MINIREVIEW

Molecular Aspects of Arterial Smooth Muscle Contraction: Focus on Rho

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The vascular smooth muscle cell is a highly specialized cell whose primary function is contraction and relaxation. It expresses a variety of contractile proteins, ion channels, and signalling molecules that regulate contraction. Upon contraction, vascular smooth muscle cells shorten, thereby decreasing the diameter of a blood vessel to regulate the blood flow and pressure. Contractile activity in vascular smooth muscle cells is initiated by a Ca^{2+}-calmodulin interaction to stimulate phosphorylation of the light chain of myosin. Ca^{2+}-sensitization of the contractile proteins is signaled by the RhoA/Rho-kinase pathway to inhibit the dephosphorylation of the light chain by myosin phosphatase, thereby maintaining force. Removal of Ca^{2+} from the cytosol and stimulation of myoson phosphatase initiate the relaxation of vascular smooth muscle. Exp Biol Med 230:829–835, 2005.

Key words: smooth muscle; contraction; vascular; calcium; signaling

Smooth muscle cells constitute the walls of various organs and tubes of the body including blood vessels, stomach, intestines, bladder, airways, uterus, and the penile and clitoral cavernosal sinuses. Smooth muscle derives its name from the fact that it lacks the striated banding pattern that is characteristic of cardiac and skeletal muscle. Smooth muscle cells receive neural innervation from the autonomic nervous system. In addition, the contractile state of smooth muscle is controlled by several hormonal and autocrine/paracrine actions. Smooth muscle cells also develop tonic and phasic contractions in response to changes in load or length. Regardless of the stimulus, smooth muscle cells use cross-bridge cycling (between actin and myosin) to develop force and calcium ions (Ca^{2+}) that initiate the underlying molecular signaling that leads to contraction.

The vascular smooth muscle cell is a highly specialized cell whose principal function is contraction. It expresses a variety of contractile proteins, ion channels, and signaling molecules that regulate contraction. On contraction, vascular smooth muscle cells shorten, thereby decreasing the diameter of a blood vessel to regulate the blood flow and pressure. However, this contractile phenotype can differentiate into a synthetic phenotype during, for instance, vasculogenesis, which is characterized by proliferation and production of extracellular matrix components of the arterial wall (1). In this minireview, we focus primarily on the contractile phenotype of vascular smooth muscle cells and describe the molecular-signaling pathways involved in regulating contraction. For a more in-depth outline of the molecular mechanism of smooth muscle contraction and its regulation, the reader is referred to several recently published reviews (2–6).

The Contractile Mechanism

In the intact body, the process of smooth muscle contraction is principally regulated by pharmacomechanic activation (i.e., activation by ligands of cell surface receptors) and electromechanic activation (i.e., stretch, intraluminal pressure) of the contractile proteins myosin and actin (7). In the latter, a depolarization of the membrane's resting potential, brought on by the firing of action potentials or by activation of stretch-dependent ion channels and voltage-operated Ca^{2+} channels in the plasma membrane, triggers the influx of Ca^{2+} and ultimately leads

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to contraction. Myogenic tone or pressure-induced constriction of arterioles and small-resistance arteries depends on this electromechanic coupling (8, 9). For contraction to occur, myosin light chain (MLC) kinase, activated by Ca^{2+} calmodulin, must phosphorylate Ser 19 of the 20-kDa regulatory MLC, enabling the molecular interaction of myosin with actin (10, 11). Energy released from ATP by myosin ATPase activity results in the cycling of the myosin cross-bridges with actin for contraction (12, 13). Thus, contractile activity in smooth muscle cells is primarily determined by the phosphorylation state of the MLC. In some smooth muscle cells, the phosphorylation of the MLC is maintained at a low level in the absence of external stimuli (i.e., no receptor or mechanical activation; Ref. 14). This activity results in what is known as smooth muscle tone, and its intensity can be varied.

