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Intercellular communication in the vascular wall: A modeling perspective

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

Department of Biomedical Engineering, Florida International University, 10555 West Flagler Street, Miami, FL 33174

Abstract

Movement of ions (Ca^{2+}, K^+, Na^+ and Cl^-) and second messenger molecules like inositol 1, 4, 5-trisphosphate inside and in between different cells is the basis of many signaling mechanisms in the microcirculation. In spite of the vast experimental efforts directed towards evaluation of these fluxes, it has been a challenge to establish their roles in many essential microcirculatory phenomena. Recently, detailed theoretical models of calcium dynamics and plasma membrane electrophysiology have emerged to assist in the quantification of these intra and intercellular fluxes and enhance understanding of their physiological importance. This perspective reviews selected models relevant to estimation of such intra and intercellular ionic and second messenger fluxes and prediction of their relative significance to a variety of vascular phenomena such as myoendothelial feedback, conducted responses and vasomotion.

Keywords

Intercellular signaling; Ca^{2+} and IP_3 fluxes; myoendothelial projections

Intercellular signaling allows for integration and coordination of responses in microcirculatory vessels, and is critical for local regulation of vascular tone and blood flow [1; 2; 3]. Cell - cell communication depends to a large extent on homocellular and heterocellular gap junction channels which form pathways for the diffusion of ionic species and second messengers. The exchange of current, calcium (Ca^{2+}) and inositol 1,4,5-trisphosphate (IP_3) through gap junctions, in particular, can readily initiate signaling events in neighboring cells and thus, these three fluxes can play a key role in vascular communication.

Ca^{2+} has been established as a key signaling molecule in the cardiovascular system that regulates a plethora of functions including tone development or the release of vasoactive mediators. It also modulates electrical properties and the membrane potential (V_m). Although Ca^{2+} homeostasis and intercellular fluxes have attracted most of the attention of investigators, other ionic species can influence Ca^{2+} dependent responses and participate in vascular signaling. K^+, Cl^-, and Na^+ concentrations, for example, determine corresponding

*Corresponding Author: Nikolaos Tsoukias, Associate Professor, Department of Biomedical Engineering, Florida International University, 10555 W. Flagler St., TEC 2674, Miami, FL 33174, [Tel]: 305 348-7291, [Fax]: 305 348-6954, tsoukias@fiu.edu.

membrane currents and thus membrane potential and the activity of voltage-operated Ca^{2+} channels (VOCC) in smooth muscle cells (SMCs) [4]. Na^+ balance has been suggested to affect intracellular Ca^{2+} via Na^+-Ca^{2+} exchange in SMCs [5].

Direct Ca^{2+} diffusion through gap junction or indirect Ca^{2+} coupling through the diffusion of IP_3 can provide a pathway for cell to cell communication. Intercellular Ca^{2+} fluxes have been suggested to synchronize SMCs in vasomotion [6] and IP_3 and/or Ca^{2+} diffusion via heterocellular gap junctions may generate a myoendothelial feedback control loop that can modulate SM responses to vasoconstrictors [7; 8]. Current carried by the three major ionic species is also a key signal for coordinated vessel behavior. Current flow within the smooth muscle or endothelial layers, as well as between the two layers, play a central role in conducted responses [3] and Cl^- currents have been suggested to coordinate SMCs during vasomotion [9]. Furthermore, experimental results indicate that intracellular ionic composition of endothelial cells (ECs) is altered after coupling to SMCs [10].

Despite several evidence that exchange of ions and second messenger occurs between vascular cells, it has been often difficult to assess their relative importance in different responses. A major limitation in investigations is the quantification of these intercellular fluxes experimentally. Thus, alternative hypotheses have often been proposed regarding the actual signaling mediator that contributes to a coordinated vessel behavior. Theoretical analyses and mathematical models can contribute in this discussion by providing estimates for the magnitude of these fluxes and their potential contribution in signaling. This perspective utilizes such approaches to examine the role of these mediators in myoendothelial communication, conducted responses and vasomotion.

Estimation of intercellular fluxes through gap junctions

Gap junctions allow for the transmission of electrical current between cells, as well as for the diffusion of second messengers such as Ca^{2+} and IP_3. Current is carried mostly by the three major ionic species (i.e. K^+, Cl^-, Na^+) and evokes changes in V_m. The fluxes of ions and second messengers depend on the electrochemical gradient as well as on the permeability and density of gap junctions between the two cells.

An unspecified electric current can be calculated from the membrane potential difference between two coupled cells *n*-th and *m*-th ($V_m^n - V_m^m = \Delta V_{gj}$) and the effective gap junction resistance (R_{gj}) [11]:

$$I_{gj} = (V_m^n - V_m^m)/R_{gj} = \Delta V_{gj}/R_{gj}. \quad (1)$$

To estimate the intercellular exchange of a species, a flux proportional to concentration difference between two cells has often been assumed [12]:

$$J_{gj,S} = P_{gj,S}([S]_i^n - [S]_i^m) \quad (2)$$

where $P_{gj,S}$ is the gap junction permeability to *S* (e.g., Ca^{2+}, IP_3, cyclic adenosine monophosphate (cAMP), cyclic guanosine monophosphate (cGMP)).

Current, however, is carried by ionic species and many second messengers are charged particles, and the simplified equations (1) and (2) do not account for exchange based on the combined electrochemical gradient. A linear model can be used to account for both chemical and electrical gradients acting on specified charged species *S* [9]:

$$I_{gj,S}=P_{gj,S}z_S F\left(\Delta[S]_{gj}+\frac{z_S F}{RT}\left[\overline{S}\right]_{gj}\Delta V_{gj}\right) \quad (3)$$

where $\Delta[S]_{gj}=[S]_i^n-[S]_i^m$, and $\left[\overline{S}\right]_{\mathrm{gj}}=\left([S]_{\mathrm{i}}^n-[S]_{\mathrm{i}}^m\right)/2$ are the concentration difference and the average concentration across the gap junction (Fig. 1).

