Heterogeneity and weak coupling may explain the synchronization characteristics of cells in the arterial wall

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Vascular smooth muscle cells (SMCs) exhibit different types of calcium dynamics. Static vascular tone is associated with unsynchronized calcium waves and the developed force depends on the number of recruited cells. Global calcium transients synchronized among a large number of cells cause rhythmic development of force known as vasomotion. We present experimental data showing a considerable heterogeneity in cellular calcium dynamics in the vascular wall. In stimulated vessels, some SMCs remain quiescent, whereas others display waves of variable frequency. At the onset of vasomotion, all SMCs are enrolled into synchronized oscillation.

Simulations of coupled SMCs show that the experimentally observed cellular recruitment, the presence of quiescent cells and the variation in oscillation frequency may arise if the cell population is phenotypically heterogeneous. In this case, quiescent cells can be entrained at the onset of vasomotion by the collective driving force from the synchronized oscillations in the membrane potential of the surrounding cells. Partial synchronization arises with an increase in the concentration of cyclic guanosine monophosphate, but in a heterogeneous cell population complete synchronization also requires a high-conductance pathway that provides strong coupling between the cells.

Keywords: arterial; vasomotion; synchronization; gap junctions

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1. Introduction

The main resistance to blood flow in the circulatory system is found in small muscular arteries and arterioles (Pries et al. 1995), making the contractile state in these vessels a central parameter in the regulation of arterial blood pressure. By adjusting the diameter, these small vessels also control local tissue perfusion. The ability to efficiently regulate microvascular flow in response to a change in local tissue demand, e.g. at the onset of physical exercise, is a fundamental property of the microcirculation (Segal 2000). This becomes clear when flow regulation is compromised due to pathological changes in the vascular wall (e.g. in diabetes or due to atherosclerosis) leading to a reduced performance capacity of tissues such as the heart. Since diseases related to elevated blood pressure (e.g. stroke) or inadequate perfusion (e.g. ischaemic heart disease; Neal et al. 2000) are the major causes of morbidity and death in the Western world, a better understanding of blood pressure control and blood flow regulation is an issue of substantial interest.

To this end, the properties of the smooth muscle cell (SMC), the contractile cell of the vascular wall, are central. By static or oscillating contractions, these cells control vessel size and hence microvascular network resistance and perfusion (Lee et al. 2002). The force contributed by a single SMC is, however, small. Only through an integrated response among a large number of coupled SMCs can the vascular wall develop sufficient force to contract against the intravascular pressure. Investigations of such inherently complex systems can be advanced substantially by performing simulation studies aimed at identifying governing mechanisms in the system and hence aid the interpretation of experimental data.

Vascular SMCs are typically in a state of partial activation. Such ‘myogenic tone’ is mainly an intrinsic reaction of the SMC to the intravascular pressure (Davis & Hill 1999). The presence of a basal tone allows for very fast modification of vessel radius to continuously meet the changing tissue demands. As in other contractile cells, the cytoplasmic calcium concentration, $[Ca^{2+}]_{cyt}$, in the SMC is central to the activation of contractile proteins in the cell interior (Davis & Hill 1999).

SMCs exhibit different types of calcium dynamics including calcium waves and global calcium oscillations (Lee et al. 2002). In many vessels, static vascular tone increases with the number of cells recruited into the wave mode (Lee et al. 2005). Regulation of static tone may therefore depend on cellular heterogeneity allowing for a graded recruitment with increasing stimulus strength. Calcium waves characteristically are uncoordinated; in different cells they move in different directions with different velocities and they do not spread between cells (Zang et al. 2001). They persist when the vessel is immersed into calcium-free medium, indicating that they depend primarily on calcium release and re-uptake related to internal cellular calcium stores, e.g. the sarcoplasmic reticulum (SR; Peng et al. 2001).

Global calcium oscillations, on the other hand, in which calcium increases uniformly in all parts of the cytoplasm, depend on calcium entry from the extracellular space (Lee et al. 2002). The frequency is generally higher than that of the waves, and they are often, though not always, synchronized among a large number of cells, in which case the wall shows rhythmic generation of force, i.e. vasomotion (Peng et al. 2001; Rahman et al. 2005).
In the vascular SMC, stimulation of a specific surface receptor, the α-adrenoceptor, with, for example, noradrenaline (NA) leads to the generation of the intracellular messenger molecule inositol triphosphate (IP₃; Katzung 1989). In turn, IP₃ causes release of calcium from the SR. In mesenteric small arteries of the rat, stimulation with NA in a low concentration causes recruitment of SMCs into the unsynchronized wave mode (Zang et al. 2001, 2006). After a variable period, spontaneous synchronization may ensue if the cell layer lining the inside of the vessel wall, the endothelium, is intact (Peng et al. 2001). During synchronized activity, all SMCs participate in the oscillation and the calcium transients change from waves to global oscillations in most cells.