Ca^{2+}-Dependent Contraction of Smooth Muscle

Contraction of smooth muscle is initiated by a Ca^{2+}-mediated change in the thick (myosin) filaments, whereas in striated muscle, Ca^{2+} mediates contraction by changes in the thin (actin) filaments. In response to specific stimuli in smooth muscle, the α_1-adrenoceptor agonist norepinephrine released from the sympathetic nerves within the arterial wall for instance, the intracellular concentration of Ca^{2+} rapidly increases and declines to a level that is elevated above basal in the continued presence of the agonist (15). This initial transient increase in cytosolic Ca^{2+} arises from Ca^{2+} released from intracellular stores (sarcoplasmic reticulum), while the latter increase arises from entry from the extracellular space through Ca^{2+} channels (receptor-operated Ca^{2+} channels), as shown by high-resolution confocal imaging (see Ref. 5 for a comprehensive review). The biochemical signals that trigger this Ca^{2+} release are documented in detail (see Refs. 16 and 17 for reviews) and will be described here only briefly. Phospholipase C (PLC) is activated by the heterotrimeric G protein $G\alpha,q$ on binding of agonists such as norepinephrine, angiotensin II, or endothelin-1 to serpentine receptors on the smooth muscle membrane (Fig. 1). PLC is specific for the membrane lipid phosphatidylinositol 4,5-biphosphate to catalyze the formation of two second messengers: inositol 1,4,5-trisphosphate (IP_3) and diacylglycerol (DAG). The binding of IP_3 to receptors on the sarcoplasmic reticulum results in the release of Ca^{2+} into the cytosol. The primary target protein of this initial rise in intracellular Ca^{2+} is believed to be calmodulin, a member of the family of EF hand Ca^{2+}-binding proteins (18). The binding of Ca^{2+} to these four EF hands causes a conformational change in the calmodulin molecule, allowing a subsequent interaction with MLC kinase. This association results in a conformational change of the calmodulin-MLC kinase complex, exposing the catalytic site. This sequence of events leads to the activation of MLC kinase and the phosphorylation of Ser 19 of the 20-kDa regulatory MLC (MLC20; Refs. 10, 11).

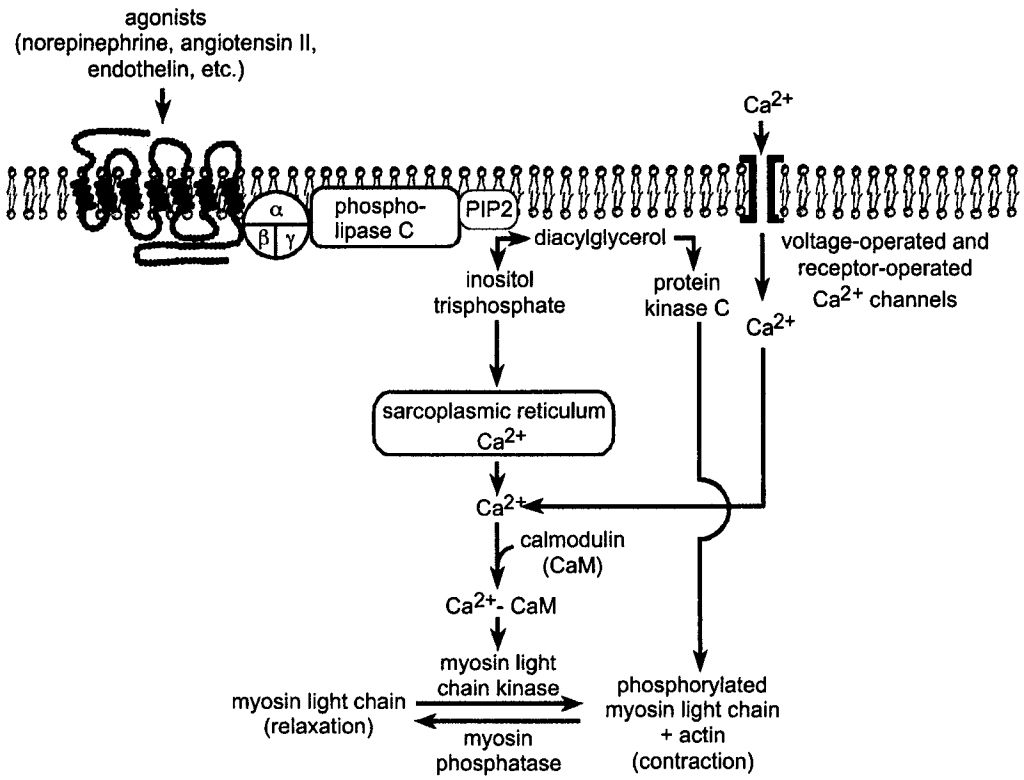

Figure 1. Molecular signaling pathways involved in agonist-induced smooth muscle cell contraction. The contractile response is initiated by a rapid and transient rise in intracellular Ca^{2+}, followed by a Ca^{2+}-calmodulin interaction to stimulate phosphorylation of the MLC. Refer to the text for a more detailed outline.

