouse Mesenteric Small Arteries: Myogenic Tone and Adrenergic Vasoconstriction." *American journal of physiology. Heart and circulatory physiology* 292(3): H1523–32.
Zalk, Ran, Oliver B Clarke, Amédée des Georges, Robert A Grassucci, Steven Reiken, Filippo Mancia, Wayne A Hendrickson, Joachim Frank, and Andrew R Marks. 2015. "Structure of a Mammalian Ryanodine Receptor." *Nature* 517(7532): 44–49.
Zamponi, Gerald W, Joerg Striessnig, Alexandra Koschak, and Annette C Dolphin. 2015. "The Physiology, Pathology, and Pharmacology of Voltage-Gated Calcium Channels and Their Future Therapeutic Potential." *Pharmacological reviews* 67(4): 821–70.
Zani, Brett G, and H Glenn Bohlen. 2005. "Transport of Extracellular L-Arginine via Cationic Amino Acid Transporter Is Required during in Vivo Endothelial Nitric Oxide Production." *Am J Physiol Heart Circ Physiol* 289: H1381–90.
Zaritsky, Joshua J, Delrae M Eckman, George C Wellman, Mark T Nelson, and Thomas L Schwarz. 2000. "Targeted Disruption of Kir2.1 and Kir2.2 Genes Reveals the Essential Role of the Inwardly Rectifying K+ Current in K+-Mediated Vasodilation." *Circulation Research* 87(2): 160–66.
Zeng, Weizhong, Joseph P Yuan, Min Seuk Kim, Young Jin Choi, Guo N Huang, Paul F Worley, and Shmuel Muallem. 2008. "STIM1 Gates TRPC Channels, but Not Orai1, by Electrostatic Interaction." *Molecular cell* 32(3): 439–48.
Zhang, David X, Suelhem A Mendoza, Aaron H Bubolz, Atsuko Mizuno, Zhi Dong Ge, Rongshan Li, David C Warltier, Makoto Suzuki, and David D Gutterman. 2009. "Transient Receptor Potential Vanilloid Type 4-Deficient Mice Exhibit Impaired Endothelium-Dependent Relaxation Induced by Acetylcholine in Vitro and in Vivo." *Hypertension* 53(3): 532–38.
Zhang, Liyan, Jong Kook Lee, Scott A. John, Nobuyuki Uozumi, and Itsuo Kodama. 2004. "Mechanosensitivity of GIRK Channels Is Mediated by Protein Kinase C-Dependent Channel-Phosphatidylinositol 4,5-Bisphosphate Interaction." *Journal of Biological Chemistry* 279(8): 7037–47.
Zhang, Y, F Vogalis, and R K Goyal. 1998. "Nitric Oxide Suppresses a Ca2+-Stimulated Cl- Current in Smooth Muscle Cells of Opossum Esophagus." *Am J Physiol* 274(5 Pt 1): G886-

-90.
Zharikov, S I, H Herrera, and E R Block. 1997. “Role of Membrane Potential in Hypoxic Inhibition of L-Arginine Uptake by Lung Endothelial Cells.” *The American journal of physiology* 272(1 Pt 1): L78-84.
Zhou, Hong, Takeshi Nakamura, Nagisa Matsumoto, Chihiro Hisatsune, Akihiro Mizutani, Takafumi Iesaki, Hiroyuki Daida, and Katsuhiko Mikoshiba. 2008. “Predominant Role of Type 1 IP 3 Receptor in Aortic Vascular Muscle Contraction.”
Zhou, Jiaguo, David D Kim, and R Daniel Peluffo. 2010. “Nitric Oxide Can Acutely Modulate Its Biosynthesis through a Negative Feedback Mechanism on L -Arginine Transport in Cardiac Myocytes.” : 230–39.
Zimmermann, K., J. K. Lennerz, A. Hein, A. S. Link, J. S. Kaczmarek, M. Delling, S. Uysal, J. D. Pfeifer, A. Riccio, and D. E. Clapham. 2011. “Transient Receptor Potential Cation Channel, Subfamily C, Member 5 (TRPC5) Is a Cold-Transducer in the Peripheral Nervous System.” *Proceedings of the National Academy of Sciences* 108(44): 18114–19.
Zobel, Carsten, Hee Cheol Cho, The-Tin Nguyen, Roman Pekhletski, Roberto J Diaz, Gregory J Wilson, and Peter H Backx. 2003. “Molecular Dissection of the Inward Rectifier Potassium Current (IK1) in Rabbit Cardiomyocytes: Evidence for Heteromeric Co-Assembly of Kir2.1 and Kir2.2.” *The Journal of physiology* 550(Pt 2): 365–72.
Zurborg, Sandra, Brian Yurgionas, Julia A Jira, Ombretta Caspani, and Paul A Heppenstall. 2007. “Direct Activation of the Ion Channel TRPA1 by Ca2+.” *Nature Neuroscience* 10(3): 277–79.