Current/flux of an ionic species via a gap junction can also be estimated from the nonlinear Goldman-Hodgkin-Katz (GHK) equation:

$$I_{gj,S}=P_{gj,S}\frac{z_S^2F^2}{RT}\Delta V_{gj}\frac{[S]_i^n-[S]_i^m\exp\left(-z_S F\Delta V_{gj}/RT\right)}{1-\exp\left(-z_S F\Delta V_{gj}/RT\right)} \quad (4)$$

where z_s, *F*, *R* and *T* are the valence of ion *S*, Faraday's constant, gas constant and temperature, respectively [13; 14]. It predicts a rectifying *I-V* relationship when ionic concentrations are unequal, with larger conductance when current flows from the side of higher concentration [15]. Under physiological range of concentration and potential differences, the GHK equation is similar to the Ohmic behavior from Eq. 3.

In most theoretical models, R_{gj} and P_{gj} are assumed constant in Eqs. 1 – 4. In general, gap junctions can be dynamically regulated by potential difference, phosphorylation and various second messengers, including Ca^{2+} [13; 16; 17; 18]. Theoretical models demonstrated that second messengers acting on gap junctions can produce 50-125 % changes in the number of recruited cells after local stimulation [12]. Modeling and patch-clamp studies also suggest that downregulation of intercellular communication is likely to be more physiologically important than upregulation due to relatively high open probabilities of gap junction channels [1]. The role of these changes in the regulation of vascular tone under control conditions remain unclear [19; 20], although some studies suggest that physiological concentrations of cytosolic Ca^{2+} can regulate the permeability of Cx43 in a calmodulin-dependent manner [21].

If the concentration of any charged particle is the same in the two cells, Eqs. 3 and 4 reduce to Eq. 1 and the flux depends only on V_m gradient. If there is no difference in V_m between the coupled cells then Eqs. 3 and 4 are reduced to Eq. 2 and intercellular flux is proportional to concentration difference between the two cells. In general, significant differences in both V_m and concentrations can appear between two cells particularly in heterocellular coupling or during asymmetric stimulations.

This approach allows us to partition the total gap junction current into the currents carried by individual ionic species:

$$I_{gj,tot} = \sum I_{gj,S} \quad (5)$$

The intracellular concentrations of K^+, Cl^- and Na^+ are much larger than that of Ca^{2+} and other charged molecules permeable through gap junctions; thus, the total electric current is mediated mostly by these three ions. If there are small concentrations gradients across the gap junction for the three major ionic species, the total current is given by:

$$I_{gj,tot} = \frac{\Delta V_{gj}}{R_{gj}} \approx \sum \left(P_{gj,S} \frac{z_S^2 F^2}{RT} [S]_i \Delta V_{gj} \right), \text{where} S = \mathrm{K}^+, \mathrm{Cl}^-, \text{and} \mathrm{Na}^+. \quad (6)$$

Vascular gap junctions are poorly selective for small ionic species *M* [1; 15], and thus, their ionic permeabilities $P_{gj,M}$ should be similar and approximately equal to a common permeability P_{gj}. P_{gj} can then be estimated from Eq. 6 [14]:

$$P_{gj,M} \approx P_{gj} \approx \frac{RT}{F^2 R_{gj} \sum \left(z_S^2 [S]_i \right)}, \text{where} S = \mathrm{K}^+, \mathrm{Cl}^-, \text{and} \mathrm{Na}^+. \quad (7)$$

Eq. 7 allows us to predict the ionic permeability from reported values of R_{gj}. Although the electrical resistance is easier to measure experimentally than the permeability, it is also associated with large uncertainty due to experimental limitations and tissue/preparation dependent variability. There are only few estimates of gap junction coupling between vascular cells, and thus the estimates for P_{gj} provide a first approximation. In general, endothelial gap junctions are more prevalent than smooth muscle and myoendothelial gap junctions.

Representative estimates for the R_{gj} often used in modeling studies are a low EC-EC resistance $(R_{gj}^{EC-EC} = 3.3 M\Omega)$, an intermediate SMC-SMC resistance $(R_{gj}^{SMC-SMC} = 87.4 M\Omega)$, and a high EC-SMC resistance per SMC $(R_{gj}^{EC-SEC} = 900 M\Omega)$ [11]. Based on these values, gap junction permeabilities to K^+, Cl^-, Na^+ and Ca^{2+} can be predicted assuming typical values of cytosolic concentrations for, K^+, Na^+ and Cl^- ($P_S^{EC-EC} = 0.4 pL/s$, $P_S^{SMC-SMC} = 0.015 pL/s$, and $P_S^{EC-SMC} = 0.0015 pL/s$, where S = K^+ Cl^-, Na^+ and Ca^{2+}).

Similar values for the gap junction permeability for IP_3 can be assumed as a first approximation, based on the similar diffusivities of IP_3 and Ca^{2+} in un-buffered solution [22]. However, lower permeability for IP_3 and other larger molecules is possible, since inside the pore, larger molecules may actually move slower than single atom ions. Fig. 2 shows ionic currents predicted by Eqs. 3 and 7 as a function of gap junctional resistance. Currents are based on an assumed electrical gradient (or equivalent concentration difference) between the two cells. An important prediction is that with similar permeabilities $P_{gj,S}$ for the four ions, the predominant current carrier will be K^+, due to its highest cytosolic concentration [13]. Contribution of Cl^- and Na^+ to electrical coupling will be approximately 3-and 12-fold smaller, respectively, based on their concentration ratio to K^+. To assess the

significance of these currents typical whole cell membrane currents under resting and stimulatory conditions are presented in Fig. 2b. Whole cell currents are predicted from our earlier theoretical models of isolated EC and SMC [23; 24]. The K^+, Cl^-, and Na^+ gap junction currents induced by a small V_m gradient (i.e. ΔV_{gj} = 3 mV) are comparable with the transmembrane currents, and therefore gap junctional currents for these three ions have the potential to induce significant V_m changes in neighboring cells.

Cytosolic Ca^{2+} concentration is about 10^5 times lower than that of K^+, and thus gap junctional Ca^{2+} current has minimum effect on V_m. A significant Ca^{2+} concentration gradient across the gap junction (i.e. $\Delta[Ca]_{gj}$ = 1 μM) results in a relative small Ca^{2+} flux (i.e. < 0.1pA) (Fig. 2a, *red line*) that is significantly lower than typical whole cell transmembrane Ca^{2+} currents in EC and SMC (Fig. 2b). Thus, the predicted magnitude of intercellular Ca^{2+} flux is rather small. In addition, the cells have the capacity to effectively absorb Ca^{2+} influx due to a significant buffering ability and the presence of effective Ca^{2+} extrusion pumps (i.e. PMCA and SERCA). Thus, these preliminary considerations question the ability of intercellular Ca^{2+} fluxes to affect global Ca^{2+} levels in neighboring cells.

