The principle functions of arterial smooth muscle cells include contraction, relaxation, and growth. Calcium signaling mechanisms govern the main functions of arterial smooth muscle and trigger specific responses. Cytosolic calcium concentrations and calcium signals are finely tuned by intracellular sources such as the sarcoplasmic reticulum, calcium-binding proteins, and plasma membrane calcium-permeable channels. In hypertension, these mechanisms are substantially modified, promoting a hypercontractile state and arterial wall remodeling. In this review, we will discuss various elements that are central to intracellular calcium handling and signaling in arterial smooth muscle cells. Emphasis will be given to most recent discoveries of components that link intracellular calcium stores to plasma membrane calcium entry channels. In addition, we will propose a novel paradigm, suggesting that in hypertension, alarm signals generated by chronic innate immune system activation and transduced by pattern recognition receptors modulate calcium signaling mechanisms in arterial smooth muscle, promoting vascular dysfunction. Finally, new research directions in the context of calcium signaling in hypertension will be addressed.

**Arterial Smooth Muscle Contractile Mechanism and Calcium Handling**

Arterial smooth muscle contraction is regulated by receptor or mechanical activation of the contractile proteins actin and myosin. Changes in the membrane potential can also initiate contraction. The phosphorylation state of the light chain of myosin determines the contractile activity of arterial smooth muscle. Specifically, for contraction to occur, myosin light chain (MLC) kinase must phosphorylate Ser 19 of the 20-kDa regulatory MLC, enabling the interaction between myosin and actin. The cycling of the myosin cross-bridges with actin is promoted by energy released from ATP by myosin ATPase activity. In some arteries, MLC is in a phosphorylated state in the absence of any external stimuli (ie, vascular smooth muscle tone).

An increase in cytosolic calcium concentration is the trigger for vascular contraction. Hypertensive patients and animal models of hypertension exhibit augmented vascular contractile responses. A defect in the regulation of calcium and calcium signaling plays a role in hypertension-associated vascular dysfunction. Figure 1 illustrates a timeline of seminal scientific observations on calcium handling in arterial smooth muscle as it relates to hypertension. Abnormal calcium handling in arterial smooth muscle cells may involve increased calcium entry, increased calcium storage, and decreased calcium extrusion. Figure 2 illustrates components of Ca²⁺ signaling in vascular arterial smooth muscle that participate in the contractile process.

**Calcium-Dependent Contraction of Arterial Smooth Muscle**

In vivo, the intracellular concentration of calcium is several orders of magnitude lower than that in the extracellular fluid and exhibits dynamic changes throughout the cell attributable to calcium flux. Strategic spatial positioning of intracellular calcium transporters and targets as well as spatial relationship of ion pumps and channels in the plasma membrane determine calcium flux and the fluctuations in intracellular calcium concentrations. The calcium/calcmodulin complex activates MLC to phosphorylate the light chain of myosin. The increase in intracellular calcium concentration in response to specific stimuli occurs in a biphasic mode. In response to receptor-dependent or mechanical stimuli, intracellular calcium concentrations increase. The initial rapid increase in cytosolic calcium is attributable to calcium release from the sarcoplasmic reticulum, whereas the latter phase of calcium increase in the intracellular space is attributable to calcium entry from the extracellular space through plasma membrane calcium channels. Ligand–receptor interaction on the plasma membrane stimulates phospholipase C, which specifically catalyzes the formation of 2 second messengers, inositol triphosphate and diacylglycerol. Inositol triphosphate binds to receptors on the sarcoplasmic reticulum (inositol triphosphate receptor) and initiates the release of calcium into the cytosol. This calcium binds to calmodulin, causing conformational changes, which allow the interaction of the calcium/calmodulin complex with the MLC. These events subsequently lead to activation of MLC kinase and phosphorylation of the regulatory MLC. Diacylglycerol, along with calcium, activates protein kinase C, which phosphorylates specific target proteins.

**Symphony of Vascular Contraction**

**How Smooth Muscle Cells Lose Harmony to Signal Increased Vascular Resistance in Hypertension**

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**Hypertension** is available at http://hyper.ahajournals.org

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contractile proteins, regulatory proteins, channels, pumps, etc) and has contraction-promoting effects.

