Activation of a cGMP-sensitive calcium-dependent chloride channel may cause transition from calcium waves to whole-cell oscillations in smooth muscle cells

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Short title: Chloride channels and rhythmic calcium elevations

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Abstract

In vitro, α-adrenoceptor stimulation of rat mesenteric small arteries often leads to a rhythmic change in wall tension, vasomotion. Within the individual smooth muscle cells of the vascular wall, vasomotion is often preceded by a period of asynchronous calcium waves. Abruptly, these low-frequency waves may transform into high-frequency whole-cell calcium oscillations. Simultaneously, multiple cells synchronize leading to rhythmic generation of tension.

We present a mathematical model of vascular smooth muscle cells that aims at characterizing this sudden transition. Simulations show calcium waves sweeping through the cytoplasm when the SR is stimulated to release calcium. A rise in cyclic guanosine monophosphate (cGMP) leads to the experimentally observed transition from waves to whole-cell calcium oscillations. At the same time membrane potential starts to oscillate and the frequency approximately doubles.

In this transition, the simulated results point to a key role for a recently discovered cGMP-sensitive calcium-dependent chloride channel. This channel depolarizes the membrane in response to calcium released from the SR. In turn, depolarization causes uniform opening of L-type calcium channels on the cell surface stimulating synchronized release of SR-calcium and inducing the shift from waves to whole-cell oscillations. The effect of the channel is therefore to couple the processes of the SR with those of the membrane. We hypothesize that the shift in oscillatory mode and the associated onset of oscillations in membrane potential within the individual cell may underlie sudden intercellular synchronization and the appearance of vasomotion.
Introduction

Cytoplasmic calcium elevations in the vascular smooth muscle cell (VSMC) exhibit a continuum of patterns. On the sub-cellular level, calcium release from the sarcoplasmic reticulum (SR) may result from coordinated opening of clusters of SR-calcium-release channels (sparks) (31). On a cellular level, more massive release causes calcium waves to sweep across the cytoplasm. Ultimately, calcium may rise simultaneously in all parts of the cytoplasm, here referred to as whole-cell oscillations (36).

Asynchronous calcium-waves in rat mesenteric small arteries are normally not associated with development of tension (50). In contrast, synchronized whole-cell calcium oscillations are associated with cyclic development of tension (50). This phenomenon, known as vasomotion, has been observed in vessels from numerous vascular beds in different species (1; 9; 13; 15; 19; 23-25; 67; 70). It has been implicated in the protection against tissue ischemia at critically low perfusion (52) but despite having been known for more than 150 years (56), the significance and physiological role of vasomotion is still not well established (43). The ubiquitous nature of vasomotion though, and the observation that its characteristics change in disease states such as hypertension (37; 48), preeclampsia (1) and diabetes (63), suggest the phenomenon has important microcirculatory functions.

In rat mesenteric small arteries α-adrenoceptor stimulation cause calcium waves to appear with a frequency of typically 3-6 min⁻¹ (50) in both intact and endothelium-denuded arteries (50; 51). These waves seem to depend on a functional SR, and are, in the short term, not dependent on calcium entry from the cell surface (50; 53). Furthermore, the waves are uncoordinated between neighboring cells, and are not associated with oscillations in membrane potential (51). After a variable period, waves in intact arteries may transform spontaneously into high-frequency whole-cell calcium oscillations associated with vasomotion (50). In endothelium-denuded vessels, the same transition can be induced by application of the membrane permeable cGMP-analogue, 8Br-cGMP.

Whole cell oscillations during vasomotion have a frequency of typically 10-20 min⁻¹ (23; 25), and are, in contrast to waves, associated with recurring changes in membrane potential (5; 24) and depend on cyclic influx and efflux of calcium across the plasma membrane (25; 50). The effect of 8Br-cGMP on endothelium-denuded vessels suggests that cGMP may be involved in the transition from waves to whole-cell oscillations also in intact arteries. Peng et al (50) suggested, that the effect of cGMP could be mediated through a cGMP-sensitive calcium-dependent chloride channel located in the smooth muscle cell membrane (41).
In endothelium-denuded vessels Rahman et al (51) demonstrated that at low concentrations of 8Br-cGMP the cells switch from waves to whole-cell oscillations, but that these oscillations are not well coordinated along the vessel. Instead small islands of cells are oscillating with individual frequencies. Only as 8Br-cGMP is increased do these islands synchronize, leading to regular vasomotion. This observation indicates that rhythmic whole-cell calcium elevations is a characteristic mode of oscillation within the individual VSMC that may precede and lead to vasomotion rather than being a consequence of it. It is therefore of interest what underlies the transition from waves to whole-cell oscillations within the individual cell. The investigation of that question is the aim of the present study.

We present a mathematical cell model in which we focus on testing possible consequences of activation of a cGMP-sensitive calcium-dependent chloride channel located in the smooth muscle cell membrane. This channel was given key characteristics similar to those found in rat mesenteric small arteries (41), and was inserted into a cell model. Full-scale simulation of the model shows that a transition from the resting state to wave-like calcium elevations can be caused by an increased cytosolic concentration of inositol triphosphate, IP₃, causing release of calcium from the SR. A further transition to whole-cell oscillations can be caused by an increased coupling between the calcium dynamics of the SR and that of the plasma membrane. This increased coupling is achieved through activation of the cGMP-sensitive calcium-dependent chloride channel and leads to a characteristic shift in frequency and onset of oscillations in membrane potential.
1. The model

1.1 Cell model: The vascular smooth muscle cell is modeled as being spindle-shaped with a certain length, \( L_{cell} \), and a certain central radius, \( r_{cell} \) (table 1). The central 1/3 of the cell is cylindrical whereas the lateral parts taper off. Fig. 1A shows the cellular compartments considered in the model. These are the cytoplasm (cyt), the SR and the plasma membrane. For simplicity other membrane bound organelles as well as specialized structures in the cytoplasm (e.g. cytoskeleton) are omitted. Fig. 1B shows the characteristic elongated shape with tapering ends of a smooth muscle cell isolated from a rat mesenteric small artery (see legend for details).

1.2 Cytoplasm model: The rate of change in the cytoplasmic calcium concentration, \( \frac{d[Ca^{2+}]_{cyt}}{dt} \), due to a specific flux, \( f \), of calcium will be denoted \( J_{Ca^{2+}-cyt,f} \). The change in intracellular calcium concentration at a given point in the cytoplasm is the sum of the contributions from diffusion from other parts of the cell, from buffering, from the SR and from calcium fluxes across the plasma membrane. The expression for the change in cytoplasmic calcium concentration therefore becomes:

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

(1)

Details of each of these terms are given in the appendix.

1.3 Sarcoplasmic Reticulum model: The SR is modeled as being diffusely distributed in the cytoplasm. Calcium is assumed to diffuse inside the interconnected SR-cisternae to eliminate concentration gradients. The expressions for calcium diffusion and calcium buffering (66) inside the SR are, except for different constants, identical to the expressions used for the cytoplasm (appendix section 4.1 & 4.2). The rate of change in free calcium concentration in the SR becomes:

\[
\frac{d[Ca^{2+}]_{SR}}{dt} = J_{Ca^{2+}-SR, diffusion} + J_{Ca^{2+}-SR, buffering} - \left( \frac{V_{cyt}}{V_{SR}} \right) \times J_{Ca^{2+}-cyt, SR}
\]  

(2)

where the ratio of cytoplasmic and SR volumes: \( \frac{V_{cyt}}{V_{SR}} \) scales the rate of concentration change in the SR to its much smaller volume, as compared to the cytoplasm.
1.4 Membrane model: Differences in membrane potential from one place to another on the cell surface are assumed to equilibrate very rapidly (i.e. instantaneously) compared to all other processes considered in the model. Thus, membrane potential, $V_m$, is assumed to be the same everywhere in the cell. It is further assumed that ion channels, pumps and carriers (Fig. 1A) are distributed across the whole cell surface, but with varying local concentration (see Perturbation section). The dynamic change in membrane potential is the sum of all trans-membrane currents (45):

$$\frac{dV_m}{dt} = -\frac{1}{C_{m,\text{cell}}} \left( I_{\text{Na}_k} + I_{\text{Ca}_P} + I_{\text{Na}_Ca} + I_{\text{Cal}} + I_{\text{Cl}_{\text{ca}}} + I_{\text{K}_e} + \sum_s I_{\text{back},s} \right), $$