The gap junction fluxes will affect membrane potential and cytosolic concentrations of ions according to the equations:

$$C_m \frac{dV_m}{dt} = \sum I_{m,k} + \sum I_{gj,S} \quad (8)$$

$$\frac{d[Ca]_i}{dt} = \frac{\sum I_{m,Ca} + \sum I_{gj,Ca}}{z_{Ca} F vol} + \sum J_{SR} + \sum J_{buffer} \quad (9)$$

$$\frac{d[S]_i}{dt} = \frac{\sum I_{m,S} + \sum I_{gj,S}}{z_S F vol} \quad (10)$$

where C_m is the cell membrane capacitance, $I_{m,k}$ represents all membrane currents, *vol* is the cell volume, J_{SR} - the Ca^{2+} exchange between the cytosol and sarco/endoplasmic reticulum, and J_{buffer} accounts for Ca^{2+} buffering.

Fluxes in homocellular coupling

Gap junctions may allow the transient exchange of intercellular signals but also a sustained flux of various intracellular species. Homocellular gap junctions, i.e., SMC-to-SMC and EC-to-EC, are most likely to mediate transient signals with a zero net flux over time, assuming an absence of steady-state gradients between cells of the same type. Transient intercellular Ca^{2+} gradients may occur from spontaneous intracellular Ca^{2+} activity, (e.g., Ca^{2+} sparks and puffs). Whether such local heterogeneities may give rise to intercellular Ca^{2+} waves, manifested with a dramatic elevation of cytosolic Ca^{2+} relative to its resting value is under investigation.

Spread of a short term local V_m perturbation will be conducted mostly by K^+. A V_m change can be accomplished without a significant modification of the cytosolic K^+, Cl^- or Na^+. The amount of extra ions (ΔN) necessary to charge the membrane capacitance to a new V_m is very small compared to the total amount of ions (N) in the cytoplasm. For example, for a ΔV_m of 5 mV only about 10^{-18} mol of K^+ are required, compared to the $N = 10^{-13}$ mol of the total cytosolic K^+:

$$\frac{\Delta N}{N} = \frac{\Delta V_m C_m / F}{vol[K]_i} \quad (11)$$

The V_m charging has a small time constant $\tau \approx R_{gj} \times C_m$ (e.g., 100 MΩ × 10 pF = 1 ms), thus the process is fast. If a V_m gradient persists beyond the time constant, an equilibrium point is reached where gap junction currents balance the total membrane current. However, at the new value of V_m the individual fluxes of ions will not be at equilibrium, and ionic concentrations will drift slowly with time to match the new V_m. For a sufficiently long perturbation (tens of minutes), a new true steady state will be reached, sustained by continuous gap junction fluxes. V_m gradients in homocellular coupling can appear during conducted responses and sustained gradients may also exist between SMCs in the vascular wall with multiple layers of smooth muscle. A radial asymmetry in the system may be created by ECs coupled to the innermost SM layer and/or by nonuniform innervations of the muscle cells.

Myoendothelial coupling

In heterocellular coupling, sustained gap junction fluxes are likely, although their presence, magnitude and significance remain controversial. Vascular ECs and SMCs express different set of membrane channels, and isolated, cultured or poorly coupled ECs have often resting V_m significantly different than SMCs [25; 26]. In guinea-pig mesenteric arterioles, the resting EC V_m is on average slightly less negative (few mV) than V_m in SMCs [10]. Furthermore, once the SM layer was removed, the resting V_m in EC layer depolarized significantly to the average of -4.2 mV. It was further suggested that ECs are dependent on SMCs not only for resting V_m but also their intracellular ionic concentration. Accordingly, the expression of Na^+-K^+ pump seems to be upregulated in isolated ECs as compared to ECs in arteries, which indicates persistent and physiologically important ionic exchange between ECs and SMCs [10]. A strong gap junction coupling in intact vessels may equilibrate EC and SMC V_m so that no significant differences can be recorded at rest [27; 28]. Different membrane composition can maintain continuous gap junction fluxes under such conditions, and a net myoendothelial current can be sometimes revealed by changes in V_m after application of gap junction uncouplers [29].

The equilibrium following acute uncoupling of EC and SMC will require balancing total membrane current as well as the balancing of transmembrane ionic fluxes for each cell. Thus, both V_m and ionic compositions in either cell are expected to change after uncoupling. Fig. 3 and Table 1 shows the predicted time course of EC and SMC V_m after gap junction uncoupling (t = 10 min) in an integrated EC-SMC model [14]. Table 1 presents predicted changes in ionic concentrations in the two cells from the same simulation. After gap junction

uncoupling, myoendothelial current is blocked instantaneously ($I_{gj,S}$=0). EC and SMC V_m change rapidly within ms (i.e. time constant, $\tau \approx R_m \times C_m$) towards their V_m in the isolated state (i.e. V_m that gives a total transmembrane current ($\Sigma I_{m,S}$) equal to zero (Eq. 8). This V_m change alters electrochemical gradients for the ions and thus, following this initial change in V_m, ionic concentrations will drift. Changes in ionic concentrations affect Nernst potentials and thus V_m will also drift until a steady-state is reached. The time course of the drift is relatively slow (Fig. 3) and depends on parameters such as cell volume, buffering, or membrane currents. Although the content of the individual ions changes significantly, their net electric charge remains constant (Table 1).

Similar fast and slow phases as well as concentration drifts may occur during cell stimulations. For example, activation of intermediate and small conductance Ca^{2+} activated K^+ (IK_{Ca}/SK_{Ca}) channels by acetylcholine rapidly hyperpolarizes EC and SMC (through the gap junctions) towards the K^+ Nernst potential until a new electric current balance is reached. If the agonist stimulation persists, the new electrochemical gradients will result in slow drifts in ionic concentrations and V_m towards a new steady state. In the simulations above we have assumed that cells do not regulate the presence or activity of the membrane components during uncoupling or stimulation. Regulatory mechanisms that will enable cells to control ionic concentrations and V_m in response to uncoupling or prolonged stimulations have not been identified at this point.

Can Ca^{2+} and IP_3 fluxes engage in propagation of conducted responses?

