Vascular Smooth Muscle
A Review of the Molecular Basis of Contractility*

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In 1653, the English physician William Harvey published the first comprehensive study of blood vessels, De Motu Cordis. This classic treatise delineated the directions of blood flow, differences between veins and arteries, functions of venous “valves,” and anatomy of the systemic and pulmonary circulations. By the 19th century, the major layers constituting blood vessels were identified and often described in the analysis of pathological specimens, such as aneurysms. However, it has been only during the past 20 years that specific biochemical mechanisms modulating contractile protein interaction in vascular smooth muscle have been described and characterized both in vitro and in living muscle. Even more recently, the endothelium has been identified as an important endocrine modulator of vascular reactivity and growth. Simultaneously, converging disciplines involving molecular and cellular biology, as well as clinical studies of atherosclerosis, have brought to bear both increased attention and research on blood vessels. This increased interest and activity virtually ensure that the next decade of research will more fully explore the integrated function of the cells that compose the blood vessel; it is pathology of the vascular wall that leads to the constellation of problems that constitute ischemic heart disease.

This review focuses on the myogenic component of blood vessels: vascular smooth muscle. Particular emphasis is given to molecular mechanisms that regulate contractile properties of this tissue.

Vascular Myocyte Structure
The tone-generating capacity of vascular smooth muscle is dependent on the interaction between the major contractile proteins, actin and myosin. An analogy to the sliding-filament model of striated muscle contraction is made by most investigators of vascular contractility. In this model, the heads of myosin molecules, which are bundled together into thick filaments, undergo a cycle of high- and low-affinity binding to actin in a reaction driven by ATP hydrolysis. Tension generation occurs as a result of translocation of the heads of myosin, which are bound to actin, from an angle of 90° to 45° with respect to the long axis of actin- and myosin-containing filaments. The direction of force is determined by the polarity of these filaments. Thus, for smooth muscle cells to shorten or develop isometric tension, filaments of opposing polarity must extend from opposite ends of an anchoring structure. In striated muscle, the organization of thick (myosin) and thin (actin) filaments is highly ordered, and banded structures corresponding to various filamentous elements are readily observed under light microscopy. The organization of filaments in smooth muscle is less apparent, and an unambiguous model of the cytoskeleton remains to be elucidated.

In smooth muscle, thin filaments comprising actin, tropomyosin, and other proteins are attached to the cell membrane via membrane plaques. These structures contain several proteins including vinculin, metavinculin, α-actinin, and talin. Within the cytoplasm, thin filaments insert into fusiform dense bodies, which contain α-actinin and other unidentified proteins. Dense bodies are functionally analogous to the Z-lines of striated muscle and are sensitive to proteolysis. It has been suggested that dense bodies are held together in a three-dimensional network by intermediate filaments comprising the proteins, vimentin, and/or desmin. Most vascular intermediate filaments are composed of desmin, although gradients of desmin and vimentin are present in the human aorta with the relative proportion of vimentin increasing in a cranial-to-caudal direction. Another protein, filamin (MW, 260,000), crosslinks or bundles some actin filaments. It is likely that these bundles of filaments serve to maintain the shape of the smooth muscle cell. The organization of thin, thick, and intermediate filaments not only imparts to vas-
cular myocytes their characteristic, elongated, fusiform shape but also gives rise to the unusual cork-screw configuration of isolated myocytes that have been stimulated to contract in vitro.14

