Ministry of Higher Education and Scientific Research
University of Diyala College of Medicine

A Review Article in:

Vascular smooth muscle cells contraction and relaxation signalling in health and diseases

Submitted to the Council of the College of Medicine, Diyala University, In Partial Fulfillment of Requirements for the Bachelor Degree in medicine and general surgery.

Submitted by

Mustafa kamal khaleel

Supervised by

Dr. Omar Jassim Katwan

2021 **2022**

Abstract

Vascular smooth muscle cells (VSMCs) are the main cell type in the arterial wall and normally adopt a quiescent, contractile phenotype to regulate vascular tone. In the arterial wall, VSMCs are exposed to multiple mechanical cues, including stretch and matrix stiffness, which regulate VSMC contraction. However, during ageing and in vascular disease, such as , hypertension , which is considered A major risk factor for many common chronic diseases, such as heart failure, myocardial infarction, stroke, vascular dementia, and chronic kidney disease. Pathophysiological mechanisms contributing to the development of hypertension include increased vascular resistance, determined in large part by reduced vascular diameter due to increased vascular contraction and arterial remodeling. These processes are regulated by complexinteracting systems. VSMC are highly plastic and in pathological conditions undergo phenotypic changes from a contractile to a proliferative state. VSM contraction is triggered by an increase in intracellular free calcium concentration ($[Ca^{2+}]i$), promoting actin–myosin cross-bridge formation. Growing evidence indicates that contraction is also regulated by calcium-independent mechanisms involving RhoA-Rho kinase, protein Kinase C and mitogen-activated protein kinase (MRP kinase) signaling, reactive oxygen species, and reorganization of the actin cytoskeleton. Activation of immune/inflammatory pathways and noncoding RNAs are also emerging as important regulators of vascular function. Vascular smooth muscle cell [Ca2+]i not only determines the contractile state but also influences activity of many calcium-dependent transcription factors and proteins thereby impacting the cellular phenotype and function. In the present review, we discuss mechanisms regulating vascular reactivity and contraction in physiological and pathophysiological

conditions and VSMCs sense , relaxation signalling pathway and respond to changes in their mechanical environment.

Introduction

VSMCs are the main cellular components of the normal blood vessel walls, interweaving with elastic fiber layers to form the vascular media that provides structural integrity. VSMCs play an important role in the regulation of blood pressure and blood distribution to various tissues of the body through dynamic contraction and relaxation in response to vasoactive stimuli such as hormones, metabolites and neurotransmitters. Morphological and biochemical studies have revealed that two distinct phenotypes of VSMCs co-exist in the vessel wall, which are the differentiated contractile and the synthetic proliferative phenotypes. These two phenotypes of VSMCs are dictated by their environmental and functional requirements and also reflect differing patterns of gene expression [1,2,3]. The contractile VSMCs are characterized by specific contractile proteins, ion channels, and cellular surface receptors that regulate the contractile process. Synthetic VSMCs, also called secretory VSMCs, are characterized by significant proliferation and migration activity, such as the production of a large amount of extracellular matrix during development, in response to the physiological changes (such as long-term exercise and pregnancy) and pathological injury (such as under the conditions of inflammation, hypertension, diabetes) [4] . In hypertension is associated with vascular changes characterized by endothelial dysfunction, increased vascular contraction, and arterial remodelling.[30][31] Vascular smooth muscle cells, which constitute the bulk of the vascular wall, are critically involved in these processes through their highly plastic and dynamic features and ability to undergo phenotypic

differentiation.[30][31] Pro-hypertensive stimuli, such as activation of the renin-angiotensin-aldosterone system (RAAS), oxidative stress, activation of the sympathetic nervous system, haemodynamic changes, and mechanical forces stimulate vascular smooth muscle cell signalling, which promotes vasoconstriction, vascular hypertrophy, fibrosis, inflammation, and calcification, processes that underlie vascular functional, structural, and mechanical changes in hypertension.[32][33] In the present review, we discuss mechanisms regulating vascular reactivity and contraction in physiological and pathophysiological conditions, with basic information on hypertension. The role of vascular smooth muscle function in vascular remodeling, and outline relaxation signalling pathways mechanism in vascular smooth muscle.

Molecular mechansims of vascular smooth muscle cell dedifferentiation in hypertension

The plastic of Vascular smooth muscle cells are specialized cells that are highly plastic and multifunctional. Physiologically vascular smooth muscle cells are quiescent and exhibit low levels of growth. Normally, they express genes and proteins important for contraction/dilation, which allows them to control systemic and local pressure through the regulation of vascular tone.[34] In hypertension and other pathological conditions associated with vascular injury, the phenotypic switch contributes to vascular dysfunction and arterial remodeling. Molecular mechanisms underlying the cellular phenotypic switch in hypertension are complex and multifactorial. Vasoactive stimuli [Angiotensin II (Ang II), norepinephrine, and endothelin-1 (ET-1)], growth factors [Insulin-like growth factor 1 (IGF-1), epidermal growth factor (EGF), platelet-derived growth factor (PDGF)], mechanical forces (stretch), and physical factors (shear stress, pressure)

are important.[35],[36] These processes induce changes in expression and function of genes that control cell membrane receptors, growth signalling pathways, extracellular matrix components, transcription factors, ion channels, and transporters, important in vascular hypertrophy in hypertension.[36][37]

Calcium dependent mechanisms of Vascular smooth muscle cells contraction

The key event in vascular smooth muscle excitation-contraction coupling is an increase in Ca+2 in response to mechanical, humoral, or neural stimuli [5][6] and calcium signaling control the key functions of vascular smooth muscle cells and are finely tuned by plasma membrane calcium permeable channels exchangers, and transporters and by intracellular sources, including the sarcoplasmic reticulum, mitochondria, and calciumbinding proteins. The extracellular concentration of calcium is 2-4 mM with a basal [Cat]; of 90–110 nM. [5][7]. Contraction of smooth muscle is initiated by a Ca^{2+}-mediated change in the thick filaments, whereas in striated muscle Ca^{2+} mediates contraction by changes in the thin filaments. In response to specific stimuli in smooth muscle, the intracellular concentration of Ca^{2+} increases, and this activator Ca^{2+} combines with the acidic protein calmodulin. This complex activates myosin light chain kinase (MLC kinase) to phosphorylate the light chain of myosin Figure1. Cytosolic Ca^{2+} is increased through Ca^{2+} release from intracellular stores (sarcoplasmic reticulum) as well as entry from the extracellular space through Ca^{2+} channels (receptor-operated Ca^{2+} channels). Agonists (norepinephrine, angiotensin II, endothelin-1, etc.) binding to serpentine receptors, coupled to a heterotrimeric G protein, stimulate phospholipase C activity. This enzyme is specific for the membrane lipid phosphatidylinositol 4,5-bisphosphate to catalyze the formation of two potent second messengers: inositol trisphosphate (IP_3) and diacylglycerol