(3)

where $C_{m,\text{cell}}$ (Table 1) is the capacitance of the cell membrane. Except for $I_{\text{Cl}_{\text{ca}}}$ (see below) details for each current are given in the appendix. The equilibrium potential, $E_x$, is defined as:

$$E_x = -\frac{RT}{zF} \ln \left( \frac{[x]_{\text{cyt}}}{[x]_o} \right), $$

(4)

where $R$ is the gas-constant, $T$ is the absolute temperature $(293.15 \text{ K})$, $z$ is the charge of the generic ion $x$ and $F$ is Faraday’s number (45). The index $o$ refers to the extra-cellular space. Except for the concentrations of calcium in cytoplasm and SR, all ion concentrations are held constant at their bulk cytoplasmic or extracellular values (Table 2), and are thus assumed to be invariant on the time scale considered in the model (few minutes).

1.5 cGMP-sensitive calcium-dependent chloride current (Table 3). A simple model of the bulk properties of this current is formulated as:

$$I_{\text{Cl}_{\text{ca}}} = g_{\text{Cl}_{\text{ca}}} P_{\text{open}} (V_m - E_{\text{Cl}}), $$

(5)

where $g_{\text{Cl}_{\text{ca}}}$ is maximum whole-cell conductance of the channel. The dynamic change in channel open probability, $P_{\text{open}}$, is assumed to follow first-order kinetics with one (invariant) time constant:
\[
\frac{dP_{\text{open}}}{dt} = \frac{P_{\text{open}} - P_{\text{open}}^{\text{ss}}}{\tau_{\text{Cl}_{\text{ca}}}}
\]

(6)

Steady state open probability of the channel is modeled as:

\[
P_{\text{open}}^{\text{ss}} = \frac{\alpha \left[ Ca^{2+}\right]_{\text{cyt}}^{n}}{\left[ Ca^{2+}\right]_{\text{cyt}}^{n} + \left[ k_{\text{Cl},n}\left(1 - \alpha\rho\right)\right]^{n}},
\]

(7)

where

\[
\alpha = \frac{[cGMP]^{n}}{[cGMP]^{n} + k_{\text{Cl},cGMP}^{n}}.
\]

(8)

The constants of Eqs. 6-8 are adjusted to fit experimental data from rat mesenteric vessels (41). Hence, \( \alpha \) gives \( P_{\text{open}} \) a dependence on [cGMP], whereas \( \rho \) (ranging from 0 to 1) determines the overall strength of the influence from cGMP.

Fig. 2A shows the model-generated open probability for the channel as a function of \( \left[ Ca^{2+}\right]_{\text{cyt}} \) at different levels of [cGMP]. Fig. 2B shows experimental current densities at different levels of [cGMP] following application of caffeine (41). Fig. 2C shows the same curves as generated by the model. Fig 3A shows the experimental whole cell current in response to caffeine stimulation in the presence of 10 µM cGMP (41) and Fig. 3B shows the corresponding model generated trace. For the simulation of Fig. 3B, the increase in \( [Ca^{2+}]_{\text{cyt}} \) in response to caffeine stimulation is assumed to follow the trace shown in Panel C (29).

2. Computational methods

2.1 Discretization: The cell is divided along its longitudinal axis into \( e \) (=120) segments. Radial concentration gradients are not considered. In the middle third of the cell the segments are cylindrical and their volume, \( v_e \), and surface area, \( S_e \), are

\[
v_e = \pi r_{\text{cell}}^2 \left( L_{\text{cell}} / e \right) \quad \text{and} \quad S_e = 2\pi r_{\text{cell}} \left( L_{\text{cell}} / e \right),
\]

respectively. Towards the ends of the cell the segments are shaped as a frustum of a cone with
volume and surface area being \( V_e = \left( \pi L_{cell} \left( R^2 + Rr + r^2 \right) / 3 \right) \) and 
\[
S_e = \pi \left( R + r \right) \sqrt{\left( R - r \right)^2 + \left( L_{cell} / \ell \right)^2},
\]
respectively, where \( R \) and \( r \) are the radii of basis and top of the frustum respectively. Each end of the tapering cell is assigned a radius \( r_{\text{min}} = r_{\text{cell}} / k_{\text{rad}} \) which is an arbitrarily chosen fraction, \( 1 / k_{\text{rad}} \), of the central cell radius (Table 1).

**2.2 Perturbation:** A smooth muscle cell is heterogeneous along its longitudinal axis since the local concentration of e.g. SR (36) and membrane proteins may vary along the cell. Spatial heterogeneity is implemented in the model by multiplying a given quantity (perturbed quantities are marked with asterisk in the tables) by a perturbation factor \( \omega = 1 + \left( (\phi - 0.5) \times k_{\text{perturb}} \right) \). \( \phi \) is a random number between 0 and 1. \( k_{\text{perturb}} \) scales the size of the perturbation; for simplicity the same value of \( k_{\text{perturb}} (0.01) \) was used for all perturbed quantities. Thus, \( \omega \) varies randomly between each segment along the cell. In each segment, \( \omega \) has an individual and independent value for each perturbed quantity. In addition to the parameters, also the initial values of the variables \([cGMP]\) and \([IP_3]\) are perturbed according to the above formulation. The values of \( \omega \) are maintained during a single simulation, but changes between simulations. Accordingly, the results vary slightly between each simulation. In simulations where \([cGMP]\) is increased, it is increased by the same amount in all segments hence preserving the differences in \([cGMP]\) between the segments.

**2.3 Wave velocity:** Average velocity is calculated by determining, during the passage of a wave, the time by which calcium first exceeds 150 nM at two randomly chosen places in the central cell region, separated by a known distance. Wave speed is high close to the point where the wave originates. It then decreases rapidly to reach a more stable level as the wave moves on. For that reason, velocity measurements are made > 4 \( \mu \)m away from the point of origin.
3. Numerical methods

3.1 Software: The program source code is written in the C-programming language (ANSI C standard), using Microsoft Developer Studio (Visual C++ 6.0, Microsoft, Seattle, WA). Cells are visualized using IDL 5.2 for Windows (Research Systems, Boulder, CO). Simulations are performed on ordinary desktop PCs (Intel Pentium D, 2.8 GHz, dual core CPU).

3.2 Initial conditions: As initial condition $\left[Ca^{2+}\right]_{cyt}$ and $\left[Ca^{2+}\right]_{SR}$ are set to 100 nM and 1 mM respectively and $V_m$ to -50 mV. The cytosolic oscillator in each segment along the cell is started in an individual state by varying randomly (between 0 and 1) the initial value of $f_{inact}$ (appendix section 4.3) along the cell.

