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A mathematical model of vasoreactivity in rat mesenteric arterioles: I. Myoendothelial communication

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Abstract

To study the effect of myoendothelial communication on vascular reactivity, we integrated detailed mathematical models of Ca^{2+} dynamics and membrane electrophysiology in arteriolar smooth muscle (SMC) and endothelial (EC) cells. Cells are coupled through the exchange of Ca^{2+} , Cl^- , K^+ , and Na^+ ions, inositol 1,4,5-triphosphate (IP_3), and the paracrine diffusion of nitric oxide (NO). EC stimulation reduces intracellular Ca^{2+} ($[\text{Ca}^{2+}]_i$) in the SMC by transmitting a hyperpolarizing current carried primarily by K^+ . The NO-independent endothelium-derived hyperpolarization was abolished in a synergistic-like manner by inhibition of EC SK_{Ca} and IK_{Ca} channels. During NE stimulation, IP_3 diffusing from the SMC induces EC Ca^{2+} release, which, in turn, moderates SMC depolarization and $[\text{Ca}^{2+}]_i$ elevation. On the contrary, SMC $[\text{Ca}^{2+}]_i$ was not affected by EC-derived IP_3 . Myoendothelial Ca^{2+} fluxes had no effect in either cell. The EC exerts a stabilizing effect on calcium-induced calcium release-dependent SMC Ca^{2+} oscillations by increasing the norepinephrine concentration window for oscillations. We conclude that a model based on independent data for subcellular components can capture major features of the integrated vessel behavior. This study provides a tissue-specific approach for analyzing complex signaling mechanisms in the vasculature.

Keywords

calcium dynamics; gap junctions; nitric oxide; EDHF; computer simulations

INTRODUCTION

The endothelium plays an important role in the regulation of microvascular tone and blood flow by modulating intracellular calcium concentration ($[\text{Ca}^{2+}]_i$) in the smooth muscle [26,58,65]. Complex bidirectional myoendothelial interactions form feedback loops and regulate Ca^{2+} responses to vasoactive agents and mechanical stimuli in blood vessels [17,20,50,77]. The vascular Ca^{2+} dynamics and the resulting vasoreactivity may differ considerably between vascular beds, but certain mechanisms seem common across many tissues. In resistance vessels, endothelium-dependent relaxation is often mediated by the action of the endothelium-derived relaxing factor (EDRF), the endothelium-derived hyperpolarizing factor (EDHF), and the presence of gap junctions [23,25].

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EDRF, now identified as nitric oxide (NO), is produced through the degradation of L-arginine by endothelial nitric oxide synthase (eNOS), which is activated by Ca^{2+} and various kinases [32,48]. NO diffuses across cell membranes and relaxes smooth muscle cells (SMCs) by modifying the contractile machinery and/or reducing $[\text{Ca}^{2+}]_i$. Endothelium-derived smooth muscle hyperpolarization can be mediated by a variety of mechanisms independently from the NO pathway [23]. In some vascular beds, including rat mesenteric arterioles (RMAs), the spread of endothelial hyperpolarization to SMCs through myoendothelial gap junctions is likely to play a central role in EDHF response [11,21,28]. Besides their role in electrotonic coupling, the myoendothelial gap junctions also allow the exchange of various ions and small polar molecules [8,10] to the extent that the intracellular ionic composition of one cell is altered after coupling with the other [75]. Secondary diffusion of second messengers may form feedback loops and moderate responses. For example, an increase in endothelial cell (EC) $[\text{Ca}^{2+}]_i$ and subsequent release of endothelium-derived factors has been documented during SM stimulation and has been attributed to Ca^{2+} and/or inositol 1,4,5-trisphosphate (IP_3) diffusion from SMCs to (ECs) via gap junctions [35,45]. Thus, myoendothelial communication plays an important role in vascular cell homeostasis and in regulating the effect of stimuli on vascular diameter.

The bidirectional myoendothelial interactions and the presence of multiple signaling cascades complicate the elucidation of the mechanisms that regulate microvascular tone. Mathematical modeling can facilitate the analysis of myoendothelial communication and untangle complex processes involved in vasoreactivity. However, theoretical analyses of vascular signaling have been limited due to the absence of detailed cellular models for ECs and SMCs. Recently, detailed models of electrophysiology and Ca^{2+} dynamics have been presented for vascular endothelial [56,61,72] and SMCs [22,36,40,51,76]. In some cases, single-cell models have been integrated into multicellular models to describe intercellular interactions and function at the vessel level [37,42,43].

Koenigsberger et al. [42,43] and Diep et al. [15] developed multicellular vessel models and investigated the effect of myoendothelial signaling on arterial vasomotion and conducted vasoreactivity, respectively. The models comprised SMCs and ECs arranged into layers and connected by homo- and heterocellular gap junctions that provided Ca^{2+} , IP_3 , and electrical coupling. In Koenigsberger et al.'s model, each SMC and EC was described by modified models of Parthimos et al. [51] and Schuster et al. [56], respectively. These previously developed, generic, cellular models simulated changes in cytosolic Ca^{2+} and IP_3 concentrations and plasma-membrane potential (V_m). The effect of NO was also incorporated. SMC stimulation by an agonist increased $[\text{Ca}^{2+}]_i$ in SMCs and subsequently in ECs through the diffusion of IP_3 and/or through stretch-activated EC Ca^{2+} channels. The resulting EC hyperpolarization was transmitted back to the SMC, decreasing SMC $[\text{Ca}^{2+}]_i$, and affecting (i.e., inducing or abolishing) vasomotion. In Diep et al.'s model [15], each EC and SMC was treated as a capacitor in parallel with a nonlinear resistor representing ionic conductance, and intercellular gap junctions were represented by ohmic resistances. The model focuses on electrical communication along skeletal muscle resistance arteries. Results suggested that myoendothelial gap junction resistance can play an important role in conducted vasoreactivity.

In this study, we develop a computational model to investigate aspects of myoendothelial communication in RMA. The main aim of this study was to investigate how macroscale vascular responses emerge from the nonlinear interaction of subcellular components and processes. Independent experimental data that characterize intracellular components and signaling cascades were integrated, and model simulations were compared against macroscale physiological responses. Thus, the study attempted to correlate data at two different scales (i.e., subcellular and tissue/vessel). This study investigated myoendothelial

interactions in vasoreactivity, but it also outlined the methodology for developing multicellular models of signaling and transport for investigations of vascular pathophysiology.

MODEL DEVELOPMENT

We approximated the endothelial and smooth muscle layers in the arteriolar wall as a two-cell system (Figure 1). For this simplification, we assumed that: (1) the arteriolar wall consisted of a single layer of SMCs surrounding the ECs, (2) the density of ECs and SMCs was equal, and (3) negligible concentration and membrane-potential gradients existed in the circumferential and longitudinal direction of the vessel. Thus, the model was meant to simulate spatially uniform stimulations of small arterioles. Simulations with three layers of SMCs (one EC and three SMCs) are also presented in the Supplement section. We have previously developed detailed mathematical models of an isolated EC [61] and SMC [40]. Schematics of these models are depicted in Figure 1. In this study, we coupled the two cell models through the diffusion of ions and IP_3 via gap junctions and the paracrine diffusion of NO. The integrated model was utilized to investigate aspects of myoendothelial communication and mechanisms of Ca^{2+} regulation in the vascular wall.

EC model

A mathematical model of plasma-membrane electrophysiology and Ca^{2+} dynamics in an isolated vascular endothelial cell has been described in detail elsewhere [61]. The model includes formulations for the major ionic channels identified in RMA ECs (Figure 1). The plasma membrane contains inward rectifying potassium channels (K_{ir}), volume-regulated anion channels (VRACs), small- (SK_{Ca}) and intermediate-conductance (IK_{Ca}) Ca^{2+} -activated potassium channels, store-operated Ca^{2+} channels (SOCs), nonselective cation channels (NSCCs), Ca^{2+} -activated chloride channels (CaCCs), Na^+ - K^+ -ATPase pumps (NaK), plasma-membrane Ca^{2+} -ATPase pumps (PMCA), Na^+ - Ca^{2+} exchangers (NCXs), and the Na^+ - K^+ - Cl^- cotransports (NaKCl). The cytosol contains a Ca^{2+} store, representing mostly the endoplasmic reticulum. Formulations account for store Ca^{2+} release via an IP_3 receptor (IP_3R)-dependent channel and for Ca^{2+} sequestration through the sarco/endoplasmic reticulum Ca^{2+} -ATPase pump (SERCA). Differential equations describe the balance of free and buffered Ca^{2+} in the store and the cytosol. A novel feature of this model is that it also accounts for the balance of the other major ionic species (Na^+ , K^+ , Cl^-) and the second messenger, IP_3 , in the cytosol. The model can capture a number of established features of EC physiology. It shows normal whole-cell resistivity and physiological resting ionic concentrations. It can also reproduce Ca^{2+} and V_m responses under a variety of conditions that include blockade of channels and agonist or extracellular K^+ challenges.