Contractile Mechanism: Regulation of Actin–Myosin Interaction

The contraction of vascular smooth muscle is regulated by Ca²⁺ in several ways. One important mechanism involves phosphorylation of the myosin molecule.15 Vascular myosin is composed of two heavy chains (MW, 200,000 and 204,000) and two sets of light-chain subunits (regulatory, or 20,000 Da, light chains (i.e., LC₂₀) and alkali, or 17,000 Da, (i.e., LC₁₇) light chains). Serine-19 of the LC₂₀ is phosphorylated by the enzyme myosin light-chain kinase (MLCK), which is activated by Ca²⁺ and the Ca²⁺-binding protein calmodulin.15 MLCK in vascular smooth muscle has a molecular weight of 160,000 and binds 1 mol calmodulin/mol enzyme.16 Phosphorylation of LC₂₀ is the signal that activates the cycling of cross-bridges and, hence, initiates contraction. Biochemical studies with subfragments of avian smooth muscle myosin in vitro have shown that LC₂₀ phosphorylation increases the rate constant of the rate-limiting step in the actomyosin ATPase cycle (i.e., inorganic phosphate release) by more than 1,000-fold.17 Moreover, studies with intact myosin molecules in vitro have shown that LC₂₀ phosphorylation induces major conformational changes in the molecule. In physiological salt solutions, myosin molecules tend to form hairpinlike structures with sedimentation coefficients of 10S. However, LC₂₀ phosphorylation induces the extended or 6S conformation of myosin which readily forms filamentous structures.18 Thus, phosphorylation of the LC₂₀ of vascular smooth muscle myosin not only may “switch on” actomyosin ATPase activity but also could facilitate the formation of thick filaments during development or in modulated phenotypes of smooth muscle cells.

The pivotal role of LC₂₀ phosphorylation in smooth muscle contraction has been demonstrated through the use of proteolytic fragments of MLCK, which are catalytically active in the absence of Ca²⁺ and calmodulin. In either chemically skinned fiber preparations or by direct microinjection into intact single cells,20 Ca²⁺-independent MLCK induces contraction in the absence of any change in Ca²⁺ concentration. Thus, it is concluded that although Ca²⁺ may modulate the contractile response through other mechanisms, activation of MLCK with subsequent phosphorylation of LC₂₀ is sufficient to induce contraction.

The state of phosphorylation of smooth muscle myosin is also regulated by the opposing action of myosin phosphatase and by modulation of MLCK activity. In vitro, MLCK can be phosphorylated by several protein kinases, including cyclic AMP (cAMP)–dependent protein kinase,21 cyclic GMP (cGMP)–dependent protein kinase,16,22 Ca²⁺/calmodulin-dependent protein kinase II,23 and protein kinase C.24 Several of the phosphorylation sites have been sequenced and assigned to the linear map of the molecule determined from the deduced amino acid sequence obtained from DNA cloning of the avian enzyme.25,26 For example, both cAMP-dependent protein kinase and Ca²⁺/calmodulin-dependent protein kinase II can phosphorylate a common serine residue (e.g., serine-512) in the calmodulin-binding domain of MLCK.23 Phosphorylation at this site diminishes the affinity of MLCK for calmodulin.16,21,23 Although it has not been established whether these particular kinases phosphorylate MLCK in intact cells or muscle, recent studies by Stull and colleagues27 have shown that MLCK is phosphorylated in tracheal smooth muscle in the course of tension development. Thus, modulation of MLCK activity via phosphorylation may prove to be an important mechanism of enzyme regulation.

Although several phosphoprotein phosphatases that dephosphorylate LC₂₀ have been isolated from vascular28 and nonvascular smooth muscles,29 none has manifested complete substrate specificity. Relatively specific phosphatases have been isolated from mammalian vascular30 and avian gizzard31 smooth muscles. Both are heterodimers comprising putative regulatory and catalytic subunits, although no known second-messenger system has been shown to modulate myosin dephosphorylation. Various inhibitors of the smooth muscle myosin phosphatase,32 including the toxin okadaic acid,33 induce contraction or prevent relaxation of skinned fiber preparations. These experiments illustrate the important role of myosin phosphatase in the reversal of LC₂₀ phosphorylation, a prerequisite for relaxation.