(DG). The binding of IP_3 to receptors on the sarcoplasmic reticulum results in the release of Ca^{2+} into the cytosol. DG, along with Ca^{2+}, activates protein kinase C (PKC), which phosphorylates specific target proteins. There are several isozymes of PKC in smooth muscle, and each has a tissue-specific role (e.g., vascular, uterine, intestinal, etc.). In many cases, PKC has contraction-promoting effects such as phosphorylation of L-type Ca^{2+} channels or other proteins that regulate cross-bridge cycling. Phorbol esters, a group of synthetic compounds known to activate PKC, mimic the action of DG and cause contraction of smooth muscle. Finally, L-type Ca^{2+} channels (voltage-operated Ca^{2+} channels) in the membrane also open in response to membrane depolarization brought on by stretch of the smooth muscle cell. In hypertension, these processes are altered leading to increased $[Ca^{2+}]_i$ and a hypercontractile state and vascular remodeling. Stimulation of vascular smooth muscle cells by prohypertensive factors such as neurohumoral stimuli (acetylcholine, norepinephrine) and vasoactive peptides (Ang II, ET-1), induces activation of receptors coupled to Phospholipase C (PLC), leading to generation of the second messengers inositol trisphosphate (IP_3) and diacylglycerol (DAG).[36][38][39] Circulating non-cellular factors such as cytokines, diffusible reactive oxygen species (ROS) (nitric oxide and hydrogen peroxide), and miRNAs and cellular-derived factors such as microparticles and endothelial progenitor cells also stimulate membrane receptors or cross the plasma membrane to regulate pathways that control $[Ca^{2+}]_i$.[40][41]

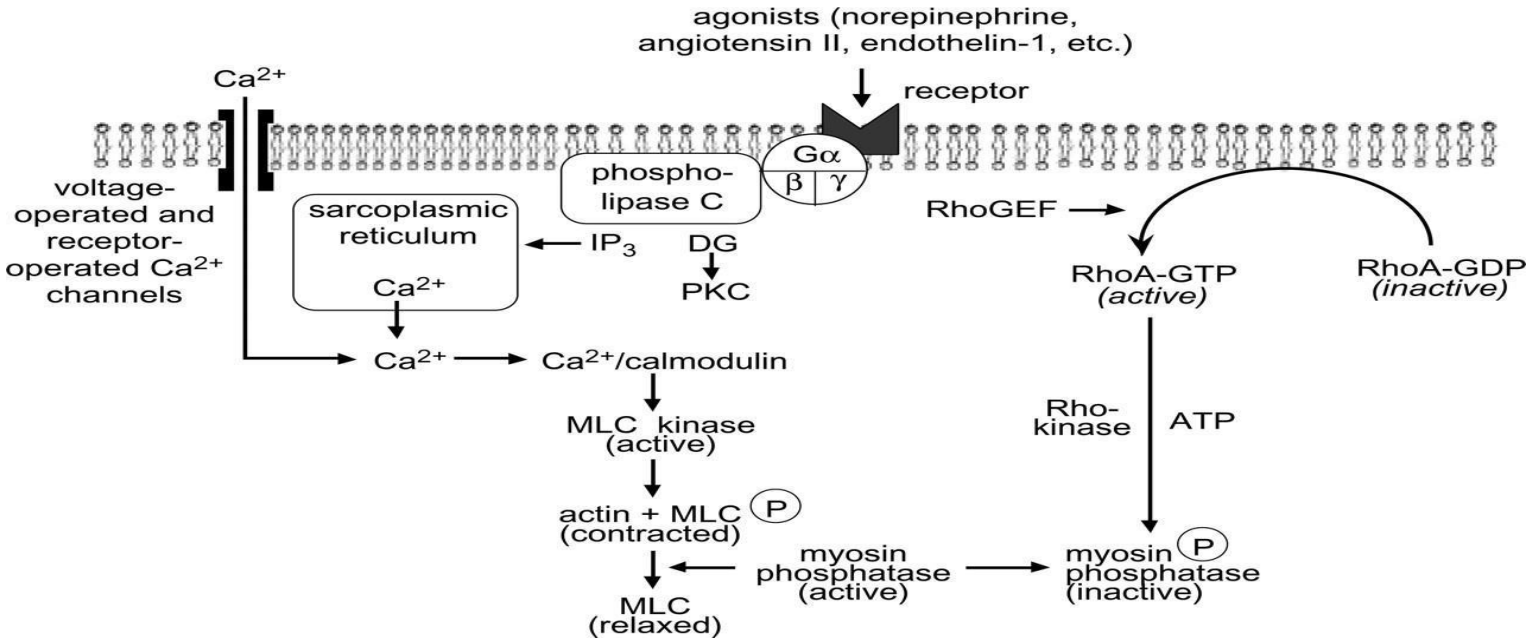

Figure 1 / Regulation of smooth muscle contraction. Various agonists (neurotransmitters, hormones, etc.) bind to specific receptors to activate contraction in smooth muscle. Subsequent to this binding, the prototypical response of the cell is to increase phospholipase C activity via coupling through a G protein. Phospholipase C produces two potent second messengers from the membrane lipid phosphatidylinositol 4,5-bisphosphate: diacylglycerol (DG) and inositol 1,4,5-trisphosphate (IP_3). IP_3 binds to specific receptors on the sarcoplasmic reticulum, causing release of activator calcium (Ca^{2+}). DG along with Ca^{2+} activates PKC, which phosphorylates specific target proteins. In most smooth muscles, PKC has contraction-promoting effects such as phosphorylation of Ca^{2+} channels or other proteins that regulate cross-bridge cycling. Activator Ca^{2+} binds to calmodulin, leading to activation of myosin light chain kinase (MLC kinase). This kinase phosphorylates the light chain of myosin, and, in conjunction with actin, cross-bridge cycling occurs, initiating shortening of the smooth muscle cell. However, the elevation in Ca^{2+} concentration within the cell is transient, and the contractile response is maintained by a Ca^{2+}-sensitizing mechanism brought about by the inhibition of myosin phosphatase activity by Rho kinase.