**Calcium Sensitization Mechanisms in Arterial Smooth Muscle**

The force of contraction induced by ligand–receptor interaction in arterial smooth muscle is greater than that predicted by the actual calcium concentration in the cells, indicating the existence of calcium-independent mechanisms. In addition to MLC kinase, MLC phosphatase has a regulatory role in MLC phosphorylation. Activation of MLC phosphatase (or myosin phosphatase) promotes arterial smooth muscle relaxation via dephosphorylation of light chain of myosin. Myosin phosphatase has 3 subunits: a 37-kDa protein phosphatase 1 catalytic subunit, a 20-kDa subunit, and a 110- to 130-kDa myosin phosphatase target subunit 1. The binding of protein phosphatase 1 catalytic subunit with the myosin phosphatase target subunit 1 inhibits the enzymatic activity of myosin phosphatase, allowing the light chain of myosin to remain phosphorylated, and thereby, promoting contraction.

The calcium-sensitizing effect has been ascribed to the activation of the small G protein, RhoA, and its downstream effector Rho kinase. RhoA cycles between an inactive GDP-bound and an active GTP-bound state in response to various stimuli. Three classes of regulatory proteins facilitate activation/inactivation of RhoA: (1) GTPase-activating proteins increase the intrinsic GTPase activity of RhoA to facilitate the return of the protein to its inactive state, (2) guanine nucleotide dissociation inhibitors sequester the GDP-bound form of RhoA and prevent its binding to the membrane, and (3) Rho-specific guanine nucleotide exchange factors enable the exchange of nucleotide to activate RhoA-GTP (active state). On activation, RhoA engages downstream effectors such as the enzyme Rho kinase, which phosphorylates the myosin-binding subunit of myosin phosphatase, inhibiting enzyme activity and promoting phosphorylation of MLC and contraction. Rho kinase is found in 2 isoforms: ROCK1 and ROCK2. Pharmacological inhibition of Rho kinase reduces blood pressure in experimental models of hypertension and induces relaxation in isolated arterial segments.

**Calcium Entry Mechanisms**

Most calcium mobilization within the arterial smooth muscle cells is modulated by calcium entry channels, such as voltage-operated channels (VOCs) and receptor-operated channels, and store-operated calcium entry (SOCE) mechanisms. Other pathways, including purinergic receptors, transient receptor membrane potential (TRP) channels, and Na+/Ca2+ exchanger, are also involved in calcium influx mechanisms, but they will not be the focus of the current review.

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**Timeline of observations on calcium handling in vascular smooth muscle**

<table>
<thead>
<tr>
<th>Year</th>
<th>Event</th>
</tr>
</thead>
<tbody>
<tr>
<td>1960</td>
<td>Role of Ca2+ in excitation-contraction coupling established (1-3)</td>
</tr>
<tr>
<td>1966</td>
<td>Arteries from hypertensive animals contain more Ca2+ and are more sensitive to the contractile effects of the cation (13,14)</td>
</tr>
<tr>
<td>1967-76</td>
<td>SR Ca2+ uptake is reduced in arteries from hypertensive rats (18-20)</td>
</tr>
<tr>
<td>1970</td>
<td>Ca2+-dependent rhythmic contractions in arteries from hypertensive rats are increased (15)</td>
</tr>
<tr>
<td>1978</td>
<td>Increased sensitivity to Ca2+ channel blockers in arteries from SHR (21)</td>
</tr>
<tr>
<td>1988</td>
<td>Transmembrane Ca2+ currents are increased in arteries from SHR (24)</td>
</tr>
<tr>
<td>1990</td>
<td>Increased vascular reactivity to Ca2+ channel agonist in SHRSP (27)</td>
</tr>
<tr>
<td>2003</td>
<td>Ca2+ sparks are less active in SHR smooth muscle resulting in less BK channel activity (31)</td>
</tr>
</tbody>
</table>

