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Modeling Ca^{2+} Signaling in the Microcirculation: Intercellular Communication and Vasoreactivity

Adam Kapela, Sridevi Nagaraja, Jaimit Parikh, and Nikolaos M. Tsoukias*

Department of Biomedical Engineering, Florida International University, Miami, FL

Abstract

A network of intracellular signaling pathways and complex intercellular interactions regulate calcium mobilization in vascular cells, arteriolar tone, and blood flow. Different endothelium-derived vasoreactive factors have been identified and the importance of myoendothelial communication in vasoreactivity is now well appreciated. The ability of many vascular networks to conduct signals upstream also is established. This phenomenon is critical for both short-term changes in blood perfusion as well as long-term adaptations of a vascular network. In addition, in a phenomenon termed *vasomotion*, arterioles often exhibit spontaneous oscillations in diameter. This is thought to improve tissue oxygenation and enhance blood flow. Experimentation has begun to reveal important aspects of the regulatory machinery and the significance of these phenomena for the regulation of local perfusion and oxygenation. Mathematical modeling can assist in elucidating the complex signaling mechanisms that participate in these phenomena. This review highlights some of the important experimental studies and relevant mathematical models that provide the current understanding of these mechanisms in vasoreactivity.

Keywords

vascular tone; smooth muscle; endothelium; vasomotion; spreading responses

I. INTRODUCTION

Calcium (Ca^{2+}) is a universal signaling molecule, and in the microcirculation it plays a central role in important physiological functions including the regulation of vascular tone. An elaborate signaling network exists that regulates Ca^{2+} concentrations in the vascular wall. This network includes intracellular signaling as well as intercellular communication with paracrine factors or diffusion of species through gap junctions. Signaling between vascular cells affects Ca^{2+} levels and coordinates vessel function. The importance of myoendothelial communication is now well-appreciated and continuing investigations reveal important aspects of how the 2 cell layers interact. Recent evidence, for example, suggests the presence of localized signaling machinery for Ca^{2+} control in specialized cellular extensions between the endothelial cell (EC) and the smooth muscle cell (SMC) (often referred to as myoendothelial projections).¹ In addition to the local signaling between the 2 cell layers, focal stimulation in some vasculatures leads to spreading Ca^{2+} and vasomotor responses along the vascular tree, and this ability of vessels to conduct signals is critical for both the rapid and long-term coordination of the vascular network.² Also, synchronization of Ca^{2+} oscillations between many cells in the vascular wall causes

coordinated rhythmic oscillations in vessel diameter (i.e., vasomotion), which plays an important role in the regulation of tissue perfusion and oxygenation.³ Experiments continuously provide new insights about this elaborate network and the mechanisms that regulate vascular function. Mathematical modeling offers an alternative tool for the analysis of this complex signaling system.

Significant progress has been made in the development of comprehensive models of Ca^{2+} dynamics for some systems such as in cardiac myocytes.^{4–8} Often these models are integrated with descriptions for membrane electrophysiology, cell mechanics, and metabolic and signal-transduction pathways, and multicellular/multiscale models have emerged for the heart.^{9–14} These models are capable of describing function at the tissue level while integrating mechanisms at the subcellular/molecular level and have been utilized to investigate physiological function as well as disease states.^{15–18} Although Ca^{2+} signaling in various cell types shows many common features and motifs,¹⁹ there are significant differences in Ca^{2+} mobilization between cardiac and vascular cells.^{20–25} Recently, similar attempts have been made in the modeling of the vasculature and cellular models of Ca^{2+} dynamics have emerged for vascular cells. These single-cell models can form the basis for integrated models of the vasculature.

This review focuses on recent efforts in the development of mathematical models to describe vascular Ca^{2+} dynamics in single cells and in multicellular vascular segments. These models investigate intercellular communication in the vessel wall and the mechanisms that regulate vasoreactivity in the microcirculation.

II. VASCULAR CELL MODELS

A series of Ca^{2+} dynamics models for vascular cells have been presented and recently reviewed.²⁶ The level of detail in these models ranges from minimal models with phenomenologic descriptions to models with detailed descriptions of transmembrane currents. Models exist that incorporate biophysical descriptions for the signaling pathways leading to or affected by Ca^{2+} mobilization. Published models also differ in the level of detail in spatial organization, from models with a single cytosolic compartment with single or multiple internal stores to models with cytosolic subcompartments and spatial gradients.

SMCs differ from the electrically nonexcitable ECs in channel and receptor composition and in their contribution to the various Ca^{2+} mobilization pathways. Though L-type Ca^{2+} channels and calcium-induced calcium release (CICR) from ryanodine receptor (RyR)–sensitive stores play a predominant role in myocytes, the phosphatidylinositol pathway and capacitative calcium entry (CCE) have a significant role in ECs. Furthermore, the type and density of membrane ion channels exhibit significant differences among species and tissues and probably change with the size of the vessel even within the same vascular bed. This heterogeneity suggests a need for cell- and vessel-specific models of Ca^{2+} signaling and dynamics in the vasculature.

II.A. Endothelial Cell Models

The first endothelial cell models were based on earlier generic models of calcium dynamics in electrically nonexcitable cells.^{27–30} Early models started with simple descriptions of Ca^{2+} handling components and few transmembrane currents. Winston and coworkers³¹ studied the effects of mechanical strain on cultured bovine pulmonary artery endothelial cells. Ca^{2+} dynamics and electrical activity of vascular ECs first were examined in a model introduced by Wong and Klassen.^{32,33}

The first in-depth model of calcium dynamics was presented by Wiesner and coworkers³⁴ for human umbilical vein endothelial cells. Their model incorporated detailed thrombin receptor kinetics, as described by Vu et al.³⁵ The model included most of the major calcium mobilization pathways including CICR, CCE, inositol (1,4,5)-trisphosphate receptor (IP₃R)-dependent store Ca²⁺ release, and Ca²⁺ buffering. The model described IP₃ and Ca²⁺ dynamics and included separate mass balances for cytosolic, store, and buffered Ca²⁺. Schuster and coworkers³⁶ developed a model to simulate changes in membrane electrical activity following bradykinin stimulation of coronary artery ECs. Experimental data provided empirical correlations for the cytosolic calcium and IP₃ changes after stimulation. The focus of the model was to predict K⁺ currents and membrane potential (V_m) changes in the presence and absence of extracellular Ca²⁺.

In a recent study, we presented a detailed EC model that integrates both EC Ca²⁺ dynamics and plasmalemmal electrical activity to investigate EC responses to various stimulatory conditions and the relationship between Ca²⁺ and V_m.³⁷ The model describes most of the major membrane channels and pumps present in EC of rat mesenteric arteries (RMAs). The plasma membrane includes kinetic descriptions for nonselective cation (NSC) channels, store-operated cation channels, small (SK_{Ca}) and intermediate (IK_{Ca}) conductance calcium-activated K⁺ channels, inward rectifier K⁺ channels (K_{ir}), volume regulated anion channels (VRAC), calcium-activated Cl channels, Na⁺-Ca²⁺ exchanger (NCX), and Na⁺-K⁺-Cl cotransporter and Na⁺-K⁺-adenosine triphosphatase (ATPase) pump. It also includes intracellular Ca²⁺ handling components such as IP₃ receptor, sarco/endoplasmic reticulum, Ca²⁺-ATPase (SERCA) and plasma membrane Ca²⁺-ATPase pumps. For the first time, we integrated balances for the major ionic species (i.e., K⁺, Cl⁻, Na⁺) in addition to the balances for cytosolic, store, and buffered Ca²⁺ and IP₃ and the Hodgkin-Huxley-type formalism for the V_m. The model reproduces experimentally observed Ca²⁺ transients during agonist stimulation.