Latch State

In intact vascular smooth muscle, the extent of LC₂₀ phosphorylation correlates best with the velocity of muscle shortening.34 This mechanical parameter, which can be measured as the time required to redevelopment tension after abrupt shortening of a contracted muscle (i.e., slack test), is an estimate of the rate of actomyosin crossbridge cycling. The cycling of crossbridges is directly related to actomyosin ATPase activity as one cycle of a crossbridge consumes one molecule of ATP. The characteristic feature of vascular contraction in response to electrical stimulation or treatment with pharmacological agonists is an initial rapid increase in LC₂₀ phosphorylation and velocity of shortening followed by a gradual decline of both shortening velocity and levels of LC₂₀ phosphorylation to near-basal or unstimulated levels.35 Investigations with the fluorescent Ca²⁺ indicator fura-2 or the photoprotein aequorin have demonstrated that intracellular Ca²⁺ concentrations parallel the changes in LC₂₀ phosphorylation.36,37 Tension, on the other hand, increases more slowly to a peak level and is maintained while LC₂₀ phosphorylation and Ca²⁺ levels decrease. This mechanical state of tension maintenance with slowly cycling crossbridges has been called the “ latch state” by Hai and Murphy.34 The latch state is energy efficient because tension is
maintained at reduced ATP consumption due to the slowly cycling actomyosin crossbridges.

The molecular mechanisms that produce the latch state are unknown, although several theories have been proposed. Hai and Murphy hypothesized that dephosphorylation of LC20 while in a high-affinity binding conformation might alter the kinetics of crossbridge detachment. Slowing of crossbridge detachment would prolong high-affinity binding and, thus, tension. However, latch bridges eventually break, resulting in dissipation of tension unless there is a process of slow reformation. Thus, even in the latch state, relatively low but activating levels of Ca²⁺ and, hence, LC20 phosphorylation are required for the reformation of high-affinity crossbridges. The latch state is dynamic, unlike the rigor state of striated muscle, and represents a new steady state in which low levels of LC20 phosphorylation are requisite to form new latch bridges that in turn slowly decompose.

Other Proteins That May Modulate Contractility

More recently, two actin-binding proteins, caldesmon and calponin, have been identified in smooth muscle and proposed as possible thin filament–based regulatory proteins. Calponin (MW, 34,000) binds to both actin and tropomyosin, the major thin filament proteins, and inhibits actomyosin ATPase activity. This inhibition is reversed by Ca²⁺ and calmodulin as well as by phosphorylation of the protein by protein kinase C or Ca²⁺/calmodulin-dependent protein kinase II. It is interesting that a GTP-binding domain has been identified in the deduced amino acid sequence from a complementary DNA (cDNA)–encoding calponin. Recently, Somlyo and colleagues demonstrated GTP-dependent Ca²⁺ sensitivity of tension development in chemically skinned smooth muscle fibers. Although untested, this raises the possibility that calponin could modulate Ca²⁺ sensitivity of smooth muscle contraction.

Caldesmon from vascular smooth muscle (MW, 145,000) inhibits actomyosin ATPase activity and can be phosphorylated in vitro by several protein kinases. Moreover, it is a weak Ca²⁺/calmodulin binding protein. Recently, avian caldesmon has been cloned, and the amino acid sequence was deduced from cDNA. The carboxyl terminus of the protein contains sequences homologous to troponin-T as well as calmodulin-binding proteins. Thus, sequence data are consistent with the known actin-, calmodulin-, and tropomyosin-binding properties of this portion of the molecule. The larger amino terminal portion of caldesmon shows a repeating pattern consistent with an α-helix terminating in a globular head. A smaller form of caldesmon (MW, 80,000) has been identified in phenotypically modulated smooth muscle cells as well as many nonmuscle cells. Protein structural studies have shown that “little” caldesmon lacks much of the central α-helical domain of the larger form but possesses homologous amino and carboxyl domains. It is interesting that “little” caldesmon is phosphorylated in cells undergoing mitosis and the phosphoprotein dissociates from actin filaments. Because both actin and myosin are thought to be involved in such processes as cytokinesis and possibly chromosomal translocation during mitosis, it is conceivable that this isoenzyme is involved in modulating these processes by a conserved capacity for interaction with actomyosin.

