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Cellular and molecular mechanisms regulating vascular tone. Part 2: regulatory mechanisms modulating Ca^{2+} mobilization and/or myofilament Ca^{2+} sensitivity in vascular smooth muscle cells

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Abstract
Understanding the physiological mechanisms regulating vascular tone would lead to better circulatory management during general anesthesia. This two-part review provides an overview of current knowledge about the cellular and molecular mechanisms regulating the contractile state of vascular smooth muscle cells (i.e., vascular tone). The first part reviews basic mechanisms controlling the cytosolic Ca^{2+} concentration in vascular smooth muscle cells, and the Ca^{2+}-dependent regulation of vascular tone. This second part reviews the regulatory mechanisms modulating Ca^{2+} mobilization and/or myofilament Ca^{2+} sensitivity in vascular smooth muscle cells—including Rho/Rho kinase, protein kinase C, arachidonic acid, Ca^{2+}/calmodulin-dependent protein kinase II, caldesmon, calponin, mitogen-activated protein kinases, tyrosine kinases, cyclic nucleotides, Cl^- channels, and K^+ channels.

Key words Vascular smooth muscle · Vascular endothelium · Intracellular Ca^{2+} concentration · Myofilament Ca^{2+} sensitivity

Introduction

Most general anesthetics threaten the functional integrity of a wide variety of cellular and molecular mechanisms that regulate the contractile state of vascular smooth muscle cells (VSMCs; i.e., vascular tone; see review [1] for details of anesthetic vascular pharmacology). In addition, in current anesthetic management, various vasoactive agents are often used to control vascular tone or reactivity, thereby maintaining hemodynamic stability and organ blood flow. Understanding the physiological mechanisms regulating vascular tone would, thus, be beneficial for anesthesiologists. This two-part review provides an overview of current knowledge about the cellular and molecular mechanisms regulating vascular tone—i.e., targets for general anesthetics, as well as for vasoactive drugs that are used in intraoperative circulatory management. The first part of the review concerns the basic mechanisms controlling cytosolic Ca^{2+} concentration ($[Ca^{2+}]c$), and the Ca^{2+}-dependent regulation of vascular tone [2]. This second part of the review covers the regulatory mechanisms modulating Ca^{2+} mobilization and/or the sensitivity of contractile myofilaments to Ca^{2+} (i.e., myofilament Ca^{2+} sensitivity) in VSMCs, these mechanism being possibly involved in the regulation of vascular tone under physiological conditions.

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Regulatory mechanisms controlling the myofilament calcium sensitivity

Following increases in $[Ca^{2+}]c$ in response to vasoconstrictor stimuli, cytosolic Ca^{2+} ions bind to calmodulin (CaM), forming a complex containing four Ca^{2+} ions for each CaM molecule [2]. This Ca^{2+}-CaM complex binds to an inactive myosin light-chain kinase (MLCK) to form an active holoenzymatic complex that catalyzes the transfer of a phosphate group from adenosine triphosphate (ATP)-Mg^{2+} to a specific serine residue at position 19 (Ser^{19}) of 20-kD myosin regulatory light chain (MLC_{20}). This MLC_{20} phosphorylation permits the activation of the myosin adenosine triphosphatase (ATPase) by actin, leading to increased ATP hydrolysis and, thus, to an increase in the crossbridge cycling rate and, consequently, to the development of force [3].

The Ca^{2+}-dependent activation of MLCK and its phosphorylation of MLC_{20} is generally considered the primary mechanism responsible for the initial development of contractile force in VSMCs [4]. However, the relation between $[Ca^{2+}]c$ and MLC_{20} phosphorylation can be variable during the subsequent development and maintenance of contractile force, indicating that the

Ca^{2+} sensitivity of MLC_{20} phosphorylation can be secondarily modulated by other signal transduction pathways [5]. During stimulation with receptor agonists, as detailed below, the Ca^{2+} sensitivity of MLC_{20} phosphorylation is increased, presumably by the inhibition of myosin phosphatase, which dephosphorylates Ser^{19} of MLC_{20}. On the other hand, there may exist some mechanisms downregulating the Ca^{2+} signal by inhibiting MLCK activity and thereby decreasing the Ca^{2+} sensitivity of MLC_{20} phosphorylation [6–8].

Mechanisms to increase myofilament Ca^{2+} sensitivity during stimulation with receptor agonists

The force/$[Ca^{2+}]$c ratio during receptor stimulation is much higher than that during membrane depolarization in VSMCs [9–11]. In addition, receptor agonists enhance the contractile response to fixed concentrations of Ca^{2+} in membrane-permeabilized VSMCs [12,13]. Thus, the Ca^{2+} sensitivity of contractile proteins is increased during stimulation with receptor agonists.

Receptor stimulation by Ca^{2+}-sensitizing agonists activates the plasma membrane-associated heterotrimeric (αβγ) guanosine-5′-triphosphate (GTP)-binding protein (G-protein) by catalyzing guanosine-5′-diphosphate (GDP)/GTP exchange on its α-subunit (Fig. 1). In membrane-permeabilized VSMCs, the agonist-induced increase in myofilament Ca^{2+} sensitivity requires the presence of GTP; is mimicked by guanosine-5′-(3-*O*-thio) triphosphate (GTPγS), a nonhydrolyzable GTP analogue that produces persistent activation of G-proteins by locking them in the active GTP-bound form; and is inhibited by guanosine-5′-(2-*O*-thio) diphosphate (GDPβS), a nonhydrolyzable GDP analogue that competitively inhibits the interaction of GTP with G-proteins (i.e., activation of G-proteins) [12–14]. Thus, receptor agonist-induced Ca^{2+} sensitization is, presumably, mediated through the activation of G-proteins.

