A mathematical model of vasoreactivity in rat mesenteric arterioles. II. Conducted vasoreactivity

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Kapela A, Nagaraja S, Tsoukias NM. A mathematical model of vasoreactivity in rat mesenteric arterioles. II. Conducted vasoreactivity. Am J Physiol Heart Circ Physiol 298: H52–H65, 2010. First published October 23, 2009; doi:10.1152/ajpheart.00546.2009.—This study presents a multicellular computational model of a rat mesenteric arteriole to investigate the signal transduction mechanisms involved in the generation of conducted vasoreactivity. The model comprises detailed descriptions of endothelial (ECs) and smooth muscle (SM) cells (SMCs), coupled by nonselective gap junctions. With strong myoendothelial coupling, local agonist stimulation of the EC or SM layer causes local changes in membrane potential ($V_m$) that are conducted electrotonically, primarily through the endothelium. When myoendothelial coupling is weak, signals initiated in the SM conduct poorly, but the sensitivity of the SMCs to current injection and agonist stimulation increases. Thus physiological transmembrane currents can induce different levels of local $V_m$ change, depending on cell’s gap junction connectivity. The physiological relevance of current and voltage clamp stimulations in intact vessels is discussed. Focal agonist stimulation of the endothelium reduces cytosolic calcium ([Ca$^{2+}$]$_i$) in the prestimulated SM layer. This SMC [Ca$^{2+}$]$_i$- reduction is attributed to a spread of EC hyperpolarization via gap junctions. Inositol (1,4,5)-trisphosphate, but not [Ca$^{2+}$], diffusion through homocellular gap junctions can increase intracellular Ca$^{2+}$ concentration in neighboring ECs. The small endothelial Ca$^{2+}$ spread can amplify the total current generated at the local site by the ECs and through the nitric oxide pathway, by the SMCs, and thus reduces the number of stimulated cells required to induce distant responses. The distance of the electrotonic and Ca$^{2+}$ spread depends on the magnitude of SM prestimulation and the number of SM layers. Model results are consistent with experimental data for vasoreactivity in rat mesenteric resistance arteries.

intercellular communication; membrane potential; calcium dynamics

FOCAL APPLICATION OF CERTAIN vasoactive agents to microvessels may cause significant vasomotor responses, both locally and at relatively distant sites. The distant responses are mediated by intrinsic signal transduction mechanisms within the vascular wall, independent of the diffusion of the stimulating agent, hemodynamic effects, or innervations. These conducted responses have been reported in different vascular beds and species (12, 14, 20, 33, 41) and may play a role in both the rapid and long-term coordination of microvascular function. Vasodilatation initiated locally by increased metabolic demand may be conducted upstream to feed arteries to allow adequate increase in blood flow (42, 43); conducted vasoconstriction may be important in the tubuloglomerular feedback mechanism of renal autoregulation (20); and theoretical simulations suggest that axial communication in the vasculature is required to suppress the generation of large proximal shunts during long-term structural adaptation of microvascular networks (38).

The underlying mechanisms of spreading responses remain poorly understood, but electrotonic transmission of membrane potential changes ($\Delta V_m$) and calcium (Ca$^{2+}$) waves through the endothelium seem to play the major role (46, 48). In some vessels, including in rat mesenteric resistance arteries (RMA), the signal is attenuated away from the stimulus site, and the vasoreactivity observed at a distant site is attributed to Ca$^{2+}$- independent passive electronic diffusion through gap junctions (20, 47). In other vascular beds, the conducted signal can spread over significant distances with minimal attenuation, and thus facilitating/regenerative mechanisms should be involved (24). A number of hypotheses have been proposed to account for the facilitation of the transmitted signal. One suggestion is that membrane hyperpolarization is enhanced by inwardly rectifying potassium ($K_{ir}$) channels and/or the sodium-potassium (NaK) pump (8). Alternatively, a wave of nitric oxide (NO) release along the arteriolar endothelium, triggered by a spread of Ca$^{2+}$, could induce spreading dilatation (4, 10). Remote Ca$^{2+}$ waves have been reported in hamster feed arteries (10, 50). A regenerative mechanism based on the activation of endothelial voltage-dependent sodium and calcium channels has also been suggested (16).

A number of theoretical studies have been performed to investigate spreading responses. Hirst and Neild (26) modeled a vessel segment as a continuous wire with uniform axial resistance and applied traditional cable theory to determine its electrical properties. Crane et al. (6) used a cable model to simulate the spread of $\Delta V_m$ in microvascular trees. The results of these simulations suggest that a thick smooth muscle layer could favor electrical conduction, but passive conduction was insufficient to explain the experimental recordings. Haug and Segal (23) predicted with a similar passive cable model that the inhibition of conducted vasodilation by $\alpha_1$- and $\alpha_2$-adrenoceptors can be explained by decreased smooth muscle cell (SMC) membrane resistance or increased myoendothelial resistance. Diep et al. (9) developed a detailed computational model of skeletal muscle resistance artery with discrete SMCs and endothelial cells (ECs). Each cell was treated as a capacitor coupled in parallel with a nonlinear resistor representing ionic conductance. Intercellular gap junctions were represented by ohmic resistances. According to the simulations, the vessel wall was not a syncytium, and electrical stimuli did not spread uniformly. The cells’ orientation and coupling resistances were the critical factors in determining the differential electrical communication within and between the endothelium and the smooth muscle.

Previous theoretical studies (6, 9, 23, 26) have focused mostly on the electrical behavior of the vessel and have provided insights into the major aspects of spreading re-

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METHODS

Our laboratory has previously developed detailed mathematical models of plasma membrane electrophysiology and Ca\(^{2+}\) dynamics in isolated EC (44) and SMC (28). Schematics of these models are depicted in Fig. 1. Both cellular models are based primarily on data from RMA. Only the salient features of these models are presented here.

EC Model

The plasma membrane of the EC includes all of the major channels identified in RMA ECs. Mathematical formulations describe ionic fluxes through the K\(_\text{v}\) channel, the voltage-regulated anion channel, the small- (SKCa), and the intermediate-conductance Ca\(^{2+}\)-activated potassium channels (IKCa), the store-operated Ca\(^{2+}\) channel, the nonselective cation channel (NSC), the Ca\(^{2+}\)-activated chloride channel, the NaK pump, the plasma membrane Ca\(^{2+}\)-ATPase pump, the Na\(^+\)/Ca\(^{2+}\) exchanger (NCX), and the Na\(^+\)-K\(^+\)-Cl\(^-\) cotransport (Na-KCl). The fluid compartment contains a Ca\(^{2+}\) store that represents the endoplasmic reticulum. Formulations account for Ca\(^{2+}\) release via an IP\(_3\) receptor (IP\(_3\),R)-dependent channel and for Ca\(^{2+}\) sequestration through the sarco/endoplasmic reticulum Ca\(^{2+}\)-ATPase pump. Differential equations account for 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. Acetylcholine...
(ACh) stimulation is simulated by IP3 generation. Thus simulation results can also refer to other Ca2+ elevating agonist that act through the IP3 pathway.

The model can capture a number of established features of EC physiology. It exhibits normal whole cell resistivity and physiological resting ionic concentrations. It can also reproduce Ca2+ and Vm responses under a variety of conditions that include blockage of channels, and agonist or extracellular K+ challenges.