To date, the majority of the EC models were compartmental in nature. Recently, Hong et al.³⁸ developed a 2-dimensional finite element model that incorporated intracellular resolution and spatial concentration gradients. This study highlighted the need for subcellular resolution in future models of EC Ca²⁺ dynamics and signaling.

II.B. Smooth Muscle Cell Models

Modeling of Ca²⁺ dynamics in SMCs has received more attention than in ECs. Early attempts include the generic models of Wong and Klassen,^{39,40} the oscillatory model of Gonzalez-Fernandez and Ermentrout,⁴¹ the IP₃-dependent Ca²⁺ release model in A7r5 cells of Fink and coworkers,⁴² and the norepinephrine (NE) diffusion model of Bennett and coworkers.⁴³ The complexity of Ca²⁺ mobilization and the many components affecting a membrane's electrical activity prompted Parthimos and coworkers⁴⁴ to develop a minimal model of an SMC. The reduced number of parameters made their model attractive for subsequent studies of vascular signaling.^{45–47} Model simulations showed chaotic behavior in Ca²⁺ levels as a result of the nonlinear interaction between a membrane oscillator and an intracellular store calcium oscillator. The model was able to reproduce behavior seen under various experimental conditions and pharmacological interventions.^{48,49}

A different approach was followed by Yang and coworkers.⁵⁰ Their aim was to develop a detailed model that will incorporate all the known significant components of the plasma membrane in SMCs for rat cerebrovascular arteries. Their study presents perhaps the first attempt at a detailed, tissue-specific vascular model of Ca²⁺ dynamics. The ability of this study to simulate macroscale responses prompted the development of subsequent models that followed a similar, detailed, tissue-specific modeling approach with significant effort to obtain current descriptions based on electrophysiological recordings.^{51,52} Combined, these

detailed models include descriptions for important transmembrane currents such as voltage-operated Ca^{2+} channels (VOCCs), voltage-dependent K^{+} channels (K_v), large conductance Ca^{2+} activated K^{+} channels (BK_{Ca}), K_{ir} channels, stress-activated NSC channels, Cl^{-} channels, NCX , Na^{+} - K^{+} -ATPase, and Ca^{2+} -ATPase pumps. Descriptions for Ca^{2+} , Na^{+} , Cl^{-} , K^{+} , and IP_3 balance have been presented. IP_3 - and Ca^{2+} -sensitive intracellular stores also have been implemented that exhibit CICR and Ca^{2+} sequestrations through SERCA. Descriptions for the α_1 -adrenoceptor and nitric oxide (NO)/cyclic guanosine monophosphate (cGMP) signaling pathways have been utilized as well as formulations for the diacylglycerol (DAG) dependence of NSC activation as a mechanism for sustained cell depolarization following adrenoceptor stimulation.^{52,53} The Ca^{2+} models have been integrated with biomechanics models capable of simulating diameter responses to Ca^{2+} mobilization.^{54,55} In one of the models,⁵¹ an intracellular subcompartment accounts for subcellular heterogeneity and the presence of subplasmalemmal microdomains. Elements from the minimal model of Parthimos⁴⁴ and the detailed approach of Yang⁵⁰ have been incorporated in an SMC model presented by Jacobsen and coworkers.⁴⁷ A significant feature of this model is that it abandons the compartmental approach of the previous modeling attempts and provides a description for cytosolic Ca^{2+} with spatial heterogeneity. This was accomplished by incorporating diffusion of Ca^{2+} within the cytosol in the longitudinal direction of the cell.

Through this process, it has become obvious that detailed models of Ca^{2+} dynamics offer many advantages in investigations of Ca^{2+} signaling. These models, however, have to deal with significant obstacles/limitations in the form of a considerable number of unknown parameters, the absence of tissue-specific quantitative data (particularly electrophysiological recordings for transmembrane currents), the structure and function of the intracellular stores, and the spatial distribution of receptors, channels, and pumps, to name a few. ECs and SMCs regulate Ca^{2+} entry and V_m by expressing an abundant and diverse collection of ion channels. In addition, considering the balance of the other major intracellular ionic species (i.e., Na^{+} , K^{+} , Cl^{-}) is essential to modeling both single-cell electrophysiology and cell-to-cell electrochemical coupling and communication. Finally, the models need to integrate relevant signal transduction pathways. Consequently, there is still a need to build upon previous modeling work to develop cellular models that will assist in the investigations of vascular tone regulation in health and disease.

III. MYOENDOTHELIAL COMMUNICATION

Myoendothelial communication plays an important role in modulating arterial tone and blood flow. Complex bidirectional pathways between the EC and SMC form tightly controlled feedback loops that regulate vascular Ca^{2+} dynamics and vasoreactivity. These pathways are often found disrupted in diseases related to altered vasoreactivity such as in hypertension.^{56–59} Many experimental studies have been conducted to elucidate myoendothelial signaling pathways, but only few theoretical models have addressed this subject.

III.A. Endothelium Derived Signals

The mechanisms of EC control of SMC tone has been a topic of intense investigation and a number of endothelium derived factors have been identified. The endothelium-derived relaxing factor (EDRF), now recognized as NO, has been well-characterized,^{60,61} whereas other pathways such as the endothelium-derived hyperpolarizing factor (EDHF), identified around the same time,⁶² still remain controversial. Ca^{2+} increase in ECs often leads to the activation of many of these endothelium-derived vasoreactive species. NO signaling seems to play a major role in vasoreactivity of larger arteries whereas EDHF-related responses are more dominant in small resistance arteries.^{63,64} Several factors have been proposed for the

role of EDHF, including electrical signaling through myoendothelial gap junctions (MEGJs), extracellular accumulation of K^+ ions, epoxyeicosatrienoic acids, hydrogen peroxide (H_2O_2), and C-type natriuretic peptide, among others, and have been reviewed by Sandow⁶⁵ and Edwards et al.⁶⁶ In most preparations the initial step for EDHF action depends on the opening of IK_{Ca} and SK_{Ca} channels.^{1,67–73}

MEGJs often play a key role in EDHF vasorelaxation. MEGJ channels are composed of proteins called *connexins* that physically connect the cytoplasm of the EC and SMC. This allows for the electrical and chemical coupling between the 2 cell layers, as shown by membrane potential and dye transfer studies.^{74,75} Their presence has been documented in many tissues that exhibit EDHF responses.^{59,76,77} Blockade or the absence of MEGJs significantly reduces and in some cases abolishes EDHF-dependent SMC hyperpolarization in many arterial beds.^{68,78–84} This evidence suggests that Ca^{2+} elevation in ECs and the resulting hyperpolarization through the activation of IK_{Ca}/SK_{Ca} channels may be transmitted through the gap junction to the smooth muscle to induce arteriolar relaxation.

The reduction or abolishment of EDHF action seen after blockade of IK_{Ca} and SK_{Ca} channels also could be attributed to the role for K^+ as an EDHF.^{68,72,85} Stimulation of the endothelium can increase K^+ efflux from IK_{Ca} and SK_{Ca} channels and increase extracellular concentration of K^+ in the interstitial space.⁷² This K^+ accumulation might be sufficient to activate certain isoforms of Na^+/K^+ ATPases and/or K_{ir} channels on the SMC membrane leading to SMC hyperpolarization.^{72,86} Mathematical modeling can make predictions for the contribution of electrical coupling through gap junctions and/or extracellular K^+ accumulation in vasoreactivity and elucidate the conditions under which these mechanisms become important.