Besides heterotrimeric G-protein, there exist a large number of small-molecular-weight (20–25 kDa) monomeric proteins that bind and hydrolyze GTP (e.g., Rho or Ras proteins). Recent studies have proposed that the activation of such small monomeric G-proteins (or small GTPases) increases myofilament Ca^{2+} sensitivity or MLC_{20} phosphorylation in VSMCs, and is possibly involved in the receptor agonist-induced Ca^{2+} sensitization [14–17].

It has been shown that the increases in force at constant $[Ca^{2+}]$c levels during receptor stimulation result from parallel changes in MLC_{20} phosphorylation, and that the inhibition of myosin phosphatase and the resultant increased MLC_{20} phosphorylation is the main mechanism underlying G-protein-coupled Ca^{2+} sensitization during receptor stimulation. Because the receptor-G-protein complex is physically separated from the myosin phosphatase that is bound tightly with myosin and is not dissociated from myosin under physiological ionic conditions, a second messenger or cascade must communicate the inhibitory signal to myosin phosphatase activity [8,18]. Rho/Rho-kinase, protein kinase C (PKC), and arachidonic acid have been proposed to

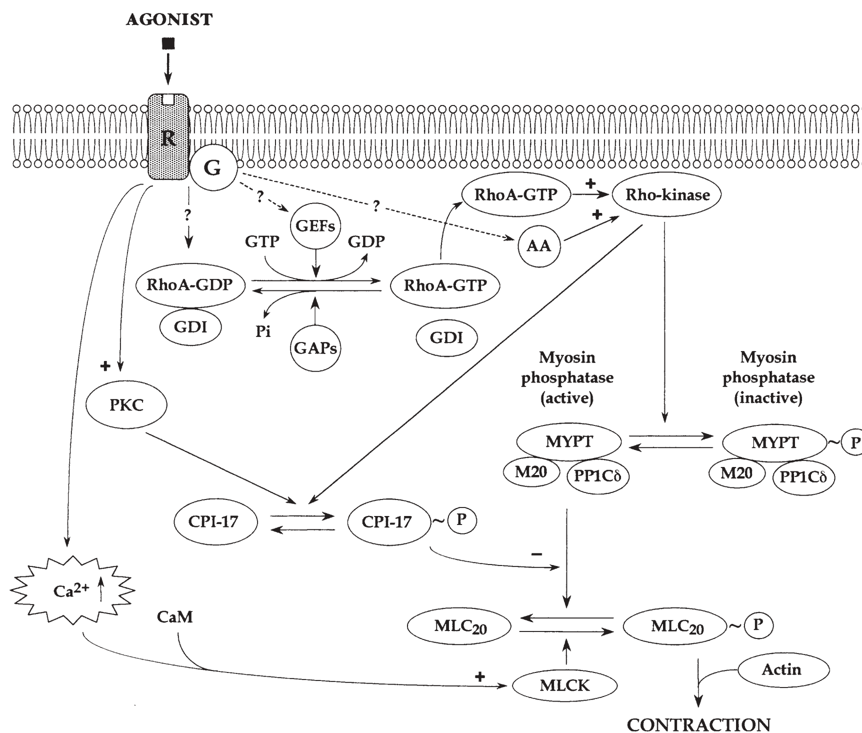

Fig. 1. Model for Rho/Rho kinase-mediated signalling (for details see text). *Plus sign*, stimulation; *minus sign*, inhibition; *question mark*, not established; *AA*, arachidonic acid; *CPI-17*, phosphorylation-dependent inhibitory protein of myosin phosphatase; *G*, guanosine-5′-triphosphate-binding protein; *GAP*, GTPase activating proteins; *GDI*, guanine-nucleotide dissociation inhibitor; *GDP*, guanosine-5′-diphosphate; *GEF*, guanine-nucleotide exchange factor; *GTP*, guanosine-5′-triphosphate; *M20*, 20-kDa subunit of myosin phosphatase; *MLC_{20}*, regulatory light chain of myosin (20 kDa); *MLCK*, myosin light chain kinase; *MYPT*, myosin phosphatase target subunit (myosin-binding subunit [MBS]); *PKC*, protein kinase C; *PP1Cδ*, catalytic subunit of myosin phosphatase (δ isoform); *R*, receptor; *RhoA-GDP*, GDP-bound RhoA; *RhoA-GTP*, GTP-bound Rho

serve as such Ca^{2+}-sensitizing messenger cascades [7,8,18]. It seems controversial whether the Rho-kinase functions upstream of, downstream of, or independently of PKC [19–21].

Rho/Rho-kinase

Myosin phosphatase, also known as SMPP (smooth muscle protein phosphatase)-1 M, is composed of three subunits: a catalytic subunit of type 1 phosphatase (δ isoform; PP1Cδ; ~37 kDa); and two noncatalytic subunits, a large 110- to 130-kDa regulatory subunit ($M_{110\text{-}130}$) and a 20-kDa subunit (M20) of unknown function [8,22]. The large subunit is a targeting subunit to which myosin binds, and is thus termed myosin phosphatase target subunit (MYPT) or myosin-binding subunit (MBS) [22,23].