SMC model

We have also developed a mathematical model for a vascular SMC [40]. The choice of model components and their mathematical formulations were based mostly on data from RMA SMCs. In this vascular bed, the following membrane components have been identified and are considered to play the most significant role in the regulation of membrane potential and ionic homeostasis: the large-conductance Ca^{2+} -activated K^+ channel (BK_{Ca}), the voltage-dependent K^+ channel (K_{v}), the Ca^{2+} -activated chloride channel (Cl_{Ca}), the NSC, the SOC, the L-type voltage-operated Ca^{2+} channel (VOCC), the NaK and PMCA pumps, the NaKCl transporter, and the NCX. We also included an unspecified K^+ leak current (K_{leak}) to account for other K^+ -permeable channels, such as the K_{ATP} channel. In the same method as for the EC model, we account for the balance of Ca^{2+} , Na^+ , K^+ , Cl^- , and IP_3 in the cytosol as well as for the store Ca^{2+} content. The sarcoplasmic reticulum (SR) contains a SERCA pump and IP_3R and ryanodine-receptor (RyR)-dependent Ca^{2+} channels. Proteins

such as calsequestrin (CSQN) in the SR and calmodulin (CM) in the cytosol regulate the concentration of free Ca^{2+} through buffering. A leak current (leak) is also included to prevent store overload. A detailed kinetic mechanism describes the signal-transduction pathway for the formation of IP_3 and diacylglycerol (DAG) following the binding of norepinephrine (NE) to α_1 -adrenoceptor (R). The formation of IP_3 leads to store- Ca^{2+} release, while DAG provides sustained membrane depolarization through its action on NSCCs. The model also simulates the effect of the vasodilator, NO. Kinetic equations describe the activation of soluble guanylate cyclase (sGC) by NO and the formation of cGMP. In our model, NO affects directly, or through the formation of cGMP, four cellular components (BK_{Ca} , Cl_{Ca} , NCX, and NaKCl) in a concentration-dependent fashion. This previous study examined the behavior of the isolated SMC. The model exhibits physiological whole-cell conductance and intracellular ionic concentrations. It also captures documented Ca^{2+} and V_m responses under various conditions that include NE, NO, and extracellular K^+ challenges or the application of channel blockers.

Myoendothelial communication

Cell coupling through gap junctions—The presence of myoendothelial gap junctions has been documented in various vascular beds [24], including in RMA [30,55], based on direct (i.e., electron microscopy) or indirect observations (i.e., dye and electrical coupling between the two cells). Gap junctions are thought to be nonselective and permeable to ions, as well as to second messengers, such as IP_3 molecules [8,10]. In the vascular wall, not all the ECs are necessarily connected with the underlying SMCs. In the model, we assume a number of gap junctions between the two simulated cells equal to the average number of these channels per cell in either layer. Thus, the intercellular permeability for ions and IP_3 is equal to the average permeability between the endothelial and the smooth muscle layer. The same applies for the electrical conductivity.

Ionic coupling—We used a novel approach to account for the electrical coupling of the two cells [39]. The detailed balances for the intracellular ionic concentrations in each cellular model enable us to partition the total current flow between the two cells into currents carried by individual ions (Equation 1). In this way, current flow and ionic exchange can be monitored simultaneously. The ionic fluxes through the gap junctions are expressed by four independent Goldman-Hodgkin-Katz equations [69], one for each ionic species (Ca^{2+} , Na^+ , K^+ , and Cl^-) (Equation 2):

$$I_{\text{gj}} = \sum_S I_{\text{gj},S} \quad (1)$$

$$I_{\text{gj},S} = P z_s^2 \frac{V_{\text{gj}} F^2}{RT} \times \frac{[S]_i^{\text{EC}} - [S]_i^{\text{SM}} \exp(-z_s V_{\text{gj}} F / RT)}{1 - \exp(-z_s V_{\text{gj}} F / RT)} \quad (2)$$

where I_{gj} is the total ionic current flowing from EC to SMC; $S = \text{Ca}^{2+}$, K^+ , Na^+ , Cl^- ; $V_{\text{gj}} = V_m^{\text{EC}} - V_m^{\text{SM}}$ is the potential drop across gap junction equal to the difference between the V_m of the EC and the SMC; $[S]_i^{\text{EC}}$ and $[S]_i^{\text{SM}}$ are the intracellular concentrations of ion S in the EC and SMC, respectively; z_s , F , R , and T are the valence of ion S , the Faraday's constant, the gas constant, and the absolute temperature, respectively. The permeability, P , is assumed to be the same for all four ions (i.e., gap junctions are nonselective).

For two limiting cases, this approach becomes identical to previous modeling attempts. When the two neighboring cells are isopotential (i.e., $V_{\text{gj}} = 0$), then Equation 2 reduces to Equation 3:

$$I_{\text{gj},S} = P z_s F ([S]_i^{\text{EC}} - [S]_i^{\text{SM}}) \quad (3)$$

An ionic flux proportional to the concentration difference has often been utilized to account for Ca^{2+} exchange between neighboring cells in different systems [9,42]. When the neighboring cells have equal intracellular concentrations (i.e., $[S]_i^{\text{EC}} = [S]_i^{\text{SM}} = [S]_i$), Equation 2 reduces to Equation 4:

$$I_{\text{gj}} = \sum_s \left(P z_s^2 \frac{V_{\text{gj}} F^2}{RT} [S]_i \right) = \frac{V_{\text{gj}}}{R_{\text{gj}}} \quad (4)$$

This equation suggests an ohmic behavior for the gap junction, and such an assumption has been often utilized in modeling current flow between cells [15,42]. Thus, our approach is more general and can account for nonlinearities that can become significant as the difference in the membrane potential and/or in the intracellular concentrations of neighboring cells increases. Further, rearranging Equation 4 yields Equation 5:

$$P = \frac{RT}{F^2 R_{\text{gj}} \sum_s Z_s^2 [S]_i} \quad (5)$$

Thus, the ionic permeability of the gap junctions (a parameter that has been arbitrarily assigned in previous studies) can be estimated from the total gap junction resistance, R_{gj} . R_{gj} can be determined experimentally, and some values exist in the literature for different vascular beds. In guinea-pig mesenteric arterioles with a single layer of SMCs, the myoendothelial gap junction resistance per single SMC was $R_{\text{gj}} = 0.9 \text{ G}\Omega$ [74]. Based on Equation 5 and the resting ionic concentrations in the two cells, this R_{gj} value yields a total cell-cell permeability ($P = 1.41 \cdot 10^{-12} \text{ cm}^3/\text{s}$).

IP₃ coupling—The IP₃ flux through a gap junction was assumed to be proportional to the IP₃-concentration difference between the two cells [42], as shown in Equation 6:

$$J_{\text{IP}_3} = P_{\text{IP}_3} ([\text{IP}_3]_i^{\text{EC}} - [\text{IP}_3]_i^{\text{SM}}) \quad (6)$$

where $[\text{IP}_3]_i^{\text{EC}}$ and $[\text{IP}_3]_i^{\text{SM}}$ are the intracellular concentrations of IP₃ in EC and SMC, respectively.

The permeability coefficient, P_{IP_3} , should be proportional to the ionic permeability, P (and thus inversely proportional to R_{gj}). This parameter has not been determined experimentally and a value of $P_{\text{IP}_3} = 0.05 \text{ s}^{-1}$ is utilized here in agreement with [42].