The second messenger, DAG, which is released on agonist-induced and/or stretch-induced PLC activation, activates protein kinase C (PKC), which phosphorylates specific target proteins. There are several isozymes of PKC in smooth muscle such as PKCα and β (those that are DAG dependent and Ca^{2+} dependent) or PKCε (which requires only DAG), and each has a tissue-specific role (e.g., vascular, uterine, intestinal). In many cases, PKC has contraction-promoting effects such as phosphorylation of many kinases including MLC kinase, ERK1/2, Rho-kinase (p160ROCK), and calmodulin-dependent protein kinase II, as well as various ion channels and ion transporters. Phorbol esters, a group of synthetic compounds known to activate PKC, mimic the action of DAG and cause contraction of smooth muscle.

Finally, L-type Ca^{2+} channels (i.e., voltage-operated Ca^{2+} channels) in the membrane also open in response to the membrane depolarization brought on by stretch of the smooth muscle cell, which is supposed to play an important role in myogenic reactivity and tone (19–21).

Ca^{2+}-Sensitization Mechanism and Contraction of Smooth Muscle

Contractility of vascular smooth muscle is not only regulated by intracellular Ca^{2+}, but also by Ca^{2+}-independent mechanisms. In addition to the Ca^{2+}-dependent activation of MLC kinase, the state of MLC phosphorylation is further regulated by MLC phosphatase, which is also known as myosin phosphatase (16, 22, 23) and removes the high-energy phosphate from the MLC to promote smooth