Caldesmon enhances the binding of myosin subfragments to actin as a result of an interaction of the amino terminal portion of the molecule with the S2 portion of myosin rod. Recently, Adam et al. showed that several pharmacological agonists that induce the latch state in vascular smooth muscle also stimulate phosphorylation of caldesmon. It is possible that phosphorylated caldesmon, acting through actin and tropomyosin, slows crossbridge detachment by altering actomyosin binding or ATPase kinetics. Identification of the specific kinase that phosphorylates caldesmon and elucidation of its regulation will be essential to test the latter hypothesis. However, the actual function of caldesmon is not known.

Contractile Protein Isoforms and Vascular Contractility

A thorough evaluation of the importance of contractile protein isoforms in modulating vascular contractility remains incomplete. However, recent studies have demonstrated the presence of at least two isoforms of myosin heavy chain in smooth muscle, two forms of the LC20 and two forms of the LC17. DNA-cloning studies have shown that isoforms of the heavy chain and the alkali light chain arise from alternative RNA splicing of a single gene, whereas LC20 forms are most likely products of separate genes. In addition, vascular smooth muscle contains a unique α-actin that is the product of a gene distinct from those that encode the sarcomeric α-actins of cardiac and skeletal muscle.

The principal structural difference between myosin heavy chains in vascular smooth muscle resides in an extended 45–amino acid carboxyl terminus of the larger of the two isoforms. Although the functional significance of this is not known, embryological studies in the rat have shown that there is developmental regulation of heavy chain isoform expression; the 200-kDa form is found in fetal vasculature, whereas the 204-kDa form appears during the neonatal period. Vasculature of mature animals contains an approximate 1:1 mixture of the myosin heavy-chain isoforms. Because it is the carboxyl terminal region of the heavy chain that forms the α-helical rod structure of the myosin molecule, which is prerequisite for filament formation, it is likely that these isoforms possess inherent differences in filament-forming capacity or in regulation of thick filament assembly or disassembly. With regard to the latter, it has recently been shown that the 204-kDa isoform of myosin heavy chain can be phosphorylated in cultured smooth muscle cells as well as in vitro with specific protein kinases. Because smooth muscle cells can readily change phenotype as a physiological
response to growth factors, phosphorylation of vascular smooth muscle myosin heavy chain may be one of the molecular mechanisms for altering myosin filament assembly or disassembly.

The LC17 subunits of smooth muscle myosin lie in proximity to the ATP-binding site in the globular head or amino terminal domain of the molecule and appear to modulate the maximal rate of actomyosin ATPase activity. Thus, it has been suggested that differential expression of the LC17 isoforms may modulate crossbridge cycling rates. Although it has been shown that aorta contains the highest content of the basic isoform of LC17 and while more muscular arteries contain the least, no systematic study of changes in expression of these isoforms in pathophysiological states such as hypertension has been undertaken.

The functional significance of LC20 isoforms is not known. Moreover, although the presence of smooth muscle α-actin has proven to be a useful marker for modulated phenotypes of vascular muscle, the functional significance of the presence of this isoform of actin in smooth muscle has not been determined.

Excitation–Contraction Coupling

Ultimately, tension generation and maintenance in vascular muscle are dependent on those processes that modulate intracellular levels of Ca²⁺. The complex nature of cytoplasmic Ca²⁺ regulation is gradually unfolding, and many similarities to striated muscle are evident. However, it is important to recognize that vascular muscle contraction is tonic in nature; unlike cardiac and skeletal muscle, many blood vessels remain in a contracted state for long periods of time. The absence of either a refractory period or fatigue of smooth muscle is a characteristic suited to its specialized function. In addition, the force maintenance mechanism, the latch state, is energy efficient. As indicated in the preceding sections, maintenance of the latch state requires Ca²⁺; although levels are considerably lower than those measured during the early phase of muscle activation.