3.3 Integration method and numerical stability: Eqs. 1-3 and their associated gating variables are integrated forward in time using the explicit Euler method with a fixed time step, $\Delta t = 5 \times 10^{-4} \text{s}$. At this $\Delta t$, based on test simulations, numerical instability is avoided (appearing as a rapid divergence towards $\pm \infty$ of $V_m$ and/or $[Ca^{2+}]_{cyt}/[Ca^{2+}]_{SR}$) and simulations can be completed within reasonable time (minutes to a few hours) with only negligible difference in the final result, as compared to simulations using smaller time steps. Reducing the time step by a factor of 10 ($\Delta t = 0.5 \times 10^{-4} \text{s}$) or 100 ($\Delta t = 0.05 \times 10^{-4} \text{s}$) and integrating the system for 600 s caused less than a 0.35% change in the computed solution.
Results

**Cytoplasm and SR model.** Fig. 4 illustrates how the isolated “cytosolic oscillator” consisting of buffered cytosol, the SR-membrane with associated calcium channels and pumps (c.f. Fig. 1) and the buffered SR interior, is able to produce autonomous oscillations (solution of Eqs. A2-A5). When the calcium release channel is unstimulated, i.e. at low [IP$_3$], calcium fluxes across the SR-membrane outbalance each other and [Ca$^{2+}$]$_{cyt}$ and [Ca$^{2+}$]$_{SR}$ stay constant. If [IP$_3$] is raised, the calcium release channel becomes more sensitive to calcium at the ambient calcium level. Consequently the efflux rate from the SR increases, rendering the system unstable. This is illustrated in Fig. 4A. A calcium peak is preceded by a slow rise in [Ca$^{2+}$]$_{cyt}$ continuing until a threshold value is reached at around 120 nM. Past this point, there is a rapid increase in [Ca$^{2+}$]$_{cyt}$ due to calcium induced calcium release (CICR). Release is terminated by the slow binding of calcium to the inactivation site on the calcium release channel. The system instability is caused by the slowness of the binding of calcium to this site, combined with the inability of the sarcoplasmic reticulum calcium ATPase (SERCA) to keep up with the massive release during CICR. Neither lateral diffusion nor the plasma membrane model is included in this simulation. Therefore, the system simulated in Fig. 4 corresponds to a closed system in which a preserved amount of calcium is transported back and forth between SR and cytoplasm. As a consequence, an inverse oscillation is seen in the local SR (Fig. 4B, note the difference in concentration scale). In this system increasing [IP$_3$] results in large amplitude oscillations occurring abruptly at [IP$_3$] = 1.1299 µM. The stable limit cycle found above this concentration has almost constant amplitude despite further increase in [IP$_3$].

Segmenting the cell along its longitudinal axis, describing the local Ca$^{2+}$ dynamics in each segment by the above model, and including calcium diffusion between the segments (solution of Eqs. A1-A5, plasma membrane model not included), a local calcium elevation will now cause release from neighboring parts of the SR, and the result will be repetitive waves sweeping through the cell. Fig. 5A shows this wave and the inverse wave in the SR (see also movie 5A1 (cytoplasm) &
5A2 (SR), suppl. material). For comparison with an *in vitro* wave, please see (50). The frequency of the waves depends on the rates at which calcium passes across the SR-membrane. An increase in $r_{\text{CICR}}$, $r_{\text{leak}}$ (Eq. A3) or $[\text{IP}_3]$ (Eq. A4) results in increased wave frequency, whereas an increase in $r_{\text{SERCA}}$ (Eq. A3) reduces wave frequency. Because of the random perturbation of the system parameters, waves will be initiated in the cytoplasmic segment along the cell having the highest intrinsic frequency. This segment will pace the other segments, and thus, it will be the site of origin of the waves and it will determine overall wave frequency.

As illustrated in Fig. 5B (and movie 5B, suppl. material) wave integrity depends on diffusion to coordinate calcium release along the cell. Furthermore, diffusion averages frequency and amplitude of the individual oscillators, allowing a smooth wave to appear. The sequence of Fig. 5B starts during a period of stable waves (first frame). At $t = 3$ s the cytoplasmic diffusion coefficient for calcium is abruptly set to zero, stopping all lateral diffusion. Immediately, the wave-pattern breaks up into uncoordinated local calcium elevations of varying frequency and amplitude and some segments stop oscillating. One minute later, the diffusion coefficient again attains its original value and the smooth wave pattern re-emerges. The speed of the waves depends on the volume fraction of SR in the cell. As shown in Fig. 6, increasing this fraction from 2% to 14% increased average wave-speed from approximately 2 µm s$^{-1}$ to 105 µm s$^{-1}$.

**Whole cell model.** With inclusion of the plasma membrane, trans-membrane fluxes of calcium come into play (solution of all described equations). These fluxes are strongly influenced by the membrane potential. The latter is uniform across the cell surface, and therefore acts to synchronize processes in different parts of the cell. Initially, with the cell in a resting state (low $[\text{IP}_3]$), $[\text{Ca}^{2+}]_{\text{cyt}}$ remain constant at 100 nM and membrane potential is stable at approximately -51.5 mV (not shown).

Fig. 7 shows the conditions necessary to induce waves and oscillations in the whole cell model. The simulation is started in a stimulated state with a moderately high $[\text{IP}_3]$ level (1.75 µM). Experimentally, $\alpha$-adreno receptor stimulation (leading to an increase in $[\text{IP}_3]$) is associated with a
depolarization that does not depend on \([\text{Ca}^{2+}]_{\text{cyt}}\) (44). Consequently, a modest depolarization (approx. 3 mV) was assumed to be associated with the increase in \([\text{IP}_3]\). In the cell model, average membrane potential can be modulated by adjusting the background conductance of either \(Na^+\), \(K^+\) or \(Cl^-\). Alternatively, average membrane potential can be altered by altering the activity of the Na/K-ATPase (see below). The effect of a change in average membrane potential only depends on the magnitude of the change, and not on the specific source of the current causing the change in potential. Under these conditions the cell shows cytoplasmic calcium waves. As shown in Fig. 7C, these are associated with only infinitesimal oscillations in membrane potential. In the absence of additional external influences, waves will continue to cross the cytoplasm with a frequency in the order of 6-7 min\(^{-1}\). In the presence of the membrane, waves are consistently initiated in the tapering ends of the cell (c.f. movie 7E, suppl. material).

Fig. 7E shows the calcium profile from two different points in the cell (marked with a point in the same color as the curve). During the wave period, frequency is low and calcium rises first at the most lateral (grey) point. Increasing the concentration of cGMP (Fig. 7A) makes the wave pattern transform abruptly into whole-cell oscillations with a frequency roughly twice that of the waves (Fig. 7E and movie 7E, suppl. material). In addition, there is an increase in amplitude. A secondary reduction in [cGMP] will lead to reemergence of the wave pattern. Whereas the waves have only minimal influence on the membrane potential, the onset of whole-cell oscillations is associated with onset of oscillations in membrane potential (Fig. 7C). The appearance of whole-cell oscillations is independent of how [cGMP] is increased (a ramp increase is used for simplicity) and of whether \([\text{IP}_3]\) is increased first and [cGMP] afterwards or vice versa. For the system to go through the wave stage though, \([\text{IP}_3]\) must increase first.

Fig. 8A shows the concentrations of cGMP at which the cell displays waves and those at which it displays whole-cell oscillations. The frequency of the whole-cell oscillations decreases with increasing [cGMP] due to its influence in the Na/K-ATPase (cf. Eq. A8, not shown). In a certain
range of [cGMP] the cell can exist in either of the two states, and it can switch between these states without any change in parameters. This bistable condition is shown in Fig. 8B where a short lasting de- or hyperpolarization (large arrows) applied to a cell in the low-frequency wave state causes resetting of the individual oscillators along the cell and initiation of sustained, high-frequency whole-cell oscillations. A depolarization of longer duration (short arrow) causes calcium flooding and reemergence of low-frequency waves as calcium is pumped back into the extracellular space.

*Frequency and amplitude:* When the cell is in the whole-cell oscillatory mode, a main determinant of frequency is the membrane potential. This is shown in Fig. 9A where average membrane potential was modulated as described in relation to Fig. 7. The figure shows a positive correlation between average membrane potential and frequency. In fact, the spectrum of whole-cell oscillation frequencies is so broad that the lowest frequencies overlap those of the preceding waves (50).