If the endothelium is removed (endothelial denudation) in NA-stimulated rat mesenteric small arteries, the situation becomes more complex. In many cases (Gustafsson & Nilsson 1994; Gustafsson et al. 1994; Mauban & Wier 2004; Rahman et al. 2005), though not all (Sell et al. 2002; Lamboley et al. 2003), such vessels do not show spontaneous onset of vasomotion. However, synchronized activity may ensue if the membrane-permeable cyclic guanosine monophosphate (cGMP) analogue, 8Br-cGMP, is applied (Rahman et al. 2005). This compound substitutes for the intracellular signal molecule cGMP, which, in the intact vessel, is produced in the SMC in response to nitric oxide (NO) released from the endothelium (Loscalzo & Vita 2000). 8Br-cGMP causes SMCs in small islands to oscillate together, but unsynchronized with other islands, sometimes giving rise to a beating oscillation in force (Rahman et al. 2005). Higher concentrations of 8Br-cGMP increase the synchronization; however, only in the presence of the endothelium is the synchronization complete with all cells oscillating in phase (Rahman et al. 2005). Characteristically, a certain fraction of the SMCs are quiescent before synchronized activity ensues, but quiescent cells are not seen after the onset of synchronization.

It should be noted, though, that while NA stimulation is generally required to initiate vasomotion in vitro, the effect of endothelial denudation or of direct or indirect manipulation of the cGMP concentration is more variable and depends on the specific experimental preparation (see §5).

In the present paper, we address the possible role of cellular heterogeneity for the function of rat mesenteric small arteries. We present new experimental data showing that SMCs of the vascular wall have individual and highly variable phenotypes. We have modified a previously published model of arterial vasomotion to reflect the observed cellular heterogeneity, and have performed simulations on large populations of SMCs. Taken together, the simulations show that several experimental observations may be explained on the basis of cellular heterogeneity.

2. Experimental methods

All procedures involving animals in this study complied with Danish animal welfare regulations and American Physiological Society principles. Animal facilities were approved by the Danish Inspectorate for Experimental Animals and the Animal Welfare Officer of the Medical Faculty of the University of Aarhus. Isolated segments of rat mesenteric small arteries (diameter approx. 200–250 μm) from

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12- to 16-week-old Wistar rats were mounted for isometric force measurement and normalized for maximal force development (Warshaw et al. 1979). The arteries were loaded with 3 μM Calcium Green-1 AM at 37°C for 60 min for confocal [Ca²⁺]⁰ imaging with an inverted confocal laser scanning microscope (Odyssey XL, Noran or LSM 5 Pascal, Zeiss). Calcium Green-1 AM was dissolved in DMSO with 0.1 per cent (w/v) Cremophor and 0.02 per cent (w/v) Pluronic F-127. Confocal imaging was carried out as described previously (Peng et al. 2001). Confocal optical sections were acquired with water-immersion objectives (Nikon or Zeiss). The emission signal from Calcium Green-1 (after excitation at 488 nm) was stored on a computer. For image analysis, the programs INTERVISION (Noran), IMAGE Space (Molecular Dynamics) and LSM IMAGE BROWSER (Zeiss) were used. The [Ca²⁺]⁰ changes within the cells were estimated as changes in the mean fluorescence intensity within the regions of interest in which all pixel values were averaged (Peng et al. 2001).

Protocol I: repeated pattern measurements. These experiments were performed on a Noran microscope using vessels with intact endothelium (from now on, intact endothelium is indicated as ‘EE’). The arteries were stimulated with 4–6 μM NA (to provide approx. 80% of maximal tone). After 3–5 min, 10 μM pinacidil (an opener of ATP-activated potassium channels that causes hyperpolarization of the cell membrane) was added, and after an additional 2–3 min an approximately 3 min long movie of the SMCs was obtained. The drugs were washed out for 30 min and the stimulation protocol was repeated twice.

Protocol II: the effects of cGMP and NA on cellular recruitment and oscillation frequency. These experiments were performed on a Zeiss LSM 5 Pascal confocal microscope. The endothelium was removed with a gentle stream of air along the lumen of the vessel for 20 s. Successful endothelial denudation (from now on, absence of the endothelium is indicated as ‘OE’) was confirmed by assessing the effect of 10 μM acetylcholine (an endothelium-dependent vasodilator) when the arteries were preconstricted with NA. The arteries were then cumulatively stimulated with increasing concentrations of NA (0.1–4 μM) for 3–5 min at each concentration, while the movie of [Ca²⁺]⁰ dynamics in the SMCs was obtained.

Table 1. Summary of the specific circumstances, experimental or simulated, in relation to each figure.

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Peng et al. 2001.
After a 30 min washout period, NA concentration–response was repeated in the presence of 300 μM 8Br-cGMP, which was applied 15 min before NA. For characterization of a calcium transient as either a wave or a global elevation, two
regions of interest in each end of the cell were chosen, and the distance between the two regions was measured. Calcium elevation was characterized as global if there was no detectable delay in the signal between the two regions. At least five SMCs were chosen for analysis in each experiment.

Summary of methods. Table 1 summarizes the specific circumstances related to each of the figures presented in §4.

Statistical analysis. All data are shown as mean ± s.e.m. Differences between means were tested either with paired Student’s t-test (when two groups were compared) or with one-way ANOVA followed by Bonferroni’s post hoc test (for multiple comparisons) with \( p < 0.05 \) being considered statistically significant.

3. The model

To simulate a system of coupled cells, we used a previously published model, a detailed account of which is given in Jacobsen et al. (2007a,b). Figure 1a,b (Jacobsen et al. 2007a) illustrates the components of the SMC and the vascular wall models (see figure caption for details). The vascular wall model does not include an endothelial cell layer. In brief, the two state variables considered in figures 3–6 of the present paper are the SMC cytoplasmic calcium concentration, \([\text{Ca}^{2+}]_{\text{cyt}}\), and the SMC membrane potential, \(V_m\).