The importance of gap junction communication in multicellular coordination has been established. Ionic (Ca^{2+}, Na^+, K^+, and Cl^-) and IP_3 fluxes exist between cells and can communicate changes upon mechanical or agonist stimulation in neighboring cells. Rapid, long-range communication of local vasodilation or vasoconstriction (i.e. conducted responses) has been observed in many vascular beds and species [30; 31; 32; 33; 34; 35]. This phenomenon is critical for matching blood perfusion to local metabolic demand.

Electrical current passing through gap junctions is considered to be the major mechanism behind conducted responses. Experimental and theoretical attempts have investigated electrical spread through gap junctions and its potential for transmitting signals along the vessel [11; 36; 37; 38; 39]. The vessel segment can be viewed as a continuous wire with uniform axial and radial resistance along the vessel's length [37; 39]. The axial and radial resistivity will determine the attenuation of the spreading hyperpolarization/depolarization. Axial resistivity depends on gap junctional resistance (R_{gj}) and the number of open gap junctions along the current path. Radial resistivity determines current loss through the cell membrane and depends on whole-cell membrane conductance and the number of cells per vessel length. Attenuation of current spread with length constants in the order of millimeters have often been measured experimentally and predicted theoretically [36; 40]. Inhibition of BK_{Ca} and K_v channels enhanced conducted vasomotor responses in isolated segments of rat mesenteric terminal arterioles, and computer simulations identified these channels as the major pathways for current dissipation across the plasma membrane [41].

In some preparations, vasodilation spreads with minimal attenuation over significant distances [42]. Passive current diffusion cannot account for such experimental observations as dissipation of current by membrane ions channels should attenuate the transmitted hyperpolarization/depolarization along the vessel. Thus, investigators have looked for mechanisms that can regenerate/facilitate the transmitted signal. Inwardly rectifying potassium (K_{ir}) channels can potentially facilitate transmission of hyperpolarization / depolarization provided that they are present in significant density and the characteristic negative conductance (i.e. negative slope of I-V curve) occurs over the V_m of interest [40]. Theoretical and experimental studies have demonstrated a positive effect of K_{ir} current in the propagation of hyperpolarization [43; 44; 45]. However, the presence of K_{ir} is tissue dependent [46; 47] and there is wide disparity in its expression among vessels [48; 49]. A non-linearity between the spread of hyperpolarization and vasodilation has been suggested by Wolfle et al. [50] to explain the inequality of their spread in arteries. Hyperpolarization may attenuate with distance from the site of stimulation; vasodilation however, remains maximal until a threshold potential is reached. The threshold potential was suggested to be the activation threshold for VOCC in SMC.

At this stage, most evidence suggests that conducted responses depend primarily on passive electrotonic spread. Theoretical [11] and experimental studies [51] have provided evidence for the importance of the endothelial layer in longitudinal signal transmission. This is attributed to the longitudinal orientation and the abundance of homocellular coupling that makes the EC layer a low resistance pathway for transmitting membrane potential changes [11]. Interestingly, mathematical models suggest ECs as the primary pathway for conduction of vasodilation as well as of vasoconstriction. SMC derived depolarization (and vasoconstriction) can be efficiently transmitted through the EC layer, provided that there is sufficient myoendothelial coupling and that enough SMCs have been stimulated to produce sufficient depolarizing current [11; 40].

A significant Ca^{2+} spread over long distances within the EC layer can provide an alternative mechanism for enhancing conduction of vasodilation/vasoconstriction along the vessel's length. Experiments have reported the activation of EC Ca^{2+} dependent pathways like NO and EDHF at distant sites following local EC stimulation[52; 53; 54]. Distant EC Ca^{2+} waves have been reported in different arteries [52; 53; 54] with speeds that cannot be accounted by IP_3 and or Ca^{2+} diffusion and the involvement of a regenerative mechanism has been suspected. Endothelium independent fast and slow speed Ca^{2+} waves have also been observed in vessels and cultured SMCs. Fast Ca^{2+} waves may result from the spread of electrical depolarization and subsequent entry of Ca^{2+} by VOCC followed by Ca^{2+} induced Ca^{2+} release (CICR) and regeneration of depolarization by chloride channels [55]. Despite these observations a consistent mobilization of EC Ca^{2+} at distant sites has not been established nor a mechanism that will allow for a regenerative Ca^{2+} spread along the vessel axis.

We have previously examined the potential of intercellular Ca^{2+} and IP_3 diffusion in conducted responses utilizing a multicellular mathematical model (Fig. 4d) [40]. In the model, electrical coupling is the only signal strong enough to spread over long distances. Local Ca^{2+} transients do not propagate significantly along the vessel and they are restricted

to only a couple of cells away from the stimulus site (Fig. 4a, *dashed line*). The limited Ca^{2+} spread was actually a result of IP_3 rather than Ca^{2+} diffusion (Fig. 4a, *dash-dot line*). This limited IP_3 mediated Ca^{2+} mobilization in neighboring cells could amplify the total current generated at the local site (Fig. 4c, *dashed* vs. *solid line*), thus contributing to the strength of the electrical signal spreading along the ECs. Thus, model simulations suggested a limited passive Ca^{2+} and IP_3 spread which cannot facilitate signal transmission along the vessel but under some conditions can enhance distant responses by increasing local stimulus strength.

Can the presence of local domains enhance the role of Ca^{2+} and IP_3 fluxes?

Theoretical considerations suggest small gap junction fluxes for Ca^{2+} and IP_3 and their limited role in spreading responses. The effect of these fluxes, however, may be amplified through a CICR mechanism. For example, weak Ca^{2+} and/or IP_3 fluxes may be amplified and cause significant Ca^{2+} events near the gap junctions in the presence of localized ryanodine receptors (RyRs) and/or IP_3Rs. Although such microdomains have not been reported around homocellular gap junctions, myoendothelial gap junctions (MEGJs) are usually colocalized with IP_3Rs on cellular extensions known as myoendothelial projections (MPs). MPs have been identified in many tissues and species with numbers that increase with decreasing vessel size, an attribute shared by MEGJ expression and EDHF action [1; 56; 57; 58]. MPs consist of an extremely small fraction of total EC volume (<1%) and create restricted spaces within the EC where ions can potentially accumulate. Recent experimental evidence shows that key molecular elements like IP_3Rs, IK_{Ca} and NaKATPases (NaK) [59; 60; 61] are localized close to MP. Localization of IP_3R in such a restricted space can potentially allow IP_3 and/Ca^{2+} diffusing from a stimulated SMC to accumulate in the MP and cause a rapid increase in local Ca^{2+} concentration. Localized Ca^{2+} events have been reported in and around these structures [61]. The proximity of such Ca^{2+} events to a localized density of IK_{Ca} channels or endothelial nitric oxide synthase (eNOS) can lead to EC hyperpolarizations that can feedback to the SMC or SMC relaxation via nitric oxide released in the EC. This myoendothelial feedback response can attenuate SMC response to vasoconstrictors.