There are a finite number of mechanisms for Ca²⁺ entry into the cytoplasmic compartment of vascular cells, including transsarcolemmal entry through L- and T-type Ca²⁺ channels, Na⁺–Ca²⁺ exchange, and receptor-operated Ca²⁺ channels as well as the release of internal Ca²⁺ stores via ryanodine receptor–like Ca²⁺ channels of sarcoplasmic reticulum and release of Ca²⁺ from other internal sites via an inositol-1,4,5-triphosphate (IP₃)–activated channel. Our understanding of these mechanisms is evolving, and more refined models of excitation–contraction coupling, such as those for cardiac muscle, remain to be elaborated. Nevertheless, it is clear that the electrochemical gradient of ions in vascular cells supports a membrane potential of −40 to −55 mV in resting cells. The sarcolemma of vascular smooth muscle cells lacks tetrodotoxin-sensitive Na⁺ channels, and possesses an inherently lower permeability to K⁺ ions compared with cardiac muscle. In response to depolarization of smooth muscle cells by electrical stimulation, potassium chloride, or various neurotransmitters, the inward current is carried by Ca²⁺ ions through voltage-dependent Ca²⁺ channels that share certain functional characteristics with L-type Ca²⁺ channels of striated muscle. Although it appears clear that endogenous neurotransmitters such as norepinephrine increase the open time of L-type Ca²⁺ channels, intermediate steps between drug–receptor interaction and the enhancement of open time of the channel have not been thoroughly clarified. Moreover, mechanical factors such as stretch can depolarize smooth muscle cells. The latter may involve a stretch-activated cation channel similar to those recently identified in endothelial cells. Whatever the mechanism, relatively small changes in myocyte membrane potential can produce significant alterations in voltage-dependent Ca²⁺ channel activity.

Vascular smooth muscle contraction can be inhibited by the drug ryanodine, indicating that Ca²⁺-release channels of sarcoplasmic reticulum participate in Ca²⁺ mobilization during activation. These have not been thoroughly characterized in smooth muscle, but if similar to those in striated muscle, one important mechanism of excitation–contraction coupling may be Ca²⁺-induced Ca²⁺ release from sarcoplasmic reticulum. Recently, a cDNA encoding the Ca²⁺-release channel of skeletal muscle sarcoplasmic reticulum has been cloned and sequenced. This cDNA encodes a large protein (MW 400,000) that contains calmodulin-binding domains, several membrane-spanning domains, and a number of sequences that suggest potential phosphorylation sites for protein kinases. These results suggest not only additional mechanisms for regulation of sarcoplasmic reticulum Ca²⁺ release but also novel conceptual avenues for pharmacological modulation of muscle contractility. It is noteworthy, however, that the sarcoplasmic reticulum of vascular smooth muscle is moderately extensive in conduit vessels but less so in smaller muscular arteries. Thus, there is probably heterogeneity in the biochemical mechanisms regulating the sarcoplasmic reticulum component of excitation–contraction coupling in vascular smooth muscle.

The sarcoplasmic reticulum of smooth muscle also contains a Ca²⁺ pump. A cDNA corresponding to this pump has been cloned from uterine smooth muscle and encodes a protein that is structurally similar to the Ca²⁺ pump of slow twitch skeletal and cardiac muscle sarcoplasmic reticulum. The smooth muscle isoform of the Ca²⁺ pump appears to arise by the process of alternative RNA splicing and contains an additional 49 amino acids at the carboxyl terminus of the protein. Furukawa et al suggested that the affinity of the sarcoplasmic reticulum Ca²⁺ pump for Ca²⁺ is enhanced by agents that increase cGMP, whereas agents that activate protein kinase C increase the maximal activity of the pump. However,
the molecular mechanisms of these effects have not been established. In addition, vascular smooth muscle contains the sarcoplasmic reticulum protein phospholamban.74 This protein possesses Ca²⁺ channel activity in lipid bilayers75 and has been implicated in cAMP-mediated enhancement of Ca²⁺ uptake by sarcoplasmic reticulum.76 In cardiac muscle β-adrenergic agonists increase intracellular levels of cAMP leading to the activation of Ca²⁺-dependent protein kinase.76 The latter enzyme phosphorylates phospholamban, resulting in enhanced uptake of Ca²⁺ into the sarcoplasmic reticulum.76 A similar cascade may account for the relaxant effects of β₁-adrenergic agonists on vascular smooth muscle.