**SMC Model**

In RMA SMCs, the following membrane components have been identified and are considered to play the most significant role in the regulation of Vm and ionic homeostasis: the large-conductance Ca2+-activated K+ channel (BKCa), the voltage-dependent K+ channel, the Ca2+-activated chloride channel, the NSC channel, the store-operated Ca2+ channel, the L-type voltage-operated Ca2+ channel, the NaK and plasma membrane Ca2+-ATPase pump, the NaKCl, and the NCX. We have also included an unspecified K+ leak current to account for other K+-permeable channels, such as the ATP-sensitive K+ channel. In the same way as for the EC model, we account for the balance of Ca2+ in the cytosol as well as for the stored Ca2+. The sarcoplasmic reticulum (SR) contains a sarco/endoplasmic reticulum Ca2+-ATPase pump, and IP3 binding to ryanodine receptor-dependent Ca2+ channels. Proteins such as calsequestrin in the SR and calmodulin in the cytosol regulate the concentration of free Ca2+ 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 IP3 and diacylglycerol following the binding of norepinephrine (NE) to αt-adrenergic receptor (receptor). Formation of IP3 leads to a release of stored Ca2+, whereas diacylglycerol provides sustained membrane depolarization through its action on NCX. The model also simulates the effect of the vasodilator, NO. Kinetic equations describe the activation of soluble guanylate cyclase by NO and the formation of cyclic guanosine monophosphate (cGMP). In our model, NO affects BKCa, Ca2+-activated chloride channel, NCX, NaKCl in a concentration-dependent fashion. The model exhibits physiological whole cell conductance and intracellular ionic concentrations. It also captures documented Ca2+ and Vm responses under various conditions that include NE, NO, and extracellular K+ challenges, or application of channel blockers.

**Multicellular Vessel Model**

A 3-mm arteriolar segment was constructed through the appropriate arrangement of ECs and SMCs (Fig. 2), similar to the study by Diep et al. (9). We assume a single layer of SMCs is surrounding the ECs. The effect of multiple layers of SMCs on conducted responses is examined in the supplemental data. (The online version of this article contains supplemental data.) The SMCs are aligned perpendicular to the ECs and the vessel axis. The vessel model was reduced to two dimensions (axial and radial) on the assumption that concentration and potential gradients in the circumferential direction are negligible (6). These gradients should be minimal during circumferentially uniform stimulations, but are small even during local stimulation with an intracellular microelectrode (26).

In this study, we assume that an EC spans 15 SMCs and vice versa. This results in an EC-to-SMC population ratio in the vascular wall of 1:1 and assumes a SMC width equal to 1/15 of the EC length. Representative cell dimensions and population ratios of ECs and SMCs from various tissues are summarized in Supplemental Table S1. The exact cell dimensions can vary significantly between different tissues and with vessel size. An EC length of 100 μm is assumed in this study to translate the number of cells through which a signal is transmitted, into a longitudinal distance. Due to the circumferential symmetry, only one SMC is implemented at each discrete axial position along the vessel (Fig. 2). We assume that each cell is connected with its neighbors in the same layer and with overlapping cells on the other layer. Only one EC at a given axial position is simulated, and identical ECs are assumed in the circumferential direction (Fig. 2). The overlapping arrangement of ECs is simplified and replaced by a serial arrangement with regular end-to-end couplings, as shown in Fig. 2. The ECs’ effect on a neighboring SMC is estimated by multiplying the myoendothelial flux into a SMC by 15 (i.e., one for each of the 15 identical ECs that overlap the SMC). The simulated vessel segment is 3 mm long and incorporates 450 ECs and 450 SMCs. (Note that only 30 of the 450 ECs are simulated, since there are 15 identical ECs in each longitudinal position.) An actual vessel segment of the same length should contain a higher number of cells that is dependent on the vessel’s diameter. The assumption of circumferential symmetry allows us to significantly reduce the number of simulated cells and thus the number of differential equations.

The electrical equivalent of the model vessel is shown in Fig. 3. We assumed electrically sealed ends (26), small intracellular and extracellular resistances, and negligible effect of tight junctions between ECs. The model assumes that the individual ECs and SMCs are isopotential (22) and without intracellular concentration gradients. For spatially uniform endothelial and/or smooth muscle stimulation, the vessel model is equivalent to a two-cell EC/SMC model, a scenario that has been examined elsewhere (29, 30).

**Intercellular Communication**

Cell coupling through gap junctions. Homocellular gap junctions are present in the endothelium and smooth muscle, but are usually more prevalent in the endothelium (17, 20, 40, 46). Myoendothelial gap junctions, connecting SMCs with ECs, are present in RMA (25, 40), although they may be absent in some other vessels (46). The homo- and heterocellular gap junctions are thought to be nonselective.
and permeable to ions, as well as to IP$_3$ molecules (3, 5). In this model, all neighboring cells within the smooth muscle or the endothelial layer are connected via homocellular gap junctions, while myoendothelial gap junctions connect overlapping EC and SMCs.

**Ionic coupling.** We used a novel approach to account for the electrical coupling of neighboring cells. The detailed balances in intracellular ionic concentrations enable us to partition the total current flow between two cells into currents carried by individual ions (Eq. 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, one for each ionic species (Ca$^{2+}$, Na$^+$, K$^+$, and Cl$^{-}$) (Eq. 2) (29, 30):

$$I_{g,j} = \sum_n I_{g,n}$$

$$I_{g,n} = P_{g,n} \frac{V_{j,n}^o - [S]^n}{S^n} \exp(-z_n F V_{j,n}/R T)$$

where $I_{g,j}$ is the total ionic current flowing from cell $n$ to $m$; $S = \text{Ca}^{2+}, \text{K}^+, \text{Na}^+, \text{Cl}^-$; $V_{j,n} = V_{m}^o - V_{m}^n$ is the potential drop across the gap junction that is equal to the difference between $V_{m}$ of cell $n$ and cell $m$; $[S]^n$ is the intracellular concentration of ion $S$ in cell $n$; and $z_n$, $F$, $R$, and $T$ represent the valence of ion $S$, the Faraday’s constant, the gas constant, and the absolute temperature, respectively. The ionic permeabilities depend on the cell’s isomorphs participating in channel formation and their phosphorylation state, but such dependencies have not been incorporated in the model at this stage. Instead, the permeability, $P$, is assumed to be the same for all four ionic species, and junctions are nonelective and represents resultant behavior of various gap junction channels. This parameter has been arbitrarily assigned in previous studies, but can be estimated from the total gap junction resistance ($R_{g,j}$), as we have recently described in Ref. 29.

$$P = \frac{RT}{F^2 R_{g,j} \sum_n (z_n S^n)}$$

$R_{g,j}$ can be determined experimentally, and some values for different vascular beds exist in the literature. Supplemental Table S2 summarizes different experimental estimates of $R_{g,j}$, as well as values utilized in previous theoretical studies. The control values utilized in this study are based on this data. Similar simulation results would be obtained if the Goldman-Hodgkin-Katz equations were replaced with an additive model, as described in Ref. 27, in which the fluxes are proportional to a linear combination of concentration and potential differences, and the gap junction permeability.

**IP$_3$ coupling.** The IP$_3$ flux through the gap junction was assumed to be proportional to the IP$_3$ concentration difference between the two cells:

$$J_{IP_3} = P_{IP_3}[IP_3]_j^o - [IP_3]_j$$

The permeability coefficient, $P_{IP_3}$, has not been determined experimentally. Koenigsberger et al. (32) utilized a value for the myoendothelial IP$_3$ permeability of 0.05 s$^{-1}$, for a gap junctional resistance per SMC of 0.9 GΩ. The value of the IP$_3$ permeability between two overlapping cells utilized in this study ($P_{IP_3}^{SMC-SMC} = 0.0033$ s$^{-1}$) corresponds to the same total cell permeability of the earlier study. (Note: the permeability has to decrease 15-fold to account for the distribution of flux into 15 overlapping cells.) The permeability of IP$_3$ should be inversely proportional to $R_{g,j}$, because an increase in the number of gap junction channels increases, in the same proportion, both the electrical conductance and permeability for larger molecules. Based on the assumed values for $P_{IP_3}^{SMC-SMC}$ (Supplemental Table S2), the intercellular permeabilities of IP$_3$ in the endothelium and smooth muscle layers were adjusted accordingly (i.e., $P_{IP_3}^{EC-EC} = 13.6$ s$^{-1}$; $P_{IP_3}^{SMC-SCMC} = 0.53$ s$^{-1}$). The IP$_3$ permeability is larger in the endothelium than in the smooth muscle due to the smaller endothelial $R_{g,j}$.