Recent evidence suggests that the inhibitory signal to myosin phosphatase activity (for Ca^{2+} sensitization) is communicated by RhoA, a small monomeric G-protein, to a Rho-kinase that phosphorylates the MYPT (i.e., MBS) of myosin phosphatase and inhibits its catalytic activity, increasing MLC_{20} phosphorylation and, hence, contraction (Fig. 1). RhoA is, like most GTPases, active when it is bound to GTP, and inactive when the bound nucleotide is GDP. Under resting conditions, the inactive form of RhoA is complexed with guanine-nucleotide dissociation inhibitor (GDI) in the cytosol. However, when the RhoA-RhoGDI complex is stimulated by guanine-nucleotide exchange factors (GEFs), GTP replaces GDP on RhoA. The active form of RhoA (i.e., RhoA-GTP) then dissociates from the complex and subsequently translocates to the plasma membrane, while GDI is retained in the cytosol (Fig. 1) [8,23]. The translocated RhoA-GTP then activates a serine/threonine-kinase, referred to as Rho-kinase (also identified as ROKα and ROCK2 [23]), which, in turn, phosphorylates the MYPT (i.e., MBS) of myosin phosphatase and thereby inhibits its catalytic activity (Fig. 1). The detailed mechanism of Rho-kinase activation by RhoA-GTP is currently unknown; however, the activation of Rho-kinase by RhoA-GTP presumably occurs upon the recruitment of both proteins to the plasma membrane [8].

Arachidonic acid

Arachidonic acid, released through the phospholipase A_2 (PLA_2)-mediated hydrolysis of arachidonyl phospholipids and/or phospholipase D (PLD) activation during receptor stimulation [24], was shown to directly (i.e., independently of RhoA-GTP) activate Rho-kinase [25]. Thus, arachidonic acid may also play a role in Rho-kinase-mediated Ca^{2+}-sensitization (Fig. 1) [8,23]. In addition to the MYPT (or MBS) of myosin phosphatase, Rho-kinase may phosphorylate and activate CPI-17, a potent inhibitor (when phosphorylated) of the catalytic subunit (PP1Cδ) of myosin phosphatase, and thereby inhibit myosin phosphatase activity (Fig. 1) [26].

Protein kinase C (PKC)

The term "PKC" refers to a family of at least 11 different closely related serine/threonine kinases. The PKC family can be divided into four major isoform groups: (1) classical group A (cPKC-α, βI, βII, and γ); (2) novel group B (nPKC-δ, ε, η, and θ); (3) atypical group C (aPKC-ζ and ι); and (4) group D (PKCμ) [7,27]. Recent studies have identified several isoforms of PKC in VSMCs, cPKCα, cPKCβ, nPKCδ, nPKCε, cPKCγ, and aPKCζ [7,28,29]. The cPKC isoforms require Ca^{2+}, diacylglycerol (DG), and phosphatidylserine (PS) for activation, while the novel PKC isoforms require DG and PS for activity and are activatable by phorbol esters [7,27]. On the other hand, the aPKC isoforms require only PS for activation, and are insensitive to Ca^{2+}, DG, or phorbol esters. Because of its unique substrate specificity—which differs from that of cPKCs, nPKCs, and aPKCs—PKCμ (PKD) is considered as a distinct relative of PKCs. The cPKC and nPKC isoforms undergo translocation from the cytosol to membranous sites upon activation with DG (in the presence of PS) or certain phorbol esters [7]. Once activated, PKC could phosphorylate serine and/or threonine substrates in target proteins in or in close association with the membrane, and thereby affect their activities and, hence, the cellular responses.

The Ca^{2+}-sensitizing agonists acting on G-protein-coupled receptors activate the phospholipase C (PLC)-mediated hydrolysis of phosphatidylinositol 4,5-bisphosphate (PIP_2), leading to the production of inositol 1,4,5-triphosphate (IP_3) and DG, which releases Ca^{2+} from the intracellular Ca^{2+} store (i.e., sarcoplasmic reticulum [SR]) and activates PKC, respectively [2]. Both PKC activators (e.g., phorbol esters) and a constitutively active PKC have been shown to increase myofilament Ca^{2+} sensitivity [7,18]. In addition, PKC has been shown to phosphorylate CPI-17, the phosphorylation-dependent inhibitory protein of myosin phosphatase, and thereby inhibit its catalytic activity (Fig. 1) [30]. Furthermore, PKC has been suggested to phosphorylate the actin-binding proteins calponin and caldesmon, eliminating their ability to inhibit the actin-activated Mg^{2+}-ATPase activity of myosin [7,29]. Therefore, PKC may play a key role in receptor agonist-induced Ca^{2+} sensitization [7,8,23]. However, the Ca^{2+} sensitizing action of receptor agonists acting on G-protein-coupled receptors has been shown to be dissociated from their Ca^{2+}-releasing action mediated by a PLC product (i.e., IP_3) [10,31], apparently excluding another PLC product (i.e., DG) as a major Ca^{2+} sensitizing messenger and leading to the general view that PKC plays only a minor or transient role in G-protein-coupled Ca^{2+} sensitization

[8]. However, DG can also be generated by the PLD-mediated hydrolysis of phosphatidylcholine. In addition, cytosolic PKC can be activated in the absence of the phosphatidylserine-containing membrane by free arachidonic acids that can be produced by the PLA_2-mediated hydrolysis of membrane phospholipids. Thus, there still remains a possibility that PKC plays a significant role in Ca^{2+} sensitization, its extent possibly depending on the agonist and/or cell type involved [7,8].