Several types of K⁺ channels have been detected in vascular myocytes, including Ca²⁺-activated K⁺ channels, delayed rectifier K⁺ channels, and ATP-sensitive K⁺ channels.62,77,78 Increases in K⁺ conductance as a result of activation of any of these channels leads to membrane hyperpolarization with subsequent inactivation of voltage-dependent Ca²⁺ channels79 and enhanced Ca²⁺ extrusion via Na⁺–Ca²⁺ exchange.80 In either case, the physiological response is relaxation or reduction of tone. The open time and frequency of opening of Ca²⁺-activated K⁺ channels are increased by 5'-GMP, the major metabolite of cGMP.81 Because nitrovasodilators, endothelial-derived relaxing factor (EDRF), and atrial natriuretic factor (ANF) increase levels of cGMP in vascular smooth muscle, they may also indirectly stimulate the Ca²⁺-activated K⁺ channel through conversion of cGMP to 5'-GMP.81 Ca²⁺-activated K⁺ channels can be inhibited by certain agents such as charybdotoxin and tetraethylammonium ions.82 ATP-sensitive K⁺ channels are activated by pharmacological agents such as pinacidil, nicorandil, minoxidil, diazoxide, and certain neuropeptides.83 On the other hand, ATP-sensitive K⁺ channels can be inhibited by sulfonamides such as glibenclamide.84 Thus, K⁺ channel subtypes not only have proven to be good targets for potential therapeutic agents but could also be implicated in the molecular pathophysiology of diseases such as hypertension.

Supporting the electrochemical gradient of ions are several ion pumps and exchangers that have been identified in vascular smooth muscle.68 For example, cloning of genes for Na⁺, K⁺, ATPase (i.e., the Na⁺–K⁺ pump) has elucidated the specific type of pump in vascular smooth muscle.85 Pharmacological induction of hypertension modulates gene-specific expression of the Na⁺–K⁺ pump in vascular tissue.86 In rats made hypertensive with angiotensin II or deoxycorticosterone, marked decreases in the amount of messenger RNA (mRNA) encoding the α₁-subunit of Na⁺, K⁺ ATPase as well as a corresponding increase in α₁-mRNA have been measured.86 The sarcolemmal of vascular smooth muscle also contains an outwardly directed Ca²⁺ pump87 as well as a Na⁺–H⁺ exchanger and a Na⁺–Ca²⁺ exchanger.88–90 Recent studies have shown that the sarcolemmal Ca²⁺ pump is stimulated by agents that increase cGMP, such as atrial natriuretic factor and nitrovasodilators.90 The specific mechanism may involve generation of phosphatidylinositol diphosphate, which has been shown to increase pump activity.90

The Na⁺–H⁺ exchanger of vascular smooth muscle has received considerable attention because its activity is modulated by contractile agonists and mitogens.91 Cellular alkalization is an early but not necessarily obligatory event in the steps leading to vascular myocyte proliferation.91 Inhibitors of the Na⁺–H⁺ exchanger, such as amiloride, possess anti-mitogenic activity.91 By extruding H⁺ and importing Na⁺, Na⁺–H⁺ exchange can indirectly increase intracellular levels of Ca²⁺. The latter occurs via exchange of the increased intracellular Na⁺ for Ca²⁺.92 Ca²⁺ entering the cell by means of the Na⁺–Ca²⁺ exchanger is available for contraction or cellular growth and proliferation. Recently, a cDNA encoding the human amiloride-sensitive Na⁺–H⁺ exchanger has been cloned and sequenced.93 The availability of this information should facilitate investigation of several problems, including the existence of tissue-specific isoforms, the molecular mechanisms of regulation, and the identification of protein domains responsible for ion and/or drug inhibitor binding.