**Theoretical considerations.** The EC (44) and SMC (28) models were modified to include a description for myoendothelial communication through the NO/cGMP pathway, as previously described (29). Simultaneous measurements of Ca$^{2+}$ and NO in agonist-stimulated ECs indicate that NO production is regulated by cytosolic calcium (2, 11, 36). EC may also release NO in a Ca$^{2+}$-independent fashion, under some conditions, but such release is not accounted by the model at this stage (1). Thus we assumed that the relative NO production rate depends only on EC Ca$^{2+}$ concentration with a sigmoidal function. Once released by the endotheium, NO can freely diffuse across cell membranes and reach the SM to exercise its vasodilatory action. Theoretical models of NO transport in arterioles (49) predict that the concentration of the endothelium-derived NO in the smooth muscle is proportional to the EC NO release rate, and the concentration profile is relatively flat in the smooth muscle in the absence of significant extravascular scavenging. Equations and parameters that relate EC intracellular Ca$^{2+}$ concentration ([Ca$^{2+}$]$i$) to the NO levels in the smooth muscle are presented in Ref. 29. The concentration of the endothelium-derived NO in the smooth muscle can affect SMC [Ca$^{2+}$]$i$, and Vm by modifying four cellular components, as described earlier (Fig. 1).

**Length Constants.** A blood vessel can be approximated from the electrical point of view as a cable with certain membrane and internal conductances (7, 26). In a passive linear cable with infinite length, the steady-state spread of a local potential change is attenuated exponentially:
\[
\Delta V_m(x) = \Delta V_{m,\text{max}} \exp\left(-x/\lambda\right), \quad x \geq 0
\]  
where \(x\) is the distance along the vessel from the stimulus site, and \(\Delta V_{m,\text{max}}\) is the maximum \(\Delta V_m\) at the local site [i.e., \(\Delta V_{m,0}\)]. The constant \(\lambda\), referred to as the cable length constant, characterizes the attenuation and quantifies the extent of spread of voltage changes and the distance that information can be transmitted. If the cable’s length \(L\) is finite (i.e., comparable to \(\lambda\)), the attenuation is not exponential. In a segment with sealed ends, the \(\Delta V_m\) profile is described by the following equation:

\[
\begin{align*}
\Delta V_{m}(x) &= \begin{cases} 
\Delta V_{m,\text{max}} \cosh\left(\frac{L - y}{\lambda}\right) \cosh\left(\frac{y}{\lambda}\right), & 0 \leq y \leq L - y \\
\Delta V_{m,\text{max}} \cosh\left(\frac{x + y}{\lambda}\right) \cosh\left(\frac{y}{\lambda}\right), & -y \leq x \leq 0
\end{cases}
\end{align*}
\]  

where \(y\) is the location of the stimulus site (i.e., \(0 < y < L\)). Equation 6 is fitted to SMC \(\Delta V_m\) profiles predicted during current and ACh stimulation, and the length constants \(\lambda_{el}\) and \(\lambda_{el,ACh}\), respectively, are estimated.

In a similar fashion, a length constant can be defined for the attenuation of the EC Ca\(^{2+}\) spread along the vessel axis following stimulation and an increase of intracellular Ca\(^{2+}\) at the local site. Fitting the Ca\(^{2+}\) profile with an exponential function yields an apparent length constant for the Ca\(^{2+}\) spread (\(\lambda_{el}\)):  

\[
\Delta [Ca^{2+}](x) = \Delta [Ca^{2+}]_{i,\text{max}} \exp\left(-x/\lambda_{el}\right), \quad 0 \leq x \leq L - y
\]  

where \(\Delta [Ca^{2+}]_{i,\text{max}}\) is the EC Ca\(^{2+}\) elevation at the ACh stimulation site [i.e., \(\Delta [Ca^{2+}](x = 0)\)]. The exponential function was used because the Ca\(^{2+}\) spread in the endothelium was much smaller than the length of the vessel examined, and despite the fact the Ca\(^{2+}\) spread is affected by the diffusion of IP\(_3\) in a nonlinear fashion.

Numerical Methods

Each EC and SMC was modeled with 11 and 26 differential equations, respectively, and the vessel segment was described by a system of 12,030 differential equations (i.e., 30 ECs \(\times\) 11 equations/EC + 450 SMCs \(\times\) 26 equations/SMC). The equations were coded in Fortran 90 and solved numerically using Gear’s backward differentiation formula method for stiff systems (IMSL Numerical Library routine). The maximum time step was 4 ms, and the tolerance for convergence was 0.0005. Equations 6 and 7 were fitted in their corresponding domains to the predicted profiles using the least squares method. Both the length constant and the maximum local response were optimized in the fittings.

RESULTS

To examine possible differences in mechanisms and properties of spreading responses induced by agonist and electrical stimulations and the physiological relevance of the latter, we performed simulations with both types of stimuli. Simulations with blocked \(K_{\text{ir}}\) channels and with multiple layers of SMCs are presented in the Supplement.

Electrical Stimulation

In Fig. 4, a hyperpolarizing current (\(-150\) pA per EC) was injected for 3 s into the ECs located at \(x = 0\). (Note that, due to circumferential symmetry, the current was injected to every EC located at \(x = 0\)). The circles and squares represent \(V_m\) in SMCs and ECs, respectively, as a function of their location along the longitudinal direction \(x\). The maximum \(\Delta V_m\) occurred at the local site and was attenuated with distance. The \(V_m\) profile is fitted well by Eq. 6 with an apparent length constant, \(\lambda_{el} = 1.6\) mm (see Supplemental Fig. S1, solid line). [Note that a simple exponential function does not adequately fit the profile and overestimates the length constant.] Low and intermediate values for \(R_{EC-SMC}\) of 70 \(\Omega\) and 525 \(\Omega\) (i.e., equivalent to 4.7 \(\Omega\) and 35 \(\Omega\) per single SMC, respectively) (40) result in almost identical EC and SMC \(V_m\). Despite the significant difference in the two resistances, there was no observable difference in the \(V_m\) profile (Fig. 4, A and B). A high \(R_{EC-SMC}\) of 13.5 \(\Omega\) (i.e., equivalent to 900 \(\Omega\) per single SMC (54)) affects the resting EC and SM \(V_m\), but does not affect \(\lambda_{el}\) (Fig. 4C). Overall, a significant increase in \(R_{EC-SMC}\) had only a moderate effect on the conduction of signals initiated in the endothelium.

In a series of simulations, the vessel was also stimulated locally at the SM side. Figure 5 presents SMC \(V_m\) as a function of longitudinal distance for two different stimulation protocols, utilizing three different values for \(R_{EC-SMC}\). A significant depolarizing current (\(15 \times 150\) pA per EC) was injected into a single SMC located at \(y = 500\) \(\mu\)m (Fig. 5, A, C, and E). Alternatively, all SMCs connected to the same EC were stimulated by current injection (150 pA per SMC) (Fig. 5, B, D, and F) (similar to the arteriolar-segment voltage-clamp protocol presented in Ref. 9). Contrary to the EC stimulation in Fig. 4, there was a biphasic response when the vessel was stimulated from the SM side. The stimulated cell(s) exhibited significant depolarization that was more pronounced when \(R_{EC-SMC}\) was high (20 mV in Fig. 5A vs. 110 mV in Fig. 5E) and when the same

**Fig. 4.** The effect of myoendothelial \(R_{EC}\) on EC (C) and SMC (C) \(V_m\) in the 3-mm-long vessel at rest and at time \(t = 3\) s, after injection of a hyperpolarizing current (\(-150\) pA per EC; for 3 s) into the ECs located at \(x = 0\). Simulations are shown for a low \(R_{EC-SMC} = 70\) \(\Omega\) (A), an intermediate \(R_{EC-SMC} = 525\) \(\Omega\) (B), and high \(R_{EC-SMC} = 13.5\) \(\Omega\) (C).
total current was injected in a single vs. a series of SMCs (20 mV in Fig. 5A vs. 9 mV in Fig. 5B). Away from the stimulus site, the length constant of the electrotonic spread was maintained in all four scenarios and was similar to the length constant observed during the EC stimulation ($\lambda_{el} = 1.6$ mm). The amplitude of distant depolarization, however, decreased as the $R_{gj}^{EC-SMC}$ value increased (i.e., at $x = 2.5$ mm, there is 3-mV depolarization in Fig. 5A vs. 0.5 mV in Fig. 5E).