Fig. 9B shows the amplitude (distance between curves) of the oscillation in intracellular calcium concentration for the same simulations as in Fig. 9A. The amplitude is virtually independent of the frequency.
Discussion

Transition from waves to whole-cell oscillations in individual cells of rat mesenteric small arteries is seen in close relation to intercellular synchronization (50). It raises the question whether this transition is caused by intercellular synchronization per se, or whether a change within the individual cell promotes sudden synchronization. On the basis of the present model we hypothesize that the latter is the case. The main findings of the simulations are that 1) a shift in oscillatory mode and 2) a shift in frequency and 3) the onset of oscillations in membrane potential, may all be explained on the basis of processes occurring within the individual cell.

*In vitro* intercellular synchronization occurs abruptly and often, on a cellular scale, across large distances (for a movie of the synchronization process please see (50)). VSMC’s are electrically coupled (54) and may influence each other through intercellular differences in membrane potential which could therefore act as the fast spreading signal causing synchronization.

Simulations of the present model show absence of membrane potential oscillations in the wave state (Fig. 7C). This is in accordance with the observation of Rahman *et al* (51) that endothelium-denuded, noradrenaline (NA)-stimulated vessels show waves, but no oscillations in membrane potential. Most likely, cells in the wave state are therefore unable to influence each other in a rhythmic fashion through the membrane potential. The shift in oscillatory mode and the onset of oscillations in potential changes this situation. Coupled cells may now rhythmically influence each other, ultimately leading to intercellular synchronization.

The present results indicate that the characteristic shift in both oscillation mode and frequency may be explained as a consequence of an increased spatial and temporal coupling between SR and plasma membrane established through the activation of a cGMP-sensitive calcium-dependent chloride channel. Indeed, estimates of [cGMP] in the mesenteric arterial tree (40) recalculated to water volume (2) indicate that the chloride channel can be regulated by cGMP within the physiological concentration range. An increase in [cGMP] will increase the sensitivity of the
channel to calcium released from the SR. Consequently, localized calcium release will now have a small, but global, depolarizing influence on the membrane, causing uniform calcium influx through L-type calcium channels and entrainment of CICR throughout the cell. Following an increase in [cGMP] the system thus enters a positive feed-back loop between chloride, L-type and SR-calcium release channels, causing cytoplasmic calcium to rise rapidly throughout the cell (Fig. 7E) and switching the pattern from low-frequency waves to high-frequency whole-cell oscillations.

In NA-stimulated endothelium-denuded preparations not showing spontaneous vasomotion, the latter can be induced by application of 8Br-cGMP (23; 24; 26; 50; 51). Furthermore, the degree of coordination between different parts of the isolated vessel has been found to increase with increasing [8-Br-cGMP] (51). In native vessels cGMP production depends on nitric oxide (NO) released from the endothelium, but endothelial denudation has a variable effect in rat mesenteric small arteries. In some studies, denuded vessels stimulated only with α-adreno receptor agonist show no vasomotion (23; 24; 26; 42; 50; 51), whereas vasomotion is found in others (35; 59). The reason for this discrepancy is not clear. Along the same line, abolition of vasomotion with inhibitors of nitric oxide synthase or guanylate cyclase (producing cGMP in response to NO) in mesenteric (24) or retinal (28) vessels is found in some studies but not in others (42; 51). Sell et al (59) found that increasing [NO] by acetylcholine stimulation abolished vasomotion whereas reducing it with L-NAME promoted rhythmic activity.

Some of these discrepancies could be due to the existence of a certain region of [cGMP] in which vasomotion, and hence synchronized whole-cell oscillations, are seen. Such a region is found in the present simulations (Fig. 8A) and could, in the experimental setting, be entered by both increasing (24; 50; 51) or reducing (59) [cGMP]. Similarly the region may be left by either reducing (24) or increasing (59) [cGMP]. Furthermore, its size and position may vary with the experimental conditions and with the presence of other substances, e.g. EDHF (42), known to play important roles in the initiation of vasomotion. Other possibilities could be that the mechanisms underlying
vasomotion differs between vascular beds or that vasomotion can arise from different mechanisms within the same vascular bed. Indeed, in iridial arterioles vasomotion may continue during voltage clamp (27), a situation which is incompatible with the present model.

A number of experimental (3; 48; 68) and model (33) studies show that myogenic contraction, a process known to be associated with membrane depolarization (57), increases vasomotion frequency. The results of the present simulations indicate that membrane potential could indeed be an important determinant of frequency (Fig. 9A). It therefore seems counterintuitive that 8Br-cGMP in fact reduces vasomotion frequency with increasing concentration (28; 51) considering its depolarizing effect via the chloride channel. One possibility is that cGMP has an opposing effect via another electrogenic membrane protein; in the present model the Na/K-ATPase. Such an effect still allows for a frequency shift at the transition from waves to whole-cell oscillations, but a larger increase in [cGMP] will reduce the frequency of the whole-cell oscillations. In support of this suggestion Gustafsson et al (24) showed that in endothelium-denuded, NA-stimulated mesenteric vessels, exposure to sodium nitroprusside (releasing NO) is associated with hyperpolarization. Furthermore, a stimulating effect of cGMP on the Na/K-ATPase has been found in pulmonary arterial smooth muscle cells (64; 65), whereas in other tissues the effect can be either absent or inhibitory (6; 60). The simulated results however, neither depend critically on which current is affected by cGMP nor on the exact characteristics of the stimulation as a function of cGMP (Eq. A8). Rather the important thing is the general effect of modulating membrane potential. Test simulations showed a similar effect on frequency, assuming a stimulatory effect on calcium activated potassium current (58). Inhibition of Na/K-ATPase activity with 1mM ouabain abolishes arterial vasomotion in both intact and endothelium-denuded rat mesenteric arteries (26; 47) and, since this effect is immediate, Gustafsson and Nilsson (26) suggested it was due to membrane depolarization. Similarly, a too strong depolarization also abolishes oscillations in the present simulations (Fig. 9A).
Critique of the model. The present model is qualitative in nature and focus is on the qualitative behavior of the system. Free parameters are unavoidable in cell models and their values must be chosen so as to give basic model components a realistic behavior when compared to experimental observations. For that reason the model cannot be said to represent a unique description of a rat intestinal VSMC. Rather it is a cell model with some features specific for this cell type.

A simplification of the present model is that micro-domains between SR and plasma membrane are not considered (for an excellent review see (36)). Ionic concentrations may here reach values not seen in the bulk cytoplasm. The highly organized ultra-structure of the SR may have implications for the regulation of the membrane potential (32). SR located in the periphery may also to some degree shield the deep parts of the cytoplasm from calcium entering the cell, a phenomenon known as “the superficial buffer barrier” (36; 55). In such areas the sodium/calcium exchanger may continuously offload calcium from the SR before it enters deeper parts of the cell (36), or it may periodically operate in reverse mode when local sodium concentration becomes high (7). Elsewhere on the cell surface the sodium/calcium exchanger may rather be in contact with the bulk cytoplasm. Such functional heterogeneity is not taken into account, and its consequences are difficult to evaluate; most likely though, forward mode operation will be the most common mode of operation, and hence, in accordance with the present formulation.

Specialized surface structures such as caveolae are not considered in the model. Caveolae may penetrate the peripheral SR and, due to the presence of voltage sensitive calcium channels, deliver calcium directly to the deep cytoplasm (36). This organization would, on the other hand, agree with the present formulation with the lack of a superficial buffer barrier.

Lack of a superficial buffer barrier for Ca$^{2+}$ may however explain some of the present results. Experimentally, calcium waves appear to be due primarily to calcium release from the SR, since they continue in calcium-free medium (50). Simulating the model without calcium fluxes across the plasma membrane (Fig. 5A) shows a similar sustained wave pattern, with a realistic wave velocity.
(Fig. 6) as compared to the values reported by Peng et al. of 36 (12-175) µm s⁻¹ (50) and Sell et al of 25.2 ± 2.6 µm s⁻¹ (59). With inclusion of calcium fluxes across the plasma membrane however, simulated wave-velocity is generally lower (2-5 µm s⁻¹) due to local extrusion of calcium from the wave. Furthermore, insufficient influx of extracellular calcium will lead to immediate disappearance of the wave pattern. One explanation for this discrepancy could be that in the living cell, calcium released to the deep parts of the cytoplasm does not come in contact with the plasma membrane as easily as is the case in the present model where radial concentration gradients are omitted (36).