Unlike skeletal muscle but similar to cardiac muscle, vascular myocytes are coupled via intercellular pathways of low electrical resistance.5 This process is mediated by connexons, or gap junctions, that are composed of a hexameric array of a 43,000-Da protein.94 A cDNA encoding the vascular gap junctional protein has been cloned and sequenced.95 The deduced amino acid sequence shows virtual identity with the cardiac connexon-43. Moreover, both cultured vascular cells of the modulated phenotype and vascular endothelial cells express RNA for connexon-43.95 Thus, intercellular coupling in cardiac and vascular tissues appears to be mediated by gap junctions of similar protein composition. Although incompletely studied, vascular gap junctions are likely to play an important role in coordinating the contraction of blood vessel segments.

**Signal Transduction**

Receptors for α- and β-adrenergic, muscarinic cholinergic, serotonin, and peptide hormones such as angiotensin are coupled to intracellular second-messenger systems through membrane-bound guanine nucleotide binding (G) proteins.96 The G-proteins consist of three subunits (α, β, and γ) with the α-subunit conferring specificity.96 For example, the β₁-adrenergic receptor is coupled to adenylate cyclase via Gs, where s is stimulatory. Many of the G-proteins and the genes encoding them have been identified through DNA-cloning techniques.97 In addition, cDNAs encoding the adrenergic and muscarinic cholinergic have been cloned and sequenced.98 On the other hand, few studies have been undertaken to thoroughly characterize molecular diversity of receptor subtypes and G-proteins in vascular
smooth muscle, although a large body of pharmacological characterization exists. Investigation of the phosphoinositide pathway and its regulation in vascular smooth muscle has established the molecular basis of "pharmacomechanical coupling" first described by Somlyo and Somlyo more than 20 years ago. Pharmacomechanical coupling is hormone- or drug-induced contraction of vascular smooth muscle in the absence of membrane depolarization. Hormone receptors, such as those for \( \alpha \)-adrenergic agonists and angiotensin II, are coupled to phosphatidylinositol hydrolysis via G-proteins. It has been speculated that the target of the activated \( \alpha \)-subunit of the G-protein complex is phospholipase C. This enzyme converts phosphatidylinositol-4,5-

**FIGURE 1.** Schematic of signal transduction mechanisms in vascular smooth muscle. \( \text{Ca}^{2+} \) mobilization is affected by many pathways in the vascular myocyte. Various hormones acting through receptors (R) coupled to G-proteins (G) activate membrane-bound phospholipase C (PLC), which converts L-\( \alpha \)-phosphatidyl inositol diphosphate (PIP\(_2\)) to diacylglycerol (DAG) and inositol-1,4,5-triphosphate (IP\(_3\)). The IP\(_3\) stimulates release of \( \text{Ca}^{2+} \) from sarcoplasmic reticulum (SR) via an IP\(_3\)-receptor protein (IP\(_3\)-R), which is a type of \( \text{Ca}^{2+} \) channel. Synthesis of DAG in the membrane and an increase in intracellular \( \text{Ca}^{2+} \) result in translocation of protein kinase C from cytoplasm (PK\(_C\)) to sarcolemma. PK\(_C\) phosphorylates many sarclemmal proteins and, via this mechanism, stimulates activity of the Na\(^+\)-K\(^+\) exchanger. For example, amiloride can indirectly modulate intracellular \( \text{Ca}^{2+} \) by inhibiting Na\(^+\)-H\(^+\) exchange or directly inhibit Na\(^+\)-Ca\(^{2+}\) exchange. Suramin, an antihelminthic drug, blocks the PDGF receptor. Pinacidil, a dihydropyridine, inhibits activity of L-type or voltage-dependent \( \text{Ca}^{2+} \) channels. Pinacidil activates ATP-sensitive \( K^{+} \) channels, resulting in membrane hyperpolarization; this leads to relaxation due to inactivation of voltage-dependent \( \text{Ca}^{2+} \) channels.