III.B. Myoendothelial Feedback

Endothelium-derived signals also can be evoked during SMC stimulation. This allows the vessel to generate a feedback response that modulates arterial constriction. SMCs can be stimulated by a range of vasoconstrictors that activate the phospholipase C (PLC) pathway, causing an increase in SMC Ca^{2+} and IP_3 along with SMC depolarization. This depolarization has been observed to spread to the EC via gap junctions.⁷⁴ EC depolarization will tend to reduce cytosolic Ca^{2+} levels by reducing electrochemical gradient for Ca^{2+} entry, but Ca^{2+} mobilization in EC after SMC stimulation has been reported.⁸⁷ Induced by SMC stimulation, elevated EC Ca^{2+} can activate EDHF and EDRF pathways and form feedback loops that moderate SMC constriction. This myoendothelial feedback may play an important role in the regulation of vasoreactivity, but many aspects of this mechanism remain unresolved.

Some studies have reported global EC Ca^{2+} events after SMC stimulation^{87–89} that are inhibited by gap junction uncouplers, whereas others have provided evidence for discrete and confined Ca^{2+} mobilization in EC.^{90–92} Some authors have suggested Ca^{2+} diffusion through MEGJs as an important mediator of the EC feedback response.^{87,89} Because gap junctions allow for the transport of small, second-messenger molecules such as IP_3 in addition to Ca^{2+} , it is possible for IP_3 to travel down its concentration gradient into the EC and cause Ca^{2+} release from Ca^{2+} stores in the endothelium. Certain studies have provided evidence that IP_3 diffusion plays an important role in generating the feedback.^{88,93,94} Ca^{2+} movement inside the cell is restricted by extensive buffering. IP_3 , on the other hand, is rapidly degraded by phosphatases present in the cytoplasm.^{42,93,95} At this stage, the relative contribution of the 2 second messengers in this feedback response has not been resolved. Furthermore, stimulating the endothelium to induce Ca^{2+} transients does not yield a corresponding increase in SMC Ca^{2+} . Differences in IP_3 kinetics and IP_3R expression

between the 2 cell types may explain the observed unidirectionality of IP₃ signaling between the 2 cell layers.⁹³

III.C. Localized Signals

Local EC Ca²⁺ mobilization after SMC stimulation has been shown to be present inside and in the vicinity of structures called myoendothelial projections or microprojections (MPs). MPs are cellular extensions from EC and/or SMC that come in close contact in the internal elastic lamina^{96,97} (Fig. 1A). Recent information regarding the functional characteristics of these structures suggests that they can play an important role as a local regulatory module in myoendothelial communication. The presence of MPs has been documented in many rat arteries such as the saphenous, mesenteric, femoral, and caudal and mouse cremaster arterioles using electron microscopy techniques.^{59,78,82,83,97–99} MPs are known to occur in different numbers and sizes, with variation in vascular beds, age, species, sex, and disease states. However, a general observation is an increase in occurrence of MPs with a decrease in vessel diameter. Highest numbers of MPs often appear in small arteries and arterioles, which are also the main site of EDHF activity.^{97,98} Most MEGJs are located on top of these projections, although almost 50% of the projections do not have gap junctions on them.^{59,98,99} Immunohistochemical labeling studies show a heavy localization of IP₃R (Fig. 1B) and IK_{Ca} channels (Fig. 1C) in MPs.^{1,68,90,94,100} Colocalization of Na⁺/K⁺-ATPases with IK_{Ca} channels around the MP has been shown.⁶⁸ This sort of arrangement creates high IP₃R density in a small volume (<1% of EC volume), which may be activated even with a small amount of IP₃ entering during SMC stimulation and cause local increase in EC Ca²⁺ by release from ER stores (Fig. 1D, 1 and 2). Ca²⁺ increases can cause nearby IK_{Ca} channels to open, leading to EC hyperpolarization, which spreads to SMC via MEGJs (Fig. 1D, 3 through 5). The localization of IP₃Rs and IK_{Ca} channels in the MP suggests that a local Ca²⁺ transient in MP rather than a global Ca²⁺ increase may be sufficient to provide feedback to SMC. In addition, these structures may provide confined, small-volume extracellular space needed for K⁺ accumulation in EDHF. Current experimental efforts are directed toward characterizing local Ca²⁺ events and the amount of hyperpolarizing feedback, as well as the localization of receptors and channels in the vicinity of these structures. Mathematical models can assist in the investigations of these mechanisms and test under what conditions some of the proposed hypotheses can have a significant impact in vascular Ca²⁺ regulation.

III.C. Models

Most electrophysiological models of vascular ECs^{34,36,37} and SMCs^{44,47,51,52,101,102} have examined V_m and Ca²⁺ dynamics in isolated cells. Brace and Anderson¹⁰³ presented a model of membrane potential changes in vascular SMCs to examine the effect of extracellular K⁺ concentration. Model simulations showed that extracellular K⁺ has the potential to act as a hyperpolarizing factor through activation of Na⁺-K⁺-ATPase. On the other hand, Schuster et al.³⁶ made theoretical calculations of extracellular K⁺ changes based on the EC IK_{Ca} and SK_{Ca} channel currents during bradykinin stimulation of cultured ECs. The model predicted that a significant increase in extracellular K⁺ is possible (10–70 mM), depending on the assumed thickness of the internal elastic lamina (1–6 μm). More recent studies have incorporated myoendothelial coupling and accounted for the effect of EC-SMC interaction in vasomotion,¹⁰⁴ conducted responses,^{105,106} and myoendothelial feedback.¹⁰⁷

We have recently presented a theoretical analysis of myoendothelial communication¹⁰⁷ by integrating an isolated EC³⁷ and an SMC⁵² model. The cells were coupled by nonselective gap junctions (permeable to Ca²⁺, K⁺, Na⁺ and Cl[−] ions and to IP₃) and the diffusion of NO (Fig. 2A). SMC was agonist-activated through α₁-adrenoceptors and NSC channels that cause membrane depolarization, opening of VOCC channels, and increase in intracellular Ca²⁺. EC agonist generated IP₃ that activates IP₃Rs and increases cytosolic Ca²⁺, which

opens SK_{Ca} and IK_{Ca} channels and hyperpolarizes EC. The model was able to capture many of the known features of EC-SMC interaction. EC stimulation increased EC Ca^{2+} but reduced SMC Ca^{2+} through the EDHF and EDRF mechanisms. The EDHF action was caused by hyperpolarizing current flowing through MEGJs and was abolished in a synergistic manner by inhibition of EC SK_{Ca} and IK_{Ca} channels. Following stimulation of either cell, the predicted myoendothelial Ca^{2+} fluxes were too small to affect bulk Ca^{2+} in the other cell. Significant gap junction permeability to IP_3 and EC IP_3R currents had to be assumed to capture the feedback response to SMC stimulation in the model.

This model was recently extended to include subcompartments accounting for MPs in the EC and an extracellular cleft to quantify the accumulation of extracellular K^+ (Fig. 2A).¹⁰⁸ K^+ accumulation was observed in the cleft during acetylcholine (ACh) stimulation of the EC. However, the extracellular K^+ concentration is limited by the diffusion of K^+ toward the bulk extracellular space and by the inhibition of the efflux of K^+ through the EC K_{Ca} channels as concentration increases. In addition, the hyperpolarizing effect of K^+ accumulation on SMC V_m through $Na^+-K^+-ATPase$ is reduced by a depolarizing effect of the changing K^+ Nernst potential in both EC and SMC. Thus, K^+ -mediated SMC hyperpolarization was rather small and transient under most of the examined scenarios. Further studies and model simulations are required to investigate the role of extracellular K^+ in the EDHF pathway.