NO/cGMP pathway—The EC model was modified to include a description for the release rate of NO. Simultaneous measurements of Ca^{2+} and NO in agonist-stimulated ECs indicate that NO production is regulated by cytosolic calcium [3,16,47]. EC may also release NO in a Ca^{2+} -independent fashion, under some conditions, but such a release is not accounted by the model at this stage (2). Thus, we assumed that the relative NO production rate depends only on EC Ca^{2+} concentration with a sigmoidal function (Equation 7)

$$\bar{Q}_{NO,SS} = \frac{Q_{NO,SS}}{Q_{NO,max}} = \frac{[Ca^{2+}]_i^n}{[Ca^{2+}]_i + K_{m,Ca}^{NO,n}} \quad (7)$$

where $\bar{Q}_{NO,SS}$ is a steady-state production rate of NO, normalized by the maximum production rate, $Q_{NO,max}$. The Ca^{2+} concentration for half-maximum release rate, $K_{m,Ca}^{NO} = 300$ nM, and the Hill coefficient, $n=4.2$, were determined by fitting Equation 7 to data for the Ca^{2+} dependence of eNOS purified from cultured bovine aortic ECs [52]. The time required for the Ca^{2+} -dependent activation/deactivation of eNOS was accounted for by a first-order differential equation (Equation 8):

$$\frac{d\bar{Q}_{NO}}{dt} = \frac{\bar{Q}_{NO,SS} - \bar{Q}_{NO}}{\tau_{eNOS}} \quad (8)$$

where $\bar{Q}_{NO}$ is the relative time-dependent NO release rate (i.e., $\bar{Q}_{NO} = Q_{NO}/Q_{NO,max}$). A time constant, $\tau_{eNOS} = 2$ seconds, was assumed based on data from [38,67].

Once released in the endothelium, NO can freely diffuse across cell membranes and reach the SM to exercise its vasodilatory action. Theoretical models of NO transport in arterioles [68] show that the concentration of the endothelium-derived NO in the SM is proportional to the endothelial NO release rate, as shown in Equation 9:

$$[NO]^{SM} = [NO]_{max} \bar{Q}_{NO} \quad (9)$$

where $[NO]^{SM}$ is the concentration of NO in the SM and $[NO]_{max}$ is the NO concentration during maximum NO release. A value of 380 nM was assumed for $[NO]_{max}$, based on measurements with a porphyrinic microsensor inserted into the lumen of an isolated rat mesenteric resistance artery [67]. Equations 7–9 relate EC $[Ca^{2+}]_i$ to the NO levels in the smooth muscle. $[NO]^{SM}$ affects SMC $[Ca^{2+}]_i$ and V_m by modifying the four cellular components described earlier.

Single-cell equations and parameter values

The equations describing each cell's behavior are described in detailed in previous publications [40,61]. The ECs and SMCs are modeled with 10 and 26 differential equations, respectively. Parameter values utilized here for the integrated model are the same as in the isolated cell models. The equations were coded in Fortran 90 and solved numerically by using Gear's backward differentiation formula method for stiff systems (IMSL Numerical Library routine). The maximum time step was four milliseconds, and the tolerance for convergence was 0.0005.

RESULTS

Resting conditions

The isolated EC and SMC exhibit different resting membrane potentials (−24.9 and −59 mV, respectively) as a result of different conductivity and kinetics of membrane components, such as pumps, exchangers, and ion channels [40,61]. Coupling via gap junctions allows current flow between the two cells. This ionic flux modified the resting membrane potential of the SMC to −52.6 mV and of the EC to −49.7 mV (Table S1). The membrane-potential difference between the SMC and EC was reduced to −2.9 mV. The significant EC repolarization and SMC depolarization, relative to the isolated state, are maintained by constant myoendothelial ionic fluxes. The effect of electrical coupling on V_m

is significant, because the EC and SMC resting membrane resistances (i.e., 2 and 3 G Ω , respectively) are larger than the gap-junction resistance (i.e., $R_{gj}=0.9$ G Ω). Following the SMC depolarization, VOCCs opened and the resting SMC $[Ca^{2+}]_i$ increased from 68.4 to 96 nM. The EC repolarization increased the electrochemical transmembrane Ca^{2+} gradient and led to the elevation of resting EC $[Ca^{2+}]_i$ from 70 to 131 nM. The addition of the NO pathway did not affect significantly the resting conditions (Table S1; values in parentheses).

NO/cGMP pathway

Figure 2A depicts the predicted SM NO concentration as a function of the EC $[Ca^{2+}]_i$ (Equations 7–9). The EC $[Ca^{2+}]_i$ concentration for half-maximum NO concentration ($K_{m,NO}^{NO}$) was 300 nM. The amount of NO required in the SMC model for half-maximum activation of sGC ($K_{m,NO}^{sGC}$) and for half-maximum cGMP-independent activation of the BK_{Ca} channel ($K_{m,NO}^{BKCa}$) are also shown for reference [40]. The NO-dependent activation of sGC stimulates the formation of cGMP, as previously described [40]. Figure 2B presents the predicted increase in the concentration of [cGMP] in the SM as a function of the EC $[Ca^{2+}]_i$. The EC $[Ca^{2+}]_i$ concentration for half-maximum cGMP formation ($K_{m,Ca}^{cGMP}$) was 200 nM. The concentration of cGMP for half-maximum activation of the BK_{Ca} channel ($K_{m,cGMP}^{BKCa}$) is also shown for reference. With the parameters utilized in this study, changes in the activity of the NO pathway occurred over a physiological range of EC $[Ca^{2+}]_i$ (100–600 nM). For resting EC $[Ca^{2+}]_i$ (i.e., 131 nM), the basal NO production and concentration were relatively low ([NO] = 10 nM), and sGC was only slightly activated, and limited activation of BK_{Ca} channels occurs mostly through the small cGMP formation. For EC $[Ca^{2+}]_i$ above 600 nM, the NO/cGMP pathway worked in a near-saturation state.

Effect of myoendothelial communication on V_m and $[Ca^{2+}]_i$

To determine the role of myoendothelial communication in the regulation of vascular-wall Ca^{2+} dynamics, we examined model responses to transient EC and SMC stimulations. Simulations were performed for three different scenarios. First, cells were only coupled electrically by the diffusion of ions. In the second scenario, ions and IP₃ were allowed to diffuse through the gap junctions. In the third scenario, the intercellular diffusion of ions, IP₃, and NO were accounted for. Blockade of the NO pathway is often utilized experimentally to examine NO contribution to the EC/SMC interactions (i.e., inhibition of eNOS by L-arginine analogs, such as N-omega-nitro-L-arginine methyl ester; L-NAME). Selective inhibition of IP₃ diffusion through the gap junctions is not feasible, and thus the first scenario (i.e., ionic coupling only) did not have an appropriate experimental analog. However, simulations with and without IP₃ diffusion allowed us to evaluate the contribution of IP₃ in EC/SMC communication.

Ionic coupling

Simulation results for the first scenario (i.e., cells are only coupled electrically) are depicted in Figure 3. Figure 3A shows changes in V_m for the EC (dashed-dotted line) and for the SMC (solid line), following the application of NE (1 μ M NE) and/or acetylcholine (ACh) ($Q_{IP3}=5.5 \cdot 10^{-8}$ M/s). Corresponding changes in the $[Ca^{2+}]_i$ of the two cells are shown in Figure 3B. Stimulation of the SMC with NE for 60 seconds ($t=60$ –120 seconds) opened NSCCs, depolarized SMC V_m from around -50 to about -40 mV (Figure 3A, solid line), activated VOCCs, and increased SMC $[Ca^{2+}]_i$ (Figure 3B, solid line). The SMC depolarization was transmitted through the gap junctions to the EC. The EC V_m follows closely the V_m change in the SMC. This EC depolarization results in a reduction of $[Ca^{2+}]_i$ in the EC. This is attributed to a less favorable potential gradient for transmembrane Ca^{2+}

entry after depolarization and the absence of voltage-gated Ca^{2+} channels in the EC. Despite a relatively large Ca^{2+} concentration gradient between the SMC and the EC, the Ca^{2+} flux from the SMC to the EC was too small to increase EC $[\text{Ca}^{2+}]_i$.