The cable length constant can be affected by the gap junction resistances in the endothelial and smooth muscle layers. Some experimental values for homocellular $R_{gj}$ have been reported in different vascular beds (Supplemental Table S2), but literature values vary significantly for the interendothelial $R_{gj}$ ($R_{gj}^{EC-EC}$). Table 1 summarizes model predictions for $\lambda_{el}$ utilizing different values for the homocellular gap junction resistances. For these simulations, the endothelium was stimulated with current injection similar to Fig. 4. An $R_{gj}^{EC-EC}$ in the lower range of the previously reported values (i.e., $3.3$ M$\Omega$) yields a cable length constant that is consistent with experiments in RMA (47). Based on this observation, we utilized this value for $R_{gj}^{EC-EC}$ in this study. Disruption of the endothelial gap junctions (i.e., $R_{gj}^{EC-EC} = \infty$) reduced the length constant by 20-fold, whereas disruption of the smooth muscle gap junctions (i.e., $R_{gj}^{SMC-SMC} = \infty$) had no significant effect on $\lambda_{el}$. In order for the SM layer to conduct changes in $V_m$ similarly to the endothelium, $R_{gj}^{SMC-SMC}$ has to be reduced to a value 15$^\text{th}$ times smaller than $R_{gj}^{EC-EC}$. This is due to the SMCs’ perpendicular orientation relative to the vessel’s axis and the number of SMCs assumed to span the length of an EC. Thus the endothelium is the major pathway for the electrotonic communication in the model. The estimated $\lambda_{el}$ does not depend significantly on the magnitude and polarity of the injected current, provided that the resulting $\Delta V_m$ remains within physiological range. Thus the vessel behaves similar to a linear cable in the physiological range of $V_m$ in agreement with experiments (Fig. 3 in Ref. 26).

**NE and ACh Stimulation**

Figure 6 presents the $\Delta V_m$ along the vessel, after 6 s of localized NE application. (Supplemental Fig. S2 shows corresponding simulations in the model with three layers of SMCs). In Fig. 6, A and B, three adjacent SMCs were stimulated with saturating concentration of NE (10 $\mu$M). In the model, NE generates a depolarizing current through activation of the NSC channels (Fig. 1). Simulations were performed for low (Fig. 6A) and high (Fig. 6B) $R_{gj}^{EC-SMC}$. The same stimulus had a significantly higher impact on the SMCs at the local site, if the NE was not well coupled to the endothelium (1.2 mV hyperpolarization in Fig. 6A vs. 15.3 mV in Fig. 6B). In both cases, the predicted effect of NE away from the stimulus site was small. In the first case (Fig. 6A), $\Delta V_m$ were conducted effectively along the vessel, but the distant response was small, because the total NE-induced transmembrane current was insufficient. In the second case (Fig. 6B), the NE-induced transmembrane current did not diffuse to other cells and produced a large local $\Delta V_m$. This change, however, was poorly conducted to distant sites. In Fig. 6, C and D, nine adjacent SMCs were

**Table 1. Predicted electrical length constant, $\lambda_{el}$ (mm)**

<table>
<thead>
<tr>
<th>$R_{gj}^{EC-EC}$, M$\Omega$</th>
<th>$R_{gj}^{EC-SMC}$, M$\Omega$</th>
<th>$V_m$/mV</th>
<th>$\lambda_{el}$ (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.3</td>
<td>1.6</td>
<td>1.6</td>
<td>3.3</td>
</tr>
<tr>
<td>17.5</td>
<td>0.67</td>
<td>0.67</td>
<td>17.5</td>
</tr>
<tr>
<td>31.8</td>
<td>0.5</td>
<td>0.5</td>
<td>31.8</td>
</tr>
<tr>
<td>$\infty$</td>
<td>1.45</td>
<td>0.46</td>
<td>$\infty$</td>
</tr>
</tbody>
</table>

$R_{gj}$, gap junction resistance; EC, endothelial cell; SMC, smooth muscle cell; $\infty$, infinity.

**Fig. 5.** EC (A) and SMC (C) $V_m$ in the 3-mm-long vessel at rest and at $t = 3$ s, after injection of a depolarizing current, 15 $\times$ 150 pA were injected for 3 s into either a single SMC (A, C, and E) or to 15 SMCs (B, D, and F). Simulations are shown for a low $R_{gj}^{EC-SMC} = 70$ M$\Omega$ (A and B), an intermediate $R_{gj}^{EC-SMC} = 525$ M$\Omega$ (C and D), and a high $R_{gj}^{EC-SMC} = 13.5$ G$\Omega$ (E and F). A large depolarization at the local site is predicted that becomes more pronounced as $R_{gj}^{EC-SMC}$ increases, and when the same total current is injected into one vs. many SMCs.
Fig. 6. EC (○) and SMC (▼) $V_m$ in the 3-mm-long vessel at rest and at $t = 6$ s, after local NE application. SMCs are stimulated for 6 s with a saturating concentration of NE (10 μM). A: three adjacent cells are stimulated, and a strong myoendothelial coupling is assumed ($R_{EC-SMC}^{E} = 70$ MΩ). B: three SMCs are stimulated, and a weak myoendothelial coupling is assumed ($R_{EC-SMC}^{E} = 13.5$ GΩ). C: nine SMCs are stimulated, and a strong myoendothelial coupling is assumed ($R_{EC-SMC}^{E} = 70$ MΩ). D: nine SMCs are stimulated, and a weak myoendothelial coupling is assumed ($R_{EC-SMC}^{E} = 13.5$ GΩ). The same agonist stimulus has a significantly larger effect at the local site, if the SM is poorly coupled to the endothelium.

Fig. 7. Model responses to local acetylcholine (ACh) stimulation in a vessel prestimulated with 200 nM of NE and with $R_{EC-SMC}^{E} = 525$ MΩ. From time $t = 2$ s, the ECs at position $x = 0$ are continuously stimulated with ACh [IP$_3$] release rate ($Q_{IP3,ss}$) = 0.55 nM/ms. A: endothelial [Ca$^{2+}$], as a function of time and distance from stimulus site; B: endothelial $V_m$ as a function of time and distance from stimulus site; C: smooth muscle [Ca$^{2+}$], as a function of time and distance from stimulus site; D: smooth muscle $V_m$ as a function of time and distance from stimulus site.