This Ca^{2+}-sensitizing mechanism is initiated at the same time that phospholipase C is activated, and it involves the activation of the small GTP-binding protein RhoA. The precise nature of the activation of RhoA by the G protein-coupled receptor is not entirely clear but involves a guanine nucleotide exchange factor (RhoGEF) and migration of RhoA to the plasma membrane. Upon activation, RhoA increases Rho kinase activity, leading to inhibition of myosin phosphatase. This promotes the contractile state, since the light chain of myosin cannot be dephosphorylated. This figure was obtained from [43].

Calcium independent mechanisms in vascular smooth muscle contraction

In the absence of external contractile stimuli, the MLC-20 light chain remains phosphorylated at a low level. This low level leads to a slower tonic form of contraction, which regulates the vascular tone [23] . A calciumindependent pathway that involves Rho/Rho-associated protein kinase (ROCK) signalling regulates VSMC tonic Contractions Figure 2. This pathway not only caters to contractile function, but also extends to smooth muscle cell migration, proliferation and apoptosis [24]. RhoA, part of the Ras superfamily, is a GTPase which can act as a molecular switch between a GTP/GDP bound state [25]. In resting conditions, the Rho GDP dissociation inhibitor targets GDP-Rho for binding, as a means to localise the GTPase from the membrane to the cytosol. However, activation of Gprotein coupled receptor GPCR receptors, in particular Ga12/13 subtypes, can catalyse GTP for GDP exchange in RhoA by binding to p115 RhoGTPase guanine nucleotide exchange factors [26]. In its GTP bound form, RhoA can interact with target proteins by utilising its Cterminal geranyl-geranylated tail to anchor itself to the plasmab membrane [26]. One of the target proteins activated by RhoA is ROCK[27]. ROCK is a member of the protein kinase A, G and C family of protein kinases, and is characterised as a serine/threonine kinase. There are two isoforms of this kinase, referred to as ROCK1 and ROCK2, with expression of both present in VSMCs [28]. Its structure is composed of an N-terminal kinase domain, a central coiled-coil domain and a C-terminal pleckstrin homology domain that associates with the Rho GTPase [26]. ROCK has many effects within VSMCs and influences actomyosin activity by two main pathways. Firstly, ROCK actively regulates cytoskeletal organisation by preventing actin filament depolymerisation [27] Secondly,

ROCK inhibits myosin light chain phosphatase (MLCP). MLCP has a structure that is composed of three subunits; a 37 kDa catalytic subunit, a variable subunit and a myosin-binding subunit [23]. The myosin-binding site is crucial for its regulation and is subject to phosphorylation, specifically at residues. Threonine-695/697 (major site), serine-849/854 and threonine850/855[26][29]. Phosphorylation prevents MLCP from regulating the MLC phosphorylation state and increases the basal phosphorylated MLC level, stimulating VSMC contraction and augmenting vascular tone.[29]