Compartmental (Fig. 2A) as well as continuum (Fig. 2B) models have been developed to account for the effect of projections and localization of channels on myoendothelial communication. Finite element method simulations, for example, allow us to capture Ca^{2+} and IP_3 concentration gradients in the MPs and predict the levels of Ca^{2+} accumulation.¹⁰⁹ Simulations predict that, after SMC stimulation, IP_3 -mediated Ca^{2+} transients are present in the MP (Fig. 2C). The potential of these transients to spread significantly into the bulk of the EC and generate a global response is rather limited. These Ca^{2+} transients can activate IK_{Ca} channels localized on/near the MP and generate hyperpolarizing current that can spread back to the SMC. Hyperpolarizing feedback of a few millivolts can be generated, which translates in a significant modulation of arterial tone. Interestingly, feedback is abolished in the absence of MPs. Thus, simulations suggest that MPs are essential structures for the EC feedback response to SMC stimulation.

IV. CONDUCTED RESPONSES

Some vasoactive agents, when applied focally to microvessels, may cause significant vasomotor responses both locally and at relatively distant sites. Such spreading responses can be conducted by intrinsic signal transduction mechanisms within the vascular wall, independently of the diffusion of the stimulating agent, hemodynamic effects, or innervations. Conducted responses have been reported in small arterioles and as far as small resistance arteries with 3 to 5 layers of SMCs from many vascular beds and species.^{110–115} The distance of spreading responses depends on vessel type, stimulation, and experimental conditions, but the mechanical length constant describing the decay of diameter changes is typically between 1 and 2 mm.¹¹³

Spreading relaxation may play a central role in the hemodynamics of microcirculation because in its absence local relaxation would not significantly increase blood flow restricted by other sections of the vessel. Thus, vasodilatation initiated locally by increased metabolic demand may be conducted upstream to feed arteries to allow adequate increase in perfusion.^{116–118} Conducted vasoconstrictions also were demonstrated experimentally and were implicated in the tubuloglomerular feedback mechanism of renal autoregulation.¹¹⁹ Theoretical simulations suggest that axial communication in the vasculature is also required

to suppress the generation of large proximal shunts during long-term structural adaptation of microvascular networks.¹²⁰

IV.A. Signal Transduction At The Local Site

Spreading relaxation can be initiated by various vasoactive substances acting through different receptors and signal transduction pathways, but the mechanisms that link local metabolic state or oxygen saturation level with spreading responses remain unclear. One possibility is ATP released by erythrocytes in response to changes in the oxygen saturation level, and by perivascular nerves or parenchymal tissue.^{121–123} ATP acting on P2Y receptors can then produce local and spreading relaxation.^{123–125} Local changes in oxygen level also can induce conducted responses independently from the effect of erythrocytes on ATP or NO.¹²⁶ Low (or high) oxygen tension induces conducted vasodilatation (or vasoconstriction) that is inhibited by glibenclamide, a blocker of ATP-sensitive K^+ channels.¹²⁷ This suggests that oxygen level may modulate K_{ATP} channels in the SM through its effect on cytosolic ATP levels.

A common feature of stimuli that are effective in initiating conducted vasodilatation is their ability to produce local hyperpolarization.¹¹⁵ Having strong hyperpolarizing effect, Ach is the agonist used most often to induce conducted dilations in a variety of preparations. Ach increases EC Ca^{2+} , activates endothelial IK_{Ca} and SK_{Ca} channels, and produces both endothelial and smooth muscle hyperpolarization.^{128–130}

IV.B. Mechanisms of Signal Conduction

Gap junctions are central elements for the ability of a vessel to produce conducted responses.^{131–133} Homocellular gap junctions are present in the endothelium (Cx40 and Cx37) and smooth muscle (Cx43 and Cx45) but usually are more prevalent in the endothelium.^{76,99,113,134,135} MEGJs, connecting SMCs with ECs, are present in many but not all vessels.^{80,99,134} The homo- and heterocellular gap junctions are thought to be nonselective and permeable to small ions and molecules such as Ca^{2+} and IP_3 , respectively.^{136,137} Electrotonic conduction of membrane potential changes through the gap junctions is considered to be the major mechanism of spreading responses in most vascular beds.^{113,133,134} The length constant of the electrical spread is similar to the mechanical length constant and in the range of 1 to 2 mm.^{113,128,138,139}

Experiments with disrupted endothelial or smooth muscle layers and genetically engineered mice with cell-specific connexin deficiencies indicate that the endothelium, rather than the smooth muscle, plays the leading role in the propagation of spreading responses in most vascular networks.^{115,123,128,133} Local V_m changes usually spread axially through the endothelial gap junctions and then radially to the SMCs through the MEGJs. In some vessels, like in RMAs, the endothelium was identified as the major conducting pathway. V_m changes, however, initiated either in the endothelium or in the smooth muscle, spread similarly, suggesting strong myoendothelial coupling and that endothelium with smooth muscle acts as an electrical syncytium.¹²⁸ In other vessels, however, SM may provide a separate pathway from the endothelium with different conduction properties.¹⁴⁰ In the mouse cremaster muscle, Ach induces vasodilatation that spreads through the endothelium, whereas adenosine activates SM K_{ATP} channels and induces distant vasodilations through the SM layer.¹²⁹

IV.C. Facilitation of Spreading Responses

In addition to passive electrotonic coupling through gap junctions, conducted responses may involve different facilitating mechanisms, depending on the type of stimulation and the vascular network.¹¹⁵ For instance, in hamster feed arteries, the conduction of

hyperpolarization was augmented during Ach stimulation compared with electric current stimulation, as indicated by an increase in the electrical length constant from 1.2 mm to 1.9 mm.¹³⁹ Membrane hyperpolarizations potentially can be enhanced by inwardly rectifying potassium channels and/or the sodium-potassium pump.¹⁴¹ K_{ir} channels acting as amplifiers in conducted dilations were demonstrated in coronary microcirculation,¹⁴² hamster retractor muscle feed arteries,¹⁴³ and mouse cremaster muscles.¹²⁹ K_{ir} also may amplify conducted responses in RMAs according to some, but not all, studies.^{128,144}

In many vessels, Ach produces distant relaxations without significant endothelial Ca^{2+} spread.^{113,114,134,145} For example, in RMA a noticeable EC Ca^{2+} increase appears only within few hundred micrometers from the Ach stimulation site.¹²⁸ NO has negligible effect on conducted responses in RMAs,¹²⁵ mouse cremaster microcirculation,¹²⁶ and rat cerebral penetrating arterioles,¹²³ presumably because of the limited EC Ca^{2+} spread that is required for its release at distant sites. However, some studies reported significant NO- and EDHF-dependent components of the conducted response and suggested an EC Ca^{2+} increase at remote sites.^{116,146–148} In some ECs, hyperpolarization itself can increase intracellular Ca^{2+} by increasing the driving force for Ca^{2+} entry.^{21,149} It was speculated that conducted hyperpolarization could trigger Ca^{2+} transients and activate NO release at distant sites to dilate the vessel.¹⁴⁷ However, there is no direct evidence for such mechanism in spreading responses, and it is not clear if such hyperpolarization-induced Ca^{2+} transient could be significant.¹⁴⁵