\((a)\) Central model components

\([\text{Ca}^{2+}]_{\text{cyt}}\) shows variation along the individual cell. The rate of change in \([\text{Ca}^{2+}]_{\text{cyt}}\) at a given point in a cell depends on the local fluxes of calcium into and out of the cytoplasm. These fluxes are due to diffusion and buffering, and fluxes across the membrane of the SR and the plasma membrane. The rate of change in \([\text{Ca}^{2+}]_{\text{cyt}}\) hence becomes

\[
\frac{d[\text{Ca}^{2+}]_{\text{cyt}}}{dt} = J_{\text{Ca}^{2+}-\text{cyt, diffusion}} + J_{\text{Ca}^{2+}-\text{cyt, buffering}} + J_{\text{Ca}^{2+}-\text{cyt, SR}} + J_{\text{Ca}^{2+}-\text{cyt, plasma membrane}}.
\] (3.1)

An important component in this part of the model is calcium-induced calcium release (CICR) from the SR. This phenomenon is described by the equations giving the steady-state open probability of the SR calcium release channel a bell-shaped dependence on \([\text{Ca}^{2+}]_{\text{cyt}}\). The maximum of this curve increases with increasing concentration of \(\text{IP}_3\) (for details see Jacobsen et al. 2007b). Curves for the steady-state open probability are shown in figure 1c. The bell shape of the curves has the consequence that calcium release will enhance itself at low \([\text{Ca}^{2+}]_{\text{cyt}}\), but inhibit itself when local \([\text{Ca}^{2+}]_{\text{cyt}}\) becomes too high. The channel is also stimulated by calcium entering the cell from the extracellular space. The system can give rise to calcium waves sweeping through the cytoplasm in one direction and is also important in the generation of global calcium oscillations. The ability to generate calcium elevations increases with increasing \([\text{IP}_3]\).
As opposed to the cytoplasmic calcium concentration, the membrane potential is assumed to be uniform over the whole cell surface. The rate of change in the membrane potential is the sum of the individual membrane currents divided by the cell membrane capacitance, \( C_m \),

\[
\frac{dV_m}{dt} = -\left( \frac{1}{C_{m,\text{cell}}} \right) \left( \sum_x I_{\text{back},x} + \sum_x I_{\text{gap},x} + I_{\text{NaK}} + I_{\text{NaCa}} + I_{\text{CaL}} 
+ I_{\text{ClCa}} + I_{\text{KCa}} + I_{\text{CaP}} \right),
\]

where \( x \) refers to a particular ion (\( \text{Na}^+, \text{K}^+, \text{Ca}^{2+} \text{ or Cl}^- \)). \( I_{\text{back},x} \) denotes an unspecific background current of \( x \). Other abbreviations are provided in the caption of figure 1.

An important component of the membrane model is a cGMP-sensitive calcium-dependent chloride channel. Opening of this channel causes membrane depolarization due to efflux of negative charge (\( \text{Cl}^- \)). Steady-state open probability of the channel was modelled as (Jacobsen et al. 2007b)

\[
\bar{P}_{\text{open}} = \frac{\alpha[\text{Ca}^{2+}]_\text{cyt}^n}{[\text{Ca}^{2+}]_\text{cyt}^n + \left[k_{\text{ClCa}}(1-\alpha)\right]^n}, \quad \text{where} \quad \alpha = \frac{[\text{cGMP}]^m}{[\text{cGMP}]^m + k_{\text{Cl,cGMP}}^m},
\]

Hence, the steady-state open probability has a sigmoid dependence on \( [\text{Ca}^{2+}]_\text{cyt} \), the steepness of which is modified by \([\text{cGMP}]\). \( k_{\text{ClCa}}, k_{\text{Cl,cGMP}}, \rho, m \text{ and } n \) are characteristic constants as given in Jacobsen et al. (2007b). Curves for \( \bar{P}_{\text{open}} \) as a function of \( [\text{Ca}^{2+}]_\text{cyt} \) at different levels of cGMP are shown in figure 1d (Jacobsen et al. 2007b). The figure shows that when \([\text{cGMP}]\) is high, the channel is extremely sensitive to an increase in \([\text{Ca}^{2+}]_\text{cyt} \), caused, for instance, by a local release of calcium from the SR. By contrast, when \([\text{cGMP}]\) is low, the channel is quite insensitive to calcium. In this way, the channel couples calcium release from the SR to depolarization of the plasma membrane with a strength that is dependent on the cytoplasmic concentration of cGMP. Neighbouring cells are electrically coupled through gap junctions, and the depolarization will therefore also spread to the neighbouring coupled cells. In turn, the membrane depolarization causes opening of voltage-sensitive calcium channels and influx of calcium, with uniform induction of CICR along the cell. Hereby, a local wave is transformed into a global calcium elevation. The global calcium elevation influences cGMP-sensitive calcium-dependent chloride channels over the whole membrane simultaneously with a consequent much stronger influence on the membrane potential. Over a few cycles, the oscillations in the membrane potential and \([\text{Ca}^{2+}]_\text{cyt} \) will reach a steady amplitude.