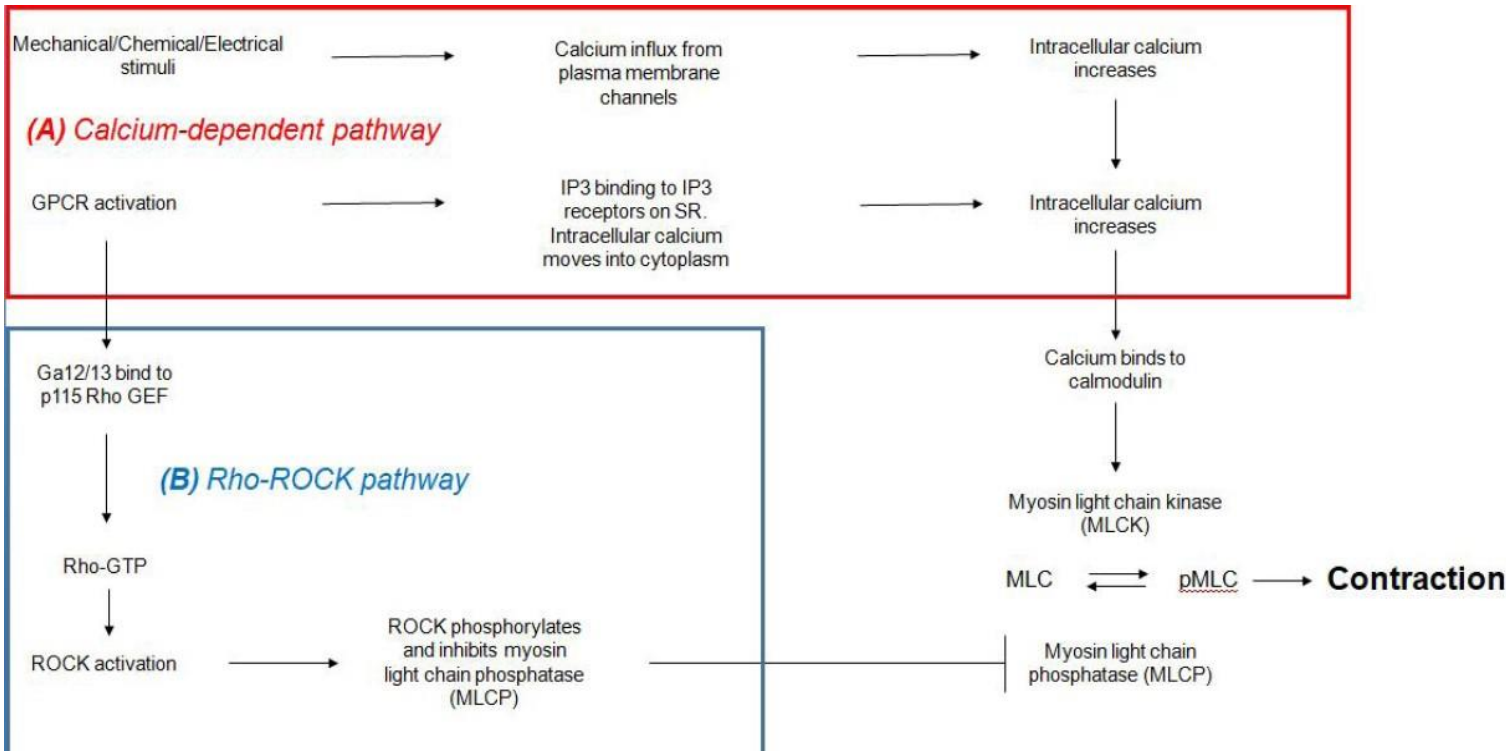

Figure 2 / Calcium dependent and independent regulation of VSMC contraction. The two pathways work synergistically. Calcium dependent regulation is associated with transient phasic contraction whereas RhoA/ROCK regulation is associated with the prolonged tonic contraction of VSMCs. GPCR: G-protein coupled receptor; IP3: inositol triphosphate; SR: Sarcoplasmic reticulum; Rho GEF: RhoGTPase guanine nucleotide exchange factors; ROCK: Rho-associated protein kinase; MLC: myosin light chain; VSMC: vascular smooth muscle cell. This figure was obtained from [44].

CALCIUM SENSITIZATION AND DESENSITIZATION

Recognition that calcium is the intracellular messenger that triggers muscle contraction [7] by binding, in striated muscles, to the Ca-binding protein troponin [8] eventually led to the realization that Ca-regulated myosin II plays major roles not only in striated, but also in smooth muscle and in non-muscle cells. Identification of other Ca-binding proteins, such as the ubiquitous calmodulin, and their effectors revealed complex, interconnected cellular signaling mechanisms, critically regulated by protein kinases [9] and phosphatases [10]. In the case of smooth muscle and non-muscle myosin II, their ATPase activity and associated motility contraction are activated by actin, but only when Ser-19 of the myosin regulatory light chain (RLC) is phosphorylated, usually by a calciumcalmodulin (Ca^{2+}/CaM)-dependent myosin light-chain kinase (MLCK) [11][12]; see Figure 3 This Ca-dependent activation of myosin II plays an essential role in a variety of processes, but regulation of cellular functions by changes in cytoplasmic Ca^{2+} concentration. Ca^{2+} is further modulated by the Ca^{2+} sensitivity of the Ca^{2+} sensors and effectors that can also be modified by factors such as the affinity (K_{CaM}) of calmodulin for MLCK and the activity of the G protein-regulated myosin phosphatase. Studies with Ca^{2+}-sensitive fluorophores suggested that, as expected [13], force developed at a given global level of $[Ca^{2+}]_i$ could vary, depending on the type of excitatory stimulus: agonist-induced force is often higher than depolarization (high K)-induced force at similar, or even lower, $[Ca^{2+}]_i$ [14][15] . Studies employing cell permeabilization methods that retained G protein-coupled receptors confirmed that the underlying mechanism is agonist-induced Ca^{2+} sensitization of the contractile/regulatory apparatus, not an artifact of the Ca^{2+} reporters. When such permeabilized muscles are activated by an agonist or guanosine 5′-*O*-(3-thiotriphosphate)

(GTPγS) while $[Ca^{2+}]_i$ is clamped, they respond with increased RLC phosphorylation and force [16]. The term *Ca^{2+}sensitization* was coined to describe this phenomenon. Interestingly, different agonists can stimulate unequal maximal Ca^{2+} sensitization [17] through yet to be identified mechanism(s), perhaps qualitatively or quantitatively variable coupling between different agonists and trimeric and monomeric (RhoA) G proteins and guanine nucleotide exchange factors (GEFs) figure 4. Ca^{2+} desensitization was also first recognized in similar experiments that showed a decline in force and RLC phosphorylation while $[Ca^{2+}]_i$ was unchanged during K^+ (depolarization)-induced contractions of intact smooth muscles [18] and was confirmed by the phasic decline in RLC phosphorylation and force in permeabilized phasic smooth muscles maintained at constant $[Ca^{2+}]_i$ [19] .Ca^{2+} sensitization and desensitization are now understood to involve the major physiological mechanisms that regulate myosin II activity: phosphorylation and dephosphorylation.