Fig. 7 shows a representative ACh-induced conducted response (Supplemental Fig. S3 shows corresponding simulations in the model with three layers of SMCs.) The simulation scenario mimics the experimental protocol from an earlier study of conducted vasoreactivity in RMA (47). The difference between the in silico and the in vitro study is that a sustained local application of the agonist is stimulated here. In experiments, transient focal stimulation is utilized to limit the diffusion of agonist away from the local site. Figure 7 shows the endothelial Ca$^{2+}$ concentration (A), the endothelial $V_m$ (B), the smooth muscle Ca$^{2+}$ concentration (C), and the smooth muscle $V_m$ (D) as a function of time and distance from the stimulation site. First, a continuous and uniform prestimulation of the vessel with 200 nM of NE is simulated until a steady-state is reached ($t = 0$). This results in an elevation of the SM Ca$^{2+}$ along the entire vessel to $\sim 200$ nM. At time $t = 2$ s, ACh is applied locally to 15 ECs located at distance $y = 400–500$ μm from the inlet of the arteriole. The concentration of ACh is not specified, but it is assumed that it increases the IP$_3$ release rate ($Q_{IP3,ss}$) from 0 to 0.55 nM/ms. IP$_3$ generation increases the [Ca$^{2+}$] in the ECs (Fig. 7A). In response to the elevation of [Ca$^{2+}$], the SKCa and IKCa channels open and hyperpolarize the cell (Fig. 7B). This hyperpolarization spreads rapidly to neighboring ECs and SMCs via gap junctions (Fig. 7, B and D). Hyperpolarization of the SMCs closes the voltage-operated Ca$^{2+}$ channels and reduces intracellular calcium (Fig. 7C).

Figure 8A depicts relative Ca$^{2+}$ changes in the SM along the vessel calculated from Fig. 7C. A 100% change denotes a reduction of Ca$^{2+}$ concentration to its resting value before NE prestimulation (i.e., from SM NE [Ca$^{2+}$] = 200 nM to SMC Rest [Ca$^{2+}$] = 130 nM). The model predicts significant Ca$^{2+}$ change throughout the vessel segment. Incorporation of the NO pathway in the simulations (Fig. 8A; dashed line) increased slightly the smooth muscle Ca$^{2+}$ reduction at the local and at the distant site. The NO/cGMP pathway activates at the local site BKCa channels in SMCs (Fig. 1), which generate hyperpolarizing currents. Because the myoendothelial coupling is strong, these currents are transmitted and enhance SM hyperpolarization at distant sites as well. The result is higher Ca$^{2+}$ reduction (i.e., relaxation). Inhibition of the interendothelial IP$_3$ diffusion impaired significantly the ACh-induced SM Ca$^{2+}$ reduction (Fig. 8A, dashed-dotted line). The inhibition of IP$_3$ diffusion abolishes EC Ca$^{2+}$ spread (Fig. 8B), which reduces the number of ECs with open SKCa and IKCa channels. Consequently, the total hyperpolarizing current generated at the local site is smaller, and the SM relaxation is impaired.
Figure 8B investigates the endothelial Ca\(^{2+}\) spread along the vessel. The difference between the basal prestimulation level and the poststimulation steady-state value of EC Ca\(^{2+}\) (i.e., Fig. 7A; \(t = 0\) and \(t = 30\) s) is depicted as a function of the distance (\(x\)) from the stimulation site. Ca\(^{2+}\) changes are normalized by the maximum change at the local site. Under control conditions (solid line), significant Ca\(^{2+}\) elevation was observed only within 300 \(\mu\)m from the stimulus site (apparent length constant, \(\lambda_{Ca} = 0.17\) mm). Inhibition of axial IP\(_3\) diffusion (i.e., \(P_{IP,EC-EC} = 0\), dash-dotted line) practically abolishes the Ca\(^{2+}\) spread. In simulation with EC gap junctions impermeable to IP\(_3\) and 100 times more permeable to Ca\(^{2+}\) than control (i.e., \(P_{Ca,EC-EC} = 100P_{IP}\), dashed line), Ca\(^{2+}\) spread is also limited (i.e., <400 \(\mu\)m, \(\lambda_{Ca} = 0.15\) mm).

Figure 9 examines the IP\(_3\)-independent Ca\(^{2+}\) electrodiffusion through the endothelial gap junctions. Figure 9, A and B, shows the poststimulation steady-state Ca\(^{2+}\) and \(\Delta V_m\), respectively, in ECs along the vessel's axis for the simulations presented in Fig. 7 (\(t = 30\) s). The Ca\(^{2+}\) flux between neighboring cells is presented as Ca\(^{2+}\) current (Fig. 9C). A positive current denotes Ca\(^{2+}\) ions flowing to the right, and a negative current to the left. There is a maximum 500 nM difference in [Ca\(^{2+}\)]\(_i\), and a maximum 0.5-mV difference in \(V_m\) between neighboring ECs at the local site. Under these conditions, the concentration gradient dominates the electrochemical gradient, and Ca\(^{2+}\) ions move away from the stimulated EC. The Ca\(^{2+}\) current through the gap junctions between the fifth and the sixth EC is 0.03 pA. Away from the stimulation site, the concentration difference between neighboring cells decreases, and so does the Ca\(^{2+}\) current. Interestingly, after the 10th EC, a very weak Ca\(^{2+}\) current flows toward the stimulation site (i.e., the current becomes negative) as the electrical field dominates the electrochemical gradient across the gap junction (Fig. 9C, inset).

**Effect of Stimulus Strength on \(V_m\) and Ca\(^{2+}\) Spread**

We investigated if the strength of agonist stimulation can affect the rate of decay of the conducted signal. Figure 10 examines the effect of NE prestimulation on the Ca\(^{2+}\) and electrotonic spread. The model vessel was stimulated uniformly with different concentrations of NE before local stimulation of the endothelium with ACh. Figure 10 shows the predicted length constants for the endothelial Ca\(^{2+}\) spread (\(\lambda_{Ca}\)) (A) and for the SM \(V_m\) attenuation \(\lambda_{gj,ACh}\) (B), for NE concentrations varying from 10\(^{-2}\) to 10\(^{2}\) \(\mu\)M. In Fig. 10A, the stimulating ACh concentration was either held constant (solid line) or was modified to produce the same local [Ca\(^{2+}\)]\(_i\) increase at each NE concentration (dashed line). The \(\lambda_{Ca}\) can increase significantly with increasing levels of NE prestimulation. This effect is reduced but is not abolished if the same local Ca\(^{2+}\) transient is preserved at each NE concentration (dotted line). On the contrary NE prestimulation can reduce \(\lambda_{gj,ACh}\) and thus the electrotonic spread (Fig. 10B). The significance of this effect is increased for high myoendothelial gap junction resistance (solid line). Large NE prestimulation also reduces the magnitude of the SMC \(\Delta V_m\) (data not shown). For endothelium initiated responses, as the concentration of ACh

![Diagram](http://ajpheart.physiology.org/)
Myoendothelial gap junction resistance plays also an important role in the model’s behavior. Sandow and Hill (40) provided an estimate for $R_{EC-SMC}^{gj}$ in proximal RMA, based on morphological observations. A resistance value of 70 MΩ per gap junction and two gap junctions per SMC was reported (corresponding to 35 MΩ per SMC). The estimate of Yamamoto et al. (54) in guinea pig mesenteric arteries was significantly higher (i.e., 900 MΩ per single SMC). The incidence of myoendothelial gap junctions may vary between vascular beds and may even be significantly smaller in proximal rather than in distal RMA (40). For these reasons, a wide range of values were utilized in the model (i.e., $R_{EC-SMC}^{gj} = 70–13,500$ MΩ cell to cell resistance or 4.7–900 MΩ total myoendothelial resistance per SMC).