Mechanisms to decrease myofilament Ca^{2+} sensitivity through inhibition of myosin light chain kinase activity

The activity of MLCK is primarily regulated by the Ca^{2+}-CaM complex. However, the phosphorylation of MLCK at a specific serine residue in the region of its CaM-binding domain (i.e., *site A*) decreases its affinity for the Ca^{2+}-CaM complex and, hence, its phosphorylating activity [6,8]. Thus, Ca^{2+}/CaM-dependent protein kinase II (CaMKII), previously reported to phosphorylate MLCK at *site A* at higher $[Ca^{2+}]$c levels, may play a physiological role in downregulating the Ca^{2+} signal by decreasing the Ca^{2+} sensitivity of MLC_{20} phosphorylation [6–8].

Feedback inhibition of Rho/Rho-kinase-mediated Ca^{2+} sensitization may also occur through the inhibition of MLCK, which may be caused either by phosphorylation by p21-activated kinase (a serine/threonine kinase activated by other Rho-subfamily GTPases, Cdc42 and Rac), or by dephosphorylation at its inhibitory site by some protein phosphatase [8,18,32].

Mechanisms to induce Ca^{2+}-independent contraction

Myosin phosphatase inhibitors (e.g., microcystin-LR), phorbol esters (PKC activators), and sphingosylphosphorylcholine (SPC; a sphingolipid generated by the N-deacylation of sphingomyelin, one of the most abundant lipids in the cell membrane) have recently been reported to induce contraction without an increase in $[Ca^{2+}]$c in VSMCs [20,33–35]. Phorbol esters can induce Ca^{2+}-independent contraction without an increase in MLC_{20} phosphorylation [27], while myosin phosphatase inhibitors induced Ca^{2+}-independent contraction with an increase in MLC_{20} phosphorylation [34,35]. The kinase(s) responsible for Ca^{2+}-independent contraction has yet to be identified and its underlying mechanisms remain controversial. The kinase(s) responsible for microcystin-LR-induced, Ca^{2+}-independent MLC_{20} phosphorylation and contraction has been proposed to be distinct from Rho-kinase, MLCK, and PKC [34,35]. On the other hand, SPC-induced, Ca^{2+}-independent contraction was recently proposed to be mediated through the activation of Rho-kinase, but not through PKC activation [20].

Mechanisms to increase myofilament Ca^{2+} sensitivity through phosphorylation of thin-filament-associated proteins

The thin-filament-associated proteins caldesmon and calponin possess actin- and CaM-binding activities, and the capability to inhibit the actin-activation of myosin ATPase activity and, hence, crossbridge cycling (i.e., myosin-actin interaction) [7,36]. When phosphorylated, their inhibitory action on actomyosin ATPase (i.e., the actin-activated, ATPase activity of myosin) can be reversed, possibly leading to enhanced crossbridge cycling and thus to an enhanced contractile response without an increase in MLC_{20} phosphorylation [7,36]. Thus, these thin-filament-associated proteins may participate in the regulation of myofilament Ca^{2+} sensitivity through their phosphorylation by mitogen-activated protein kinases (MAPKs) and/or other kinases such as PKC, CaMKII, or p21-activated kinase. However, their precise roles in the regulation of VSM contraction remain to be clarified [7,18,36].

Other important regulatory mechanisms

Ca^{2+}/calmodulin-dependent protein kinase II

Ser19 of MLC_{20} also can be phosphorylated by multifunctional CaMKII; however, this occurs only at a very slow rate and probably does not contribute to the physiological initiation of contraction [18]. However, as mentioned above, CaMKII, activatable only at higher $[Ca^{2+}]$c levels, can phosphorylate MLCK, thereby decreasing its affinity for CaM (i.e., its phosphorylating activity) and, hence, the Ca^{2+} sensitivity of MLC_{20} phosphorylation [6,7,18]. Furthermore, CaMKII may activate a pathway by which the activation of MAPK leads to MLC_{20} phosphorylation via the activation of MLCK [37]. Thus, CaMKII seems to play some important roles in the regulation of vascular tone.

Tyrosine kinases and mitogen-activated protein kinases

Tyrosine kinases, present abundantly in VSMCs, may affect both Ca^{2+} mobilization and myofilament Ca^{2+} sensitivity in VSMCs [38,39]. Tyrosine kinase-catalyzed protein tyrosine phosphorylation has been reported to regulate the activity of voltage-operated Ca^{2+} channels (VOCCs), store-operated Ca^{2+} channels, K^+ channels, PLC, PLD, and MAPKs in VSMCs [24,38,40]. PLD hydrolyzes phosphatidylcholine to generate DG, leading to the activation of PKC, while MAPKs phosphorylate caldesmon to attenuate its inhibitory action on actomyosin ATPase activity [7]. Thus, tyrosine phosphorylation might be involved in the regulation of myofilament Ca^{2+} sensitivity through the effects on PLD

and/or MAPKs [38]. However, the precise roles of tyrosine kinases and MAPKs in the physiological regulation of vascular tone are not fully understood yet.