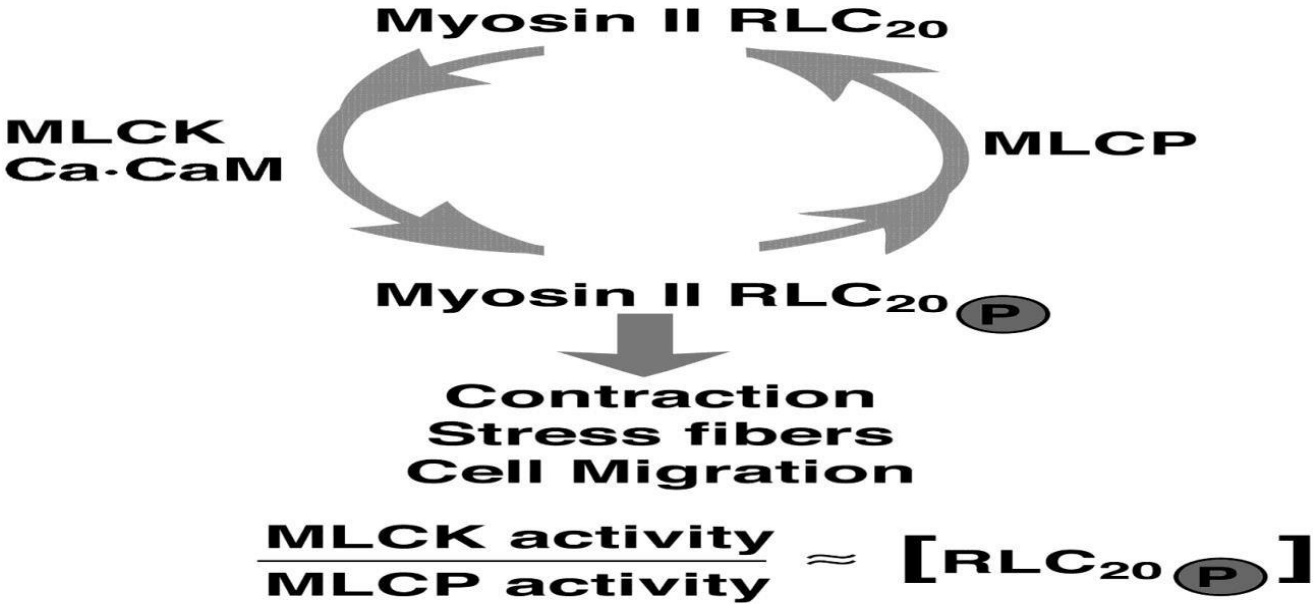

Figure 3 /Regulation of contraction, stress fiber formation, and cell migration through phosphorylation/dephosphorylation of the regulatory light chain (RLC_{20}) of myosin II. Activation of myosin light-chain kinase (MLCK) by Ca^{2+} binding to calmodulin (CaM) leads to phosphorylation of the RLCs of myosin II to switch on cross-bridge cycling and force development by actin-activated myosin. The ratio of kinase to phosphatase activities determines the level of RLC phosphorylation and the extent of activation. [Ca^{2+}]-independent modulation of the activities of MLCK and/or myosin light-chain phosphatase (MLCP) provides additional mechanisms for regulation of RLC_{20} phosphorylation. This figure was obtained from [45].

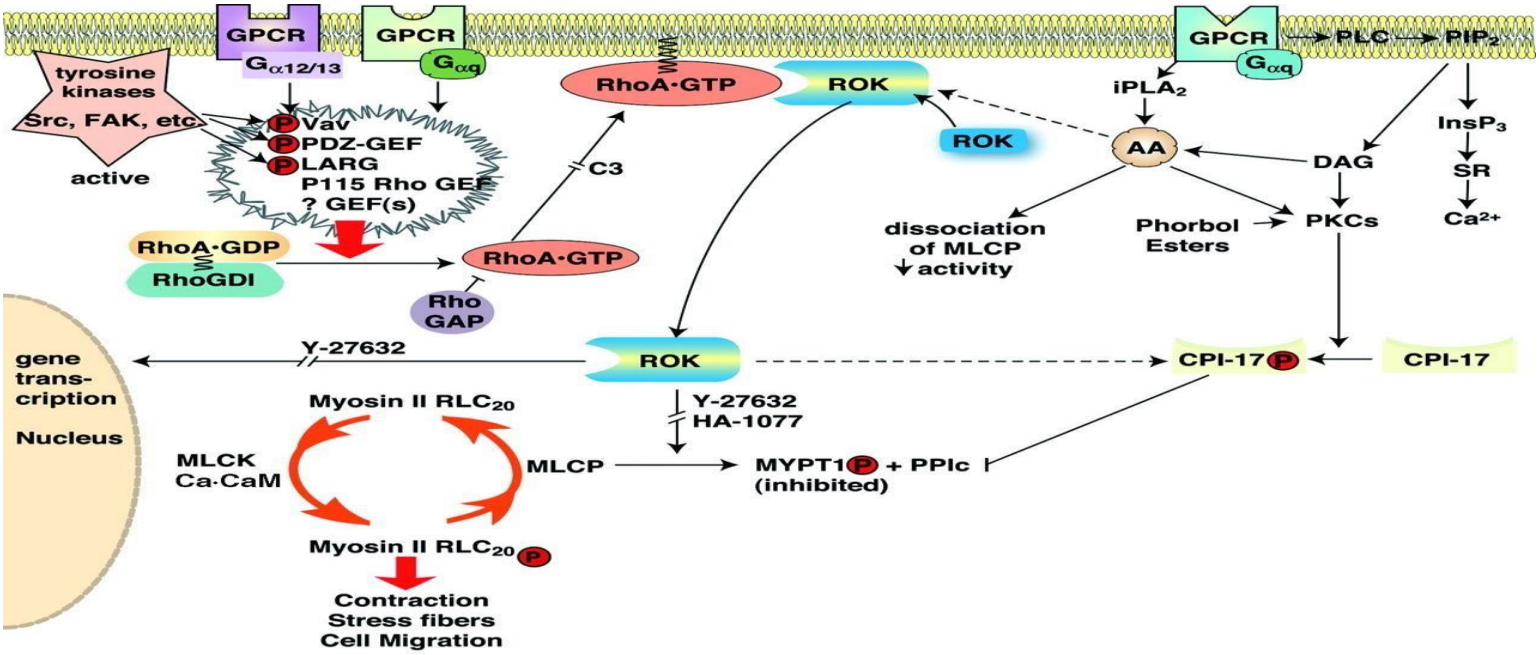

Figure 4 / Signaling pathways for Ca^{2+} sensitization in smooth muscle. Different smooth muscles respond to a large number of different agonists including catecholamines, muscarinic agonists, thromboxane, histamine, serotonin, as well as the sphingolipids: sphingosine 1-phosphate and sphingosylphosphorylcholine. Activation of their receptors initiates signaling through the illustrated cascades that inhibit myosin phosphatase (MLCP), increase RLC_{20} phosphorylation and contraction, stress fiber formation, and/or cell migration. The Rho/ROK pathway can also lead to activation of smooth muscle differentiation marker gene expression. GPCR, G protein-coupled receptors; GEF, guanine nucleotide exchange factor(s); RhoGDI, GDP dissociation inhibitor; RhoGAP, GTPase activating protein; ROK, Rho-kinase, ROKα/ROCK II, ROKβ/ROCK I; C3, clostridial C3 exoenzyme ADP ribosylates and inactivates RhoA; $iPLA_2$, Ca^{2+}-independent phospholipase A_2; CPI-17, PKC-potentiated inhibitor protein of 17 kDa; MYPT1 and PP1c, myosin phosphatase regulator and catalytic subunits; MLCK, myosin light-chain kinase; DAG, diacylglycerol; PKC, protein kinase C; AA, archidonic acid; SR, sarcoplasmic reticulum; $InsP_3$, inositol 1,4,5-trisphosphate; PLC, phospholipase C; PIP_2, phosphatidylinositol 4,5bisphosphate. This figure was obtained from [45].