EC stimulation with ACh ($t=180$ – 240 seconds) induced store Ca^{2+} release and an increase in $[\text{Ca}^{2+}]_i$ (Figure 3B, dashed-dotted line). This activated SK_{Ca} and IK_{Ca} channels, leading to EC hyperpolarization from around -50 to -70 mV (Figure 3A, dashed-dotted line). The hyperpolarization was transmitted electrotonically to the SMC, and SMC V_m followed closely the EC V_m , approaching -70 mV (Figure 3A, solid line). SMC $[\text{Ca}^{2+}]_i$ slightly decreases during ACh stimulation (Figure 3B, solid line) as a result of the effect of hyperpolarization on the VOCCs and despite a large Ca^{2+} concentration gradient from the EC to SMC that favors Ca^{2+} diffusion into the SMC. The EC stimulation was repeated in the presence of $1 \mu\text{M}$ of NE (Figure 3; $t=420$ – 480 seconds). The EC repolarizes from -40 to around -65 mV. The SMC V_m cannot “keep up” this time with the EC hyperpolarization and repolarizes only to -51 mV (a value close to the resting V_m). This new SMC V_m reduces SMC $[\text{Ca}^{2+}]_i$ to a value that falls within the concentration window for oscillations.

Ionic and IP_3 coupling

In Figure 4, the same stimulations were repeated after allowing for IP_3 exchange between the two cells. NE stimulation ($t=60$ – 120 seconds) caused SMC depolarization (Figure 4A, solid line) and a Ca^{2+} increase (Figure 4B, solid line) similar to those observed in Figure 3. This time, however, a significant increase in EC $[\text{Ca}^{2+}]_i$ could also be seen (Figure 4B, dashed-dotted line). NE-induced SMC-derived IP_3 diffuses into the EC, activates IP_3 receptors, and produces this EC Ca^{2+} increase. This higher $[\text{Ca}^{2+}]_i$ activates SK_{Ca} and IK_{Ca} channels and repolarizes the EC (Figure 4A, dashed line). The EC repolarization affects the SMC V_m because of the electrical coupling of the two cells.

Stimulation of the EC with an IP_3 -releasing agonist is also simulated ($t=180$ – 240 seconds). A comparison of Figures 3 and 4 demonstrated a minor effect of IP_3 diffusion. In the same way as before, EC stimulation increased EC Ca^{2+} and hyperpolarized both cells to around -70 mV. Interestingly, Ca^{2+} and IP_3 generated in the EC diffuse to the SMC, but they cannot increase SMC Ca^{2+} , which actually slightly reduces as a result of the effect of hyperpolarization on VOCCs. As shown in Figure 4, ACh was also applied after the vessel was “precontracted” with NE ($t=420$ – 480 seconds). IP_3 coupling amplified the ACh-induced EC Ca^{2+} transient in the presence of NE (40% increase in maximum $[\text{Ca}^{2+}]_i$ in Figure 4B vs. 3B). This amplification enhanced EC hyperpolarization, SMC repolarization, and SMC Ca^{2+} reduction. Diffusion of IP_3 from the NE-prestimulated SMC sensitizes the EC by increasing its intracellular IP_3 levels prior to stimulation. This results in an EC that can generate a larger Ca^{2+} response for the same stimulus.

Ionic, IP_3 , and NO couplings

In Figure 5A and B, the effects of the NO pathway are taken into account. SMC responses to NE were significantly moderated by the EC ($t=60$ – 120 seconds). A comparison of Figures 4 and 5 shows that SMC V_m and Ca^{2+} changes were suppressed to almost half in the presence of NO signaling. As IP_3 is generated in the SMC, it diffuses and stimulates the EC. The increase in EC Ca^{2+} leads to hyperpolarization and NO generation. Both of these signals feed back to the SMC via the ionic flow through gap junctions and the effect of the EC-derived NO on the SMC BK_{Ca} channels.

EC stimulation in the presence of NO coupling (Figure 5; $t=180$ – 240 seconds) results in a slightly higher SMC hyperpolarization (-68 mV in Figure 4 vs. -72 mV in Figure 5) and similar SMC Ca^{2+} reduction. After termination of the EC stimulation, SMC V_m returns to

the resting value, slower in the presence of NO, because of the slow sGC inactivation. In the case of EC stimulation following SMC pre-constriction with 1 μM of NE (Figure 5; $t=420\text{--}480$ seconds), NO coupling amplified SMC repolarization and $[\text{Ca}^{2+}]_i$ reduction.

Corresponding experimental data are presented in Figure 5C–5E, as reproduced from [17,45,50]. Data show the parallel increase in EC and SMC $[\text{Ca}^{2+}]_i$ during NE stimulation (Figure 5C) and the significant increase in EC $[\text{Ca}^{2+}]_i$ alone during stimulation with ACh (Figure 5D and E).

EC sensitivity to stimulation

The ability of the EC to feed back and modulate SMC responses depends on the stimulatory status of the cell. In Figure 6, a weak EC stimulation (ACh = 0.5 a.u.; $Q_{\text{IP}_3} = 2.73 \cdot 10^{-8} \text{ M/s}$) increased intracellular IP_3 levels prior to NE application. This increase was small and did not induce any significant release of Ca^{2+} , EDHF, or NO. The application of NE causes IP_3 generation and diffusion toward the EC, as described previously. However, the effect of IP_3 is now amplified due to the increased basal EC IP_3 level, and the release of Ca^{2+} , EDHF, and NO is much stronger. As a result, the endothelium can have a larger impact in limiting SMC depolarization and Ca^{2+} increase (compare Figure 5, $t=60\text{--}120$ seconds, and Figure 6). The model suggests that there is an optimal level of EC prestimulation for maximum regulatory control of SMC contractility. In the presence of a strong EC agonist, stimulation (data not shown) of NE increases EC IP_3 and Ca^{2+} , but the EDHF and NO responses did not change significantly because of saturation of the two pathways. Thus, EC sensitivity is maximal during weak basal EC prestimulation.

Endothelium-derived hyperpolarizing factor

Figure 7A shows the contribution of the EC Ca^{2+} -activated potassium channels to the EDHF response. After the blockade of the NO pathway, 0.6 μM of NE depolarized the SMC from resting at -52 mV to -41 mV . Subsequently, the EC was stimulated with increasing concentrations of ACh, and the resulting changes in V_m are depicted in Figure 7A. We assume that changes in the ACh concentration result in proportional changes in IP_3 release and assign the value of 1 a.u. to an ACh concentration that yields the IP_3 release rate of $Q_{\text{IP}_3} = 5.46 \cdot 10^{-8} \text{ M/s}$. Simulations are presented under control conditions (open circles), after the blockade of the SK_{Ca} channels (solid circles), after the blockade of the IK_{Ca} channels (solid triangles), and after the blockade of both the SK_{Ca} and IK_{Ca} channels (solid squares).

Figure 7A shows that the blockade of both channel types is required to completely abolish the EDHF response, and that the blockade of both channels has a larger effect on the SMC V_m than the sum of the effects of individual channel blockade. Thus, simulations suggest a synergistic effect in the simultaneous blockade of both channels. In the model, selective blockade of the SK_{Ca} channels was more effective in reducing SMC hyperpolarization at weak EC stimulation, while selective blockade of IK_{Ca} channels reduced hyperpolarizations at strong EC stimulations.

Figure 7B shows the effect of myoendothelial resistance on the EDHF response after the blockade of the NO pathway and in the presence of 0.6 μM of NE. The EC was stimulated with ACh (2 a.u.), and the resulting changes in SMC V_m are depicted in Figure 7B. Simulations are presented for the model with one SMC (open circles), three SMCs (asterisks), and nine SMCs (pluses). A myoendothelial R_{gi} of 900 $\text{M}\Omega$ allows strong EDHF when there is a single SMC, but not when multiple layers of SMCs are considered. Reduction of myoendothelial resistance to 35 $\text{M}\Omega$ is required to produce similar EDHF response in the model with three SMCs. In the model with nine SMCs, we observed a

differential hyperpolarization of the SMC layers, and only the SMCs closer to the endothelium exhibited significant hyperpolarizations.