Cyclic nucleotides

An increase in the cytosolic level of cyclic guanosine 3′,5′-monophosphate (cGMP) or cyclic adenosine 3′,5′-monophosphate (cAMP) in VSMCs is considered one of the major mechanisms that mediate vasodilation under physiological conditions. Particularly, cGMP mediates the action of endogenous vasodilators that play an important role in the control of vascular tone, such as nitric oxide (NO), carbon monoxide (CO), or natriuretic peptides [41]. The major cellular mechanisms for the increase in cGMP levels include the inhibition of various phosphodiesterase subtypes, the activation of soluble guanylyl cyclase (by NO or CO), and the activation of membrane-bound guanylyl cyclase (by natriuretic peptides) [41–47]. The increase in cGMP levels leads to the activation of cGMP-dependent protein kinase (protein kinase G; PKG), which, in turn, reduces both [Ca^{2+}]c and myofilament Ca^{2+} sensitivity in VSMCs, causing vasorelaxation [12,41]. Multiple mechanisms have been proposed to explain the PKG-mediated reduction of [Ca^{2+}]c, including: (1) activation of Ca^{2+} uptake by the intracellular stores (via the phosphorylation of phospholamban and the activation of SR Ca^{2+}-ATPase), (2) increased Ca^{2+} efflux (via the stimulation of plasma membrane Ca^{2+}-ATPase and Na^+/Ca^{2+} exchanger), (3) the inhibition of Ca^{2+} release from the SR (via PKG-mediated phosphorylation of the SR IP_3 receptor and/or inhibition of IP_3 synthesis), (4) membrane hyperpolarization via direct and/or indirect activation of K^+ channels, and (5) direct inhibition of VOCCs (via dephosphorylation of the VOCCs, due, in turn, to the phosphorylation of protein phosphatase 2A; Fig. 2) [3,41]. However, the relative contribution of these mechanisms to the [Ca^{2+}]c-lowering action of PKG seems to be variable, depending on VSM type, species, and contractile stimulus [41]. The PKG-induced reduction of myofilament Ca^{2+} sensitivity is, presumably, due to a reduction in the Ca^{2+} sensitivity of MLC_{20} phosphorylation, possibly via accelerated MLC_{20} dephosphorylation due to the upregulation of myosin phosphatase (Fig. 2) [8,18,41].

Similarly, the increase in cAMP levels exerts profound influences on cellular Ca^{2+} mobilization through the activation of cAMP-dependent protein kinase (protein kinase A; PKA), including inhibition of the activation of PLC and Ca^{2+} channels, stimulation of plasma membrane Ca^{2+}-ATPase and the Na^+/Ca^{2+} exchanger, stimulation of Ca^{2+} uptake into the SR (via PKA-mediated phosphorylation of phospholamban), and activation of K^+ channels (Fig. 2), [5,48,49]. In addition, the increase in cAMP levels reduces myofilament Ca^{2+} sensitivity, presumably by phosphorylating MLCK and thereby decreasing its affinity for the Ca^{2+}-CaM complex [5,49]. Furthermore, cross-activation of PKG by cAMP may also be involved in its vasodilator action [5,41]. Conversely, cGMP may also cross-activate PKA (Fig. 7) [41,49].

Chloride channels

Chloride (Cl^-) channels, abundantly distributed in the VSM membrane, would appear to play an important role in the regulation of [Ca^{2+}]c in VSMCs [50]. In VSMCs, the intracellular Cl^- concentration and equilibrium potential for Cl^- are 57 mM and −26 mV, respectively [51], the latter of which is high enough to activate L-type VOCCs. There exist at least two types of Cl^- channels in VSMCs, i.e., Ca^{2+}-dependent Cl^- (Cl_{Ca}) and volume-regulated Cl^- (Cl_v) channels [50].

Because the Cl_v channel can be activated not only by low osmotic pressure but also by mechanical stretch, vascular distension caused by a rise in blood pressure would lead to the activation of Cl_v channels and membrane depolarization, which, in turn, activate VOCCs and thereby cause vasoconstriction. Thus, the Cl_v channel may play a protective role in maintaining tissue integrity against mechanical stretch [50].

The Cl_{Ca} channels have been reported to be activated by an increase in [Ca^{2+}]c caused by either Ca^{2+} release from the SR [50,52] or plasmalemmal Ca^{2+} influx [50,53]. In some vascular beds (e.g., portal vein [52] and small mesenteric arteries [53]), the Cl_{Ca} channels probably contribute to the contractile response to receptor agonists such as norepinephrine, particularly in its tonic phase.

Potassium channels

In VSMCs, potassium (K^+) channels situated in the plasmalemma play a fundamental role in maintaining the membrane potential, a major determinant of vascular tone, particularly in systemic resistance vessels [54,55]. The membrane potential is determined by membrane permeability to several ions, including K^+, Ca^{2+}, Na^+, and Cl^- ions. Other ion transport systems such as the Na^+/K^+ pump or anion transporters can contribute as well. Among these conductances, the K^+ channels play the most prominent role in regulating the membrane potential. Thus, changes in their activity significantly alter the membrane potential and, hence, vascular tone [55]. Specifically, the blockade of K^+ channels results in membrane depolarization and increased Ca^{2+} influx through VOCCs, leading to vasoconstriction. Conversely, the activation of K^+ channels results in plasmalemmal K^+