Vascular smooth muscle cells actin cytoskeleton reorganization

The role of filamention actin in the activation of myosin ATPase activity and cross-bridge cycling is well established, and actomyosin cross-bridge cycling is recognized as the fundamental mechanism for tension development and shortening in all forms of muscle, as well as in contractile non-muscle cells. The activation of myosin by a contractile stimulus enables myosin filaments to crawl along actin filaments through the ATPase activity of the myosin head, thus resulting in shortening or tension generation by the cell. This well-established paradigm for smooth muscle contraction has relied on the assumption that the structure and organization of filamentous actin remains relatively constant during a contractile event, and that actin filaments anchored at adhesion sites at the plasma membrane and at dense bodies within the cytosol provide a fixed and stable network on which the myosin or thick filaments move during shortening and tension development. Recent studies have documented a critical role for actin polymerization and cytoskeletal dynamics in the regulation of active tension development in smooth muscle. There is mounting evidence that smooth muscle contraction requires the polymerization of actin filaments and a range of other cytoskeletal processes that extend well beyond the actomyosin interaction and cross-bridge cycling. A complex set of cytoskeletal events is triggered concurrently with activation of the actomyosin system that appear to play a fundamental role in the mechanical response of the muscle tissue. This has prompted the formulation of new paradigms for smooth muscle contraction to encompass observations that the activation of the actomyosin system is not the only cellular mechanism involved in the

regulation of smooth muscle contraction and tension development. These dynamic cytoskeletal processes may underlie the unique adaptive properties of many smooth muscle tissues that enable them to modulate their contractile and mechanical properties to accommodate to changes in their surrounding environment. Growing evidence suggests that the cytoskeletal processes that occur during the contractile. activation of smooth muscle cells may have much in common with the cytoskeletal mechanisms that govern cell motility and migration, and that tension generation in smooth muscle requires a more complex array of physiological processes than previously supposed.

THE CONTRACTILE MECHANISM

activation of smooth muscle cells may have much in common with the cytoskeletal mechanisms that govern cell motility and migration, and that tension generation in smooth muscle requires a more complex array of physiological processes than previously supposed. In the intact body, the process of smooth muscle cell contraction is regulated principally by receptor and mechanical (stretch) activation of the contractile proteins myosin and actin. A change in membrane potential, brought on by the firing of action potentials or by activation of stretchdependent ion channels in the plasma membrane, can also trigger contraction. For contraction to occur, myosin light chain kinase (MLC kinase) must phosphorylate the 20-kDa light chain of myosin, enabling the molecular interaction of myosin with actin. Energy released from ATP by myosin ATPase activity results in the cycling of the myosin cross-bridges with actin for contraction. Thus contractile activity in smooth muscle is determined primarily by the phosphorylation state of the light chain of myosin—a highly regulated process. In some smooth muscle cells, the phosphorylation of the light

chain of myosin is maintained at a low level in the absence of external stimuli (i.e., no receptor or mechanical activation). This activity results in what is known as smooth muscle tone and its intensity can be varied Figure 5.

SMOOTH MUSCLE RELAXATION

Smooth muscle relaxation occurs either as a result of removal of the contractile stimulus or by the direct action of a substance that stimulates inhibition of the contractile mechanism (e.g., atrial natriuretic factor is a vasodilator). Regardless, the process of relaxation requires a decreased intracellular Ca^{2+} concentration and increased MLC phosphatase activity Figure 5 [20][21]. The mechanisms that sequester or remove intracellular Ca^{2+} and/or increase MLC phosphatase activity may become altered, contributing to abnormal smooth muscle responsiveness. A decrease in the intracellular concentration of activator Ca^{2+} elicits smooth muscle cell relaxation. Several mechanisms are implicated in the removal of cytosolic Ca^{2+} and involve the sarcoplasmic reticulum and the plasma membrane. Ca^{2+} uptake into the sarcoplasmic reticulum is dependent on ATP hydrolysis. This sarcoplasmic reticular Ca,Mg-ATPase, when phosphorylated, binds two Ca^{2+} ions, which are then translocated to the luminal side of the sarcoplasmic reticulum and released. Mg^{2+} is necessary for the activity of the enzyme; it binds to the catalytic site of the ATPase to mediate the reaction. The sarcoplasmic reticular Ca, MgATPase is inhibited by several different pharmacological agents: vanadate, thapsigargin, and cyclopiazonic acid. Sarcoplasmic reticular Ca^{2+}-binding proteins also contribute to decreased intracellular Ca^{2+} levels. Recent studies have identified calsequestrin and calreticulin as sarcoplasmic reticular Ca^{2+}-binding proteins in smooth muscle. The

plasma membrane also contains Ca, Mg-ATPases, providing an additional mechanism for reducing the concentration of activator Ca^{2+} in the cell. This enzyme differs from the sarcoplasmic reticular protein in that it has an autoinhibitory domain that can be bound by calmodulin, causing stimulation of the plasma membrane Ca^{2+} pump. Na^{+}/Ca^{2+} exchangers are also located on the plasma membrane and aid in decreasing intracellular Ca^{2+}. This low-affinity antiporter is closely coupled to intracellular Ca^{2+} levels and can be inhibited by amiloride and quinidine.Receptor-operated and voltage-operated Ca^{2+} channels located in the plasma membrane are important in Ca^{2+} influx and smooth muscle contraction, as previously mentioned. Inhibition of these channels can elicit relaxation. Channel antagonists such as dihydropyridine, phenylalkylamines, and benzothiazepines bind to distinct receptors on the channel protein and inhibit Ca^{2+} entry in smooth muscle Figure 5 .