Ca²⁺ oscillations

Oscillations generated by increasing concentrations of NE in the presence of ionic, IP₃, and NO coupling are shown in Figure 8A. Sustained oscillations were generated through the calcium-induced calcium release (CICR) mechanism when the mean SMC Ca²⁺ was above the threshold value of ~160 nM. The effect of EC on the oscillations is shown in Figure 8B. A bifurcation diagram is shown for the isolated SMC model (dotted line) and for the integrated model in the absence (solid line) and presence of a strong EC stimulus (dashed-dotted line). For a given NE, each bifurcation diagram shows the maximum (upper branch) and minimum (lower branch) SMC [Ca²⁺]_i during oscillations or the steady-state SMC [Ca²⁺]_i in the absence of oscillations. Although the Ca²⁺-concentration window for oscillations remains the same for all three scenarios ([Ca²⁺]_i=160 nM– to 290 nM), the range of NE concentrations that can induce the necessary Ca²⁺ elevation for oscillations changes. Interestingly, the coupled model predicts an oscillatory period (solid line) that decreases with stimulus strength (i.e., NE concentration). Similar SMC Ca²⁺ oscillations can be also induced by extracellular K⁺, and representative simulations are presented in the Supplement section.

DISCUSSION

Ionic coupling

Previous theoretical studies of intercellular communication have modeled gap junctions as an ohmic resistance and the exchange of Ca²⁺ ions as a linear function of the intercellular concentration gradient [15,42]. Jacobsen et al. [37] have recently provided a more detailed model of coupled arterial SMCs. Their intercellular Ca²⁺ exchange was proportional to a linear combination of concentration and potential differences, and the assumed value for the gap-junction permeability. Equations 1 and 2 unify intercellular ionic diffusion and cell electrical coupling into an expression for ionic exchange, based on the electrochemical gradient. This approach defines currents carried by individual ions and allows for monitoring the exchange of different ionic species between the coupled cells. The permeability parameter can be estimated from the electrical resistance (Equation 5), and the need for a separate estimate for Ca²⁺ permeability is eliminated. It requires, however, detailed cellular models that can account for the balance of the four major ionic species. In cellular models that account for the balance of cytosolic Ca²⁺ only, the intracellular concentrations of the other major ionic species can be assumed to have some control values in order to apply the same approach.

An important consequence of Equations 1 and 2 is that for the same or similar permeabilities for the four ions (P_s ; $S = K^+, Na^+, Cl^-, Ca^{2+}$), the current is carried predominately by the K⁺ ions due to their highest cytosolic concentrations [37,69]. In model simulations, the contribution of Cl⁻ and Na⁺ to electrical coupling was approximately 3- and 12-fold, respectively, smaller than K⁺. [Ca²⁺]_i is about 10⁶ times lower than [K⁺]_i, and thus the Ca²⁺ current does not contribute significantly in electrical communication. The small Ca²⁺ fluxes can potentially have a role in modulating [Ca²⁺]_i. For this to happen, however, myoendothelial Ca²⁺ flux has to be comparable to the transmembrane Ca²⁺ currents.

The magnitude of myoendothelial Ca²⁺ flux depends on the gap-junction resistance and on [Ca²⁺]_i and V_m intercellular gradients. Figure 9 shows the intercellular Ca²⁺ current ($I_{gj,Ca}$) calculated from Equation 2 as a function of gap-junction resistance (R_{gj}) under physiologically relevant Ca²⁺ and V_m gradients. The permeability P in Equation 2 was

estimated from R_{gj} , according to Equation 5. The intracellular Ca^{2+} concentrations in Equation 2 were set to 100 nM in one cell (simulating an unstimulated cell) and 500 nM in the other cell (simulating a stimulated cell). Potential difference across the gap junctions in Equation 2 was set to 0 mV (solid line), 10 mV in the direction of the $[\text{Ca}^{2+}]_i$ gradient (dotted line), and 10 mV against the $[\text{Ca}^{2+}]_i$ gradient (dashed line). A gap-junction resistance $R_{gj}=0.9 \text{ G}\Omega$ (i.e., control value used in the simulations [74]) yields a Ca^{2+} current (10^{-4} pA) that is over three orders of magnitude smaller than typical transmembrane Ca^{2+} currents in EC (red shaded area) and SMC (green shaded area). For the given Ca^{2+} and V_m gradients, the myoendothelial Ca^{2+} current increases when the R_{gj} is reduced. However, even for the wide range of previously reported R_{gj} values (35–900 $\text{M}\Omega$ [55,74]), we predicted $I_{gj,\text{Ca}}$ (yellow shaded area) smaller than the typical Ca^{2+} transmembrane currents. Thus, we concluded that the movement of Ca^{2+} between the two cell types is negligible and cannot significantly affect global $[\text{Ca}^{2+}]_i$ in either cell under most conditions.

Experimental data indicate that Ca^{2+} communication via homocellular gap junctions is not essential [45,54], and theoretical analyses have often neglected intercellular calcium fluxes [41,42,64]. Koenigsberger et al. [44], however, found that weak, but significant, Ca^{2+} diffusion between SMCs was crucial for the synchronization of Ca^{2+} oscillations in a theoretical model of vasomotion. According to our model, significant Ca^{2+} exchange is possible when gap-junction resistance is below 1 $\text{M}\Omega$.

Theoretical and experimental evidence suggest that the flow of ions through gap junctions occurs even at rest. This is a result of the different membrane electrophysiology of the two cells. Resting membrane potentials recorded in intact mesenteric arterioles have been a few millivolts more negative in the SMCs than in ECs [75] (Table S1). According to the Nernst equation, a 1.5-fold Ca^{2+} -concentration difference between the SMC and the EC is required to compensate for a 5-mV potential drop. Such compensatory differences in ionic concentrations are unlikely, and the electrical gradient between the two cells probably induces significant ionic exchange at rest. During endothelial or smooth muscle stimulation and, particularly, during simultaneous stimulation of the two layers, the potential difference may increase even further. Thus, the model predicts a continuous flow of ions through the gap junctions under most conditions. Equilibration of the ionic concentrations in the two cells is not likely. Since ionic exchange is governed by electrochemical, and not simply by concentration, gradients, and the intracellular ionic balance depends on V_m , concentration differences between the two cells may even increase after coupling. Thus, coupling of the cells induces changes in V_m and in the intracellular ionic concentrations. The resulting V_m difference between the two coupled cells and their intracellular ionic concentrations are difficult to predict, and it requires detailed description of membrane currents and the initial equilibrated state of each cell. A more detailed analysis of the dynamics of the myoendothelial ionic exchange during coupling is presented in the Supplement section.

The role of IP_3

Simulations in Figure 4 with the ionic and IP_3 communication compare well with experimental data in isolated vessels after the blockade of the NO pathway. The application of 0.6 μM of phenylephrine (PE) to isolated third-order RMA evoked sustained SMC depolarization from approximately -53 to -42 mV [12,13], and this depolarization was transmitted to the ECs [73]. Stimulation of the endothelium with ACh hyperpolarized RMA SMCs from resting at -52 mV to a maximum of -75 mV [12,13]. Similar responses are predicted by the model (Figure 4; $t=60$ – 120 and 180 – 240 seconds, respectively). In the presence of 0.6 μM PE, ACh hyperpolarized RMA SMC from -42.0 to -71 mV and induced a 97% relaxation [12,13]. In the model, a nearly 100% SMC $[\text{Ca}^{2+}]_i$ reduction and 20-mV SMC hyperpolarization is predicted in the representative simulation and compares well with the experiments (Figure 4; $t=360$ – 480 seconds).

Experimental studies have also demonstrated that following stimulation, the resulting Ca^{2+} elevation in the smooth muscle is associated with an increased $[\text{Ca}^{2+}]_i$ in the endothelium [17,45]. Ca^{2+} diffusion from the smooth muscle may be responsible for the secondary Ca^{2+} transient in the endothelium [17]. However, studies in second-order RMA with inhibitors of IP_3 production or IP_3 -induced Ca^{2+} release suggest that IP_3 , rather than Ca^{2+} , is the diffusing molecule [45]. In the model, the Ca^{2+} flux into the EC was negligible and did not generate secondary Ca^{2+} increase (Figure 3B). Only after the addition of IP_3 diffusion, EC $[\text{Ca}^{2+}]_i$ increases in response to SMC stimulation (Figure 4B and 5B) in agreement with the experimental recording in Figure 5C (reproduced from [45]). Thus, the model suggests a significant role for IP_3 diffusing from the SMC into the EC.