**Figure 1.** Timeline of observations on calcium handling in arterial smooth muscle related to hypertension (1960–2013). A clear role for calcium (Ca2+) as the activator for contraction in arterial smooth muscle was established in early 1960s. In the mid-1960s, observations by several investigators demonstrated that arteries from hypertensive animals are more sensitive to the contractile effects of the cation. During the following 5 decades, specifics about signaling cascades regulating intracellular Ca2+ in arterial smooth muscle of hypertensive animals and human subjects have been defined in greater detail. References for each observation are in parentheses. BK indicates large conductance Ca2+-activated potassium channel; SHR, spontaneously hypertensive rats; SHRSP, stroke-prone SHR; SR, sarcoplasmic reticulum; STIM, stromal interaction molecule; and TRPC, transient receptor potential cation channel, subfamily C.
Voltage-Operated Calcium Channels

The function of VOC is regulated by membrane potential. Membrane hyperpolarization leads to VOC closure, whereas depolarization results in VOC opening and promotes contraction.44 L-type VOCs regulate the majority of agonist-induced calcium entry and depolarize in response to stretch, contributing to agonist-induced vasoconstriction and development of myogenic response and vascular tone, respectively. Increases in intracellular calcium concentration following VOC activation result in stimulation of calcium release from intracellular sources (eg, sarcoplasmic reticulum) via activation of ryanodine receptors. This event leads to membrane depolarization, which further activates VOC and promotes calcium influx and constriction. Activation of cGMP-dependent protein kinase and further increases in intracellular calcium concentration act as negative feedback mechanisms that lead to the inhibition of VOC and cease of constriction.

Receptor-Operated Calcium Channels

Receptor-operated channels are defined as channels in which molecules are separate from the ligand-binding protein, are capable of activating a range of G protein–coupled receptors via circulating ligands, and are neither VOCs nor store-operated calcium channels.45 Following ligand binding, G protein–coupled receptors, which are coupled to phospholipase C, promote calcium release from the sarcoplasmic reticulum and protein kinase C–associated activation of MLC kinase.46,47 Members of the TRP channel family, including transient receptor potential cation channel, subfamily C, member 3 (TRPC3), TRPC6, and TRPC7, have been shown to be components of receptor-operated channels.48,49

Store-Operated Calcium Entry

The term SOCE describes a cellular mechanism by which depletion of calcium content in the endoplasmic/sarcoplasmic reticulum stimulates calcium influx via activation of plasma membrane calcium channels, replenishing intracellular calcium stores. In this mechanism, the endoplasmic/sarcoplasmic reticulum acts as a capacitor.50 In nonexcitable cells, SOCE is mediated by a highly calcium-sensitive, non–voltage-gated, inwardly rectifying current termed calcium release–activated calcium current (CRAC or \(I_{\text{CRAC}}\)). Earlier studies demonstrated the existence of a diffusible messenger, calcium influx factor. Calcium influx factor production is restricted in the endoplasmic reticulum, and its release is triggered by calcium depletion and a drop in intraluminal free calcium concentration. On its release from the endoplasmic reticulum, calcium influx factor induces displacement of inhibitory calmodulin from a plasma membrane variant of calcium-independent phospholipase A2 (iPLA2b), which transduces the signal to store-operated channels, leading to their opening and activation of SOCE. Another pathway that has been shown to contribute to SOCE activation is Na+/Ca2+ exchanger. When this exchanger is operating in the reverse mode, it contributes to intracellular calcium depletion and subsequent activation of SOCE.51

Stromal interaction molecule 1 (STIM1) was first identified as a calcium sensor.52,53 Currently, 2 members of the STIM family have been identified and characterized: STIM1 and STIM2. On calcium depletion, STIM1 and STIM2 translocate toward junctional areas of the endoplasmic reticulum known as puncta formations, which are in close proximity (10–25 nm) to the plasma membrane.54 STIM translocation is followed by activation of calcium release–activated calcium channels and SOCE in the plasma membrane. STIM2 plays a role in maintaining basal levels of calcium in the endoplasmic reticulum in the absence of agonist stimulation.55 In the presence of increasing agonist concentration, SOCE is mediated initially by STIM2 and incrementally by STIM1.56

Orai1 is a plasma membrane protein, an essential pore subunit of the CRAC channel,57,58 and the main means of
communication between STIM1 and plasma membrane. The association of STIM1 with Orai1 triggers calcium influx, increases $I_{\text{CRAC}}$, and is enhanced by thapsigargin, an inducer of calcium depletion in endoplasmic/sarcoplasmic reticulum. However, the role of direct conformational coupling between STIM1 and Orai1 in SOCE activation and puncta formation has been challenged by evidence showing that Orai1 is not necessary for the accumulation of STIM1 in puncta because STIM1 accumulates in the absence and presence of Orai1. These recent data suggest the existence of additional intermediate elements. Accordingly, STIM1 and Orai1 interact with TRPC channels, and TRPC channels may act as store-operated calcium channels in smooth muscle cells.