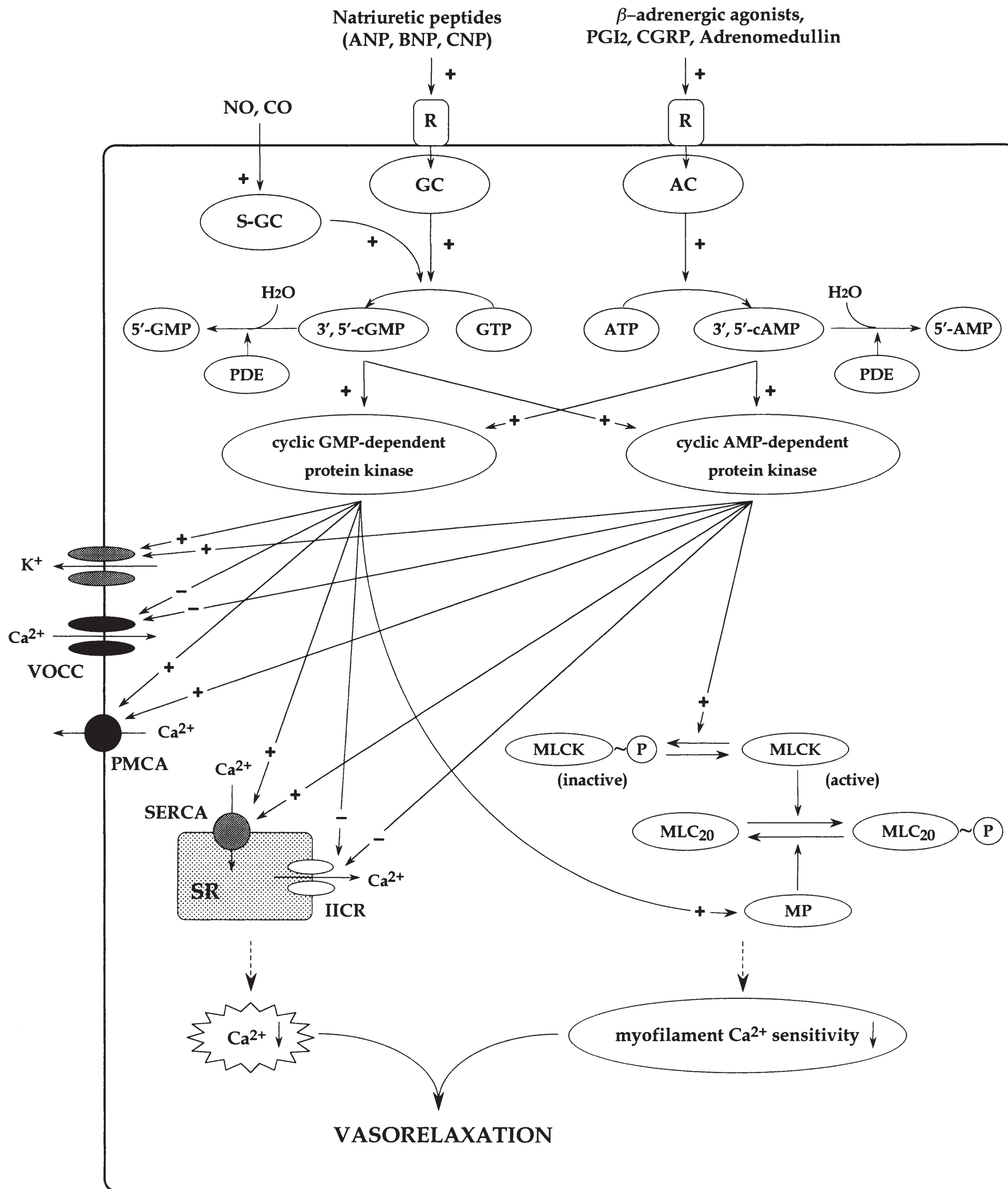

Fig. 2. Proposed mechanisms of cyclic nucleotide-mediated vasorelaxation (for details see text). *Plus sign*, stimulation; *minus sign*, inhibition; *AC*, adenylyl cyclase; *ANP*, A-type/atrial natriuretic peptide; *5′-AMP*, adenosine 5′-monophosphate; *ATP*, adenosine 5′-triphosphate; *3′, 5′-cAMP*, cyclic adenosine 3′, 5′-monophosphate; *BNP*, B-type/brain natriuretic peptide; *CNP*, C-type natriuretic peptide; *CGRP*, calcitonin gene-related peptide; *CO*, carbon monoxide; *GC*, guanylyl cyclase; *5′-GMP*, guanosine 5′-monophosphate; *GTP*, guanosine 5′-triphosphate; 3′, *5′-cGMP*, cyclic guanosine 3′, 5′-monophosphate; *IICR*, inositol 1, 4, 5-triphosphate (IP_3)-induced Ca^{2+}-release; *MLC_{20}*, regulatory light chain of myosin (20 kDa); *MLCK*, myosin light chain kinase; *MP*, myosin (light chain) phosphatase; *PDE*, phosphodiesterase; *NO*, nitric oxide; *PGI_2*, prostacyclin; *PMCA*, plasma membrane Ca^{2+}-ATPase; *R*, receptor; *SERCA*, sarcoplasmic reticulum Ca^{2+}-ATPase; *SR*, sarcoplasmic reticulum; *S-GC*, soluble guanylyl cyclase; *VOCC*, voltage-operated Ca^{2+} channel

efflux, membrane hyperpolarization, and reduced Ca^{2+} influx through VOCCs, leading to vasodilation [54,55]. In addition, recent evidence suggests that the inhibition of phosphoinositide metabolism and myofilament Ca^{2+} sensitivity also underlies hyperpolarization-induced vasodilation [56,57]. Because the relationship between the level of membrane potential and arterial tone is quite steep, changes in membrane potential of only a few mV can be associated with large changes in arterial diameter [58,59].

K^+ channel activity has been shown to be altered by various physiological factors, such as intracellular Ca^{2+}, G-proteins, cyclic nucleotides, pH, or protein kinases. In addition, K^+ channels are targets for endogenous vasodilator substances, including NO, prostacyclin, endothelium-derived hyperpolarizing factor (EDHF), and calcitonin gene-related peptide (CGRP). Thus, K^+ channels would appear to play a key role in the physiological regulation of vascular tone, and their opening is considered as one major mechanism that mediates vasodilation under physiological conditions. Altered activities of K^+ channels may explain or reflect the alterations in vascular tone observed in some disease states (e.g., shock, hypertension) [54,55].