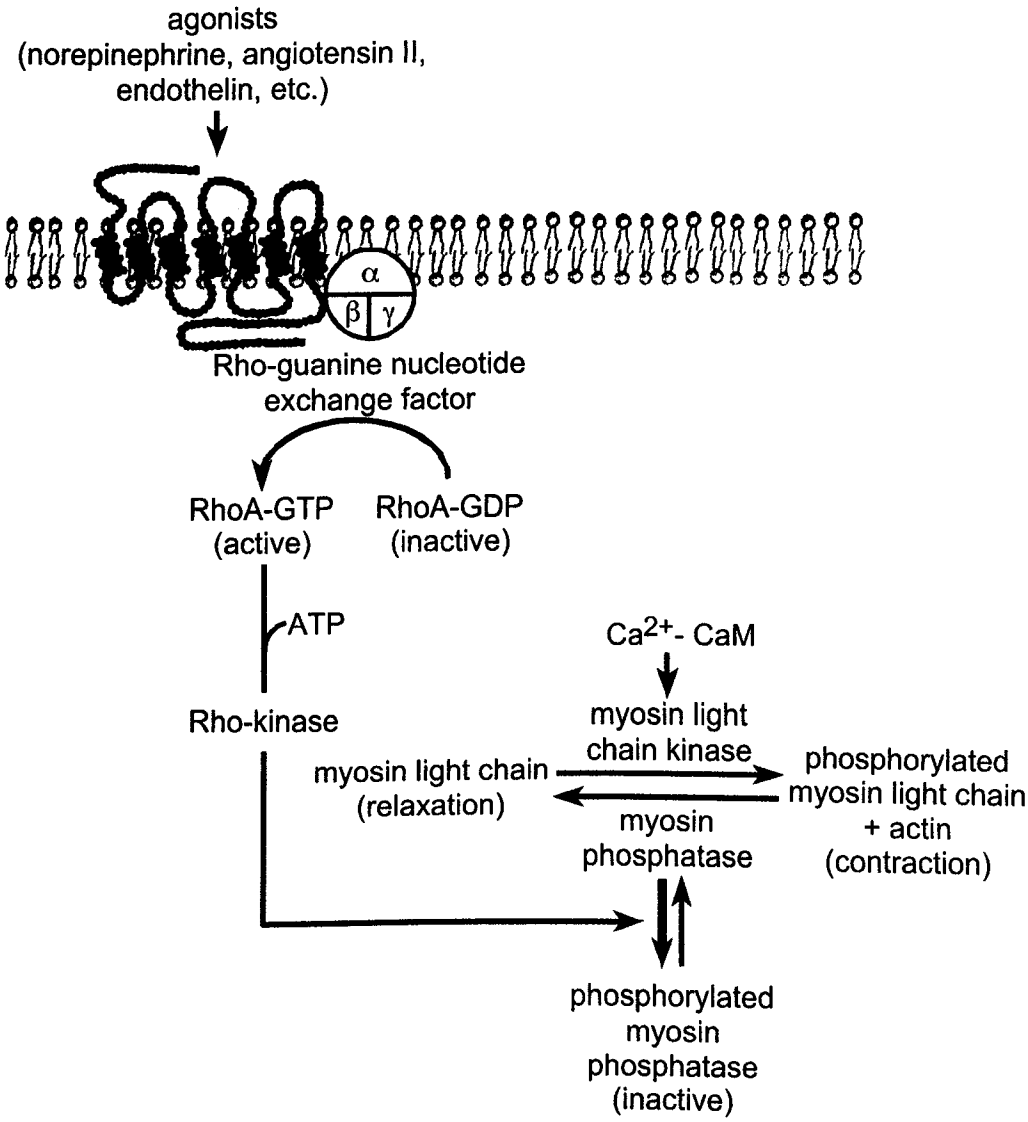

Figure 2. Ca^{2+}-sensitization mechanism and contraction of smooth muscle. Contractility of smooth muscle can also be regulated by Ca^{2+}-independent mechanisms involving G-protein–coupled receptor binding. When agonists bind to serpentine receptors on the outer membrane of the smooth muscle, the Rho-GTP is activated by Rho-guanine nucleotide exchange factors. This activated RhoA-GTP binds to and activates Rho kinase, which subsequently phosphorylates the myosin-binding subunit of MLC phosphatase and inhibits its activity, thus promoting the phosphorylated state of MLC that leads to contraction.

muscle relaxation (Fig. 2). There are three subunits of MLC phosphatase: a 37-kDa catalytic subunit (PP1c), a small noncatalytic subunit of unknown function (24), and a 110-kDa to 130-kDa myosin-binding subunit (MYPT1). The binding of MYPT1 to PP1c inhibits the enzymatic activity of MLC phosphatase, allowing the MLC to remain phosphorylated and, thereby, promoting contraction (25). Experiments have shown that activated G-proteins are involved in the signaling pathway for this Ca^{2+} sensitization because GTPγS, a nonhydrolyzable GTP analog, increases MLC phosphorylation and potentiates contraction. GTPγS was also demonstrated to decrease the rate of MLC dephosphorylation, which is consistent with an overall decrease in MLC phosphatase activity (26). Subsequent experiments have shown that the small G protein RhoA and its downstream target Rho kinase play an important role in the regulation of MLC phosphatase activity (27). RhoA is a monomeric G-protein that can be activated by several agonists including angiotensin II (28). Its activity is regulated by the binding of GTP, a transition facilitated by Rho-guanine nucleotide exchange factors (Rho-GEFs) that enable the exchange of nucleotide to activate RhoA-GDP to RhoA-GTP (29). This activated RhoA-GTP binds to and activates Rho kinase, a serine/threonine kinase (30), which subsequently phosphorylates the myosin-binding subunit of MLC phosphatase (MYPT1) and inhibits its activity (31) and, thus, promotes the phosphorylated state of the MLC (Fig. 2). A translocation of Rho kinase and RhoA to the cell membrane has been shown to occur on agonist activation (16, 32); however; how Rho kinase might regulate the dephosphorylation of myosin in the core of the cell is unknown. It might involve dissociation of MYPT1 and PP1c on the membrane, thereby decreasing MLC phosphatase activity (33). Pharmacologic inhibitors of Rho kinase, such as fasudil, Y-27632, and HA-1077, block its activity by competing with the ATP-binding site on the enzyme (34–36). Rho kinase inhibition induces the relaxation of isolated segments of smooth muscle that are contracted by a variety of agonists (37, 38). In the intact animal, the pharmacologic inhibitors of Rho kinase have been shown to cause relaxation of smooth muscle in arteries, resulting in a blood pressure–lowering effect (27, 39).