Simulating the intricate 3D-structure of the SR with associated micro-domains in a whole cell would probably resolve some of this discrepancy, but this will require a more elaborate model, with a heavy computational burden. The present simpler approach allows simulation of single cells and large systems of coupled cells while still retaining the characteristic ability of the system to generate waves based on CICR.

Another simplification is that CICR is based on a single calcium release channel, although two kinds of channels are known to coexist in the VSMC (55). Both show CICR and inhibition at high [Ca²⁺]ₙ. The IP₃-receptor channel (IP₃r) is sensitive to calcium at its bulk cytoplasmic concentrations, whereas the ryanodine-receptor channel (Ryr) requires micro-molar concentrations (71). In VSMC of the rat mesentery, waves are seen after α-adreno receptor stimulation leading to generation of IP₃, indicating a central role for the IP₃r. In the same cells though, blockade of Ryr inhibits waves (50). In fact, Boittin et al (8) found that in isolated portal vein myocytes both kinds of receptors are necessary for generating normal waves. As pointed out by Sanders (55), in a mixed population of release channels, it is most likely that the two channel types mutually stimulate each other. Despite being based around a single release channel, the CICR mechanism of the present simple model still reproduces the experimentally observed amplitude and frequency characteristics of calcium elevations in the bulk cytoplasm (Fig. 4-9). This has been a primary goal of the model.
In conclusion, the present simulations indicate that the transition from waves to whole-cell calcium oscillations and the associated increase in frequency observed at the onset of vasomotion may be explained on the basis of processes within the individual cell. These processes include activation of a cGMP-sensitive calcium-dependent chloride channel that couples the calcium dynamics of the SR with that of the membrane. We hypothesize that the onset of membrane potential oscillations associated with this process promotes intercellular synchronization.

Acknowledgments

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**Legend**

Fig. 1. Panel A: Cell model. The compartments considered in the model are the plasma membrane, the cytoplasm and the SR. The picture shows the components related to each of these compartments: 1) Na⁺/K⁺-ATPase, 2) Na⁺/Ca²⁺-exchanger, 3) Plasma membrane Ca²⁺-ATPase (PMCA), 4) Sarcoplasmic reticulum Ca²⁺-ATPase (SERCA), 5) SR calcium release channel, 6) cytoplasmic calcium buffer, 7) SR calcium buffer, 8) cGMP-sensitive calcium-dependent chloride channel, 9) Calcium activated potassium channels and 10) Voltage sensitive calcium channel (L-type calcium channel). Panel B: Isolated spindle shaped vascular smooth muscle cell. For isolation procedure, please see (10).

Fig. 2. Properties of the cGMP-sensitive calcium-dependent chloride channel. Panel A: Model: Open probability as a function of cytosolic calcium concentration at different cGMP-concentrations. Panel B (reproduced from *The Journal of General Physiology 123: 121-134, 2004* by copyright permission of The Rockefeller University Press): Experimental data: Current density to caffeine application in mesenteric vascular smooth muscle cells. Panel C: Model: Peak current density to caffeine application (peak calcium concentration 600 nM), holding potential –60 mV, E_Cl= -25mV.

Fig. 3. Whole cell cGMP-sensitive calcium-dependent depolarizing current in response to caffeine. Panel A (reproduced from *The Journal of General Physiology 123: 121-134, 2004* by copyright permission of The Rockefeller University Press): Experimental data: Holding potential –60 mV. Panel B: Simulated response to caffeine. Holding potential –60 mV, E_Cl= -25mV. Panel C: Assumed cytoplasmic calcium concentration following caffeine stimulation (29) and underlying the simulated trace of Panel B.

Fig. 4. The cytosolic oscillator. Panel A: Calcium oscillations in the cytoplasm. Panel B: Inverse oscillations in the SR from the same place in the cell. Note different scales on y-axis. See text for details.

Fig. 5. Panel A: The cytosolic oscillator with lateral diffusion of calcium. Left column of pictures shows a calcium wave sweeping through the cytoplasm of a spindle shaped cell. The wave starts to the left in the cell. Right column of pictures shows the inverse wave in the SR. Time scale shown to the right is linear. Color-code of the calcium concentration shown to the right is in relative units. In the cytoplasm calcium varies within 100 – 350 nM, whereas in the SR calcium oscillates with a low
amplitude around 1 mM. Panel B: Diffusion in the generation of cytoplasmic calcium waves. Initially the cell displays a stable wave-pattern (direction indicated by the arrow to the right). At \( t = 3 \) s the cytoplasmic diffusion coefficient for calcium is set to zero, and the wave pattern immediately breaks up into uncoordinated localized calcium elevations. At \( t = 65 \) s the diffusion coefficient attains its original value and the waves reemerge.

Fig. 6. Wave speed in the plasma membrane free model. Wave speed increases with the fraction of the cell occupied by SR. Each point represents 10 simulations given as mean ± S.E.M.

Fig. 7. Calcium waves and whole-cell oscillations in the stimulated cell. Panel A: The ramp increase in [cGMP] applied to the system. Panel B: The cell is in a stimulated state with \([\text{IP}_3] = 1.75 \) µM. Panel C: As [cGMP] increases, the cell enters a mode of fast whole-cell oscillations and the membrane potential starts to oscillate. Panel D: Small oscillations are seen in \( [Ca^{2+}]_{\text{SR}} \) during the wave phase. They increase in frequency and amplitude as the cell enters whole-cell oscillations. Panel E: \( [Ca^{2+}]_{\text{cyt}} \) at two different points in the cytoplasm (grey and black points marked on the insert). The graph shows the traces from these points. In the wave state, calcium oscillates out of phase as waves sweep across the cell from left to right. When the cell enters whole-cell oscillation mode the calcium oscillations synchronize at the two points and frequency increases.

Fig. 8. Transition between waves and whole-cell oscillations. Panel A: Ranges where waves and whole-cell oscillations are seen as a function of [cGMP]. At the lowest levels of [cGMP] membrane potential is adjusted to avoid calcium depletion. A short depolarizing pulse (potential clamp for 100 ms at -30 mV) is applied to reset the individual oscillators along the cell and induce whole-cell oscillations. Sustained whole-cell oscillations can be induced only as [cGMP] exceeds 4.7 µM and not as [cGMP] exceeds 6.8 µM. Waves are not seen as [cGMP] exceeds 5.3 µM. In the overlap region, the cell can exist in both states for the same set of parameters. Panel B: Forced transition between waves and whole-cell oscillations, with cytoplasmic [cGMP] = 5 µM in the overlap region of Panel A. At \( t = 50 \) s, a 100 ms potential clamp at -30 mV causes transition from waves to whole-cell oscillations. A depolarizing pulse of longer duration (10 s) causes the system to switch back (following calcium flooding of the cytoplasm). A 100 ms hyperpolarizing potential clamp at -60 mV at \( t = 150 \) s once again switches the system to the fast oscillation mode.
Fig. 9. Frequency and amplitude of whole cell oscillations. Panel A: Frequency as a function of average membrane potential (see text for details). Relative hyperpolarization causes the system to oscillate with a low frequency (comparable to that of waves). Depolarization causes a substantial acceleration in frequency. Panel B: Same simulations as in panel A. The amplitude (distance between curves) of the cytosolic calcium oscillations is insensitive to oscillation frequency.
Appendix

Constants are defined in the table referred to in each subsection below.