Recently distant EC Ca^{2+} waves were observed in hamster feed arteries^{150,151} and cremaster muscle arterioles in transgenic mice.² The mechanism behind the propagating Ca^{2+} wave along the endothelium remains unclear. Experimental data suggest that direct Ca^{2+} communication via homocellular gap junctions is not essential for Ca^{2+} waves.¹⁵² In mice cremaster muscle arterioles, Ca^{2+} waves were significantly faster than can be accounted for by the diffusion of IP_3 or Ca^{2+} , suggesting an underlying active/regenerative mechanism (i.e., a regenerative release of IP_3 triggered by Ach).² A regenerative mechanism based on the activation of endothelial voltage-dependent sodium (Na_v) and calcium (Ca_v) channels also has been suggested,¹⁴⁸ but mepivacaine, a blocker of Na_v channels, had no effect in another study.¹²⁹ The role of Ca^{2+} waves in conducted responses also has been questioned by Welsh et al.¹⁵³

Spreading vasodilations can be influenced significantly by the strength of vessel precontraction. Haug and Segal¹⁵⁴ reported that activation of SMC α_1 - and α_2 -adrenoceptors in feed arteries of the hamster retractor muscle inhibits conducted vasodilation, possibly by decreased SMC membrane resistance or increased myoendothelial resistance. Gustafsson and Holstein-Rathlou¹⁵⁵ showed experimentally that angiotensin II increased the electrical length constant in RMA and hypothesized that this was a result of increased cell-to-cell coupling or membrane resistance. Experimentation with hamster feed arteries led Uhrenholt et al.¹⁵⁰ to suggest that SM tone could increase basal EC $[Ca^{2+}]_i$ and sensitize the endothelium for Ca^{2+} wave propagation. Conducted responses also were reported to change under pathological conditions. In cerebral arterioles, ischemia augmented conducted vasodilation, presumably to improve cerebral perfusion.¹⁵⁶ Conducted responses in RMAs were significantly attenuated in spontaneous hypertensive rats compared with Wistar Kyoto rats, although the rate of decay with distance did not change.¹⁴⁴

IV.D. Theoretical Models

A number of theoretical models described the electrical aspect of spreading responses. Hirst and Neild¹³⁸ modeled a vessel segment as a continuous wire with uniform axial resistance and applied traditional cable theory to determine its electrical properties and cable length

constant. A general equation for steady-state spread of V_m changes in a short segment of blood vessel stimulated with current source has been derived by Crane and Neild.¹⁵⁷ The effect of branching on the passive spread of electrical signals was examined by Segal and Neild¹⁵⁸ and Crane et al.¹⁵⁹ The simulation results suggested that a thick smooth muscle layer could favor electrical conduction, but passive conduction was insufficient to explain the experimental recordings. Haug and Segal¹⁵⁴ used a passive cable model to demonstrate that the experimentally observed inhibition of conducted vasodilation by activation of SM α_1 - and α_2 -adrenoceptors can be explained by decreased smooth muscle cell membrane resistance or increased myoendothelial resistance.

Diep et al.¹⁰⁵ developed a computational model of skeletal muscle resistance artery with discrete SMCs and ECs. Each cell was represented by a nonlinear resistor and a capacitor equivalent to the whole-cell membrane conductance and capacitance, respectively. The gap junctions were assumed to be ohmic resistors with 3 M Ω between ECs, 90 M Ω between SMCs, and 900 M Ω per SMC between ECs and SMCs. In the simulations, the vessel wall was not a syncytium and electrical stimulations of ECs and SMCs conducted differentially. The axial orientation of ECs and low EC-to-EC resistance, as opposed to circumferential arrangement of SMCs and higher SMC-SMC resistance, were the major factors for the preferential conduction of V_m changes along the endothelium. In a later study, Jantzi and coworkers¹⁴³ modified the model of Diep et al.¹⁰⁵ to examine the role of K_{ir} current in the facilitation of conducted responses. A K_{ir} -like current was added (with minimal activation at -40 mV, maximal activation at -60 mV [1.5 pA], and reversal at -80 mV), as well as a second layer of SMCs. The model demonstrated that, because of the negative slope conductance of the K_{ir} current, it can enhance hyperpolarization of SM induced by local current injection and reduce hyperpolarization decay along the artery compared with the responses with blocked K_{ir} and in the agreement with corresponding experimental data.

Previous theoretical studies of spreading responses focused on the electrical behavior of the vessel wall.^{105,138,154,159} In a recent study¹⁰⁶ we developed a computational model of spreading responses that integrates detailed cellular models with tissue-specific descriptions of Ca^{2+} and ionic dynamics and plasma membrane electrophysiology (Fig. 3A). Discrete ECs and SMCs were coupled in a proper arrangement by homocellular and heterocellular gap junctions permeable to Ca^{2+} , K^+ , Na^+ , and Cl^- ions and IP_3 . ECs released NO, in a Ca^{2+} -dependent manner, which could reduce cytosolic Ca^{2+} in their overlapping SMCs. Representative responses are presented in Fig. 3B–D following focal application of Ach in a row of ECs at $x=0$. Local Ca^{2+} transients do not propagate significantly along the vessel (Fig. 3B). This limited spread of EC Ca^{2+} to neighboring ECs was mediated by diffusion of IP_3 . Hyperpolarization, however, can spread significantly through the endothelial layer (Fig. 3C), and through MEGJs this distant hyperpolarization is transmitted to the SMCs to reduced SMC Ca^{2+} (Fig. 3D) and subsequently arteriolar tone.

When the assumed myoendothelial resistance was small (below 70 M Ω), EC and SMC agonists could produce similar spreading responses that were conducted primarily through the endothelium. Larger myoendothelial resistances compromised spreading responses initiated in SMCs but increased sensitivity of SMCs to local stimuli. The IP_3 -mediated limited Ca^{2+} spread to neighboring ECs can amplify the total current generated at the local site by the stimulated ECs and thus affect distant dilations. The amplitude and distance of spreading responses also depended nonlinearly on local stimulus strength and SM prestimulation levels. The study did not identify any facilitating/regenerative mechanisms that presumably exist in some vessels. Nevertheless, it proposes a theoretical framework for developing other tissue-specific models that can account for such effects.

The electrical resistances and permeabilities of the homocellular and heterocellular gap junctions are likely to have a major effect on the robustness and differential properties of spreading responses, but they remain unknown for most vessels. Therefore, tissue-specific measurements of the gap junction properties would greatly enhance the understanding and modeling of spreading responses because the incidence of MEGJs may vary significantly between vascular beds and may even be smaller in proximal than distal vessels.⁹⁹ The physiological justification for the differential response to endothelial and smooth muscle agonists in some but not all vessels is unclear. According to theoretical analysis, as myoendothelial coupling decreases, the ability of the SMCs to generate a distant signal decreases, but the sensitivity of these cells to agonist stimulation locally increases. The hemodynamic consequences of a strong local constriction instead of a weaker spreading constriction over a larger vascular segment could be studied in the future models that integrate fluid, Ca^{2+} , and V_m dynamics. Detailed mathematical models integrating tissue-specific Ca^{2+} dynamics and membrane channels may assist experimentalists in the elucidation of the controversial role of Ca^{2+} waves and regenerative mechanisms in conducted responses.