In contrast to nonexcitable cells, where SOCE is mediated by $I_{\text{CRAC}}$, nonselective cation channels, SOCE and agonist-induced contractile responses are mediated by nonselective cation channel. The presence of auxiliary mediators, such as iPLA2b and its lypospholipid products, is required for signal transduction from STIM1 to plasma membrane store-operated calcium channels. Activation of nonselective cation store-operated calcium channels promotes calcium entry not only by mediating SOCE but also by playing the role of a depolarizing trigger for a secondary activation of VOC. This allows for further increases in calcium influx augmenting vasoconstriction.

The interaction of STIM1 and Orai1 plays a critical role in vascular contraction in various vascular beds and vessel types (ie, aorta, coronary, and cerebral arteries). RNA silencing of STIM1 and Orai1 reduced phenylephrine- and urotensin II–induced contractions in transfected coronary artery rings, whereas depolarization-induced contractions were not affected by downregulation of either Orai1 or STIM1. Furthermore, thapsigargin-induced aortic contractions were attenuated following ex vivo treatment with Orai1 and STIM1 antibodies. Genetic manipulation of STIM1 further supports these data. Smooth muscle–targeted STIM1 knockout mice had a 26% reduction in $\alpha_1$-adrenergic–induced aortic contraction in the absence of any effect on depolarization-induced contractile responses.

Aortae from spontaneous hypertensive stroke-prone rats exhibited increased isometric force responses during the calcium-loading period on the depletion of intracellular calcium stores. Others have shown that the sarcoplasmic reticulum calcium store is larger in aortae from these animals attributable to augmented aortic contractility of hypertensive rats. Force generation in aortae from genetically hypertensive rats was greater in males compared with females, but this difference was abolished in the presence of antibodies against STIM1 and Orai1. Furthermore, expression of these proteins was greater in male compared with female hypertensive rats. These studies were performed mostly in conduit arteries, and the relevance of our findings to resistance vessels in the context of hypertension needs to be examined.

**Toll-Like Receptors, Arterial Smooth Muscle Dysfunction, and Regulation of Calcium Handling in Hypertension: A New Paradigm**

Long-term experimental efforts by several investigators, including our laboratory, have shed invaluable insight into the physiological mechanisms that are responsible for the pathogenesis of hypertension. Accordingly, previous studies have demonstrated evidence in support of renocentric, neurocentric, and vasculocentric views of the cause of hypertension. These views need not be mutually exclusive. Most recently, low-grade inflammation and activation of the adaptive arm of the immune system have been implicated in hypertension, offering a potential link among the previously tested hypotheses and a new explanation for the multisystem effects of hypertension.

Recent studies show that host-derived molecules released to the extracellular space attributable to cell injury and death (damage-associated molecular patterns (DAMPs)) can trigger an inflammatory response via activation of the innate immune system. DAMPs include extracellular matrix components, plasma membrane, nuclear, and cytosolic proteins, and elements of damaged/fragmented organelles. Similar to pathogen-associated molecular patterns, DAMPs stimulate pattern recognition receptors of the immune system, such as the Toll-like receptors (TLRs), eliciting an inflammatory response.