On the contrary, when the EC is stimulated with a IP_3 -elevating agonist, SMC $[\text{Ca}^{2+}]_i$ does not increase (in Figure 4B and Figure 5B; $t=180\text{--}240$ seconds; SMC $[\text{Ca}^{2+}]_i$ actually decreased). Thus, diffusion of IP_3 from the EC to the SMC does not seem to play an important role. This asymmetrical behavior has been documented experimentally and attributed to a smaller volume of the endothelial layer relative to the smooth muscle [17] (Figure 5D). In the model, as well as in arterioles with one or two layers of SMCs, however, the endothelial and smooth muscle volumes are similar [50]. Model simulations provide an alternative explanation. The predicted unidirectionality results from different sensitivities of EC and SMC to IP_3 and different IP_3 release and degradation rates in the two cells. Localization of the EC IP_3 receptor close to the myoendothelial junction may also contribute to this phenomenon [33]. The model, at this stage, does not account for spatial heterogeneity. Incorporation of myoendothelial projections, microdomains, and localized expression of subcellular components in a new version of the model will allow us to assess the importance of localized expression of IP_3 receptors close to the MEJ.

Myoendothelial signaling through the gap junctions has been also studied in a vascular cell coculture model composed of an endothelial and a smooth muscle monolayer [35]. The Ca^{2+} communication was significant and bidirectional, while IP_3 only contributed to signaling from SMCs to ECs. Contrary to the vessel studies, EC stimulation in this system increased SMC Ca^{2+} . This discrepancy was attributed to reduced expression of L-type and T-type Ca^{2+} channels in cultured vascular SMCs, which unmasked Ca^{2+} diffusion into the SMCs. According to the present model, bidirectional Ca^{2+} signaling could be generated also by the overexpression of gap junctions in this coculture system and excessive myoendothelial permeability.

The role of NO

Model simulations with intact gap junctions and active NO signaling are presented in Figure 5. NE-induced SMC Ca^{2+} elevation and contraction were associated with increased endothelial Ca^{2+} and NO release ($t=60\text{--}120$ seconds), in agreement with experiments (Figure 5C reproduced from [45], and Figures 3 and 9 in [66]). Relative to the predictions in the absence of NO (Figure 4), the plateau SMC $[\text{Ca}^{2+}]_i$ induced by $1\text{ }\mu\text{M}$ of NE was suppressed approximately 2-fold by the NO pathway. In third-order RMA ($100\text{--}300\text{ }\mu\text{m}$ in diameter), L-NAME increased PE- and NE-induced contractions by 2-fold [20,70]. According to the model and in agreement with experimental observations, the enhanced contractile responsiveness of RMA after eNOS inhibition is, at least in part, due to the loss of BK_{Ca} channel activation by NO and smooth muscle depolarization (7). NO affects only the second phase of SMC Ca^{2+} response to NE because of the delay in Ca^{2+} -dependent eNOS activation (Equation 8), and because the initial Ca^{2+} release from the stores is NO independent, in agreement with [18].

Different responses to ACh have been observed in isolated large versus small RMA [31]. In superior (i.e., conduit) mesenteric arteries (diameter, $0.65\text{--}1.2\text{ mm}$), NO contribution is

significant [31,62,66]. In small RMA (diameter, 200–450 μm), EDHF plays a more prominent role, and the role of NO is less clear. Inhibition of eNOS had no significant effect on SMC hyperpolarization and relaxation in small RMA induced by ACh [27]. However, L-NAME administration right-shifted the SMC repolarization curve and reduced the maximum relaxation of the same vessels to ACh in [70]. This suggests that NO can modulate SMC V_m , but also that it contributes to the relaxation by a V_m -independent mechanism. Further, the relative contribution of EDHF and NO may depend on the ACh and NE concentrations applied. In small RMA precontracted with 4 μM NE, EDHF was the major mediator of the relaxation induced by low physiological concentrations of ACh (i.e., less than 0.1 μM), but EDHF and NO contributed similarly to the relaxation induced by intermediate concentrations of ACh (i.e., less than 0.3 μM) [31]. In rat aorta, ACh-induced relaxations resistant to L-NAME were unmasked at lower concentrations of NE and were mediated, possibly, by EDHF [29]. Various explanations have been proposed for the variable contribution of NO to ACh-induced relaxations, and some insights can be made from the present model.

Representative model simulations in Figures 4 and 5 show that the blockage of NO signaling increases the depolarizing and Ca^{2+} -elevating effects of 1 μM of NE on the SMC. In general, however, the contribution of NO to the SMC Ca^{2+} changes will depend on the magnitude of ACh and/or NE stimulation. At low SMC and large EC stimulations, the EDHF alone can repolarize the SMC sufficiently to induce significant Ca^{2+} reduction and an almost complete relaxation. (Small SMC depolarization and limited increases in membrane conductance can be easily balanced by the hyperpolarizing current through the gap junctions.) NO can further enhance SMC repolarization without a major effect on SMC Ca^{2+} that has returned to near resting levels (Figure 5; $t=180\text{--}360$ seconds suggests an insensitivity of SMC $[\text{Ca}^{2+}]_i$ to V_m below -50 mV). At large SMC and/or low EC stimulations, SMC Ca^{2+} remains above resting levels and sensitive to repolarization. The effects of EDHF and NO are more likely to be additive now and can be distinguished experimentally.

EC sensitivity to stimulation

According to the model, the EC sensitivity to stimulation and its ability to generate EDHF and NO-related responses depends on the stimulatory state of the cell. The nonlinear, sigmoidal, dependence of the endothelial store Ca^{2+} release on intracellular IP_3 makes cell responses very sensitive to basal IP_3 . This small IP_3 concentration may be a result of prior EC stimulation by the IP_3 -elevating agonist and/or flow or simply by the diffusion of IP_3 from stimulated SMCs. The EC prestimulation and/or IP_3 diffusion may have an unnoticeable effect on V_m and $[\text{Ca}^{2+}]_i$ and still may modulate vessel responses to different stimuli. For example, the moderating effect of the EC on the SMC contractility may be enhanced by weak EC prestimulation (e.g., in the presence of flow or ACh) (Figure 6). EC responses to agonist stimulation can be amplified when IP_3 diffuses into the EC from NE-stimulated SMC (compare Figures 3 and 4; $t=420\text{--}480$ seconds). This sensitization results in a higher EC $[\text{Ca}^{2+}]_i$, more hyperpolarized ECs and SMCs and a lower SMC $[\text{Ca}^{2+}]_i$ after ACh stimulation of the precontracted vessel. Thus, the amount of produced Ca^{2+} -dependent vasorelaxing factors could depend on smooth muscle stimulation and vessel precontraction (compare EC $[\text{Ca}^{2+}]_i$ transients at Figures 4 and 5 in the absence and presence of NE). Experimental evidence exists to support some of these predictions. 3 μM of ACh induced significantly larger NO concentration in the presence of 1 μM of NE than in the absence of NE ($[\text{NO}] = 16 \pm 2$ vs. 10 ± 2 nM, respectively) in rat superior mesenteric artery [62].

Altered Ca^{2+} regulation may play an important role in different pathological conditions. In hypertension, for example, the EDHF and EDRF may be compromised [11,23,48] and the reactivity to vasoconstrictors may be increased [4,5]. Model simulations suggest that basal

endothelial stimulation (i.e., low doses of EC agonist) may compensate compromised EDHF or EDRF responses or hyper-reactivity to vasoconstrictors. Such a therapeutic strategy may be more advantageous over a strategy that focuses exclusively on smooth muscle relaxation (direct or indirect), which will be insensitive to the contractile state of SMCs and thus prone to insufficient or excessive actions.