**Cellular heterogeneity.** As described in Jacobsen et al. (2007a,b), a number of parameters are subject to a small random variation both along the individual cell and between the cells. In the present simulations, there is an additional and substantial variation in cellular phenotype induced by varying randomly between 1 and 11 per cent the SR volume fraction among the cells.
SMCs of the vascular wall vary in phenotype. This is shown in figure 2 (experimental data) where vessels placed in a confocal microscope are stimulated with NA. The SMCs of the vascular wall react to stimulation by generating calcium waves with an individual frequency in each cell. Figure 2a (+E) shows a histogram of wave frequencies from randomly selected cells ($n=37$) in four different vessels. The figure is based on the first NA stimulation of each vessel. Each bar represents the number of cellsoscillating within the frequency interval covered by the bar, with the first bar covering the interval 0–0.5 min$^{-1}$. The heterogeneity among the cells is considerable, and approximately one-quarter of the cells remain quiescent (hatched part of the first bar).

4. Results
Figure 2b (+E) shows typical traces of intracellular calcium in four different cells. Stimulation periods are separated by periods of washout. The uppermost traces (red) are from a quiescent cell (only noise), whereas the three other traces are from the cells showing waves of different frequencies.

Figure 2c (+E, data from 4 vessels, 37 cells, 3 stimulations) shows that a given cell tends to produce waves with nearly the same frequency during each subsequent stimulation. The histogram shows the number of cells in which the difference in frequency between sequential stimulations (i.e. from the first to the second and from the second to the third) is maximally the number given under each bar. In 34 of the 37 cells (92%), the shift in frequency between sequential stimulations is less than 1.33 min⁻¹.

Figure 2d (protocol II, +E, 7 vessels, n=35 cells) shows the effect of increasing [NA] in the bath. Even without application of NA, approximately 60 per cent of the cells show calcium waves. With increasing [NA], more cells are recruited into an oscillatory mode; hence, the percentage of quiescent cells declines, reaching statistical significance at 4 µM.

Collectively, figure 2a–d points to the presence of a substantial SMC heterogeneity in the vascular wall. The characteristics of the individual SMC are preserved over time, indicating that intercellular variation is caused by the differences in SMC phenotype, rather than being a consequence of stochastic processes.

Simulated cellular heterogeneity. In the following simulations, the variation in phenotype was implemented by randomly varying the SR volume fraction of the cells between 1 and 11 per cent, thus making each cell phenotypically distinct.
Figure 3a (÷ E) shows simulated recruitment in a heterogeneous population of 45 cells corresponding to the experimental data shown in figure 2d. Increasing [IP₃] uniformly in the cells (to mimic an increase in [NA] in the bath) leads to recruitment of quiescent cells (these are shown in light blue colour). The recruited cells display either waves or global calcium oscillations of variable frequency, and little or no synchronization is seen between the cells. Note that in the simulations it is possible to have zero or very low levels of IP₃, whereas in real cells there will always be some IP₃ present intracellularly. This probably explains why, even in the absence of NA, some cells are found to be active (cf. figure 2d).

Figure 3b (÷ E, experimental data) shows the fraction of the active cells that display global calcium oscillations with increasing [NA]. No cells display global calcium oscillations if NA is not present in the solution. Hence, in this situation, all active cells display waves. If NA is increased in the absence of 8Br-cGMP (solid line: 7 vessels, 35 cells), a few cells will show global calcium oscillations, but this fraction remains low. By contrast, in the presence of 300 μM 8Br-cGMP (dashed line: 8 vessels, 40 cells), there is a rapid increase in the fraction of cells displaying global calcium oscillations; furthermore, these cells are entrained into synchrony when [NA] exceeds 1.0 μM. Hence, during vasomotion, the majority of cells, but not all, will show global calcium transients. Also, in the simulated cell population, an increase in [IP₃] (to mimic an increase in [NA]) will lead to an increasing fraction of cells showing global oscillations (picture frames in figure 3a), and at every level of [IP₃] this fraction will increase with increasing [cGMP] (not shown).

The types of calcium dynamics that can be seen in a given cell depend on the SR volume fraction. This is illustrated in figure 4 where three different uncoupled cells (÷ E) with SR volume fractions of 0.001, 0.04 and 0.11 (shown at the r.h.s.) are exposed to increasing [cGMP]. Figure 4a shows the increase in [cGMP] in the course of the simulation. Figure 4b shows calcium traces from the three activated cells ([IP₃] = 1.75 μM). The cell with the lowest SR fraction (0.001) is unable to produce either waves or global transients even at high levels of [cGMP]. The cell with medium SR fraction starts to oscillate as [cGMP] is increased. In this cell, the calcium oscillations grow in amplitude with increasing [cGMP], but the amplitude remains modest even at high [cGMP]. The complementary trace of the membrane potential (figure 4c) shows similar characteristics. By contrast, the cell with high SR volume fraction displays waves when [cGMP] is relatively low. As [cGMP] is increased, the oscillatory pattern shifts to global oscillations also in this cell. The corresponding curve for the membrane potential shows that a simulated high SR fraction is associated with larger amplitude of the membrane potential oscillations and, hence, increased ability to induce synchronization if the cell is electrically coupled to other cells (see below).