Several, at least five distinct, types of K^+ channels exist in VSMCs, although their distribution and regulation by a variety of factors vary depending on the blood vessel type [54,55]. These channels would seem to play unique roles in regulating vascular tone, depending on the vasoconstrictor or vasodilator stimulus, or the vascular bed [54].

The molecular identity or nature of the K^+ channels and their detailed electrical properties (e.g., single-channel conductance) are beyond the scope of this article, and will not be covered here.

Voltage-gated K^+ channels

Voltage-gated K^+ (K_V) channels are found in a variety of vascular tissues, and are typified by delayed rectifier K^+ (K_{DR}) and transient outward K^+ (K_{tran}) channels [55,60]. Their activity increases with membrane depolarization, but in most instances they show inactivation with sustained depolarization, although the rate varies, depending on the subtype of K_V channels [54,61]. The K_{DR} channels normally inactivate very slowly, producing sustained outward K^+ current, while the K_{tran} channels inactivate completely at membrane potentials positive to approximately –60 mV, producing transient outward K^+ currents [60,61]. The K_V channels have been reported to be inhibited by intracellular Ca^{2+} and Mg^{2+}, while being activated by intracellular ATP [54]. These channels can be pharmacologically blocked by 4-aminopyridine (>0.1 mM), tetraethylammonium (TEA; >10 mM), and Ba^{2+} (>1 mM) [54,55,60]. The molecular identity of K_V channels in VSMCs is poorly defined [60].

The K_V channels have been proposed to play several roles in the control of vascular tone, although their physiological roles are not clarified. The K_V channels may contribute to the steady-state resting membrane potential [54,55]. Because the K_V channels are activated by depolarization, they may also serve to limit membrane depolarization in response to vasoconstrictor stimuli [54]. In addition, their activation may be involved in the vasodilator response to NO, EDHF, or prostacyclin [60]. Conversely, their inhibition has been suggested to be involved in hypoxic pulmonary vasoconstriction, histamine-induced vasoconstriction in coronary VSMCs, and myogenic vasoconstriction in resistance vessels [54,59].

Ca^{2+}-activated K^+ channels

Ca^{2+}-activated K^+ (K_{Ca}) channels are present in virtually all excitable cells. Vascular K_{Ca} channels are activated by membrane depolarization and intracellular Ca^{2+}. K_{Ca} channels of different conductances have been identified in various tissues, including small (10–15 pS), intermediate (20–60 pS), and large (200–300 pS) conductance channels. Small- and large-conductance K_{Ca} channels have been reported to be present in vascular tissues.

Large-conductance, Ca^{2+}-activated K^+ channels

The most abundant K^+ channel in VSMCs is the large-conductance (200–250 pS), K_{Ca} ("BK" or "Maxi-K") channel. Its activity increases with membrane depolarization and the elevation of intracellular Ca^{2+} over the physiological ranges of membrane potential (–60 to –30 mV) and intracellular Ca^{2+} concentration (100–600 nM) [54]. Recent evidence suggests that the BK channels in VSMCs are regulated by localized bursts of Ca^{2+} (Ca^{2+} sparks) derived from the SR just under the sarcolemma [54]. The BK channels can be pharmacologically blocked by TEA, charybdotoxin, and iberiotoxin, the latter two of which are considered to be highly selective BK_{Ca} channel blockers. The molecular identity of the vascular BK channel is established (see review [60] for details).

The BK channels conceivably play an important role as a negative feedback mechanism to limit membrane depolarization and, hence, vasoconstriction [55]. Indeed, they are upregulated in hypertensive subjects, constituting an important homeostatic mechanism for buffering the increased arterial reactivity [62]. In addition, depending on the vascular beds, some endogenous vasodilators (e.g., adenosine, NO, prostacyclin, EDHF) may act, at least in part, through the activation of BK channels [54,60]. In coronary arteries, adenosine probably activates BK channels by increasing the cAMP

level and activating PKA [54]. In contrast, NO presumably activates BK channels by increasing the cGMP level and activating PKG [54]. Namely, these vasodilators seem to activate BK channels by phosphorylating the channels or their regulatory subunits through the action of either PKA or PKG [60]. NO was also shown to directly activate BK channels in VSMCs [63]. Conversely, their inhibition may underlie the contractile responses to angiontensin II or the stimulation of thromboxane A_2 receptors [54,60].

Small-conductance, Ca^{2+}-activated K^+ channels

Both pharmacological and molecular biological evidence suggests that apamin-sensitive, small-conductance (5–20 pS) K_{Ca} ("SK") channels are present in VSMCs [54]. The SK channel has been proposed to mediate EDHF-induced hyperpolarization and vasorelaxation in some arterial beds [54,55]. This channel, recently cloned from brain, shows a membrane topology similar to that of the voltage-gated channels [55]. However, the molecular identity and properties of the conductance(s) affected by apamin in smooth muscle are ill defined [60].

ATP-sensitive K^+ channels

ATP-sensitive K^+ (K_{ATP}) channels were first identified in cardiac myocytes [64], and have subsequently been found in various cell types, including VSMCs in a variety of blood vessels (e.g., cerebral, coronary, pulmonary, mesenteric, and renal arteries) [54]. The K_{ATP} channel is inhibited by exposure of the intracellular face of the cell membrane to ATP (Ki ≈ 50–500 μM), and its sensitivity to ATP can be altered by changes in the ADP/ATP ratio or intracellular pH. In addition, MgATP, cAMP, and/or G-proteins may participate in the activation of K_{ATP} channel, and seem to be required to sustain its activity. The K_{ATP} channel is only weakly voltage-dependent [54,55,61].