Endothelium-derived hyperpolarizing factor

In many vascular beds, EDHF is abolished synergistically by the blockade of SK_{Ca} and IK_{Ca} channels, and the selective blockade of either channel type has no or only partial effect [11,23]. The model predicts a similar behavior. The endothelium-dependent SMC hyperpolarization is abolished by the simultaneous blockade of SK_{Ca} and IK_{Ca} channels, since other channels are not involved in EC hyperpolarization. This EDHF inhibition is stronger than the sum of EDHF inhibitions caused by individual SK_{Ca} and IK_{Ca} blockades (Figure 7A and Figure 3C in [12]). In simulations with ACh. 1 a.u., the selective blockade of SK_{Ca} channels has no effect (Figure 7A, solid circles) because EC V_m is saturated at the K^+ Nernst potential by the large IK_{Ca} current and is insensitive to changes of the SK_{Ca} current. The selective blockade of IK_{Ca} channels has a significant effect on EDHF (Figure 7A, solid triangles), because a weaker SK_{Ca} current does not saturate EC V_m . In general, the EDHF saturation and the synergistic effect of SK_{Ca} and IK_{Ca} blockade increase with stronger ACh stimulation and larger whole-cell SK_{Ca} and IK_{Ca} conductances. (The relative contribution of SK_{Ca} and IK_{Ca} channels to EDHF is further discussed in the Supplement section.)

The model also shows how the EDHF depends on the myoendothelial resistance and the number of SMC layers (Figure 7B and S4–S6). The model predicts a different requirement for gap-junction density in single versus multilayered arterioles for an effective role of ionic coupling in EDHF. However, in the model with many SMCs, the EDHF was transmitted only to the first few SMCs, even when a low EC-SMC R_{gj} was assumed. The EDHF response was also reduced by stronger NE stimulation, because the agonist activates NSCC channels and increases the leakiness of the SMCs. In general, the EDHF is more effective with small EC-SMC and SMC-SMC resistances, high SMC input resistance, and a small number of SM layers.

Ca^{2+} oscillations and the phenomenon of vasomotion

In the isolated SMC model, the nonlinear release and the slow refilling of the intracellular store can generate spontaneous intracellular Ca^{2+} oscillations [40]. Because the myoendothelial coupling does not interfere with this mechanism, the isolated and integrated models generated Ca^{2+} oscillations with similar amplitude and period. However, the necessary stimulus that will bring SMC $[Ca^{2+}]_i$ within the concentration window for oscillations changed (Figure 8B). Model predictions and experiments in first- and second-order RMA [59] show that the presence of the EC exerts a stabilizing effect on the oscillations (i.e., oscillations can be maintained over a wider range of agonist concentrations). This effect is predicted to become more dramatic upon stimulation of the EC.

The predicted K^+ and NE concentration windows (see also the Supplement section for K^+ -induced oscillations) were similar to the concentrations inducing vasomotion in isolated segments of second-order resistance RMA (300 μm in diameter) [57] (Figure S2; online only). In the experiments, the period of vasomotion was ~25 seconds for KCl and ~six seconds for NE stimulation. In the model, however, Ca^{2+} oscillations did not depend on the stimulus type, but they were affected by the stimulus strength (i.e., decreasing from 30 to 15 seconds as the stimulus increases; Figure 8B). Subsequent investigations should examine parameters that modulate oscillatory frequency and its physiological importance.

Oscillations generated by other mechanisms were not considered and can be also further explored.

The present model can be extended to a population of ECs and SMCs in order to study the phenomenon of vasomotion and to address limitations of earlier theoretical analyses. Vasomotion in RMA is believed to originate from synchronized intracellular Ca^{2+} oscillators in SMCs [1,49]. A previous theoretical study has suggested that the purely electrical coupling of SMCs has a desynchronizing effect, and Ca^{2+} diffusion can override it and synchronize nearby cells [42]. However, the gap junctional Ca^{2+} permeability was assigned arbitrarily and independently from the electrical resistance, while these parameters are directly related. Therefore, it is unclear whether the synchronizing effect of Ca^{2+} diffusion is not offset by the desynchronizing effect of electrical coupling. It has also been proposed that the endothelium-derived NO may affect vasomotion by increasing SMC cGMP and activating cGMP-dependent Cl_{Ca} channels [1,49,53]. According to a theoretical model, cGMP-dependent activation of Cl_{Ca} channels may promote the onset of vasomotion in small RMA by coupling sarcoplasmic reticulum and plasma-membrane Ca^{2+} dynamics, V_m oscillations, and intercellular electrical synchronization [37]. However, this earlier model did not account for the cGMP-dependent activation of hyperpolarizing BK_{Ca} channels, which may counteract the depolarizing effect of the Cl_{Ca} channel activation.

Model limitations

The present model accounts for many, but not all, of the established mechanisms of Ca^{2+} regulation in the vascular wall. Further, simulation results depend on tissue-specific parameter values, and some of them have not been adequately characterized, including various channel conductances, the myoendothelial IP_3 permeability, the NO production, and consumption rates. A detailed discussion on the limitations of the cellular models is presented in our previous publications [40,61].

The high Ca^{2+} concentration for half-maximum activation of IK_{Ca} channels ($K_{\text{IKCa,Cai}} = 740 \text{ nM}$) is based on data from mouse aortic ECs and predicts the lack of IK_{Ca} activation at rest (see the Supplement section for details). The model does not account for the accumulation of K^+ ion in the interstitial space and does not include the α_2 and α_3 isoforms of the Na^+/K^+ pump nor the K_{ir} channels in the SMC description. The two isoforms, which can be activated by a moderate K^+ elevation, are expressed in RMA SMCs but are not well characterized [71]. The K_{ir} channels may be expressed in RMA SMC (6), but studies suggest that they are not functionally significant [14,63]. Future versions of the model that will account for the additional NaK isoforms and the presence of microdomains/projections with heterogeneous channel distribution will investigate this role of K^+ .

Recent studies have demonstrated the presence of endothelial projections toward the nearby SMCs and have argued for an important physiological role of this structure in myoendothelial signaling [19,46]. Localized expression of IP_3R to the EC side of the myoendothelial junctions and fast EC-derived IP_3 degradation in SMC before receptor binding may play a central role in the unidirectional IP_3 communication [33]. Incorporation of such microdomains in the model can potentially affect the predicted role of Ca^{2+} and IP_3 in intercellular communication. Negligible Ca^{2+} diffusion with respect to the whole cell can produce significant Ca^{2+} elevations in microdomains in the vicinity of gap junctions. Local transients could then induce cytosolic Ca^{2+} waves through the CICR mechanism. Different densities of IP_3 receptors or PLC near gap junctions in ECs and SMCs could give rise to the asymmetric effect of IP_3 in the EC/SMC communication independently of the whole-cell sensitivities to IP_3 . The present lumped cellular models do not incorporate this level of detail, but they provide a starting point for further development that will test the physiological significance of some of these hypotheses.

Myoendothelial gap-junction coupling may differ among vascular beds. For example, poor coupling has been documented in mouse cremasteric arterioles [34,60]. Because of such differences in gap-junction coupling as well as differences in membrane-channel composition, caution must be taken when extrapolating the results from this study, which focused on RMA, to other vessel types.

This study highlights the most significant experimental observations that characterize the behavior of the particular microvessels. Model simulations were in agreement with most of the experimental findings, but in some cases, contradictory data have been reported. Experimental discrepancies can be attributed mostly to biological variability and differences in experimental protocols. Future experimental studies are required to address these discrepancies, and this will promote further model development.

CONCLUSIONS

An integrated model utilizes independent experimental data from cellular studies to describe reactivity and signaling in rat mesenteric microvessels. Model simulations were in good agreement with experiments in isolated vessels, verifying hypotheses for the role of myoendothelial communication in vascular control. The model quantifies the effect of ionic flow and second messengers in signal transmission between the two layers. It shows that many of the observed vessel responses can be explained by integrating actions of the assumed subcellular components. The model predicts (1) altered ionic homeostasis after gap-junction coupling; (2) insufficient myoendothelial Ca^{2+} exchange for inducing global intracellular Ca^{2+} changes in the adjacent cell after stimulation of either layer; (3) unidirectional IP_3 signaling generated as a result of different cellular kinetics; and (4) store-generated SMC Ca^{2+} oscillations with frequencies observed in vasomotion and a stabilizing effect of the EC. Model simulations also show how the ability of myoendothelial current to produce EDHF response depends on the myoendothelial resistance and the number of SMC layers. Finally, representative simulations show how nonlinearities can affect responses in a fashion that cannot be predicted through a qualitative conceptual rationale. Nonlinear effects of vessel prestimulation and stimulus strength on Ca^{2+} responses may explain variability and contradictions in previous experimental data.