Despite the general agreement for the presence of myoendothelial feedback mechanism to SMC stimulation, several aspects of this response are still under investigation. An increasing amount of evidence supports a local rather than global myoendothelial feedback mechanism and a significant role of MPs in this response [62]. Theoretical simulations with finite element method models corroborate this opinion [63]. We also extended our integrated EC-SMC model [14] to incorporate an extra compartment in the EC representing a MP with localized IP_3R and IK_{Ca} channels. Representative simulations in the presence and absence of the MP are shown in Fig. 5 following stimulation of the SMC with NE. A significant feedback response (i.e. few mV) is observed only when MP is present (Fig. 5a, *solid line*) and as a result SMC is less depolarized to NE in comparison with simulations without a MP (Fig. 5a, *dashed line*). This difference is attributed to a significant Ca^{2+} response in the confined space of the MP with high density of IP_3Rs and IK_{Ca} channels (Fig. 5b, *solid line*). The amount of feedback depends on the assumed density of IK_{Ca} and IP_3Rs in the MPs and on R_{gj}. Thus, quantification of IP_3R and IK_{Ca} in the MPs will allow us to make better predictions for the myoendothelial feedback response. It also suggests that tissues and

disease dependent differences in channel localization can affect the ability of the endothelium to modulate SMC constriction.

Does Ca^{2+} or IP_3 mediate the EC feedback response to SMC stimulation?

The SMC originating signal that initiates Ca^{2+} mobilization in the MP has not been determined. Ca^{2+} [7; 64] and IP_3 [8; 65; 66] diffusion have been suggested to initiate this response (Fig. 6). Although Ca^{2+} ions and IP_3 have similar MEGJ permeabilities, the intercellular Ca^{2+} flux is probably not sufficient to mediate the feedback response (Fig. 6b). Our model simulations (unpublished data) suggest that IP_3 is more likely to be the mediator because of the localization of IP_3Rs in the MP (Fig. 6a) (i.e. feedback is lost after blockade of intercellular IP_3 diffusion but not of Ca^{2+}).

No favorable RyR localization has been reported in MP (and limited presence of RyR overall is likely in ECs) to amplify the small Ca^{2+} influx by CICR. The diffusing Ca^{2+} could however induce CICR through IP_3Rs which exhibit both Ca^{2+} dependent activation as well as inhibition [67]. However, for Ca^{2+} influx to induce a Ca^{2+} event in the MP, basal IP_3 levels need to be present (Fig. 6c). The concentration of IP_3 needs to be at a level adequate for significant opening of the receptor and to not induce significant CICR prior to stimulation. Resting MP Ca^{2+} levels should remain below the activation levels of the IP_3R and at the same time the IP_3Rs need to be sensitized so that a weak Ca^{2+} flux can cause significant CICR. It is not known if all of these conditions can exist at the same time. Most importantly, recent experimental data in hamster skeletal muscle arterioles provide evidence for IP_3 mediated feedback and corroborate the theoretical predictions. When the smooth muscle was stimulated with an IP_3 releasing vasoconstrictor (PE) a feedback response could be inhibited by blocking of EC IP_3Rs (Xestospongin C). In contrast, depolarization and vasoconstriction with a voltage dependent potassium channels (K_v) blocker, 4-aminopyridine (4-AP) remain unchanged after similar blockade of IP_3Rs in the endothelium [62].

Recent experimental data have shown local rather than global Ca^{2+} events in the endothelium upon SMC stimulation [62; 68; 69]. Mathematical models (unpublished data) corroborate these findings. The weak Ca^{2+} and IP_3 fluxes described above can be amplified in the restricted volume of the MP (Fig. 5b, *solid line*); however, their concentrations quickly dissipate in the bulk cytosol. The rapid buffering of Ca^{2+} and the degradation of IP_3 by cytosolic phosphatases [22; 66] are both unfavorable to their respective intracellular movement and weaken the possibility of a global EC response following SMC stimulation. Passive diffusion without a mechanism that will allow a regenerative Ca^{2+} wave seems to be insufficient for global Ca^{2+} mobilization.

Can intercellular IP_3 and Ca^{2+} fluxes contribute to cell synchronization in vasomotion?

Although the physiological significance of the phenomenon of vasomotion is not completely understood, it is speculated that oscillation of arteriolar diameter might increase flow conductance and in some cases may improve oxygenation as compared to steady flow

through the same average diameter [70]. Initiation of vasomotion requires the SMC's intrinsic ability for Ca^{2+} oscillations. In addition, a synchronizing mechanism needs to be in place that will enable a coordinate response in tone and diameter.

Electrical current through gap junctions can provide a signal that can spread rapidly across many cells, and thus represents the best candidate mechanism for synchronization in a vascular segment. Could Ca^{2+} or IP_3 coupling also play a role in synchronization? Considerations presented above suggest that passive diffusion across gap junction yields weak Ca^{2+} and IP_3 fluxes and their ability for global Ca^{2+} mobilization at distant sites is probably limited (at least in the absence of an amplification mechanism). However, the limited role that these fluxes may have in conduction does not necessarily mean a limited role in synchronization. A synchronizing signal has to affect only the phase of coupled self-sustained oscillators, and thus it can be relatively weak and is not required to produce forced Ca^{2+} oscillations or waves in neighboring cells. Local coordination of immediate neighbors can then synchronize larger population of SMCs. Furthermore, IP_3 and/or Ca^{2+} fluxes may generate stronger effect in immediate neighbors than electrical coupling dissipated over multiple cells [71].