K_{ATP} channels can be selectively inhibited by sulfonylurea agents such as glibenclamide (Ki ≈ 20–100 nM) or tolbutamide (Ki ≈ 350 μM). K_{ATP} channels can also be inhibited by the nonselective K^+ channel blocker TEA (Ki ≈ 6.2 mM), while they can be activated by a number of pharmacological vasodilators (e.g., cromakalim, pinacidil, nicorandil) [54,55].

K_{ATP} channels are believed to play an important physiological role in mediating vasodilator responses to a variety of endogenous vasodilators as well as to changes in metabolic activity. The endogenous vasodilators previously reported to activate K_{ATP} channels include calcitonin gene-related protein (CGRP), vasoactive intestinal peptide, adenosine, prostacyclin, NO, and β-adrenergic agonists [54,61,65]. In the coronary circulation, K_{ATP} channels appear to be active under resting conditions, contributing to the maintenance of resting membrane potential and, hence, resting vascular tone [54]. Theoretically, any interventions to reduce mitochondrial ATP generation would activate the K_{ATP} channels and thereby cause vasodilation [54,61]. Thus, the activation of K_{ATP} channels is, presumably, involved in the vasodilation observed during hypoxia or shock [54,55,61]. In the coronary circulation, the activation of K_{ATP} channels has been proposed to mediate vasodilation during hypoxia, reactive hyperemia, and acidosis [66–68]. In contrast, the inhibition of K_{ATP} channels seems to be involved in vasoconstrictor responses to endothelin, vasopressin, or angiotensin II [54].

Inward rectifying K^+ channels

Electrophysiological evidence indicates that inwardly rectifying K^+ (K_{ir}) channels are present in a variety of vascular beds, including cerebral, mesenteric, and coronary arteries [54]. As is conceivable from its name, the K_{ir} channel passes inward current more readily than outward K^+ current, due to its activation by hyperpolarization rather than depolarization [54,55]. K_{ir} channels can be inhibited by Ba^{2+} (Ki ≈ 2.2 μM at −60 mV) and by Cs^{-1} (Ki ≈ 1.6 mM at −50 mV) ions, but they are relatively unaffected by other K^+ channel blockers [54].

K_{ir} channels are activated by increases in extracellular K^+ [54,69]. For example, in cerebral arteries, elevation of extracellular K^+ from normal levels to only 7–10 mM K^+ causes large hyperpolarization and vasodilation, which is sensitive to low concentrations (≤10 μM) of Ba^{2+} [54]. The activation of K_{ir} channels may be involved in the cerebral vasodilation observed during increased neuronal activity, hypoxia, ischemia, or hypoglycemia, each of which is associated with elevations of extracellular K^+ [69]. In resistance vessels and endothelial cells, K_{ir} channels probably contribute to the maintenance of the resting membrane potential [54,55,69].

Cyclic GMP-gated K^+ channels

A novel K^+ channel activated by cGMP, but not by cAMP, was recently cloned from rabbit and found to be expressed in various tissues, including aorta [70]. Gene analysis suggests that a similar channel exists in humans [71]. This channel might be a specific target for the endogenous vasodilator agents that increase cGMP via the activation of guanylyl cyclase (e.g., NO) and are possibly involved in the physiological regulation of vascular tone. However, it remains to be determined whether this cGMP-gated channel is expressed and is functional in resistance vessels, and whether its activation by cGMP is via direct binding or via PKG-dependent phosphorylation [55].

Summary

Changes in $[Ca^{2+}]c$ are the principal mechanisms that regulate the contractile state of VSMCs. In response to vasoconstrictor stimuli, Ca^{2+} is mobilized from intracellular stores and/or the extracellular space to increase $[Ca^{2+}]c$ in VSMCs. The increase in $[Ca^{2+}]c$, in turn, activates the Ca^{2+}-CaM-MLCK pathway and stimulates MLC_{20} phosphorylation, leading to myosin-actin interactions and, hence, the development of contractile force. The sensitivity of contractile myofilaments or MLC_{20} phosphorylation to Ca^{2+} can be secondarily modulated by other signaling pathways. During receptor stimulation, the contractile force is greatly enhanced by the inhibition of myosin phosphatase. Rho/Rho kinase, PKC, and arachidonic acid have been proposed to play a pivotal role in this enhancement; however, their precise roles are not fully clarified.

Increases in cyclic nucleotides (i.e., cGMP, cAMP) and the activation of K^+ channels could be major mechanisms that mediate vasodilation under physiological conditions—i.e., the vasodilation that occurs through the inhibition of both Ca^{2+} mobilization and myofilament Ca^{2+} sensitivity in VSMCs.

Besides the above-described mechanisms that play pivotal roles in the regulation of vascular tone, there exist numerous mechanisms that could play important modulatory roles in the regulation of vascular tone through their effects on Ca^{2+} mobilization and/or myofilament Ca^{2+} sensitivity in VSMCs—such as CaMKII, caldesmon, calponin, MAPKs, tyrosine kinases, or Cl^- channels. However, their precise physiological roles remain to be clarified.

Better understanding of the cellular and molecular mechanisms involved in the regulation of vascular tone—i.e., the targets for general anesthetics, as well as for vasoactive agents used in general anesthetic practice—would lead to better circulatory management during general anesthesia, as well as to better designs for research in general anesthetic vascular pharmacology.

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