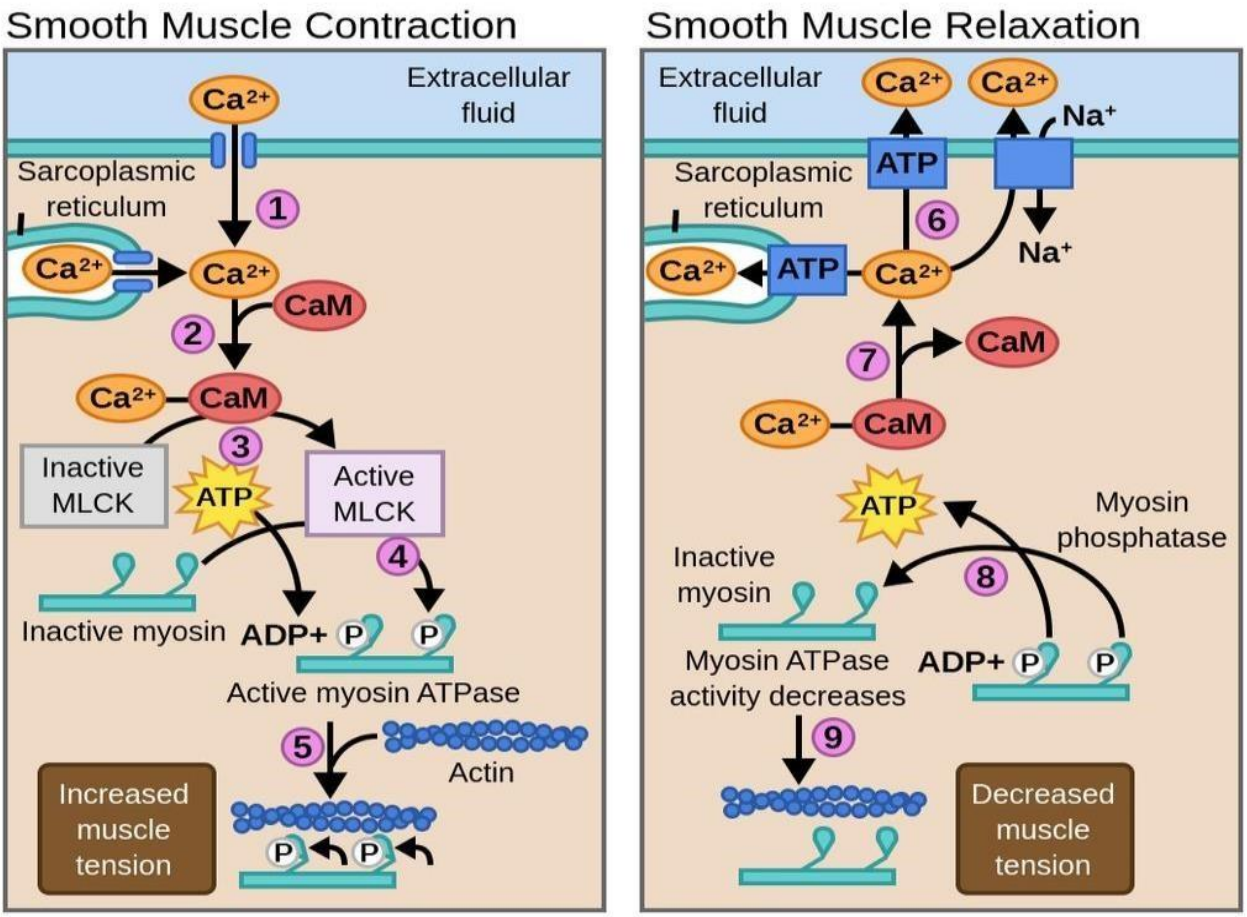

***Figure5*/ Smooth muscle contraction/relaxation mechanism. Smooth muscle contraction (left) requires five steps to perform: After the increase of intracellular Ca^{2+}concentrations from either the extracellular fluid or the sarcoplasmic reticulum (1), these ions bind to a protein called calmodulin (2). This complex activates a protein called myosin light-chain kinase (3), which subsequently phosphorylates light chains of myosin heads, increasing the myosin ATPase activity (4). Finally, active myosin cross-bridges slide along actin and create muscle tension to contract the cell. Once the contraction finishes, some events occur to relax the cell (steps 6 to 9 on the right).This figure was obtained from [46].**

Conclusions

Vascular smooth muscle cells are highly differentiated and normally maintain a contractile phenotype. Vascular contraction/relaxation is regulated by many processes that are both calcium-dependent and independent and involve calcium channels and signalling pathways such as IP_3-PKC-DAG and ROCK. In hypertension, these processes are dysregulated, and signalling pathways not typically associated with contraction, such as MAPKs, tyrosine kinases, and transcription factors are activated. These phenomena lead to a hypercontractile state and dedifferentiation of vascular smooth muscle cells to a proliferative/migratory phenotype with consequent vascular remodeling. Emerging evidence also implicates a role for the immune/inflammatory system and the non-coding genome in vascular dysfunction in hypertension. Unravelling the complex interactions between traditional pro-contractile calcium-regulated signaling pathways and non-traditional contractile mechanisms will provide better insights into processes underlying the vascular phenotype in hypertension.

Reference

1. Metz RP, Patterson JL, Wilson E. Vascular smooth muscle cells: Isolation, culture, and characterization. Methods in Molecular Biology. 2012;843:169-176

2. Shanahan CM, Weissberg PL. Smooth musc cell heterogeneity: Patterns of gene expression in vascular smooth muscle cells in vitro and in vivo. Arteriosclerosis, Thrombosis, and Vascular Biology. 1998;18:333-338

3. Weissberg PL, Cary NR, Shanahan CM. Gene expression and vascular smooth muscle cell phenotype. Blood Pressure. Supplement. 1995;2:68-73

4. Lv P, Zhang F, Yin YJ, Wang YC, Gao M, Xie XL, Zhao LL, Dong LH, Lin YL, Shu YN, Zhang DD, Liu GX, Han M. SM22alpha inhibits lamellipodium formation and migration via Ras-Arp 2/3 signaling in synthetic VSMCs. American Journal of Physiology. Cell Physiology. 2016;311:C758-C767

5. Allen BG, Walsh M.. The biochemical basis of the regulation of smooth-muscle contraction. Trends Biochem Sci 1994; 19:362– 368.

6. Ikebe M. Regulation of the function of mammalian myosin and its conformational change. Biochem Biophys Res Commun 2008; 369:157–164.

7. Walsh M. Vascular smooth muscle myosin light chain diphosphorylation: mechanism, function, and pathological implications. IUBMB Life 2011; 63:987–1000.
8. Heilbrunn LV and Wiercinski FJ. The action of various cations on muscle protoplasm. J Cell Comp Physiol 29: 15-32, 1947.
9. Ebashi S, Ebashi F, and Kodama A. Troponin as the C++ receptive protein in the contractile system. J Biochem 62: 137-138, 1967

10. Cohen P. The origins of protein phosphorylation. Nat Cell Biol 4: E127-E130, 2002.
11. Cohen PT. Protein phosphatase 1—targeted in many directions. J Cell Sci 115: 241-256, 2002.

12. Bustelo XR. Regulatory and signaling properties of the Vav family. Mol Cell Biol 20: 1461-1477, 2000.
13. Somlyo AP and Somlyo AV. Signal transduction and regulation in smooth muscle. Nature 372: 231-236, 1994.