A number of experimental and theoretical studies have investigated potential mechanisms for synchronization. In a model for vasomotion in a population of SMCs by Jacobsen et al. [9], a cGMP sensitive chloride (Cl^-) current was suggested to enhance the coupling between SMC V_m and Ca^{2+}. SMC V_m depolarization can spread to adjacent cells and coordinate Ca^{2+} elevation in those cells by activation of VOCCs. The effect of Ca^{2+} diffusion was negligible in these models. Experimental evidence supports a role for cGMP (through Cl_{Ca} channel activation) in promoting synchronization. In an experimental study by Peng et al. [72], a synchronization mechanism based on a Ca^{2+} and cGMP-activated inward current was shown for endothelium denuded vessels. In a more recent experimental study, transfecting rat mesenteric small arteries *in vivo* with siRNA specifically targeting bestrophin-3, inhibited cGMP dependent Cl_{Ca} current and abolished vasomotion in isolated arteries [73]. However, in another study from the same group [74], 4,4′-Diisothiocyano-2,2′-stilbenedisulfonic acid (DIDS) and Zn^{2+}, both blockers of $Cl_{Ca,cGMP}$ channels, did not inhibit vasomotion in intact small rat mesenteric arteries. The vasomotion was inhibited by Cl^- substitution, suggesting that cGMP-independent Cl^- channel may participate in synchronization. Cl^- currents remain the only mechanism of synchronization tested through experimentation. Nevertheless, the role of Cl^- in synchronization in intact vessels needs to be further elaborated upon, and possible contribution of other pathways should be also investigated.

The effect of Ca^{2+}, IP_3, and electrical coupling on synchronization in vasomotion was studied in multicellular mathematical models by Koenigsberger et al. [75; 76]. Intercellular Ca^{2+} fluxes have been suggested to be involved in synchronization of cells in close range. Homocellular and heterocellular gap junctions in a population of SMCs and ECs were simulated by combination of formulae (1, 2). In the models, Ca^{2+} flux between SMCs was necessary to override desynchronizing effect of large conductance calcium activated potassium channels (BK_{Ca}) channels. Although the model did not incorporate Ca^{2+} activated

Cl^- channels and did not identify any electrically mediated synchronizing pathway, it demonstrated that local Ca^{2+} coupling can synchronize a larger population of SMCs.

Both electrically and diffusion mediated synchronizing pathways were implemented in our multicellular ECs-SMCs model [77]. Previously recognized Cl^- and Ca^{2+} synchronizing mechanisms were evaluated in a system of increased complexity and under different stimulatory scenarios. The study suggests two alternative pathways for synchronization in addition to Cl^- current and direct Ca^{2+} diffusion. Intercellular diffusion of oscillatory IP_3 (Fig. 7) and pulsatile current generated by nonselective cation channels has the potential to affect synchronization. Thus, coordination is achieved or lost as a result of the competition between synchronizing and desynchronizing factors, and there can be several mechanisms that work individually, in synergy or redundancy, depending on stimulatory conditions.

Summary

Intercellular communication is essential for the coordination of microcirculatory reactivity. Continuous electrical and ionic movement occurs between coupled cells which affects resting cell states and enables transmission of signals. Based on available measurements for gap junction resistances and expected intercellular gradients of different ions and IP_3, we can estimate the magnitudes of these fluxes in different scenarios. Electrical current through gap junctions (carried predominantly by K^+ ions) is the primary signal that enables spreading responses. Ca^{2+} and IP_3 fluxes are small and thus, their passive diffusion should have a limited effect on Ca^{2+} mobilization at distant sites. These weak fluxes may be adequate, however, to amplify local current in conducted responses and to promote synchronization of oscillations in neighboring SMCs and vasomotion. The effect of Ca^{2+} and IP_3 diffusion can be amplified by the presence of molecular components like RyRs, IP_3Rs and IK_{Ca} channels in microdomains close to the gap junctions. Such localized signaling machinery exists in myoendothelial projections and enables an endothelial feedback response that moderates SMC constriction. Myoendothelial IP_3 diffusion is more likely than Ca^{2+} to mediate this response. Theoretical analyses can assist experimentation in elucidation of the complex mechanisms that regulate microcirculatory reactivity.

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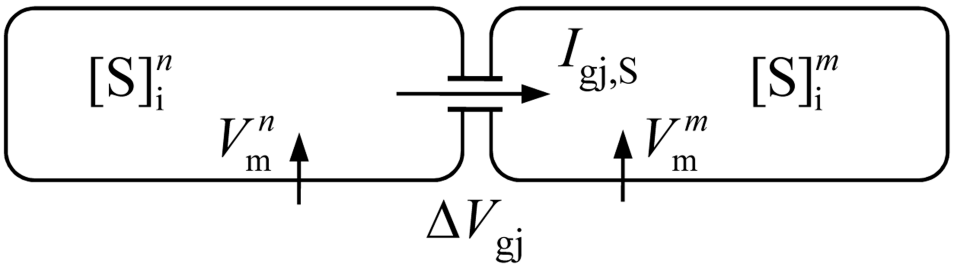

Fig. 1.

Schematic diagram of two cells, *n* and *m*, connected by nonselective gap junctions. The gap junctions are permeable to various ionic species and small molecules, *S*. The intercellular fluxes of individual species vary according to their corresponding concentration gradients and V_m difference between the two cells.

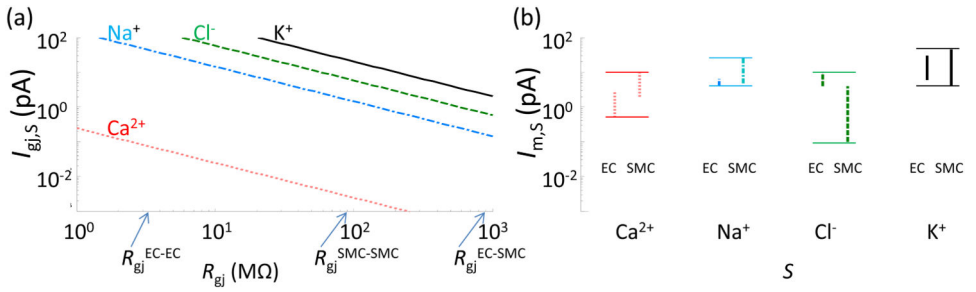

Fig. 2.

a) Gap junction K^+ (*solid line*), Cl^- (*dashed line*), Na^+ (*dash-dot line*), and Ca^{2+} (*dotted line*) currents as a function of R_{gj} predicted from Eq. 4 and 7; K^+, Cl^- and Na^+ currents are estimated with ΔV_{gj} = 3 mV (or equivalent $\Delta[K]_{gj}$ = 17 mM, $\Delta[Cl]_{gj}$ = 5 mM, $\Delta[Na]_{gj}$ = 1.2 mM), and ΔV_{gj} = 21 mV (or equivalent $\Delta[Ca]_{gj}$ = 1 μM) for Ca^{2+} current. b) Range of total membrane currents in isolated EC and SMC at rest and during agonist stimulation predicted by theoretical models [23; 24].