Several recent studies suggest a role for additional regulators of MLC kinase and phosphatase (16, 23, 40). The small protein CPI-17, once phosphorylated, is able to bind to the catalytic subunit of MLC phosphatase (PP1c) to inhibit the enzyme's activity (41, 42). CPI-17 is expressed in mammalian vascular smooth muscle (43, 44), which also serves as a substrate for both Rho-kinase and PKC (44). Thus, Ca^{2+} sensitization may involve Rho-kinase–mediated and/or PKC-mediated phosphorylation of CPI-17 to increase MLC20 phosphorylation and prolong smooth muscle contraction (Fig. 3).

Calmodulin-dependent protein kinase II promotes smooth muscle relaxation by decreasing the sensitivity of MLC kinase for Ca^{2+}. Additionally, the 17-kDa protein telokin mediates Ca^{2+} desensitization through activation of myosin phosphatase in smooth muscle cells, which leads to a decreased MLC phosphorylation and smooth muscle contraction (45, 46; Fig. 3).

An important question facing the smooth muscle physiologist is: what is the link between receptor occupation

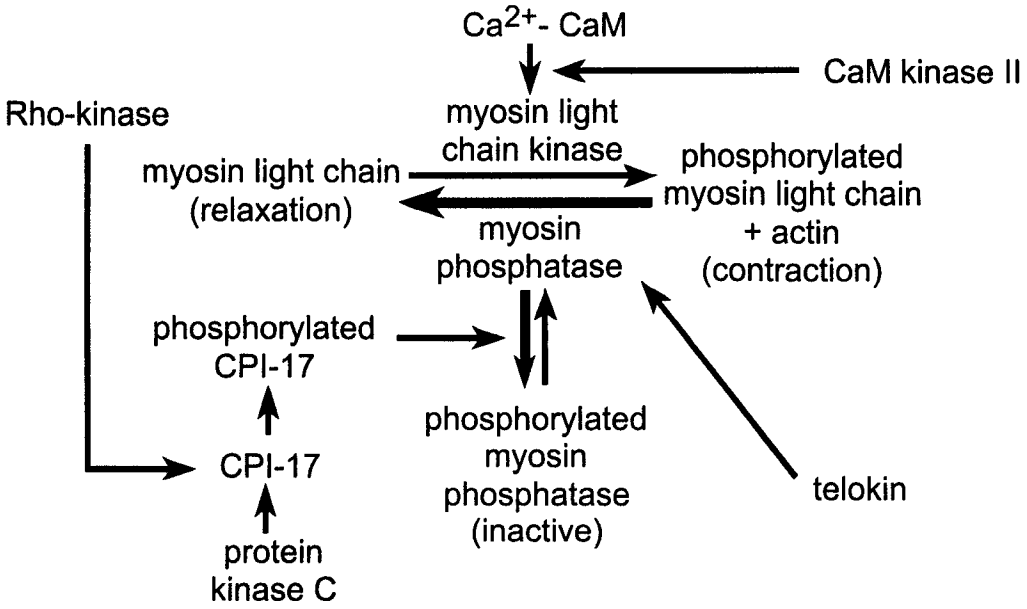

Figure 3. Additional regulators of MLC kinase and phosphatase. Phosphorylated CPI-17 is able to bind to the catalytic subunit of myosin phosphatase to inhibit the enzyme's activity, resulting in prolonged smooth muscle contraction. Both Rho-kinase and protein kinase C can activate CPI-17. Furthermore, calmodulin-dependent protein kinase II promotes smooth muscle cell relaxation by decreasing the sensitivity of MLC kinase for Ca^{2+}. The small protein telokin mediates Ca^{2+} desensitization through activation of myosin phosphatase, leading to a decrease in MLC phosphorylation and, therefore, promoting smooth muscle cell relaxation.