Ca^{2+} dynamics in the vascular wall depends on the presence of myoendothelial gap junctions, the underlying electrophysiology of the cell membrane, and the exchange of various ions and second messengers. The study outlines a methodology for the development of a tissue-specific theoretical framework for elucidating these interactions. Further model development is required to examine vascular signaling in different diseases and tissues and to incorporate additional cellular components and microdomains. This will allow the validation and/or generation of novel hypotheses about vascular tone regulation in health and disease.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

Schematic diagram of integrated Ca^{2+} dynamics in EC and SMC. Cells are coupled by nitric oxide (NO) and myoendothelial gap junctions permeable to Ca^{2+} , Na^{+} , K^{+} , and Cl^{-} ions and IP₃. K_{IR}, inward rectifier K^{+} channel; VRAC, volume-regulated anion channel; SK_{Ca}, IK_{Ca}, and BK_{Ca}, small-, intermediate-, and large-conductance Ca^{2+} -activated K^{+} channels; SOC, store-operated channel; NSC, nonselective cation channel, CaCC and Cl_{Ca}, Ca^{2+} -activated chloride channel; NaK, Na^{+} - K^{+} -ATPase; PMCA, plasma-membrane Ca^{2+} -ATPase; NCX, Na^{+} / Ca^{2+} exchanger; NaKCl, Na^{+} - K^{+} - Cl^{-} cotransport; K_v, voltage-dependent K^{+} channel; K_{leak}, unspecified K^{+} leak current; VOCC, voltage-operated Ca^{2+} channels; SR/ER, sarco/endoplasmic reticulum; IP₃R, IP₃ receptor; RyR, ryanodine receptor; SERCA, SR/ER Ca^{2+} -ATPase; CSQN, calsequestrin; CM, calmodulin; R, receptor; G, G protein; DAG, diacylglycerol; PLC, phospholipase C; sGC, soluble guanylate cyclase; cGMP, cyclic guanosine monophosphate.

Figure 2.

Steady-state dependence of SMC NO (A) and cGMP (B) concentrations on EC Ca^{2+} concentration. $K_{m,\text{NO}}^{\text{BKCa}}$ $[\text{NO}]$ for half-maximum cGMP-independent activation of BK_{Ca} channel; $K_{m,\text{NO}}^{\text{sGC}}$ $[\text{NO}]$ for half-maximum activation of sGC; $K_{m,\text{cGMP}}^{\text{BKCa}}$ $[\text{cGMP}]$ for half-maximum activation of the BK_{Ca} channel; $K_{m,\text{Ca}}^{\text{cGMP}}$ $\text{EC } [\text{Ca}^{2+}]_i$ for half-maximum SMC $[\text{NO}]$; $K_{m,\text{Ca}}^{\text{cGMP}}$ $\text{EC } [\text{Ca}^{2+}]_i$ for half-maximum $[\text{cGMP}]$. The activity of the NO/cGMP pathway is low at rest ($\text{EC } [\text{Ca}^{2+}]_i \approx 131 \text{ nM}$), changes significantly in the physiological range of $\text{EC } [\text{Ca}^{2+}]_i$ (i.e., 100–600 nM), and saturates for $\text{EC } [\text{Ca}^{2+}]_i$ above 600 nM.

Figure 3.

Predicted NE (1 μM)-induced and ACh (1 a.u.)-induced changes of membrane potentials (A) and intracellular Ca^{2+} (B) in SMC (solid lines) and EC (dashed-dotted lines) coupled by the ionic fluxes only (K^+ , Cl^- , Na^+ , Ca^{2+}). NE stimulation depolarizes SMC and EC, increases SMC $[Ca^{2+}]_i$, and reduces EC $[Ca^{2+}]_i$. ACh stimulation repolarizes EC and SMC, increases EC $[Ca^{2+}]_i$, and reduces SMC $[Ca^{2+}]_i$.

Figure 4.

Predicted NE (1 μM)-induced and ACh (1 a.u.)-induced changes of membrane potentials (A) and intracellular Ca^{2+} (B) in SMC (solid lines) and EC (dashed-dotted lines) coupled by the ionic and IP_3 fluxes. NE stimulation depolarizes SMC and EC, and increases SMC and EC $[Ca^{2+}]_i$. ACh stimulation repolarizes EC and SMC, increases EC $[Ca^{2+}]_i$, and reduces EC $[Ca^{2+}]_i$. The shaded area indicates an increase in EC Ca^{2+} transient, as compared to Figure 3.

Figure 5.

NE (1 μM)- and ACh (1 a.u.)-induced changes of V_m (A) and $[Ca^{2+}]_i$ (B) in SMC (solid lines) and EC (dashed-dotted lines) with the ionic, IP_3 , and NO couplings. (C) SM and EC Ca^{2+} measured in isolated second-order RMA after the application of 0.8 μM of NE, reproduced with permission from [45] (copyright 2005, Elsevier). (D) SM and EC Ca^{2+} in isolated hamster cheek pouch arterioles ($58.8 \pm 1.6 \mu m$ in resting diameter) after the application of ACh ($10^{-4} M$), reproduced with permission from [17] (copyright 1997, National Academy of Sciences, USA). (E) SM and EC Ca^{2+} in strips dissected from the superior part of the main branch of the mesenteric artery, in the presence of 1 μM of PE and after the application of 10 μM of ACh, reproduced with permission from [50] (copyright 2001, Elsevier). In both simulations and experiments, NE stimulation increases SMC and EC $[Ca^{2+}]_i$, and ACh stimulation increases EC $[Ca^{2+}]_i$ and reduces SMC $[Ca^{2+}]_i$.

Figure 6.

NE (1 μM)-induced Ca^{2+} transient in SMC (solid line) and EC (dashed-dotted line) in the presence of weak Ach (0.5 a.u.; $Q_{\text{IP}_3} = 2.73 \cdot 10^{-8} \text{ M/s}$) prestimulation. Comparison with Figure 5 ($t=60\text{--}120$ seconds) shows that the prestimulation has little effect on resting EC and SMC Ca^{2+} , but it significantly amplifies the EC Ca^{2+} transient and limits SMC Ca^{2+} response.

Figure 7.

(A) The contribution of the EC Ca^{2+} -activated potassium channels to the EDHF response. Change in SMC membrane potential as a function of increasing ACh stimulations (simulation results obtained with NO pathway blocked) and 0.6 μM of NE prestimulation.

(B) The effect of myoendothelial resistance on the EDHF response in the model with one SMC (open circles), three SMCs (asterisks), and nine SMCs (pluses). Only the first, last, and middle SMCs appear in the nine-cell simulation. All SMCs were prestimulated with 0.6 μM of NE, and the EC was stimulated with ACh (2 a.u.).

Figure 8.

(A) Simulations of NE-induced SMC Ca^{2+} oscillations in the integrated model with ionic, IP_3 , and NO coupling. Increasing NE depolarized the SMC, opened VOCCs, and raised SMC $[Ca^{2+}]_i$. When the mean Ca^{2+} exceeded the threshold value of ~ 160 nM, sustained Ca^{2+} oscillations in the SMC were generated through the CICR mechanism. (B) Bifurcation diagrams of SMC Ca^{2+} vs. NE for the isolated SMC model (dashed line) and for the integrated model in the absence (solid line) and presence of a strong EC stimulus (dashed-dotted line). The Ca^{2+} -concentration window for oscillations is the same in each case, but the corresponding range of NE changes. The oscillatory period (T) decreases with stimulus strength.

Figure 9.

Predicted intercellular Ca^{2+} current ($I_{\text{gj,Ca}}$) (Equation 2) as a function of gap-junction resistance (R_{gj}) (Equation 5) under physiologically relevant Ca^{2+} and V_{m} gradients. $[\text{Ca}^{2+}]_{\text{i}}$ was set to 100 nM in one cell and 500 nM in the other cell. Intercellular V_{m} difference was set to 0 mV (solid line), 10 mV in the direction of the $[\text{Ca}^{2+}]_{\text{i}}$ gradient (dotted line), and 10 mV against the $[\text{Ca}^{2+}]_{\text{i}}$ gradient (dashed line). SMC and EC I_{Ca} indicate range of transmembrane Ca^{2+} currents in the model SMC and EC. Reported myoendothelial R_{gj} values yield Ca^{2+} currents (yellow shaded area) smaller than transmembrane Ca^{2+} currents in the EC (red shaded area) and SMC (green shaded area) model.