4.1. Diffusion (table 1). In a cytoplasmic segment of volume \( v_{cyt} = v_{segment} - \beta v_{segment} \), where \( \beta \) is SR volume fraction (62), calcium diffuses between neighboring segments through an area of:

\[ A_{trans} - \beta A_{trans} \] on each side of the segment. \( A_{trans} \) is the local cell transsectional area and thus:

\[
J_{Ca^{2+}-cyt, diffusion} = - \sum_{\text{segment boundaries}} \frac{D_{cyt}}{v_{cyt}} \frac{d[Ca^{2+}]}{dx} (A_{trans} - \beta A_{trans})
\]

(A1)

\( D_{cyt} \) is the cytoplasmic diffusion coefficient (section 4.2) and \( \frac{d[Ca^{2+}]}{dx} \) is the local concentration gradient. Eq. A1 also applies to diffusion inside the SR, with local volume \( \beta v_{segment} \) and local diffusion area \( \beta A_{trans} \).

4.2. \( Ca^{2+} \) buffering (table 4). \( Buff \) is a general immobile calcium buffer (61), with a single calcium binding-site and uniform concentration throughout the cell. Formation of buffer-calcium complex is:

\[
Buff + Ca^{2+} \rightleftharpoons \frac{k_{buff}}{k_{buf}} \cdot Buff \equiv Ca^{2+}
\]

and thus:

\[
J_{Ca^{2+}-cyt, buffering} = k_{buff} \cdot \left[ Buff \equiv Ca^{2+} \right] - k_{buf} \cdot Buff \cdot \left[ Ca^{2+} \right]
\]

(A2)

A realistic value of \( Buff \) is obtained by increasing its value until a calcium diffusion wave (with \( D_{cyt} = 233 \times 10^{-12} \ m^2 s^{-1} \) (4)) moves through the cytoplasm with the same speed as if there is no buffering and \( D_{cyt} = 13 \times 10^{-12} \ m^2 s^{-1} \) (4). Eq. A2 also applies to SR buffering (table 4).

4.3 SR-calcium uptake and release (table 5). SR-calcium is modeled as being released through a common release channel sensitive to calcium at bulk cytoplasmic concentrations and to \([IP_3]\) (see discussion). Calcium-induced calcium-release, \( CICR \), occurs at low \( [Ca^{2+}]_{cyt} \) whereas higher concentrations inhibit further release (16; 71). Calcium is taken up into the SR through the SR
calcium-ATPase (SERCA). With \( r \) denoting rate-constants, total calcium flux related to the SR is modeled as (16):

\[
J_{Ca^{2+}\text{--cyt}, \text{SR}} = k_{SR} \left( r_{CICR} P_{\text{open}} + r_{\text{leak}} \right) \left( \left[ Ca^{2+} \right]_{\text{SR}} - \left[ Ca^{2+} \right]_{\text{cyt}} \right) - k_{SR} r_{\text{SERCA}} \frac{\left[ Ca^{2+} \right]_{\text{cyt}}^p}{\left[ Ca^{2+} \right]_{\text{cyt}}^p + k_{\text{SERCA}}}.
\]

\( k_{SR} = \beta / (1.0 - \beta) \) accounts for changes in SR-membrane area with SR-volume-fraction. Release-channel open probability, \( P_{\text{open}} \), has a biphasic dependence on \( \left[ Ca^{2+} \right]_{\text{cyt}} \), increasing at low \( [Ca^{2+}] \), and decreasing at higher \( [Ca^{2+}] \) (16). In addition, \( P_{\text{open}} \) is modified by \([IP_3]\) and the filling state of the SR (17; 30). It is modeled as (17; 30):

\[
P_{\text{open}} = \left( f_{\text{act}} \times (1 - f_{\text{inact}}) \right) \times \frac{\left[ IP_3 \right]^{n_{\text{IP}}} + k_{\text{IP}}}{\left[ IP_3 \right]^{n_{\text{IP}}} + k_{\text{IP}}} \times \frac{\left[ Ca^{2+} \right]^{n_{\text{SR}}} + k_{\text{SR}}}{\left[ Ca^{2+} \right]^{n_{\text{SR}}} + k_{\text{SR}}}. \tag{A4}
\]

\( f_{\text{act}} \) and \( f_{\text{inact}} \) are the fractions of the channel population occupied by calcium at the activation and inactivation sites respectively. Since it is fast compared to inactivation, \( f_{\text{act}} \) is assumed to follow steady-state kinetics (38). Hence (with bar referring to steady state):

\[
f_{\text{act}} = \bar{f}_{\text{act}} = \left[ Ca^{2+} \right]_{\text{act}}^{\text{bar}} / \left( \left[ Ca^{2+} \right]_{\text{act}}^{\text{bar}} + k_{\text{act}}^{\text{bar}} \right). \tag{A5}
\]

The dynamic change in \( f_{\text{inact}} \) is modeled as following first-order kinetics (in analogy with Eqs. 6 and A5).

### 4.4 Calcium flux across the plasma membrane

The sum of these calcium fluxes are:

\[
J_{Ca^{2+}\text{--cyt, plasma membrane}} = -\frac{1}{2zF \psi_{\text{cyt}}} \left( 2I_{\text{CaP}} - 2I_{\text{NaCa}} + I_{\text{CaL}} + I_{\text{Ca,back}} \right) \tag{A6}
\]

\( z \) is the charge on the calcium ion (+2). Details of each current are given below.

### 4.5 Ion transporters

The present model includes three ion transporters.
4.5.1 The Na⁺/K⁺-ATPase (table 6) has stoichiometry $3Na^+:2K^+$ and hyperpolarizes the membrane. The whole-cell current, $I_{NaK}$, is modeled as previously (39; 45; 69):

$$I_{NaK} = I_{NaK,\, max} \frac{[K^+]_{in}}{[K^+]_{in} + k_{NaK,K^+}} \times \frac{[Na^+]_{cyt}^{3/2}}{[Na^+]_{cyt}^{3/2} + k_{NaK,Na^+}^{3/2}} \times \frac{V_m + 0.15}{V_m + 0.20},$$

(A7)

cGMP is modeled as having a stimulating effect on Na⁺/K⁺-ATPase activity (18; 65) and hence maximum whole-cell current, $I_{NaK,\, max}$ is modeled as:

$$I_{NaK,\, max} = k_{1-NaK-cGMP} \times [cGMP] - k_{2-NaK-cGMP}.$$  

(A8)

4.5.2 The plasma membrane calcium-ATPase (PMCA, table 7) (34) extrudes calcium from the cytosol in exchange for $H^+$ (stoichiometry probably $1Ca^{2+}:1H^+$ (46), but the ratio may depend on pH (21; 22)). Its activity depends on local $[Ca^{2+}]_{cyt}$ (20; 39; 45; 46) modulated by membrane potential as modeled by Parthimos et al. (49). Hence:

$$I_{CaP} = I_{CaP,\, max} \times \frac{[Ca^{2+}]_{cyt}}{[Ca^{2+}]_{cyt} + k_{CaP}} \times \left(1 + \frac{V_m - k_c}{k_c} \right),$$

(A9)

4.5.3 The Na⁺/Ca²⁺-exchanger (table 8) has stoichiometry $3Na^+:1Ca^{2+}$ and extrudes calcium in the forward mode of operation (7). The current, $I_{NaCa}$, was modeled according to Parthimos et al. (49) as:

$$I_{NaCa} = g_{NaCa} \times \frac{[Ca^{2+}]_{cyt}}{[Ca^{2+}]_{cyt} + k_{NaCa}} \times (V_m - E_{Na/Ca}),$$

(A10)

where $E_{Na/Ca} = 3E_{Na} - 2E_{Ca}$.