and activation of the Ca^{2+}-sensitizing activity of the RhoA/Rho kinase-signaling cascade? Currently, it is thought that receptors activate a heterotrimeric G protein that is coupled to RhoA/Rho kinase signaling *via* RhoGEFs. Because RhoGEFs facilitate the activation of RhoA, they regulate the duration and intensity of signaling *via* heterotrimeric G protein–receptor coupling. There are approximately 70 RhoGEFs in the human genome (47) and three RhoGEFs have been identified in smooth muscle: PDZ-RhoGEF, LARG (leukemia-associated RhoGEF), and p115-RhoGEF. Increased expression and/or activity of RhoGEF proteins could augment contractile activation of smooth muscle and, therefore, play a role in diseases such as hypertension and asthma, where an augmented response contributes to the pathophysiology. Recent observations from our laboratory showed an involvement of reactive oxygen species in Ca^{2+} sensitization by activation of RhoA and a subsequent increase in Rho kinase activity, providing a direct link between reactive oxygen species and the RhoA/Rho kinase signaling pathway in the pathogenesis of hypertension (48).

Other Mechanisms of Smooth Muscle Cell Contraction

It has been proposed that actin filament rearrangement or remodeling may play a role in smooth muscle cell contraction (49, 50). The hypothesis assumes that actin-myosin interactions cause the initial development of force and a "gluing" of actin filaments to attachment sites where they assemble in dense bodies to form a cytoskeletal scaffold that maintains tension in the absence of further cross-bridge cycling. Indeed, disruption of actin filaments has been associated with reduced agonist-induced Ca^{2+} signaling (51). Caldesmon and calponin are actin-associated proteins that have been shown to inhibit actomysin ATPase activity (52, 53). Caldesmon has proven capable of regulating force in smooth muscle (52). Furthermore, the inhibitory effect of caldesmon on the actomysin ATPase activity can be reversed by phosphorylation, indicating a regulation mechanism of its inhibitory action (54). Others have shown that both extracellular signal-related kinase (ERK) MAP kinase and p38 MAP kinase are activated during agonist-induced smooth muscle stimulation. The activation of ERK MAP kinase leads to the phosphorylation of caldesmon (55), while p38 MAP kinase increases the phosphorylation and activation of heat-shock protein 27 (HSP27; Ref. 56). Interestingly, HSP27 is a known regulator of actin polymerization (57), suggesting that actin rearrangement is an important event in the mechanism of force maintenance during smooth muscle contraction.

Other cytoskeletal proteins such as microtubules have been demonstrated to play a role in vascular smooth muscle contraction. Microtubule depolymerization was shown to enhance agonist-induced contraction of isolated aortic rings (58) and increased myogenic tone in isolated cremaster arterioles (59). Our group found that Rho-kinase activation facilitated this enhanced contraction (60, 61). Overall, these data indicate that cytoskeletal rearrangement is crucial during prolonged smooth muscle contraction and that these events might play an important role in arterial remodeling during vascular pathologies, such as hypertension.

Concluding Remarks

Layers of smooth muscle cells line the walls of various organs and tubes, and the contractile function of smooth muscle is under voluntary control. The contractile response is initiated by a rapid and transient increase in intracellular Ca^{2+} followed by a Ca^{2+}-calmodulin interaction to stimulate phosphorylation of the MLC. To enable force maintenance, a Ca^{2+} sensitization of the contractile proteins is signaled by the RhoA/Rho kinase pathway, in concert with other proteins like CPI-17, to inhibit the dephosphorylation of the MLC phosphatase. Understandably, removal of Ca^{2+} from the cytosol and stimulation of myosin phosphatase initiates the process of smooth muscle relaxation.

Alterations in the regulatory processes maintaining intracellular Ca^{2+} and MLC phosphorylation have been proposed as possible sites that contribute to the abnormal contractile events in smooth muscle cells of various organs and tissues. Increases in the activity of RhoA/Rho kinase signaling lead to increased contractile responses that may contribute to erectile dysfunction (62) in the penis and clitoris. Increased activity of the RhoA/Rho kinase signaling pathway may also contribute to augmented contraction or spastic behavior of smooth muscle in diseases such as hypertension, asthma, or atherosclerosis (27, 39, 63–66).

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