Experimentally, it was found that both in vessels with (cf. figure 2a,b) and without (cf. figure 2d) the endothelium, a certain fraction of the cells may remain quiescent even at high [NA]. However, when and if the vessels show vasomotion, either spontaneously in intact vessels or following application of 8Br-cGMP in endothelium-denuded vessels, all cells are active and participate in the synchronized oscillation. This is illustrated in the experimental data of figure 5a (÷ E, 8 vessels, 40 cells). In the presence of 300 μM 8Br-cGMP, the fraction of quiescent cells vanishes completely when [NA] reaches 1 μM and synchronization (vasomotion) ensues. This characteristic enrolment of quiescent cells is further
Figure 4. Simulated calcium and membrane potential oscillations in three isolated (uncoupled) cells with a very low (0.001), low (0.04) or high (0.11) SR volume fraction (shown at the r.h.s.). For a simulated cell to generate spontaneous oscillations, the volume fraction of the SR must be at least 0.04. (a) The increase in [cGMP] in the course of the simulation. (b) The difference in oscillation amplitude of the cytoplasmic calcium concentration in three cells with different SR volume fractions. The isolated cell with very low SR fraction (0.001) displays neither calcium waves during low [cGMP] nor global oscillations when [cGMP] is increased. The cell with an intermediate SR fraction only becomes active as [cGMP] is increased. By contrast, the cell with a high SR volume fraction displays spontaneous calcium waves when [cGMP] is low. This is seen as a phase shift between curves obtained from two different points in the cell (indicated by two circles in the inserted cell; the colours of the circles correspond to the colours of the curves). As cGMP is increased, the oscillatory pattern shifts to global oscillations and the phase shift vanishes. The amplitude of the calcium elevations is larger when the SR fraction is high. (c) The oscillations in membrane potential in the three cells. The amplitude is the largest in the cell with the high SR volume fraction due to the interaction between calcium released from the SR and the plasma membrane. Large potential oscillations enhance the ability of coupled cells to synchronize.
Figure 5. Quiescent cells disappear at the onset of vasomotion. (a) Experimental: the percentage of quiescent cells with increasing [NA] in the presence of 300 µM 8Br-cGMP. As synchronization ensues at [NA] = 1 µM, all cells are enrolled into synchronized oscillation. (b) Experimental traces of two cells in the vascular wall before and during the onset of vasomotion representing the enrolment shown in (a). Initially, in the presence of 300 µM 8Br-cGMP and 0.6 µM NA, one cell is quiescent and the other displays waves. As the concentration of NA is increased to 1 µM, vasomotion is initiated and both cells enter synchronized oscillations. (c(i)–(iii)) Simulated traces of cytoplasmic calcium and membrane potential during an increase in [cGMP] in three cells in a vessel wall of 45 cells (these three cells are indicated with numbers on the picture frames during low (left) and high (right) cGMP in (i)). SR volume fractions of the cells are shown at the r.h.s. Only the cell with a volume fraction of 0.06 is capable of generating spontaneous waves. As [cGMP] is increased, all cells are enrolled into synchronized oscillations. Oscillations in cytoplasmic calcium and membrane potential in the previously quiescent cells are caused by the collective driving force from synchronized oscillations in membrane potential.
illustrated in the experimental data of figure 5b (\(\equiv E, 300 \mu\text{M} \ 8\text{Br-cGMP}\)) showing calcium traces of two SMCs in the vascular wall. In each cell, two regions of interest are observed. Initially, in the presence of 0.6 \(\mu\text{M} \text{NA}\), one cell is quiescent (uppermost traces) and the other displays waves (bottom traces). The latter traces are therefore slightly phase shifted. As synchronization develops following an increase in [NA] to 1 \(\mu\text{M}\), the quiescent cell is enrolled into global calcium oscillations and the phase shift in the wave-generating cell vanishes as it changes into a dynamic mode characterized by global oscillations.

Figure 5c (simulated, \(\equiv E\)) shows a possible scenario for enrolling quiescent cells in a heterogeneous cell population as the [cGMP] increases. In the uncoupled state, the cells with a low fraction of SR (below approx. 4\%) remain quiescent during high [IP_3] also when [cGMP] is increased (cf. figure 4). However, since SMCs in the vessel wall are electrically coupled through gap junctions, quiescent cells can be made to oscillate through forcing by neighbouring SMCs. As the [cGMP] increases, the cells with medium SR fraction begin to show global oscillations, and the cells with the highest SR fractions change from a pattern of waves to one also of global calcium oscillations (cf. figure 4). The global calcium oscillations are associated with oscillations in the membrane potential (cf. figure 4). The remaining quiescent cells will then be driven by the surrounding...

Figure 6. Simulated partial and complete synchronization. Arrows indicate time point of a 30-fold increase in intercellular coupling. Curves are from two different cells within each of the three different regions of interest indicated with red circles in (a). Initially, cGMP is low and the cells display waves or remain quiescent. An increase in cGMP leads to partial intercellular synchronization with a phase shift between different regions (emphasized by the first grey line on the curves and shown in (b)). An abrupt increase in intercellular coupling leads to complete synchronization with no discernable phase shift (the second grey line and (c)).
active cells, where the induced oscillations in the membrane potential cause the onset of rhythmic influx of calcium across the plasma membrane. In turn, $[Ca^{2+}]_{cyt}$ may rise above the threshold for CICR, also in cells incapable of producing spontaneous waves. Furthermore, the electrical coupling will allow the individual oscillating cells to interact, and the onset of vasomotion is therefore associated with synchronized oscillations in the membrane potential across the cell population. Figure 5c(ii)(iii) shows the curves for the membrane potential and $[Ca^{2+}]_{cyt}$ from three different cells in a simulated vessel segment of 45 cells, respectively. Only the curve from the cell with the highest SR fraction displays spontaneous waves, but as [cGMP] increases (uppermost curve), all cells are enrolled in the oscillation by the driving force of the synchronized oscillations in the membrane potential caused by cells with medium or high SR fraction. In the cell with a very low SR fraction, the variation in $[Ca^{2+}]_{cyt}$ is caused solely by calcium fluxes across the plasma membrane.