In simulations utilizing high $R_{EC-SMC}^{gj}$, conduction of a signal initiated in SMCs was limited compared with a signal initiated in ECs (Figs. 4C vs. 5, E and F). Such differential electrical communication is consistent with responses seen and simulated in some vascular beds, for example, in hamster feed arteries of the retractor muscle (43) and in Ref. 9. Experimental data suggest, however, that, in RMA, the endothelial and smooth muscle layers function as an electrical syncytium, and $\Delta V_m$ initiated in the ECs and SMCs are conducted similarly (47). Thus a value of $R_{EC-SMC}^{gj}$ in the lower range of Supplemental Table S2 is more likely in RMA. Indeed, for $R_{EC-SMC}^{gj}$ between 70 and 525 MΩ, similar hyperpolarization/depolarization appear away from the stimulus site, regardless of whether the vessel is stimulated in the endothelium or smooth muscle side (at $x = 2.5$ mm, $\Delta V_m = \sim 3$ mV in Fig. 4, A and B, and Fig. 5, B and D). However, even for low $R_{EC-SMC}^{gj}$, there is a difference between stimulation in the two sides of the vessel wall. There is a significant local $\Delta V_m$ when the current is injected in the SMC(s) that attenuates rapidly over the next few SMCs. This change is more pronounced if the same current is injected in a single vs. an array of neighboring cells (Figs. 5, A–D), because, in the latter case, the effective resistance between the stimulus site and the endothelium is smaller.

Current vs. Voltage-clamp Stimulations

Our simulation results are in agreement with model results presented by Diep et al. (9). This earlier study investigated spreading responses after ECs or SMCs were clamped at a given $V_m$ (Δ$V_m,max = 15$ mV), rather than current clamp used in the present study. Their model predicted that SM-initiated responses conducted poorly along the vessel, and a substantial voltage response in the endothelium could only be generated when a sufficient number of SMCs were clamped simultaneously. The authors attributed this behavior to poor coupling of the SMC with adjacent cells and their perpendicular orientation to the vessel axis. Our simulations using the same effective myoendothelial resistance (Fig. 5, E and F) show similar trends for SM-initiated responses. Our simulations, however, point out that a larger total injected current is required to clamp an EC vs. a SMC or many vs. few SMCs, to a given $V_m$. When the resistivity with neighboring cells is high, a large $\Delta V_m$ of the stimulated cell can be achieved with a relatively small injected current (i.e., the current does not diffuse and causes a larger depolarization/hyperpolarization; Figs. 5A vs. 5E). Thus, if the same $V_m$ clamp is applied to an EC and a SMC, the injected current will be higher in the

DISCUSSION

Role of Gap Junctions in $V_m$ Spread

Gap junctions composed of specific connexins are central to the propagation of dilations (18, 53). Electrotonic conduction of $\Delta V_m$ through gap junctions is considered to be the major mechanism of spreading responses in a number of vascular beds (20). The endothelium, rather than the SM, seems to be the major conducting pathway in RMAS, as supported by experiments with disrupted endothelial layer (47). Model simulations with electrical and agonist stimuli confirm these findings. Simulations demonstrate that the cable length constant, $\lambda_{EC}$, is inversely proportional to the square root of $R_{EC}^{gj}$. (Note: in a passive linear cable, the length constant exhibits the same dependence on axial resistance). The exact value of $R_{EC}^{gj}$ in RMA is not known. However, a value of 3.3 MΩ was estimated by Liddington et al. (34) for rat skeletal muscle. Based on this value, the predicted $\lambda_{EC}$ was in close agreement with the experimental $\lambda_{EC}$ value determined in guinea pig small-intestine arterioles (26), hamster feed arteries (15), and with dilatation data from RMA (20, 47). After disruption of the endothelial gap junctions, the ability of the model vessel to carry spreading responses was minimized, as indicated by a large decrease in $\lambda_{EC}$ (Table 1).
well-coupled endothelium. The two studies combined suggest that distal responses are more sensitive to the total current injected at the local site and less to the achieved membrane depolarization/hyperpolarization at the local site.

Both the current and the voltage clamp, however, introduce a bias in evaluating conduction when applied to cells with different gap junction connectivity. A voltage clamp will favor conducted responses in well-coupled systems (i.e., a higher stimulating current is introduced). A current clamp will overestimate conduction in poorly coupled systems (i.e., by not accounting for the effect of $V_m$ on agonist-induced transmembrane currents). Simulations presented in Fig. 6 are independent of the bias introduced by the choice of the stimulation protocol (i.e., voltage or current clamp). Simulations of agonist-induced reactivity are physiologically more relevant and differ from both voltage and current clamps. They can also be utilized to predict how many cells need to be stimulated in vivo to produce an observable effect at distant sites. Simulations show that a saturating concentration of NE applied to three SMCs induces a small response away from the stimulus site (Fig. 6A). The ability of the SM to initiate a conducted electrotonic signal increases as the myoendothelial coupling increases (Fig. 6, A vs. B) and when more SMCs are stimulated (Fig. 6, C vs. A). Interestingly, the local $V_m$ response to NE depends significantly on the myoendothelial connectivity, and SMCs that are not well coupled produce significantly higher local depolarization to NE. Thus, as coupling decreases, the ability of the SMCs to generate a distant signal decreases, but the sensitivity of these cells to agonist stimulation locally increases.

In vascular beds, where SMCs are not well coupled with neighboring ECs and SMCs, the stimulation of a few SMCs can produce large, localized changes in arteriolar tone. On the other hand, stimulation of a number of ECs or SMCs has the potential to generate spreading responses and regulate vasoactivity over larger vascular segments (9). The physiological importance of the vessel’s ability for SMC-initiated localized constriction needs to be further investigated. For example, what is the difference in the regulation of blood supply by a strong, local constriction instead of a weaker, spreading constriction over a larger vascular segment?

**Physiological Relevance of Stimulatory Protocols**

Figure 6 demonstrates that saturating concentrations of agonist can impose different levels of maximum depolarization depending on the homo/heterocellular connectivity of the cells and the number of the stimulated cells. Thus the physiological relevance of a voltage-clamp stimulation protocol is difficult to assess and should be different in intact vessels from isolated cells. In isolated SMCs, saturating NE concentration should depolarize $V_m$ up to $-40$ to $-30$ mV, and thus voltage clamps in that range are considered physiological for isolated SMCs. As the homo- and heterocellular coupling of the SMC with neighboring cells increases, the maximum physiological value should decrease. In addition, model simulations with saturating concentration of agonist in either layer reveal maximum transmembrane currents in the order of a few tens of picoamperes (i.e., determined mostly by the maximum conductance of $K_{Ca}$ channels in ECs and the NSC in SMCs). Since the level of homo- and heterocellular coupling affects agonist-induced $\Delta V_m$, the maximum transmembrane currents that occur in vivo will be affected as well. Thus, for both the voltage and the current clamp, the maximum physiological magnitude will depend on the connectivity of the stimulated cell.

These considerations suggest supraphysiological current injections per single EC in Figs. 4 and 5 and potentially in the majority of experimental studies of conducted vasoreactivity that utilize current injection in a single cell with intracellular microelectrodes. The use of a significant intracellular current injection (hundreds of picoamperes) in experimental studies is necessary to evoke robust responses, e.g., 1–3 nA in vessels 25–60 $\mu$m in diameter (26). Similar total current is predicted by the model. Because of circumferential symmetry, in a vessel with 42 $\mu$m diameter, 150 pA per EC will correspond to a total injected current of 3 nA ($\sim$20 ECs in the circumferential direction).

Although such voltage clamps and current injections remain a useful experimental approach to characterize the electrical behavior of a vessel segment, this comparison suggests that, in vivo, multiple cells need to be stimulated to generate a significant conducted response.