Diphosphate to diacylglycerol and IP\(_3\) stimulate the IP\(_3\)-responsive \( \text{Ca}^{2+} \) channel in the sarcoplasmic reticulum of vascular smooth muscle releases sarcoplasmic reticulum \( \text{Ca}^{2+} \) stores, resulting in contraction. Somlyo and colleagues have shown that addition of IP\(_3\) to either detergent- or enzymatically permeabilized vascular smooth muscle preparations results in \( \text{Ca}^{2+} \) release and contraction. Recently, an IP\(_3\) receptor protein has been purified from vascular smooth muscle. A cDNA encoding the cerebellar IP\(_3\) receptor has been cloned and sequenced. An interesting feature of the deduced amino acid sequence of the 130,000-Da protein is homology with the \( \text{Ca}^{2+} \)-release channel of skeletal muscle sarcoplasmic reticulum.
Diacylglycerol, in concert with Ca\(^{2+}\) ions and phosphatidylserine, activates the enzyme protein kinase C.\(^{106}\) In parallel with the IP\(_3\) pathway described above, protein kinase C is translocated from the cytoplasm of vascular smooth muscle cells to the sarcolemma in response to several pharmacological agents, including phorbol esters, which are direct activators of the enzyme.\(^{106}\) α-Adrenergic agonists angiotensin II, carbachol, and histamine, which increase Ca\(^{2+}\) and diacylglycerol, also stimulate processes such as Na\(^+\)–H\(^+\) and Na\(^+\)–Ca\(^{2+}\) exchange.\(^{107}\) This may be due to phosphorylation of the exchangers by protein kinase C. Quite recently, direct phosphorylation of the amiloride-sensitive Na\(^+\)–H\(^+\) antiporter has been demonstrated in living tissue.\(^{108}\) Protein kinase C may also enhance L-type Ca\(^{2+}\) channel activity via phosphorylation.\(^{109}\)

Although protein kinase C has been shown to phosphorylate several contractile or regulatory proteins in vitro, such as the myosin LC\(_{2b}\), myosin light-chain kinase, and caldesmon,\(^{110}\) there is little supporting evidence that such phosphorylation occurs in intact muscle.\(^{10}\)

**Conclusions**

Investigation of the mechanisms that modulate blood vessel tone comes at a time when there is both need and demand for novel approaches aimed at preventing or reducing the complications of diseases such as atherosclerosis, hypertension, and myocardial infarction. In the broadest sense, vascular tone is affected by a wide range of factors, including prostanoids,\(^{111}\) neuropeptides,\(^{112}\) endothelium-derived vasoactive substances,\(^{113}\) and growth factors.\(^{107}\) In view of both the complexity and multiplicity of factors that modulate vascular contractility, it is not surprising that specific molecular mechanisms underlying the etiology of hypertension remain unidentified in the majority of patients with this disease. A recent report of abnormalities in EDRF-dependent relaxation of vascular tone in some hypertensive patients is just one example of a new avenue to explore.\(^{114}\) Fertile areas for future investigation of molecular pathophysiology or potential targets for therapeutic agents will undoubtedly include ion channels of both the vascular sarcolemma and sarcoplasmic reticulum. The coexistence of several pathways for Ca\(^{2+}\) entry into the cytoplasmic compartment of the vascular myocyte as well as the unique mechanism of tone maintenance that requires relatively low levels of internal Ca\(^{2+}\) are well described but incompletely understood. Thus, although a host of agents that stimulate or inhibit vascular tone are available, the future should yield more specific substances as the models for excitation–contraction coupling and signal transduction for vascular muscle are refined.

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Vascular Smooth Muscle 389


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