4.6 Ion channels

4.6.1 Voltage sensitive calcium channel (table 9). Current through this channel is modeled as (69):

$$I_{CaL} = g_{CaL}d_Lf_L (V_m - E_{Ca}).$$

(A11)
\( d_L \) and \( f_L \) are the dynamic activation and inactivation gates respectively and \( \overline{d_L} \) and \( \overline{f_L} \) denotes the corresponding steady-state gates (14). The latter are modeled as following a Boltzmann distribution (69) (with \( x \) symbolizing either \( d_L \) or \( f_L \) and \( a \) and \( i \) referring to activation and inactivation):

\[
\overline{x} = \left(1 + e^{(V_{th} - V(x)) / k_{d,i}}\right)^{-1}.
\] (A12)

\( d_L \) is assumed to follow steady state kinetics (69). Since the inactivation process may include a non-inactivating component (69), \( f_L \) is modelled as: \( f_L = 0.74f_f + 0.26 \) (69) where \( f_f \), being the dynamic component of the inactivation gate, is assumed to follow first-order kinetics (c.f. Eq. 6 ) with time constant (69):

\[
\tau_{CaL} = k_A e^{-((V_{th} + k_d)/k_i)^2} + k_0.
\] (A13)

4.6.2 Voltage- and calcium-sensitive potassium current (table 10) is modeled according to a lumped model for an arterial cell by Parthimos et al (49) as:

\[
I_{K_{Ca}} = g_{K_{Ca}} P_{open} (V_m - E_k)
\] (A14)

Dynamic open probability is assumed to follow first-order kinetics (c.f. Eq. 6) with steady state open probability given by:

\[
\overline{P}_{open} = \frac{[Ca^{2+}]^2_{cyt}}{[Ca^{2+}]^2_{cyt} + \delta e^{-((V_{th} - V_{th,K_{Ca}})) / R_k}}.
\] (A15)

4.7 Background currents (table 11). \( I_{back,x} \), of the ion \( x \) with background conductance, \( g_{back,x} \) and equilibrium potential \( E_x \) is:

\[
I_{back,x} = g_{back,x} (V_m - E_x).
\] (A16)
### Table 1

**Cellular parameters**

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Definition</th>
<th>Value</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>$L_{cell}$</td>
<td>Cell length</td>
<td>$90 \times 10^{-6} \text{ m}$</td>
<td>(11)</td>
</tr>
<tr>
<td>$r_{cell}$</td>
<td>Central cell radius</td>
<td>$3 \times 10^{-6} \text{ m}$</td>
<td>(11)</td>
</tr>
<tr>
<td>$k_{rad}$</td>
<td>Radius at cell ends, fraction of $r_{cell}$</td>
<td>100 (no dimension)</td>
<td>Present model</td>
</tr>
<tr>
<td>$C_{m, cell}$</td>
<td>Whole-cell capacitance</td>
<td>$17 \times 10^{-12} \text{ F}$</td>
<td>(41)</td>
</tr>
<tr>
<td>$\beta$</td>
<td>SR volume fraction</td>
<td>0.05 (no dimension)</td>
<td>(62)</td>
</tr>
</tbody>
</table>

### Table 2

**Ionic concentrations**

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Definition</th>
<th>Value</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>$[\text{Ca}^{2+}]_o$</td>
<td>Extracellular calcium concentration.</td>
<td>$2 \text{ mol} \times \text{m}^{-3}$</td>
<td>(69)</td>
</tr>
<tr>
<td>$[\text{Na}^+]_o$</td>
<td>Extracellular sodium concentration.</td>
<td>$140 \text{ mol} \times \text{m}^{-3}$</td>
<td>(69)</td>
</tr>
<tr>
<td>$[\text{K}^+]_o$</td>
<td>Extracellular potassium concentration.</td>
<td>$5 \text{ mol} \times \text{m}^{-3}$</td>
<td>(69)</td>
</tr>
<tr>
<td>$[\text{Cl}^-]_o$</td>
<td>Extracellular chloride concentration.</td>
<td>$112.5 \text{ mol} \times \text{m}^{-3}$</td>
<td>Present model (12; 41)</td>
</tr>
<tr>
<td>$[\text{Ca}^{2+}]_{\text{cyt}}$ (initial)</td>
<td>Initial cytoplasmic calcium concentration.</td>
<td>$100 \times 10^{-6} \text{ mol} \times \text{m}^{-3}$</td>
<td>(69)</td>
</tr>
<tr>
<td>$[\text{Ca}^{2+}]_{\text{SR}}$ (initial)</td>
<td>Initial SR calcium concentration.</td>
<td>$1 \text{ mol} \times \text{m}^{-3}$</td>
<td>(66)</td>
</tr>
<tr>
<td>$[\text{Na}^+]_{\text{cyt}}$</td>
<td>Cytoplasmic sodium concentration.</td>
<td>$6 \text{ mol} \times \text{m}^{-3}$</td>
<td>(45; 69)</td>
</tr>
<tr>
<td>$[\text{K}^+]_{\text{cyt}}$</td>
<td>Cytoplasmic potassium concentration.</td>
<td>$120 \text{ mol} \times \text{m}^{-3}$</td>
<td>(45; 69)</td>
</tr>
<tr>
<td>$[\text{Cl}^-]_{\text{cyt}}$</td>
<td>Cytoplasmic chloride concentration.</td>
<td>$41.8 \text{ mol} \times \text{m}^{-3}$</td>
<td>(12)</td>
</tr>
</tbody>
</table>
### Table 3

cGMP-sensitive calcium-dependent chloride channel

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Definition</th>
<th>Value</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>$g_{Cl,e} \ (\ast)$</td>
<td>Maximum whole-cell conductance of chloride channel</td>
<td>$3.8 \times 10^{-9}$ S</td>
<td>(41)</td>
</tr>
<tr>
<td>$k_{Cl,e}$</td>
<td>Constant of chloride channel calcium-sensitivity</td>
<td>$400 \times 10^{-6}$ mol m$^{-3}$</td>
<td>Present model</td>
</tr>
<tr>
<td>$\rho$</td>
<td>Determinant of how strongly cGMP influences open-probability</td>
<td>0.9</td>
<td>Present model</td>
</tr>
<tr>
<td>$n$</td>
<td>Hill-coefficient of chloride channel calcium-sensitivity</td>
<td>3.0</td>
<td>Present model</td>
</tr>
<tr>
<td>$m$</td>
<td>Hill-coefficient of chloride channel cGMP-sensitivity</td>
<td>3.3</td>
<td>Present model</td>
</tr>
<tr>
<td>$k_{Cl,cGMP}$</td>
<td>Constant of chloride channel cGMP-sensitivity</td>
<td>$6.4 \times 10^{-3}$ mol m$^{-3}$</td>
<td>(41)</td>
</tr>
<tr>
<td>$\tau_{Cl,e}$</td>
<td>Time-constant of chloride channel</td>
<td>$50 \times 10^{-3}$ s</td>
<td>Present model</td>
</tr>
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</table>

### Table 4

Calcium buffering

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Definition</th>
<th>Value</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>[Buff]</td>
<td>Initial buffer concentration</td>
<td>0.023 mol m$^{-3}$ (cytoplasm) 10 mol m$^{-3}$ (SR)</td>
<td>Present model (66)</td>
</tr>
<tr>
<td>$k_{buff}$</td>
<td>Rate-constant of formation of buffer-calcium complex</td>
<td>$10^3$ m$^3$ mol$^{-1}$ s$^{-1}$ (cytoplasm) 100 m$^3$ mol$^{-1}$ s$^{-1}$ (SR)</td>
<td>(66) (66)</td>
</tr>
<tr>
<td>$k_{--buff}$</td>
<td>Rate-constant of dissociation of buffer-calcium complex</td>
<td>$10$ s$^{-1}$ (cytoplasm) 100 s$^{-1}$ (SR)</td>
<td>(66) (66)</td>
</tr>
</tbody>
</table>
Table 5