**Intercellular coupling.** In endothelium-denuded vessels, application of intermediate (30–100 μM) concentrations of 8Br-cGMP results in partial intercellular synchronization; thus small islands of SMCs oscillate together, but unsynchronized with other cell islands in the vascular wall. This frequently gives rise to a beating oscillation in force (Rahman et al. 2005). Endothelial cells are more tightly coupled compared to the SMCs (Diep et al. 2005) and, in addition, there are electrical couplings between the endothelial cells and the underlying SMCs through the so-called myoendothelial junctions (Diep et al. 2005). The endothelium therefore constitutes a low-resistance pathway between SMCs, and endothelial denudation reduces the overall electrical coupling in the vessel wall. To investigate the role of intercellular coupling on the degree of synchronization, we performed a series of simulations where the magnitude of intercellular coupling was varied. Figure 6 ( adapté) illustrates such partial synchronization in the model and its dependence on intercellular coupling. The simulation is from a heterogeneous cell population with initially weak intercellular coupling (as normally found between SMCs and mimicking the state in an endothelium-denuded vessel). At the time point corresponding to the arrows, the intercellular coupling is increased 30-fold (mimicking the presence of an endothelium). Figure 6a–c shows the characteristic calcium dynamics in three different periods. The curves shown in the lower part of the figure are the calcium concentrations in three regions of interest indicated in figure 6a (red circles), through the same periods. Initially, [cGMP] is low and the cells either display low-frequency waves or remain quiescent. A few cells display global oscillations. As [cGMP] is increased (to a maximum of 6.5 μM), the cells shift to a partially synchronized state (figure 6b) with neighbouring cells oscillating together, but out of phase with more distant cells (indicated by the first grey line). When the intercellular coupling is abruptly increased, all cells enter synchronized oscillations (figure 6c) and no phase shift is discernable (second grey line).

5. Discussion

In the present paper, we addressed the question of how variation in SMC phenotype affects calcium dynamics, recruitment and synchronization in the vascular wall, using a combination of experiments and mathematical modelling.

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We found that the introduction of variation in cellular phenotype into the mathematical model leads to convergence between simulated and experimentally observed behaviour in a population of SMCs. The results therefore indicate an important role for cellular variation in normal vascular physiology.

Small resistance arteries and arterioles exist in a state of continuous partial contraction. This is in part caused by a myogenic contraction to the intravascular pressure head (Davis & Hill 1999), and myogenic tone is associated with calcium waves in a fraction of the SMCs (Miriel et al. 1999). An increase in static tone can be induced by increasing concentrations of vasoconstrictors such as NA or (acting on the same receptor) phenylephrine, in which case the level of tone correlates with the number of cells displaying asynchronous calcium waves (Zang et al. 2001). In the present study, we find such recruitment of cells with increasing [NA] (cf. experimental data in figure 2d, \( \div E \)), and this is reproduced in the model simulations (cf. figure 3a). It is interesting that the gradual recruitment is only seen when a substantial cellular heterogeneity is introduced into the model. In a previous study of the present model, but without the introduction of substantial cellular heterogeneity, we found that all cells are recruited at the same IP\(_3\) concentration (Jacobsen et al. 2007a, b). Koenigsberger et al. (2004) observed a similar gradual recruitment in their model of a population of coupled SMCs when cellular heterogeneity was introduced through a Gaussian perturbation of the membrane conductances. Hence, cellular heterogeneity may be an efficient mechanism for inducing a graded response in the vascular wall.

The introduction of phenotypical heterogeneity into the model leads to variation in cellular calcium dynamics similar to that observed experimentally. As shown in figure 3a, both silent cells, those with waves of variable frequency and those showing global oscillations, are present simultaneously in the simulated cell population. Experimentally, we find quiescent cells both in the presence of (cf. figure 2a, \(+E\)) and in the absence of (cf. figure 2d, \( \div E \)) pinacidil-induced hyperpolarization. Without pinacidil, a certain fraction of the SMCs will display calcium transients even before NA is added (the non-quiescent cells shown in figure 2d), and these transients are in the form of waves (cf. figure 3b, \( \div E \)). Zang et al. (2001) also found such spontaneously active SMCs (in intact vessels), but at a substantially lower percentage (12.7%). As [NA] is increased, a minor fraction of the oscillating SMCs will display global calcium oscillations, but in the presence of 8Br-cGMP, this fraction increases dramatically (figure 3b, \( \div E \)). Similar behaviour is found in the simulated system in which increasing [IP\(_3\)] causes both recruitment and a shift from waves to global oscillations (cf. picture frames of figure 3a, \( \div E \)). Increasing [cGMP] independently induces the same shift from calcium waves to global calcium oscillations in the simulations.