We recently reported that TLR4 was upregulated in resistance arteries of spontaneous hypertensive rats and that TLR4-augmented activation contributed to increased contractile responses to norepinephrine and to elevated blood pressure levels in this rat model of hypertension. The cellular mechanism for these events was related to a cyclooxygenase

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**Figure 3. Damage-associated molecular patterns (DAMPs) and hypertension.** We hypothesize that in the prehypertensive state, necrotic cell injury during hypoxic and ischemic events leads to the local release of DAMPs. DAMPs activate Toll-like receptors (TLRs) in the arterial smooth muscle cell leading to disturbed $Ca^{2+}$ signaling. This altered $Ca^{2+}$ pattern leads to increased vasoconstriction and reduced vasodilation contributing to overt hypertension. In addition, activation of TLRs leads to a generalized inflammatory state, characteristic of hypertension. Considerable evidence indicates that activation of TLRs in the kidney and central (CNS) and sympathetic nervous system contribute to hypertension. Thus, activation of the innate immune response provides a common pathway to explain renocentric, neurocentric, and vasculocentric views of the cause of hypertension.
smooth muscle cells and deletion of NRP2 increased contractile responses of bladder smooth muscle. However, the presence and functional importance of NRP2 has not been investigated in the vasculature of hypertensive animals or subjects.

**Guanyl Cyclase or Nucleotidyl Cyclase**

Most studies on isolated arteries from hypertensive animals and humans indicate that sodium nitroprusside–induced relaxation does not differ from normotensive values. This has been interpreted to indicate that soluble guanylyl cyclase (sGC) activity is unchanged in hypertension. However, the role of guanylyl cyclase in vasoconstrictor events may need to be re-evaluated. Chan et al88 observed that hypoxia-induced contraction of porcine coronary arteries was inhibited by inhibition of sGC. Hypoxia also caused contraction in arteries treated with exogenous cyclic inosine 3′,5′-monophosphate but not cGMP.89 Using liquid chromatography–mass spectrometry, an increased level of cyclic inosine 3′,5′-monophosphate level was measured in arteries exposed to hypoxia. Altitude-induced systemic hypertension is related to hypoxia, and it may be that second messengers in vascular arterial smooth muscle generated by a dysregulated sGC contribute to an exaggerated vasoconstriction. A recent study by Beste et al90 demonstrates that sGC has a broader activity and may more correctly be termed nucleotidyl cyclase. They observed that purified recombinant rat sGC was capable of synthesizing 7 cyclic purine and pyrimidine nucleotides. Study of the specificity of nucleotidyl cyclase activity in arteries from hypertensive animals may identify unique contractile signaling molecules related to this enzyme.

**Conclusions/Perspectives**

Recent evidence from our laboratory supports that in hypertension, elevated levels of DAMPs activate TLRs, which then signal directly or through cells of the adaptive immune response to elicit an inflammatory process in organs and systems that regulate blood pressure (Figure 3).80–82

**Future Research Directions: Promising Therapeutic Targets in Hypertension**

**Fibrocytes**

From a traditional viewpoint, resident or adventitial fibroblasts have been thought to be activated by proinflammatory stimuli to proliferate and migrate to sites of vascular injury where they secrete collagen.81 However, recent work describes an important role for fibrocytes, cells that are implicated in chronic inflammation, fibrosis, and wound healing. These cells are derived from bone marrow and are present in atherosclerotic lesions. They have inflammatory features of macrophages and vascular remodeling properties of fibroblasts. In addition, fibrocytes develop α-actin expression and a contractile phenotype in tissue culture. Keeley et al84 observed increased circulating levels of fibrocytes in patients with hypertension, and there was a strong correlation between left ventricular mass index and fibrocyte number (total and activated). Circulating fibrocytes are also increased in patients with pulmonary hypertension and are associated with remodeling of pulmonary vessels. In the cochlea, elevation of calcium in fibrocytes surrounding regulatory vessels of the spiral ligament results in propagation of a calcium signal in neighboring vascular cells.85 Fibrocytes may play an important role in vascular remodeling in systemic hypertension, and they may contribute to disturbances in cellular calcium handling in the vascular wall.

**Neuropilin**

Neuropilins (NRPs) are transmembrane glycoprotein receptors for class III semaphorins and vascular endothelial growth factor family members, which together with coreceptor plexins are involved in regulation of angiogenesis and axonal guidance.86 It was recently revealed that the adult expression of NRP2 is enriched in smooth muscle, where it mediates cytoskeletal rearrangement and a negative regulation of the Rho/ROCK pathway. Thus, NRP2 activation via semaphorin stimulation decreased active RhoA and phosphorylation of MLC in arterial

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**Disclosures**

None.
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