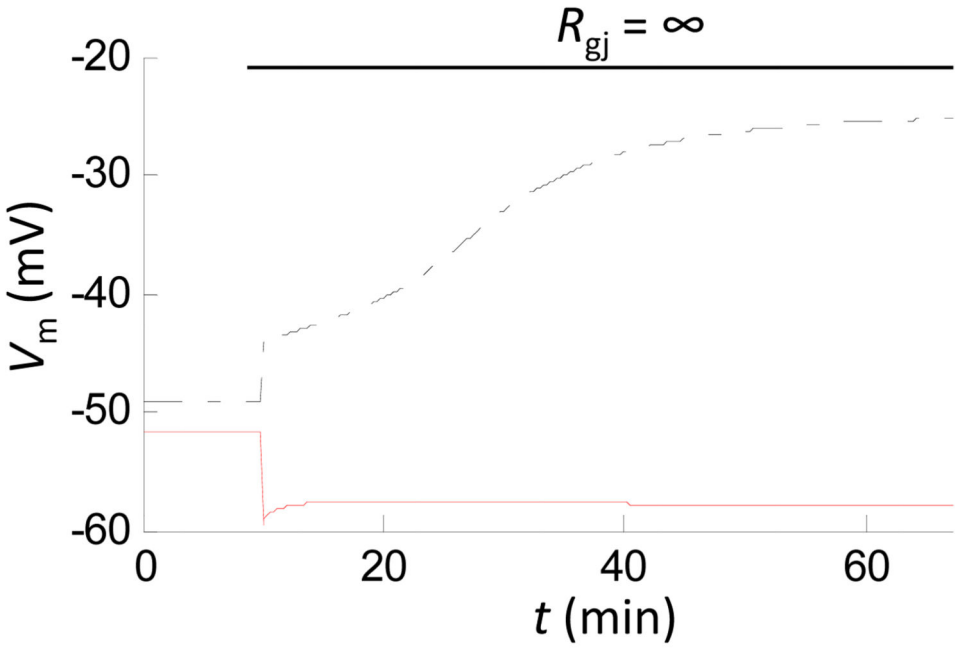

Fig. 3.

Changes in EC V_m (*dashed-dotted line*) and SMC V_m (*solid line*) after gap junction uncoupling (t = 10 min), predicted by theoretical model of EC-SMC communication utilizing Eqs. 8-10 [14]. EC and SMC V_m change rapidly after the uncoupling, indicating presence of steady myoendothelial ionic and current exchange under control conditions. Unbalanced ionic fluxes in the isolated cells cause slow drift of the cytosolic ion concentrations and V_m until a new steady state is reached.

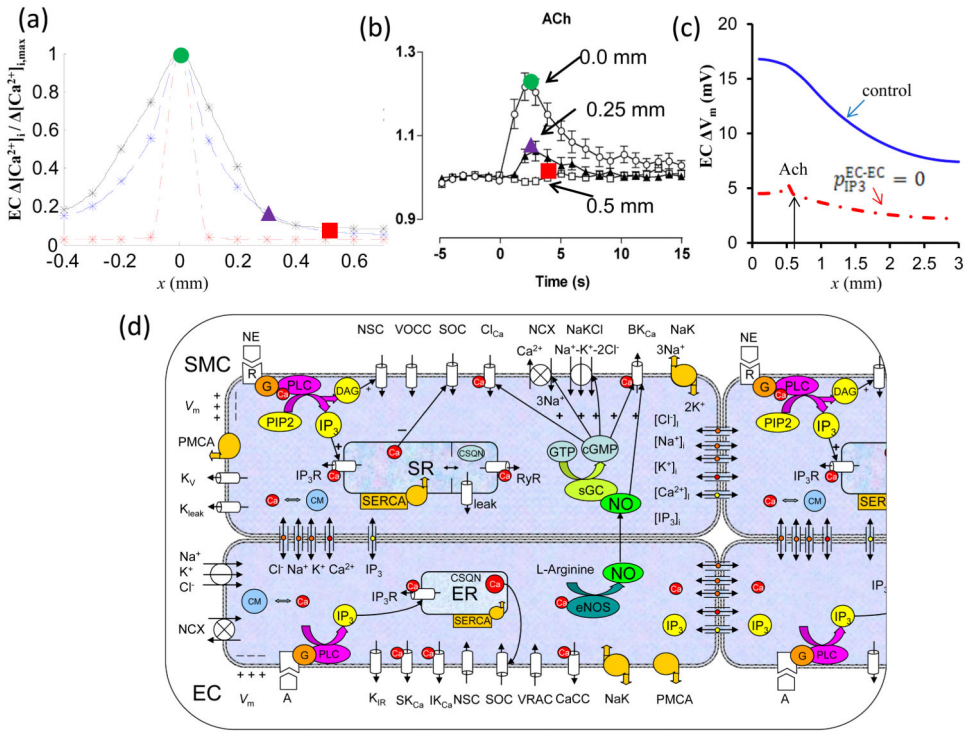

Fig. 4.

a) Normalized steady state endothelial Ca^{2+} profiles during local stimulation of 1 EC with Ach at x = 0 mm. Under control conditions (*dashed line*), the Ca^{2+} spread was limited to 300 μm (three ECs). Inhibition of axial IP_3 diffusion (EC-EC $p_{IP3} = 0$) practically abolished the Ca^{2+} spread (*dash-dot line*). One hundred fold greater permeability of endothelial gap junctions to Ca^{2+} extended Ca^{2+} spread to 400 μm (*black line*). b) Ca^{2+} spread to Ach stimulation observed in an experimental study by Takano et al. [78] which is in agreement with predictions from the model. c) Predicted changes in EC V_m with respect to resting V_m during local Ach application to one EC with (*solid line*) and without (*dashed line*) endothelial IP_3 diffusion (EC-EC $p_{IP3} = 0$) in a vessel prestimulated with NE. d) Schematic diagram of coupled ECs and SMCs used in the vessel model reproduced from [40]. Cells are coupled by nitric oxide (NO) and myoendothelial gap junctions permeable to Ca^{2+}, Na^+, K^+, and Cl^- ions, and IP_3. 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; IP_3R – IP_3 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.