14. Uehata M, Ishizaki T, Satoh H, Ono T, Kawahara T, Morishita T, Tamakawa H, Yamagami K, Inui J, Maekawa M, and Narumiya S. Calcium sensitization of smooth muscle mediated by a Rho-associated protein kinase in hypertension. Nature 389: 990-994, 1997.

15. Bradley AB and Morgan KG. Alterations in cytoplasmic calcium sensitivity during porcine coronary artery contractions as detected by aequorin. J Physiol 385: 437-448, 1987.
16. Himpens B and Casteels R. Different effects of depolarization and muscarinic stimulation on the Ca2+/force relationship

during the contraction-relaxation cycle in the guinea pig ileum. Pflügers Arch 416: 28-35, 1990.

17. Kitazawa T, Kobayashi S, Horiuti K, Somlyo AV, and Somlyo AP. Receptor-coupled, permeabilized smooth muscle. Role of the phosphatidylinositol cascade, G-proteins, and modulation of the contractile response to Ca2+. J Biol Chem 264: 5339-5342, 1989.
18. Himpens B, Kitazawa T, and Somlyo AP. Agonist-dependent modulation of Ca2+ sensitivity in rabbit pulmonary artery smooth muscle. Pflügers Arch 417: 21-28, 1990.
19. Himpens B, Matthijs G, and Somlyo AP. Desensitization to cytoplasmic Ca2+ and Ca2+ sensitivities of guinea-pig ileum and rabbit pulmonary artery smooth muscle. J Physiol 413: 489-503, 1989.
20. Kitazawa T and Somlyo AP. Desensitization and muscarinic resensitization of force and myosin light chain phosphorylation to cytoplasmic Ca2+ in smooth muscle. Biochem Biophys Res Commun 172: 1291-1297, 1990.
21. Morgan K. The role of calcium in the control of vascular tone as assessed by the Ca++ indicator Aequorin. Cardiovasc Drugs Ther 4: 1355–1362, 1990.
22. Somlyo AP, Wu X, Lalker LA, and Somlyo AV. Pharmacomechanical coupling: the role of calcium, Gproteins, kinases and phosphatases. Rev Physiol Biochem Pharmacol 134: 201–234, 1999.
23. Webb RC. Smooth muscle contraction and relaxation. Adv Physiol Educ 2003;27:201-6.

24. Schwartz MA. Integrins and extracellular matrix in mechanotransduction. Cold Spring Harb Perspect Biol 2010;2:a005066.
25. Bar-Sagi D, Hall A. Ras and Rho GTPases: a family reunion. Cell 2000;103:227.
26. Fukata Y, Amano M, Kaibuchi K. Rho-Rho-kinase pathway in smooth muscle contraction and cytoskeletal reorganization of non-muscle cells. Trends Pharmacol Sci 2001;22:32-9.
27. Amano M, Nakayama M, Kaibuchi K. Rho-kinase/ROCK: a key regulator of the cytoskeleton and cell polarity. Cytoskeleton (Hoboken) 2010;67:545-54.
28. Liao JK, Seto M, Noma K. Rho kinase (ROCK) inhibitors. J Cardiovasc Pharmacol 2007;50:17-24.
29. Murányi A, Derkach D, Erdodi F, Kiss A, Ito M, et al. Phosphorylation of Thr695 and Thr850 on the myosin phosphatase target subunit: inhibitory effects and occurrence in A7r5 cells. FEBS Lett 2005;579:6611-5.
30. Savoia C , Burger D, Nishigaki N, Montezano A, Touyz RM. Angiotensin II and the vascular phenotype in hypertension. Expert Rev Mol Med2011; 13:11–20.
31. Montezano AC , Tsiropoulou S, Dulak-Lis M, Harvey A, Camargo LDL, Touyz RM. Redox signaling, Nox5 and vascular remodeling in hypertension. Curr Opin Nephrol Hypertens2015; 24:425–433.
32. Touyz RM , Schiffrin EL. Signal transduction mechanisms mediating the physiological and pathophysiological actions of angiotensin II in vascular smooth muscle cells. Pharmacol Rev2000; 52:639–672.

33. Endemann DH , Schiffrin EL. Endothelial dysfunction. J Am Soc Nephrol2004; 15:1983–1992.
34. Coll-Bonfill N , de la Cruz-Thea B, Pisano MV, Musri MM. xNoncoding RNAs in smooth muscle cell homeostasis: implications in phenotypic switch and vascular disorders. Pflugers Arch2016; 468:1071–1087.
35. Kennedy AJ , Yang P, Read C, Kuc RE, Yang L, Taylor EJ, Taylor CW, Maguire JJ, Davenport AP. Chemerin elicits potent constrictor actions via chemokine-like receptor 1 (CMKLR1), not G-protein-coupled receptor 1 (GPR1), in human and rat vasculature. J Am Heart Assoc2016; 5:pii: e004421.
36. Wynne BM , Chiao CW, Webb RC. Vascular smooth muscle cell signaling mechanisms for contraction to angiotensin II and endothelin-1. J Am Soc Hyperten2009; 3:84–95.
37. Montezano AC , Zimmerman D, Yusuf H, Burger D, Chignalia AZ, Wadhera V, van Leeuwen FN, Touyz RM. Vascular smooth muscle cell differentiation to an osteogenic phenotype involves 38. TRPM7 modulation by magnesium. Hypertension2010; 56:453–462.
39. Allen BG , Walsh M. The biochemical basis of the regulation of smooth-muscle contraction. Trends Biochem Sci1994; 19:362–368.
40. Walsh M. Vascular smooth muscle myosin light chain diphosphorylation: mechanism, function, and pathological implications. IUBMB Life2011; 63:987–1000.
41. Hill MA , Meininger GA. Small artery mechanobiology: roles of cellular and non-cellular elements. Microcirculation2016; 23:611–613.

42. Burger D , Turner M, Munkonda MN, Touyz RM. Endothelial microparticle-derived reactive oxygen species: role in endothelial signaling and vascular function. Oxid Med Cell Longev2016;2016:1.

43. SMOOTH MUSCLE CONTRACTION AND RELAXATION by R. Clinton Webb
44. Vascular smooth muscle cell contractile function and mechanotransduction by Sultan Ahmed , Derek T. Warren.
45. Andrew p. Somlyo and Avril v. Somly
46. Labster Theory Pages