V. VASOMOTION

Many small blood vessels, under appropriate conditions, exhibit spontaneous diameter oscillations called *vasomotion*. Vasomotion results from coordinated contractile activity of many SMCs within the vessel wall and is independent from pulsatile blood flow or other periodic stimuli. The period of diameter oscillations varies from seconds to minutes, depending on the vessel and experimental conditions.^{3,160,161}

Vasomotion has been reported in intact animals as well as isolated vessels in various species and tissues including RMA^{162–165}; various skeletal muscles¹⁶⁶; basilar artery¹⁶⁷; cerebral microcirculation of humans, rats, rabbits, and cats^{168–171}; human umbilical and placental veins¹⁷²; and human skin.¹⁷³ Vasomotion has been observed directly through microscopic techniques and indirectly through methods like laser Doppler flowmetry, pressure measurement techniques, near infrared spectroscopy, reflected and scattered light, optical imaging spectroscopy, electrophysiology, and cellular Ca^{2+} imaging techniques.¹⁷⁴ Though most studies associate vasomotion with normal physiological conditions, some suggest that it occurs only under pathological conditions such as reduced perfusion.^{166,175,176} Hypertensive animals also show enhanced vasomotion in most of the studies.¹⁷⁷ The physiologic or pathophysiologic roles of vasomotion are under investigation, and several hypotheses have been proposed.³ Diameter oscillations may increase flow conductance and, under certain conditions, oscillations of oxygen tension may improve oxygenation compared with steady flow through the same average diameter.¹⁷⁸ Vasomotion can enhance tissue perfusion¹⁷⁹ and may also contribute to maternal-fetus blood flow and fetal nutrition and development.¹⁷² Desynchronized vasomotion and fiber activation have been shown to enhance oxygenation in a model of skeletal muscles.¹⁸⁰ However, vasomotion may be best understood as a network phenomenon modulated by information transfer between various vascular segments to ensure optimal tissue perfusion.¹⁷⁴

V.A. Mechanisms of Oscillations

In most vessels, the oscillations of the vascular tone underlying vasomotion originate from synchronization of the spontaneous activity in the majority of SMCs. The small remaining fraction of SMCs may exhibit oscillatory behavior as a result of coupling with the other cells, but distinct pacemaker activity driving the oscillations has not been identified to date. The variation of SMC tone results from intracellular Ca^{2+} changes, which can be generated through different mechanisms depending on vessel type and experimental conditions.

Vasomotion may originate from cytosolic oscillators and membrane oscillators.^{163,181,182} The most common mechanism giving rise to self-sustained regular Ca^{2+} oscillations comprises internal Ca^{2+} stores and RyRs or IP₃Rs.^{3,161} Cytosolic oscillators have been suggested in many vascular beds, including human umbilical and placental veins.¹⁷² In rat mesenteric arterioles, a cytosolic oscillator is the predominant mechanism for oscillations, but inhibition of SERCA gives rise to SR-independent vasomotion with a significantly longer period (~1 min) and larger amplitude.¹⁸³ It has been suggested that in this case, Ca^{2+} oscillations are generated by a membrane oscillator comprised of VOCC and BK_{Ca} channels.^{3,161} This may hold true for some vessels (e.g., rat basilar arteries) under control conditions as well.¹⁸⁴

V.B. Synchronization Mechanisms

For the initiation of vasomotion a synchronization mechanism is required in addition to the spontaneous ability of SMCs for oscillations. This mechanism needs to compensate for the biological variability of the individual Ca^{2+} oscillators and their intrinsic frequencies and for noise-induced phase fluctuations. Gap junctions are thought to play a key role for the synchronization because gap junction uncouplers consistently inhibit vasomotion.^{133,172} However, the identity, origin, and mechanism of the synchronizing signal remain uncertain.

1. Electrical Coupling—Electrical current has been suggested as the only signal fast enough (>1 cm/s) to synchronize several millimeters-long vessels and to mediate long-range coordination in gastric and lymphatic smooth muscle.^{160,185,186} Experimental data now suggest that electrical coupling directly through SMC-SMC gap junctions and/or indirectly through the endothelium may also mediate Ca^{2+} synchronization in vascular SMCs. Several experiments have demonstrated that V_m oscillates during vasomotion with the amplitude of a few millivolts, and these oscillations are highly correlated to the Ca^{2+} , diameter, and force oscillations.^{3,184,187} In vessels where vasomotion is driven by the membrane oscillators, Ca^{2+} and V_m oscillations are in phase and both have significant amplitudes. Thus, electrical coupling through gap junctions can have a direct synchronizing effect. However, Ca^{2+} oscillations produced by cytosolic oscillators do not require V_m oscillations. Secondary V_m oscillations are generated by the dependence of V_m on membrane channels regulated directly or indirectly by cytosolic Ca^{2+} . Thus, V_m oscillations may have an arbitrary phase shift and amplitude depending on the Ca^{2+} - V_m coupling mechanism, conductance, and type of the channels. In this scenario, electrical coupling through gap junctions can mediate both synchronizing and desynchronizing effect.

In rat mesenteric arterioles, vasomotion seems to depend on Ca^{2+} -activated Cl^- channels. Cl_{Ca} channels depolarize V_m in phase with local Ca^{2+} and thus can mediate synchronization by modulating VOCCs in coupled SMCs (Fig. 4). Initially, only cGMP-dependent Cl_{Ca} channels were implicated in the synchronization because application of a membrane-permeable analogue of cGMP, 8Br-cGMP, induced vasomotion.^{163,188} Later studies showed that cGMP-independent Cl_{Ca} channels may also be involved, but the absence of highly specific Cl_{Ca} inhibitors makes it difficult to assess their relative contributions.¹⁸⁹ On the other hand, activation of BK_{Ca} channels has a hyperpolarizing effect opposite to the Cl_{Ca} effect. Indeed, blocking BK_{Ca} with IbTx or K_v with 4-AP did not eliminate vasomotion in Mauban and Weir.¹⁹⁰ Furthermore, blockade of K⁺ channels promotes vasomotion in some vessels. The exact mechanism is unclear, but reduced membrane conductance or tetraethylammonium-induced formation of gap junctions have been suggested as potential explanations.³

2. Ca^{2+} Coupling— Ca^{2+} diffusion through nonselective SMC-SMC gap junctions could be the most direct way to coordinate both cytosolic and membrane Ca^{2+} oscillators (Fig. 4).

In a series of theoretical studies of vasomotion, Ca^{2+} fluxes proportional to intercellular concentration differences are the only significant synchronizing signals.^{45,46,102,104} However, there is no strong experimental evidence to support such a role for Ca^{2+} , and a number of studies have argued that Ca^{2+} is not the major synchronizing agent. Sell et al.¹⁹¹ estimated Ca^{2+} propagation velocity in single SMCs of 25 $\mu\text{m/s}$ and concluded that is too slow for intercellular Ca^{2+} synchronization. Jacobsen et al.¹⁰¹ also argued that the diffusion of Ca^{2+} or other second messengers is too slow and the quantitative movement of Ca^{2+} is too small to have a major effect.