Experimentally, we observe the disappearance of quiescent cells at the onset of vasomotion. In the presence of 300 \( \mu \text{M} \) 8Br-cGMP, this happens when [NA] reaches 1 \( \mu \text{M} \) (figure 4a, \( \div E \)). Somewhat surprisingly, however, we found that the onset of vasomotion is not associated with all cells entering a mode of global calcium oscillations, but rather some 40 per cent of the SMCs continue to display waves synchronized with the rest of the cells (figure 3b, \( \div E \)). The presence of waves in some cells during vasomotion has been noted before, but only as few cells showing waves unsynchronized with the main oscillation (Rahman et al. 2005). By contrast, during complete synchronization in the model, all cells display global oscillations.
It has previously been shown that during continuous α-adrenoceptor stimulation, wave frequency and, hence, average cytoplasmic calcium concentration decline (Mauban et al. 2001; Zang et al. 2001), probably due to receptor desensitization. However, the present experiments show that, after a sufficiently long period of washout, the SMCs regain almost the same level of average activity when stimulated again and, importantly, a given cell tends to preserve the frequency at which it generates calcium waves (cf. figure 2c, +E). We therefore suggest that the observed variation in wave frequency and the presence of quiescent cells most likely reflect an underlying phenotypical heterogeneity in the cell population.

In a vessel segment displaying vasomotion, the contraction often extends for several millimetres along the vessel and in-phase oscillations may spread even beyond branch points (Meyer et al. 1987). Such coordinated contraction against the intravascular pressure requires a large number of SMCs to act in concert. The question is what mechanisms underlie the cellular entrainment in such a population at the onset of vasomotion? Besides the cell populations being heterogeneous, the SMCs of the vascular wall are probably weakly coupled by gap junctions (Rummery & Hill 2004), and such a cell population is likely to be difficult to entrain. Hence, the SMCs of the vascular wall are probably best characterized as weakly coupled oscillators. However, the ability of the SMCs to synchronize may be determined not only by the intercellular coupling (cf. figure 6) but also by the membrane potential oscillation amplitude. The latter may, as shown in the simulations of figure 4 (±E), increase with increasing [cGMP] and increasing volume fraction of SR. The SMCs therefore constitute a special class of weakly coupled oscillators, since the apparent coupling in the system is determined in part by the state of the individual cell. As illustrated in figures 4–6, the present model points to this regulation of the functional coupling as a central player in the synchronization.

In the simulations of figure 5c (±E), a possible way of entraining quiescent cells is shown. High [NA] without 8Br-cGMP (figure 2d, ±E), or high [8Br-cGMP] without NA (figure 5a, ±E), is associated with a substantial fraction of quiescent cells. The presence of the two compounds in combination reduces the number of quiescent SMCs (figure 5a, ±E). Since the onset of vasomotion in endothelium-denuded arteries is initiated when either [NA] (figures 3b and 5a, ±E) or [8Br-cGMP] (Rahman et al. 2005) is increased, it is in principle not possible to say whether the enrolment of a quiescent cell is due to recruitment followed by synchronization or if synchronization per se enrols the quiescent cell, and there is no obvious way of resolving this question experimentally. It is a characteristic experimental finding, however, that after the onset of synchronization, quiescent cells are never seen. Moreover, this state is associated with a sharp onset of synchronized oscillations in the membrane potential. Considering the heterogeneity of the vascular wall where each cell reacts with an individual sensitivity to NA and, possibly too, to 8Br-cGMP, it is doubtful that the absence of quiescent cells is caused alone by a sudden complete recruitment of all cells. Rather, as indicated by the simulations shown in figure 5c, it seems likely that persistently quiescent cells are forced into cyclical activity during vasomotion by the synchronized oscillations in the membrane potential.

The endothelium. Endothelial denudation has variable effects on vasomotion. In some cases, it causes inhibition (Peng et al. 2001; Mauban & Wier 2004; Rahman et al. 2005), or attenuation (OMOTE & Mizusawa 1993), of vasomotion, whereas
this is not the case in other preparations (Haddock et al. 2002; Lamboley et al. 2003). The same is the case for procedures aimed at manipulating the concentration of cGMP within the vascular wall. Inhibition of endothelial NO synthase or guanylate cyclase (the latter of which produces cGMP in response to NO) may either inhibit vasomotion (Gustafsson et al. 1993; Hessellund et al. 2006) or leave it more or less unaffected (Mauban & Wier 2004). Whereas in the present mesenteric vessel preparation we consistently find that the addition of 8Br-cGMP promotes synchronization (Gustafsson et al. 1993; Peng et al. 2001; Rahman et al. 2005), Haddock et al. (2006) did not find this effect on adding dibutryryl-cGMP to endothelium-denuded rat cerebral arteries. The most straightforward explanation for these discrepancies is the presence of micro-vascular heterogeneity (Hill et al. 2001) between species and vascular beds with the coexistence of different vasomotion mechanisms. As we have speculated earlier (Jacobsen et al. 2007b), another possible explanation is the presence of an oscillatory domain within the cGMP concentration space. Interventions (e.g. endothelial removal, addition of l-NAME or 8Br-CGMP) that move the system across the borders of this domain could, depending on the starting conditions, either initiate or abolish vasomotion. A similar scheme related to cytosolic calcium rather than to cGMP was previously suggested by Koenigsberger et al. (2005).