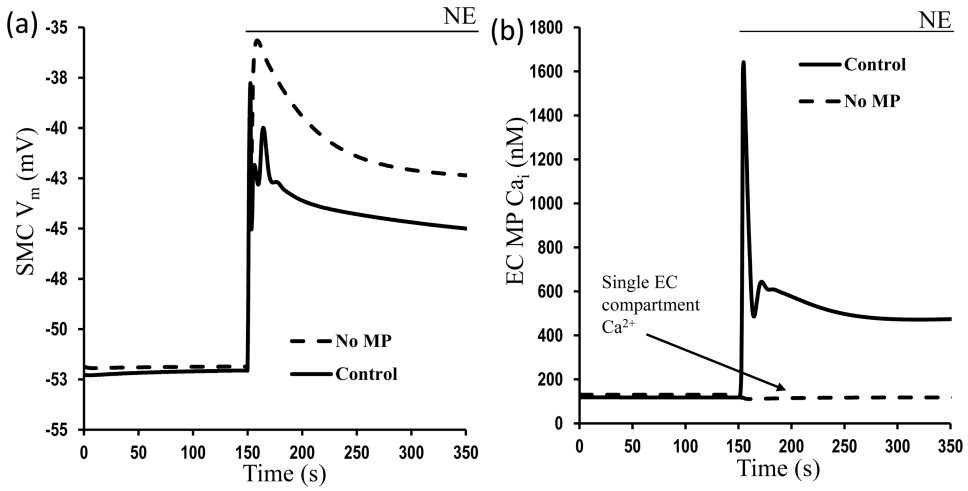

Fig. 5.

Changes in a) SMC V_m and b) EC projection Ca^{2+} after NE stimulation of SMC in the presence (*solid line*) and absence (*dashed line*) of MP in the EC.

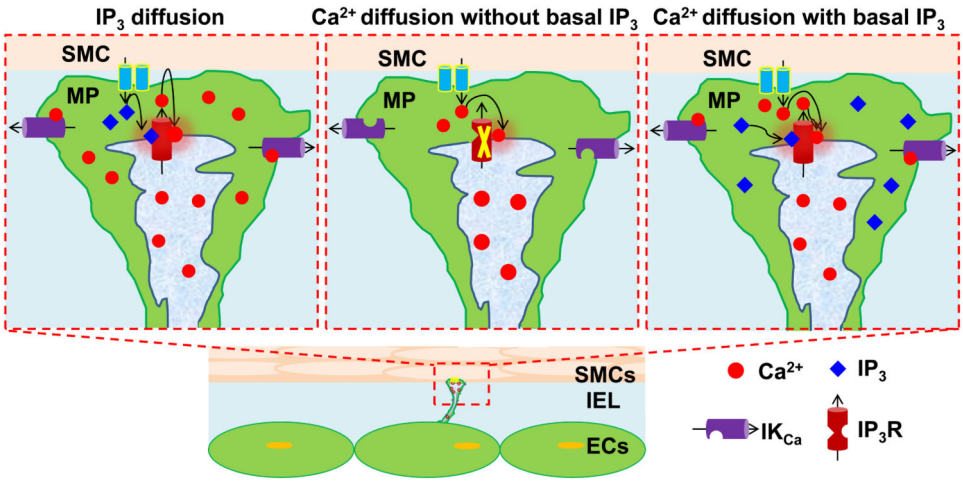

Fig. 6.

Role of IP_3 and Ca^{2+} diffusion from SMC to EC in myoendothelial feedback during a) IP_3 diffusion b) Ca^{2+} diffusion without no basal IP_3 in EC and c) Ca^{2+} diffusion with basal IP_3 in EC. Ca^{2+} requires the presence of some basal IP_3 to activate IP_3R and both the diffusing Ca^{2+} as well as the Ca^{2+} released from store can potentially cause further release from localized IP_3Rs.

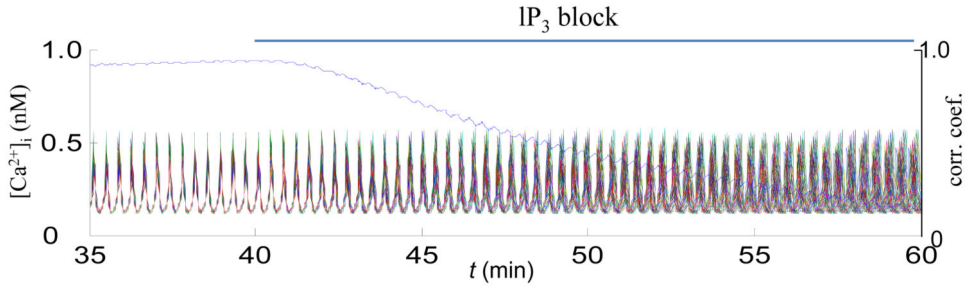

Fig. 7.

Simulations in a population of eighty SMCs and eighty ECs arranged into a cylinder. Each SMC within the cylinder is coupled to its four neighbors through R_{gj} = 87.4 MΩ, and to underlying ECs through the total myoendothelial R_{gj} = 900 MΩ per SMC. SMCs are stimulated by NE (0.8 μM), and ECs are stimulated by acetylcholine (1 a.u.). Shown are Ca^{2+} oscillations in the SMCs, and mean correlation coefficient indicating degree of synchronization. Under control conditions, SMCs are coordinated, but inhibition of intercellular IP_3 diffusion desynchronizes Ca^{2+} oscillations.

Table 1

Representative changes in V_m and ionic concentrations after gap junction uncoupling, predicted by theoretical model of EC-SMC communication [14].

	R_{gj} = 900 MΩ		R_{gj} = ∞	
	SMC	**EC**	**SMC**	**EC**
V_m (mV)	-52	-49	-59	-25
$[Ca^{2+}]_i$ (nM)	99	130	68.4	70
$[Na^+]_i$ (mM)	9.4	18.7	8.3	9.4
$[K^+]_i$ (mM)	121	116	126	152
$[Cl^-]_i$ (mM)	42	46.3	46	73
$[Na^+]_i+[K^+]_i-[Cl^-]_i$	88.4	88.4	88.4	88.4