3. IP_3 Coupling—Movement of IP_3 , rather than Ca^{2+} , between cells seems to play more significant role in Ca^{2+} wave propagation, and IP_3 diffuses faster than Ca^{2+} .¹⁹² IP_3 diffusing to other cells could influence their Ca^{2+} oscillations by modulating activity of IP_3Rs . IP_3 -mediated synchronization also would require that IP_3 concentration oscillates together with Ca^{2+} (Fig. 4). Ca^{2+} oscillations are accompanied by IP_3 oscillations in many cell types, including airway SMCs.¹⁹² In many, but not all, instances, IP_3 oscillations are generated by Ca^{2+} dependence of agonist-activated PLC and/or the agonist-independent isoform PLC- δ . PLC- δ was identified experimentally in rat mesenteric arteriole SMCs.^{88,193} Although there is no direct evidence for IP_3 oscillations in RMAs, EC Ca^{2+} oscillations in phase with SM Ca^{2+} oscillations during vasomotion is most likely attributed to SMC-to-EC communication mediated by IP_3 .^{88,89}

V.C. Role of Endothelium

The role of endothelium in vasomotion remains controversial because endothelium can both promote and inhibit vasomotion.^{3,163,165,188,190,191} The differential effect of endothelium can be explained in part by the modulatory role of the endothelium-derived factors on mean SM Ca^{2+} and transitions between oscillatory and nonoscillatory domains.^{102,104,187,194} However, the inconsistent role of endothelium may also result from multiple competing effects on the synchronization, with their net result being sensitive to experimental conditions.

cGMP produces only partial synchronization in endothelium-denuded RMA, whereas intact endothelium allowed full synchronization even with L-NAME and ODQ inhibition.¹⁸⁸ Thus, a synchronizing effect of the endothelium, through some unidentified mechanism, has been suggested. Endothelium has relatively low electrical resistance and thus can provide global coupling for SMCs through MEGJs. This, however, can either enhance or attenuate synchronization depending on the type of Ca^{2+} oscillators and the phase delay between SMC V_m and Ca^{2+} .

A modulatory role for vasomotion was assigned to NO and cGMP by Mauban and Weir.¹⁹⁰ NO/cGMP may affect vasomotion, however, in a complex way because of their potential effects on many cellular components, including components that can induce depolarizing and hyperpolarizing currents. In cerebral rat arteries, asynchronous propagating Ca^{2+} waves were recorded under control conditions, and synchronous Ca^{2+} oscillations and vasomotion were observed after NO inhibition.¹⁸⁷ In this study, the inhibitory role of NO for vasomotion was attributed to soluble guanylyl cyclase-independent effects on RyRs and VOCCs. Some studies report that during vasomotion EC Ca^{2+} is irregular and unsynchronized with SM Ca^{2+} , whereas others suggest that they oscillate effectively in phase and synchrony.^{88,89} If NO also oscillates (i.e., because of the Ca^{2+} dependence of endothelial NO synthase), then its effect on synchronization will be even more complex.

V.D. Mathematical Models

1. Single-Cell Models of Oscillations—Gonzalez-Fernandez and Ermentrout⁴¹ presented a model of vasomotion where oscillations originating from a membrane oscillator. In this model the interaction of VOCC and BK_{Ca} channels gives rise to regular Ca²⁺ oscillations. It also incorporated Ca²⁺-dependent myosin light chains phosphorylation, crossbridges formation, and the mechanics of a thick-walled cylinder. Changes in transmural pressure were assumed to modulate VOCC channels and influence the rate and amplitude of vessel oscillations. Model results were similar to the vessel pacemaker activity reported by Meyer et al.¹⁹⁵ The minimal model of Parthimos and coworkers⁴⁴ investigated arterial chaos generated by coupled cytosolic and membrane oscillators. Nonlinear interactions between the 2 oscillators produced chaotic behavior, similar to irregular arteriolar contractions in rabbit ear arteries. Subsequent work investigated observed changes in histamine-induced chaotic vasomotion after inhibition of endothelial NO.⁴⁸ The effect of L-NAME was simulated by reducing the maximum rate of Ca²⁺ uptake into the store. The original model was also modified to study the effect of various pharmacological interventions on vasomotion in isolated rat cerebral arteries.⁴⁹ The membrane oscillator was eliminated in the new implementation, and regular Ca²⁺ oscillations were generated only by CICR via RyR or IP₃Rs. Careful phase-space and parametric analyses were performed and compared with experiments. Experimental data could be simulated by the model by using a low-order 3-variable system that incorporates nonlinear interactions between dynamical control variables. Their model predicted that inhibition of PLC by U73122 terminates vasomotion mostly by preventing DAG production and activation of DAG-dependent NSC channels, which leads to V_m hyperpolarization and Ca²⁺ reduction below oscillatory domain. This prediction was confirmed by a recent study that suggests that the NSC channels are encoded by PLC-activated TRPC1 channels, which, by passing Na⁺ and Ca²⁺ currents, depolarize V_m, activate VOCC, and initiate vasomotion.¹⁹⁶ Spontaneous Ca²⁺ oscillations also appeared in our isolated SMC model.⁵² The oscillations were generated by CICR through RyRs and the slow refilling of the stores whenever average Ca²⁺ levels shifted within a concentration window. The Ca²⁺ levels could be increased by agonist-activated NSC channels or elevated extracellular K⁺ and reduced by exogenous NO. In the integrated EC-SMC model, the endothelium-derived NO and EDHF desensitized SMC to agonist stimulation and increased the range of agonist concentration, inducing Ca²⁺ oscillations.¹⁹⁷ Analogous stabilizing effect of the endothelium was reported in experiments.¹⁹⁴ The concentrations of NE and extracellular K⁺ producing Ca²⁺ oscillations in the model and vasomotion in isolated RMA were comparable.¹⁶⁴

2. Multicellular Models of Synchronization—Individual SMC Ca²⁺ oscillations or waves need to be synchronized for the occurrence of macroscopic diameter changes; hence, multicellular models are needed to account for intercellular interactions and examine the mechanisms that enable coordination of SMCs in vasomotion.

Koenigsberger et al.^{45,46} proposed a multicellular 2-dimensional model to study recruitment and synchronization of SMCs in rat mesenteric arterial segments. The description of each individual cell was based on the single cell model of Parthimos et al.,⁴⁴ but was modified to account for Ca²⁺ oscillations only from the stores, agonist and Ca²⁺-dependent IP₃ formation, and differences in channel conductances. Variability between SMCs was introduced by Gaussian noise added to membrane conductances. The cells were coupled by electrical current proportional to intercellular V_m difference and Ca²⁺ and IP₃ fluxes proportional to concentration differences. It was argued that the electrical coupling has a desynchronizing effect caused by the hyperpolarizing action of BK_{Ca}. Ca²⁺ diffusion was necessary to override the influence of noise and BK_{Ca}, whereas oscillatory IP₃ generated by Ca²⁺-dependent PLC-δ did not play a role.

The effect endothelium and wall stress on vasomotion was examined in subsequent publications.^{102,104} A layer of ECs was coupled to the SMCs by electrical current and IP₃ diffusion. The dynamics of Ca²⁺, IP₃, and V_m in each EC was taken from the earlier EC model of Schuster et al.³⁶ Agonist stimulation of the smooth muscle layer increased SMC Ca²⁺ and EC Ca²⁺ through diffusion of IP₃ from SMCs to ECs and/or opening of stretch-activated EC Ca²⁺ channels. Endothelial K_{Ca} channels activated by the indirect EC Ca²⁺ elevation generated EDHF, which fed back on the smooth muscle and moderated its Ca²⁺ elevation. The controversial effect of endothelium and wall stress on vasomotion was ascribed mostly to their modulatory effect on the Ca²⁺ levels in the smooth muscle. Thus, removal of endothelium or increase in pressure can move the average smooth muscle Ca²⁺ into or out of the oscillatory window and induce or terminate vasomotion depending on the initial conditions. A synchronizing mechanism through active stress modulating stretch-activated Ca²⁺ channels in neighboring SMCs through the vessel wall was identified, but it was too weak to synchronize Ca²⁺ oscillations in the absence of gap junctions.