**Role of EC Ca$^{2+}$ Spread**

In hamster feed arteries, the conduction of hyperpolarization was augmented during ACh stimulation compared with electric current stimulation (15). This augmentation was indicated by an increase in the length constant from $\lambda_{el} = 1.2$ mm to $\lambda_{el,ACH} = 1.9$ mm. In the model, stimulation with ACh did not significantly increase the length constant ($\lambda_{el} \sim \lambda_{el,ACH} \sim 1.6$ mm). This lack of facilitation was due to limited endothelial Ca$^{2+}$ spread following ACh stimulation (Figs. 7 and 8B). However, the limited but considerable ACh-induced Ca$^{2+}$ spread amplified significantly the hyperpolarization at the local and distant sites. Elevation of Ca$^{2+}$ in ECs near the stimulation site activates SKCa and IKCa channels, which contribute to the total current generated at the local site. Because the maximum current generated by individual cells is limited, EC Ca$^{2+}$ spread increases the ability of focal stimuli to induce conducted response with physiologically relevant magnitude. The amplification of the current is approximately equal to the ratio of the distance of Ca$^{2+}$ spread to the length of the stimulation site. In the simulations, inhibition of EC IP$_3$ and Ca$^{2+}$ spread impaired the SMC relaxation (Fig. 8A) by reducing local hyperpolarization from 10.7 to 3 mV. EC Ca$^{2+}$ spread can also increase the number of BK$_{Ca}$ channels in SMCs activated by the NO/cGMP pathway (Fig. 1) and further enhance the total hyperpolarizing current. However, this may have a small effect on the relaxation in the presence of strong endothelium-derived hyperpolarizing factor, and negligible NO effects on conducted responses have been reported in RMAAs (52).

The predicted endothelial Ca$^{2+}$ spread agrees with experimental studies on RMAAs (47, 52) and certain other vessel types (12, 19, 46). For example, Fig. 5 in Ref. 47 shows that a noticeable EC Ca$^{2+}$ increase appears only within a distance of 500 $\mu$m from the ACh stimulation site. On the other hand, studies in other vessels reported significant NO- and endothelium-derived hyperpolarizing factor-dependent components of the conducted response and suggested EC Ca$^{2+}$ increase at remote sites (4, 13, 16, 43). These findings have been challenged by others (19, 51). Recently distant EC Ca$^{2+}$ waves
were observed in hamster feed arteries (10, 50) and transgenic mice cremaster muscle arterioles (48). Our simulations do not negate the possibility of different signal transduction mechanisms in spreading responses in various vessel types.

IP$_3$-mediated Ca$^{2+}$ Spread vs. Direct Interendothelial Ca$^{2+}$ Diffusion

The mechanism responsible for the generation of a propagating Ca$^{2+}$ wave along the endothelium remains unclear. In the model, the limited yet noticeable endothelial Ca$^{2+}$ spread was mediated by axial IP$_3$ diffusion and subsequent Ca$^{2+}$ release from the stores. The relative importance of IP$_3$ and Ca$^{2+}$ diffusion depends on the assumed values for the gap junction permeabilities ($p_{ij}$ and $P_{Ca}$). These parameters have not been experimentally determined, and previous theoretical studies have used arbitrary values. In this study, $P_{EC-EC}$ was inversely related to $R_{ij}$ (Eq. 3).

For the control value of $R_{ij}$, direct Ca$^{2+}$ diffusion (<0.04 pA in Fig. 9C) was much smaller compared with other transmembrane Ca$^{2+}$ fluxes (~1 pA) and thus had a negligible effect on the global Ca$^{2+}$ transfer. $P_{EC-EC}$ has to increase 100-fold to result in noticeable direct Ca$^{2+}$ spread, but even then it was limited to 400 μm (Fig. 8B). The predicted contributions of IP$_3$ and Ca$^{2+}$ agree with the experimental data, which indicate that direct Ca$^{2+}$ communication via homocellular gap junctions is not essential for Ca$^{2+}$ waves (39). Some theoretical models of IP$_3$-mediated intercellular Ca$^{2+}$ waves have assumed negligible direct intercellular Ca$^{2+}$ diffusion (31, 45). 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 mechanism [e.g., a regenerative release of IP$_3$ triggered by ACh (48)].

Effect of Intercellular and Transmembrane Potential Gradients

Inhibition of endothelial K$_{Ca}$ channels unmasked Ca$^{2+}$-dependent, slow-conducted vasodilatation in hamster feed arteries (10). We used the model to examine if blocking conducted hyperpolarization can facilitate direct Ca$^{2+}$ waves along the vessel axis and tested the hypothesis that the physiological $V_m$ gradient inhibits longitudinal Ca$^{2+}$ diffusion. The predicted intercellular $V_m$ gradients (Fig. 9) agree with experimental recordings (47). Near the stimulation site, the electrical field across the gap junctions had a minimal effect on the Ca$^{2+}$ spread, and the Ca$^{2+}$ concentration gradient was the major determinant of a rather limited intercellular Ca$^{2+}$ flux. The electrical field becomes dominant only far away and can reverse the diffusion of Ca$^{2+}$ in a direction toward the stimulus site. The magnitude of this electrically driven intercellular Ca$^{2+}$ flux is minimal and cannot affect [Ca$^{2+}$]$_i$.

In some EC types, hyperpolarization itself can increase [Ca$^{2+}$]$_i$, by increasing the driving force for Ca$^{2+}$ entry (35, 37). Therefore, it was speculated that conducted hyperpolarization could trigger Ca$^{2+}$ transients that can activate NO release and dilate the vessel at distant sites (4). However, there is no direct evidence for the existence of such mechanism in spreading responses. It is also not clear if such hyperpolarization-induced Ca$^{2+}$ changes would be adequate for distant dilatation (19). Our laboratory has previously investigated the controversial effect of V$_m$ on [Ca$^{2+}$]$_i$ in the isolated EC model (44) and showed that resting and plateau Ca$^{2+}$ levels were rather insensitive to Δ$V_m$. The magnitude of hyperpolarization associated with conducted responses in the model could not elicit a large Ca$^{2+}$ transient. Consequently, conducted hyperpolarization did not trigger an endothelial Ca$^{2+}$ wave and did not induce NO release, activation of SK$_{Ca}$ and IK$_{Ca}$ channels, or facilitated spreading responses.

Effect of Stimulus Strength

Simulation results (Fig. 10) indicate that the length constants of the Ca$^{2+}$ and V$_m$ spread may depend on the concentration of the applied agonist or the presence of other stimuli. These effects cannot be predicted in simplified cable models. The cable length constant depends on axial and radial resistances. Thus it can be modulated by agents that change the gap junction and cell membrane resistances. In the model, prestimulation with NE reduced $\lambda_{L,ACh}$ by opening NSC channels and reducing SM membrane resistance (Fig. 10B). Haug and Segal (23) reported that activation of $\alpha_1$- and $\alpha_2$-adrenoceptors in feed arterioles of the hamster retractor muscle inhibits conducted vasodilation. Using a cable model, they also showed that decreased SM membrane resistance or increased myoendothelial resistance could account for the inhibition. Gustafsson and Holstein-Rathslo (21) showed experimentally that angiotensin II increased the electrical length constant in RMA. They hypothesized that this was a result of increased cell-to-cell coupling or membrane resistance. Increased membrane resistance could result from agonist-induced inhibition of potassium channels in SMCs, but this mechanism was not incorporated into the model due to lack of appropriate direct experimental evidence. Our data suggest that SM prestimulation can significantly affect the radial resistivity of a vessel, which, in turn, can alter the observed distance of electrical conduction.

Experimentation with hamster feed arteries led Uhrenholt et al. (50) to hypothesize that SM tone could increase basal EC [Ca$^{2+}$], and sensitize the endothelium for Ca$^{2+}$ wave propagation. A similar phenomenon was observed in the model by an increase in the length constant of the EC Ca$^{2+}$ spread following SM prestimulation (Fig. 10A). NE prestimulation generated IP$_3$ in the SM, which diffused to the ECs. The presence of a basal, subthreshold concentration of IP$_3$ sensitized the endothelium to IP$_3$ and ACh. This is due to the nonlinearity of store Ca$^{2+}$ release (i.e., Ca$^{2+}$ released from the stores is a sigmoidal function of IP$_3$ concentration). Following NE stimulation and EC sensitization, the same concentration of ACh induces a larger Ca$^{2+}$ response and an IP$_3$-dependent Ca$^{2+}$ wave with a larger $\lambda_{Ca}$ (Fig. 10A, solid line). Even imposing the same increase in IP$_3$ at the local site gives a more dramatic Ca$^{2+}$ increase in neighboring cells (sensitized by prestimulation). The result is an IP$_3$-dependent Ca$^{2+}$ wave with a larger $\lambda_{Ca}$ in the presence of NE (Fig. 10A, dashed line). Figure 10A also indicates that, at a given NE concentration, $\lambda_{Ca}$ increases with stronger ACh stimulation. On the other hand, the electrical length constant, $\lambda_{L,ACh}$, does not depend on the concentration of the stimulating agent (i.e., ACh) (data not shown), indicating that the vessel can be approximated from an electrical point of view as a linear cable.