Using the coupled SMCs model, we have previously addressed the observation of Rahman et al. (2005) that 8Br-cGMP in intermediate concentrations may give rise to partial synchronization in the vascular wall (Jacobsen et al. 2007a). We found that in a more homogeneous SMC population than the present, there is a characteristic threshold concentration of cGMP above which synchronization is complete and below which there may be partial synchronization (Jacobsen et al. 2007a). We also found that as long as intercellular coupling exceeded a certain minimal value, its magnitude did not influence the synchronization process (Jacobsen et al. 2007a). In a strongly heterogeneous SMC population like the present, however, the picture is more complex. As shown in the simulations of figure 4 (÷E), the oscillations in the membrane potential increase in amplitude with both [cGMP] and the volume fraction of SR, and in this situation the intercellular coupling strength influences the ability of the system to synchronize. As shown in the simulations of figure 6 (÷E), an increase in [cGMP] gives rise to partial synchronization when coupling strength is low, but if the coupling strength is abruptly increased at a maintained level of cGMP, synchronization becomes complete. We suggest that an increase in coupling strength (an increase in the general gap-junctional permeability coefficient) may be equivalent to the presence of a low-resistance, possibly endothelial, pathway along the vessel. Based on meticulous measurements of cell dimensions and estimates of gap-junction plaque density, Haas & Duling (1997) have previously concluded that the endothelium may in fact constitute such a low-resistance pathway. Similar conclusions were reached by Haddock et al. (2006) in experiments on isolated branches of the rat basilar artery. In these vessels, the endothelium seems to synchronize SMC activity by connecting electrically distant cells, an effect mediated by myoendothelial gap junctions. The longitudinal electrical resistance of the tunica media may be substantial (Yamamoto et al. 2001), explaining the difficulty of obtaining complete synchronization in endothelium-denuded vessels. By contrast, the interfaces between endothelial cells are densely invested with gap junctions (Yamamoto et al. 2001; Rummery & Hill 2004). Under normal

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circumstances, the endothelium may therefore convey current over longer distances and, thus, have a smoothing and synchronizing effect on the underlying ‘driving’ oscillators. Rather than being a source of the oscillations _per se_, the endothelium seems to play an important permissive and modulating role in vasomotion. As a medium for studying these phenomena, the vascular wall offers a unique opportunity, since the endothelium can be selectively removed whereby the underlying oscillators are unmasked.

In the present model, we have placed the main source of heterogeneity on the cellular fraction of SR. However, tissue heterogeneity resides on all levels from the expression of critical proteins to macroscopic variation in tissue composition. Our choice of the SR fraction is based on the central role of the SR in the normal generation of vasomotion in rat mesenteric arteries (Gustafsson & Nilsson 1993; Peng _et al._ 2001). Other sources of intercellular variation are equally possible, and may reproduce experimental behaviour equally well. Most likely multiple sources of variation coexist in the vascular wall.

We have assumed that the presence of an endothelium can be mimicked by reducing the intracellular resistance between the SMCs and, hence, that the endothelium behaves as a simple resistance. Clearly, this is a crude approach since, although not excitable (Nilius & Droogmans 2001), endothelial cells in their plasma membrane host a number of different ion channels, some of which may show voltage sensitivity (Nilius & Droogmans 2001). Furthermore, the presence of low-resistance myoendothelial gap junctions that couple the endothelial cell and the SMC layer is a prerequisite for an electrically synchronizing effect of an endothelium. The conclusion from figure 6 should therefore be restricted to the observation that, in the simulations, the presence of a low-resistance pathway along the vascular wall may lead to long-distance synchrony.

In conclusion, we have presented data showing the existence of considerable cellular heterogeneity in the vascular wall. We introduced similar heterogeneity into a model of coupled SMCs. The simulated results indicate that the collective driving force from cyclic variation in the membrane potential at the onset of vasomotion may entrain previously quiescent cells. Furthermore, simulations indicate that the partial synchronization observed in endothelium-denuded vessels is influenced by the presence of cellular heterogeneity, and that complete synchronization depends on the presence of a low-resistance, possibly endothelial, pathway along the vessel. Despite our present focus only on the properties of rat small mesenteric arteries, the SMC is in fact a very common cell type in mammals, and constitutes a main fraction of the wall of most internal hollow organs (Burkitt _et al._ 1993). Although SMCs have variable properties depending on their origin, they do share common morphological and biophysical characteristics (Burkitt _et al._ 1993). In a wider perspective, therefore, cellular recruitment and vasomotion in the arterial wall may serve as a well-established and easily accessible model for force development in a population of SMCs. Furthermore, SMC tissue is the target for a large number of drugs, the final action of which is to cause either an increase or a decrease in SMC tone, and hence change, for a specific purpose, the luminal size of structures such as airways, blood vessels, uterus and bladder (Katzung 1989). As opposed to, for example, skeletal muscle tissue, where force development relies on nervous stimulation, the degree of activation of the SMC is an integrated response to a large number of stimuli (Burkitt _et al._ 1993). Here we point to some specific mechanisms that may lead to static or dynamic force development in the arterial wall, but the number of such mechanisms

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can in fact be large. Simulation studies of complex systems like the present may facilitate the identification of such mechanisms, deepening our understanding of the system and advance the development of drugs that control SMC tone.

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