The SMC model of Jacobsen and coworkers^{47,101,198} examines the role of cGMP-sensitive Cl_{Ca} in the transition of intracellular Ca²⁺ waves to whole-cell oscillations and in the intercellular coordination of the oscillations. A spindle-shaped SMC was modeled and discretized along its longitudinal axis. A cGMP-sensitive Cl_{Ca} current was formulated based on experimental data from rat mesenteric small arteries.¹⁸⁵ Other membrane and intracellular components were taken from earlier models. Each segment could give rise to Ca²⁺ oscillations through IP₃Rs, but Ca²⁺ waves were generated by the segment with the highest intrinsic frequency determined by random perturbation of parameters along the cell. Activation of Cl_{Ca} by cGMP increased coupling between Ca²⁺ dynamics and V_m, leading to V_m oscillations and transition from Ca²⁺ waves to whole-cell oscillations. To examine intercellular synchronization, 15 cells were arranged into a tube-shaped layer. The cells were coupled by electrical current and Ca²⁺ flux proportional to concentration and potential difference. It was concluded that cGMP promotes vasomotion in RMAs through activation of Cl_{Ca} channels and the coupling of V_m depolarization with Ca²⁺ elevation. The depolarization is transmitted to neighboring cells by the gap junctions, activates VOCC, and coordinates Ca²⁺ elevation. The intercellular Ca²⁺ diffusion had no effect on the synchronization.

Inhibition or activation of various cellular components often has an inconsistent effect on vasomotion. Different mechanisms may participate in the initiation of vasomotion in different vascular beds and species. Multiple mechanisms (competing or compensating) also may be involved even within a particular vascular bed. Thus, the importance of a specific signaling pathway for vasomotion may be difficult to assess and can be sensitive to experimental conditions, such as the presence and concentration of various agonists and blockers. The 2 published multicellular models of vasomotion predict fundamentally different synchronization pathways (intercellular Ca²⁺ diffusion vs. electrical coupling) and can account for different subsets of experimental data from rat mesenteric arterioles.^{45,101}

Future theoretical studies of vasomotion that will be sufficiently comprehensive and will integrate tissue-specific, relevant cellular components have the potential to identify alternative mechanisms and investigate their relative contributions at different experimental conditions. Figure 4 shows selected signaling pathways that may influence Ca²⁺ synchronization based on experimental data, mathematical models, and our preliminary simulations. DAG-activated NSC channels may be involved in vasomotion, not only through their depolarizing effect necessary for the transition from resting to oscillatory domain, as reported from experiments¹⁹⁶ and theoretical models,⁴⁹ but also by their synchronizing effect similar to Cl_{Ca} channels (Fig. 4, solid green lines). In our model, an oscillatory activity of NSC channels results from Ca²⁺ dependence of PLC, which modulates

concentrations of DAG and IP₃. DAG-activated transient receptor potential channel-like nonselective channels are expressed in RMA SMCs,⁵³ and synchronous oscillations of DAG, IP₃ and Ca²⁺ were measured in Chinese hamster ovary cell culture.¹⁹⁹ The oscillatory IP₃ also could mediate Ca²⁺ coordination, given sufficient permeability of gap junctions and/or density of IP₃Rs (Fig. 4, dashed line). More detailed models potentially can account for the spatial distribution of RyRs and IP₃Rs and their differential compartmentalization with membrane channels,¹⁶¹ a stochastic origin for the Ca²⁺ oscillations,²⁰⁰ the transition of unsynchronized intracellular Ca²⁺ waves to global oscillations at the onset of vasomotion,¹⁶³ and for the intercellular Ca²⁺ waves associated with propagation of vasomotion.²⁰¹

VI. SUMMARY

Experimentation has revealed a complex network of intra- and intercellular signaling pathways that regulate Ca²⁺ levels in the vascular wall. Recently, mathematical models have emerged that can integrate these complex pathways and describe cellular behavior and how vessel function emerges from coordination of responses in many cells. The first models of Ca²⁺ dynamics offer many advantages in investigations of Ca²⁺ signaling. However, further development is needed to identify tissue-specific parameters and incorporate recently acquired knowledge about the function and spatial distribution of cellular components (i.e., stores, receptors, channels, or pumps). Integration of these cellular models enables us to elucidate important aspects of vessel function. Some of the early attempts that were reviewed in this article outline a methodology for cell integration and show how multicellular models can provide important physiological insights and assist in elucidation of experimental findings.

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FIGURE 1.

Experimental characterization of microprojections (MP) and their functional role in myoendothelial feedback. (A) Electron microscopy images of an endothelial microprojection in rat saphenous arteries. Reproduced with permission from Sandow et al.⁷⁸. Copyright 2004, John Wiley & Sons, Inc. (B, C) Immunohistochemical labeling studies in rat mesenteric artery showing localization of IK_{Ca} channels and IP₃R receptors on the projections. Reproduced with permission from Ledoux et al.⁹⁰. Copyright 2008, National Academy of Sciences. (D) Proposed mechanism of myoendothelial feedback involves IP₃ diffusion from smooth muscle cell (SMC) to endothelial cell (EC) MP (1), activation of IP₃R and increase of Ca²⁺ in MP (2), activation of IK_{Ca} channels (3), and hyperpolarizing current in EC (4) and SMC (5).

FIGURE 2.

(A) Schematic diagram of compartmental endothelial cell (EC)-smooth muscle cell (SMC) model with endothelial MPs showing membrane and cytosolic components and their localization as implemented by Kapela et al.¹⁰⁸ (B) Two-dimensional mesh of the finite element method model with endothelial MPs and extracellular cleft. (C) Predicted Ca^{2+} transients in the MP during norepinephrine stimulation of SMC.¹⁰⁹

FIGURE 3.

Computational model of spreading responses. (A) Assumed arrangement of discrete endothelial cells (ECs) and smooth muscle cells (SMCs) into an isolated vessel segment. (B) Predicted EC Ca^{2+} elevation induced by focal acetylcholine (Ach) stimulation at $x=0$ had limited spread from the local site. (C) Local Ach-induced EC hyperpolarization was conducted to distant ECs through endothelial gap junctions. (D) SMC Ca^{2+} was reduced at local and distant sites by hyperpolarizing current transmitted from ECs through myoendothelial gap junctions. Reproduced with permission from Kapela et al.¹⁰⁶ Copyright 2010, The American Physiological Society.

FIGURE 4.

Schematic diagram of possible Ca^{2+} synchronization pathways in vascular smooth muscle cells (SMCs). Ca^{2+} increase in first cell (*left*) can activate directly Cl_{Ca} and BK_{Ca} channels, and indirectly nonselective cation (NSC) channels through Ca^{2+} -sensitive phospholipase C (PLC) and diacylglycerol (DAG). The activation of NSC and/or Cl_{Ca} depolarizes V_m (*solid green lines*), although their effect may be moderated by BK_{Ca} channels (*solid red line*). The depolarization spreads through gap junctions to neighboring cells (*right*), activates voltage-operated Ca^{2+} channels and increases in-phase cytosolic Ca^{2+} . Ca^{2+} elevation in one cell can increase Ca^{2+} in neighboring cells by direct diffusion of Ca^{2+} through the gap junctions. Oscillatory IP_3 , generated by Ca^{2+} -dependent PLC and diffusing to other cells, may also have synchronizing effect by acting on IP_3 receptors.