The Effect of Multiple Layers of SMCs

The addition of multiple layers of SMCs into the vessel model did not change qualitatively the mechanisms and properties of conducted responses. However, multiple layers of
SMCs increase radial current leak from ECs to SMCs, which reduces the magnitude and length constant of the responses conducted through the endothelium. For example, three layers of SMCs reduced the length constant and the maximum hyperpolarization in ACh-induced response by 36 and 20%, respectively (Fig. 9B vs. Supplemental Fig. S3B). In case of SM stimulation by NE or the NO/cGMP pathway, the additional layers of SMCs can generate more current and compensate for the increased radial leak at the distant sites (Supplemental Fig. S3, A and B vs. C and D). To allow effective current diffusion from the stimulated SMCs to the endothelium, the myoendothelial and radial SMC-to-SMC resistances should be low (Supplemental Fig. S2). Increasing the number of SMC layers does not eliminate the need for endothelium in mediating spreading responses. It follows from the symmetry that, if a single layer of SMCs conducts local stimulation poorly, then addition of another layer with identical parameters and stimulation will have no effect. This is because the net axial and radial resistances decrease in the same proportions and the cable length constant does not change.

Spread of Relaxation

The model does not include at this stage a description for Ca\(^{2+}\)-induced force development and for the resulting changes in vessel diameter. Assuming that constriction and relaxation are proportional to Ca\(^{2+}\) changes in the smooth muscle, a preliminary comparison can be made between our simulation results and experimental data on spreading relaxations. SMC Ca\(^{2+}\) profiles predicted by the model and the actual relaxation profile obtained from RMA (47) show significant responses at distances 1.5 mm away from the stimulus site. The mechanical/dilatation length constant, determined by fitting an exponential to diameter changes, is typically between 1 and 2 mm, depending on the vessel type (20). The model gives SM Ca\(^{2+}\) and hyperpolarization profiles with length constants in the range of 0.9–1.6 mm (Fig. 10), depending on the NE prestimulation level.

Model Limitations

The model captures the major aspects of conducted responses in RMA and makes predictions about the parameters that can affect the transmission of information along the vessel. However, the study does not account for behavior seen in other vessels, such as Ca\(^{2+}\) waves over considerably greater distances and spreading vasodilation in the absence of \(\Delta V_n\). The simulation results do not negate these experimental findings, and they suggest different type and expression levels of ion channels and Ca\(^{2+}\) handling machinery in those vessels. Spreading vasoreactivity depends also on vessel size. Single and multiple layers of SMCs were incorporated into the model to simulate arterioles and resistance arteries, but quantitative information for the subcellular components at each vessel size is not available.

The predictions are further limited by the uncertainty in a number of parameter values and by a number of simplifying assumptions. The EC length depends on the vessel type and size, and a representative value was chosen for this study. More general predictions can be made if the reported distances are normalized with respect to the assumed EC’s length. The assumed arrangement of ECs may also influence spreading responses. ECs may overlap and form tortuous conduction pathways. The assumed circumferential symmetry and the regular end-to-end EC coupling may lead to an overestimate of the length constants. [Notice, however, that the overlapping arrangement of ECs is equivalent to the serial arrangement (both depicted in Fig. 2), if no current is leaking from the endothelium.] Circumferential heterogeneity would also arise from coupling each SMC to different underlying ECs, and it was reported that, on average, only two ECs are coupled to the same SMC (40). In Ref. 9, this was simulated by randomly connecting each SMC with 2 out of 16 underlying ECs. However, this heterogeneity in the myoendothelial coupling should have a negligible effect on axial signaling. The number of ECs coupled to each SMC was accounted for in this model by distributing the total gap junction permeability equally to all of the underlying cells in contact.

The \(R_{\text{EC-EC}}\) is a critical parameter for conducted responses and has not been determined in RMA. Simulation results suggest a \(R_{\text{EC-EC}}\) close to the lower end of previously reported values. The permeability of gap junctions to IP\(_3\) is not known. In this study, \(p_{\text{IP}}\), \(p_{\text{IP}_{\text{EC-EC}}}\), and \(p_{\text{IP}_{\text{SMC-SMC}}}\) were assumed to be inversely proportional to \(R_{\text{EC-EC}}\) and \(R_{\text{SMC-SMC}}\), respectively. In an earlier study, \(p_{\text{IP}_{\text{EC-EC}}} \times R_{\text{EC}} = 0.05 \text{s}^{-1}\), and \(R_{\text{EC-EC}} = 900 \mu\Omega\) per SMC were assumed, and the same product for these two values was maintained here. Although this parameter does not directly affect the axial communication, the excessive myoendothelial IP\(_3\) diffusion at larger \(p_{\text{IP}_{\text{EC-EC}}}\) may indicate that the \(p_{\text{IP}}\), \(p_{\text{IP}_{\text{EC-EC}}}\) product and/or the EC and SMC models are inaccurate.

Limitations in the isolated EC and SMC models have been discussed previously (28, 44). Uncertainty in parameter values and the absence of spatial resolution in the EC and SMC models present two of the most serious limitations. Parameters that affect transmembrane currents in particular can have an impact on predictions regarding passive and facilitated conduction, while parameters affecting the intracellular balance of IP\(_3\) and Ca\(^{2+}\) can determine the predicted Ca\(^{2+}\) spread. The lack of subcellular resolution may be acceptable from the electrical point of view (real cells are essentially isopotential), but the intercellular Ca\(^{2+}\) waves may depend on spatial distribution of ryanodine receptors and/or IP\(_3\)Rs and Ca\(^{2+}\)-induced Ca\(^{2+}\) release from the SR along the cells. A multicellular SM model with intracellular Ca\(^{2+}\) waves was proposed recently to study synchronization (27), but the development of EC and SMC models with Ca\(^{2+}\) waves, sparks, or pulsars is restricted by the absence of relevant tissue-specific parameter values.

Conclusions

A computational model of a vessel segment was developed to investigate spreading responses in RMAs and arterioles. The study advances previous theoretical work by using detailed models of EC and SMC and by accounting for changes in the concentration of intracellular species. Simulation results corroborate experimental findings that spreading vasorelaxation in RMAs mainly reflects Ca\(^{2+}\)-independent, passive conduction of hyperpolarization along the endothelium. The model predicts that intercellular IP\(_3\) diffusion is more important than direct intercellular Ca\(^{2+}\) diffusion, and it can play a role in modulating spreading responses. Endothelial IP\(_3\) diffusion mediated limited but significant Ca\(^{2+}\) spread that amplified total current generated at the local site. The length constant of...
voltage or Ca\(^{2+}\) propagation depends on the presence and concentrations of stimulating agonists. Simulations demonstrate that intercellular uncoupling attenuates conducted responses but sensitizes cells to local agonist and electrical stimuli. Voltage clamp is a more appropriate experimental analog of local agonist stimulation in cells that are weakly coupled and current clamp in cells that are well coupled. Overall, the mechanisms that modulate conducted vasoreactivity are complex and are often difficult to assess using a reductionist approach and qualitative syllogisms. The development of detailed computational models holds promise for the elucidation of nonlinear interactions between system components and their potential effect on signal transmission.

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DISCLOSURES

No conflicts of interest are declared by the author(s).

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