SR calcium release and uptake

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Definition</th>
<th>Value</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>$r_{\text{CICR}}$ (*)</td>
<td>Rate-constant for agonist and calcium stimulated SR calcium release</td>
<td>7.5 $\text{s}^{-1}$</td>
<td>Present model</td>
</tr>
<tr>
<td>$r_{\text{leak}}$ (*)</td>
<td>Rate-constant for passive SR calcium leak</td>
<td>2 $\text{s}^{-1}$</td>
<td>Present model</td>
</tr>
<tr>
<td>$r_{\text{SERCA}}$ (*)</td>
<td>Rate-constant for the SERCA</td>
<td>1.88 $\text{mol m}^{-3} \text{s}^{-1}$</td>
<td>Present model</td>
</tr>
<tr>
<td>$n_{\text{IP}_3}$</td>
<td>Hill-coefficient for calcium-release channel IP$_3$-sensitivity</td>
<td>4</td>
<td>Present model, (16)</td>
</tr>
<tr>
<td>$k_{\text{IP}_3}$</td>
<td>Constant for calcium-release channel IP$_3$-sensitivity</td>
<td>$0.65 \times 10^{-3} \text{mol m}^{-3}$</td>
<td>Present model, (16)</td>
</tr>
<tr>
<td>$n_{\text{SR}}$</td>
<td>Hill-coefficient for calcium-release channel SR-calcium-sensitivity</td>
<td>2</td>
<td>(17)</td>
</tr>
<tr>
<td>$k_{\text{SR}}$</td>
<td>Constant for calcium-release channel SR-calcium-sensitivity</td>
<td>2 $\text{mol m}^{-3}$</td>
<td>Present model</td>
</tr>
<tr>
<td>$x_{\text{act}}$</td>
<td>Hill-coefficient for CICR</td>
<td>4</td>
<td>(17)</td>
</tr>
<tr>
<td>$k_{\text{act}}$</td>
<td>Constant for calcium-activation of calcium-release channel</td>
<td>$130 \times 10^{-6} \text{mol m}^{-3}$</td>
<td>Present model, (16)</td>
</tr>
<tr>
<td>$x_{\text{inact}}$</td>
<td>Hill-coefficient for calcium-inhibition of calcium-release channel</td>
<td>4</td>
<td>Present model, (16)</td>
</tr>
<tr>
<td>$k_{\text{inact}}$</td>
<td>Constant for calcium-inhibition of calcium-release channel</td>
<td>$350 \times 10^{-6} \text{mol m}^{-3}$</td>
<td>Present model, (16)</td>
</tr>
<tr>
<td>$\tau_{\text{inact}}$</td>
<td>Time-constant for calcium-release channel inactivation</td>
<td>6.0 $\text{s}$</td>
<td>(38)</td>
</tr>
<tr>
<td>$p$</td>
<td>Hill-coefficient for SERCA calcium-sensitivity</td>
<td>2.5</td>
<td>Present model, (16; 17)</td>
</tr>
<tr>
<td>$k_{\text{SERCA}}$</td>
<td>Constant for SERCA calcium-sensitivity</td>
<td>$70 \times 10^{-6} \text{mol m}^{-3}$</td>
<td>Present model, (16)</td>
</tr>
</tbody>
</table>
### Table 6

**Na⁺/K⁺-ATPase**

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Definition</th>
<th>Value</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>$k_{NaK, K^+}$</td>
<td>Exchanger dependence on extracellular potassium</td>
<td>$1 \text{ mol} \times m^{-3}$</td>
<td>(45; 69)</td>
</tr>
<tr>
<td>$k_{NaK, Na^+}$</td>
<td>Exchanger dependence on cytoplasmic sodium</td>
<td>$11 \text{ mol} \times m^{-3}$</td>
<td>(45; 69)</td>
</tr>
<tr>
<td>$k_{1-\text{NaK}--\text{cGMP}}$</td>
<td>Parameter in exchanger dependence on cytoplasmic cGMP</td>
<td>$30 \times 10^{-9} \text{ A/(mol} \times m^{-3})$</td>
<td>Present model</td>
</tr>
<tr>
<td>$k_{2-\text{NaK}--\text{cGMP}}$</td>
<td>Parameter in exchanger dependence on cytoplasmic cGMP</td>
<td>$30 \times 10^{-12} \text{ A}$</td>
<td>Present model</td>
</tr>
</tbody>
</table>

### Table 7

**Plasma-membrane Ca²⁺-ATPase**

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Definition</th>
<th>Value</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>$I_{CaP, \text{max} (\ast)}$</td>
<td>Maximum whole-cell current through PMCA</td>
<td>$0.09 \times 10^{-12} \text{ A}$</td>
<td>Present model</td>
</tr>
<tr>
<td>$k_{CaP}$</td>
<td>Determinant of PMCA calcium sensitivity</td>
<td>$200 \times 10^{-6} \text{ mol} \times m^{-3}$</td>
<td>(39)</td>
</tr>
<tr>
<td>$k_{\beta}$</td>
<td>Determinant of PMCA voltage sensitivity</td>
<td>$-0.1 \text{ V}$</td>
<td>(49)</td>
</tr>
<tr>
<td>$k_{\alpha}$</td>
<td>Determinant of PMCA voltage sensitivity</td>
<td>$0.250 \text{ V}$</td>
<td>(49)</td>
</tr>
</tbody>
</table>

### Table 8

**Na⁺/Ca²⁺-exchanger**

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Definition</th>
<th>Value</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>$g_{\text{NaCa} (\ast)}$</td>
<td>Maximum whole-cell conductance of exchanger</td>
<td>$75 \times 10^{-12} \text{ S}$</td>
<td>Present model</td>
</tr>
<tr>
<td>$k_{\text{Na/Ca}}$</td>
<td>Determinant of exchanger sensitivity to cytosolic calcium</td>
<td>$500 \times 10^{-6} \text{ mol} \times m^{-3}$</td>
<td>(49)</td>
</tr>
</tbody>
</table>
### Table 9

L-type calcium channel

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Definition</th>
<th>Value</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>$g_{Cal}$</td>
<td>Maximum whole-cell conductance of L-type calcium channel</td>
<td>$10.0 \times 10^{-9}$ $S$</td>
<td>Present model</td>
</tr>
<tr>
<td>$V_{1/2a}$</td>
<td>Potential of half-maximal activation</td>
<td>$-4.36 \times 10^{-3}$ $V$</td>
<td>(69)</td>
</tr>
<tr>
<td>$V_{1/2i}$</td>
<td>Potential of half-maximal inactivation</td>
<td>$-27.9 \times 10^{-3}$ $V$</td>
<td>(69)</td>
</tr>
<tr>
<td>$k_{slopea}$</td>
<td>Activation slope-factor</td>
<td>$6.29 \times 10^{-3}$ $V$</td>
<td>(69)</td>
</tr>
<tr>
<td>$k_{slopei}$</td>
<td>Inactivation slope-factor</td>
<td>$-6.87 \times 10^{-3}$ $V$</td>
<td>(69)</td>
</tr>
<tr>
<td>$k_{3i}$</td>
<td>Parameter in expression for voltage-dependent inactivation time</td>
<td>$0.3$ $s$</td>
<td>(69)</td>
</tr>
<tr>
<td>$k_{4i}$</td>
<td>Parameter in expression for voltage-dependent inactivation time</td>
<td>$-4.7 \times 10^{-3}$ $V$</td>
<td>(69)</td>
</tr>
<tr>
<td>$k_{5i}$</td>
<td>Parameter in expression for voltage-dependent inactivation time</td>
<td>$0.113$ $V$</td>
<td>(69)</td>
</tr>
<tr>
<td>$k_{6i}$</td>
<td>Parameter in expression for voltage-dependent inactivation time</td>
<td>$23.2 \times 10^{-3}$ $s$</td>
<td>(69)</td>
</tr>
</tbody>
</table>

### Table 10

Calcium-activated potassium current

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Definition</th>
<th>Value</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>$g_{Kca}$</td>
<td>Maximum conductance of calcium activated potassium current</td>
<td>$7 \times 10^{-9}$ $S$</td>
<td>Present model</td>
</tr>
<tr>
<td>$\delta$</td>
<td>Determinant of voltage sensitive open-probability at a given [Ca$^{2+}$]_o</td>
<td>$0.13 \times 10^{-6}$ $\left(\text{mol} / \text{m}^3\right)^2$</td>
<td>(49)</td>
</tr>
<tr>
<td>$V_{1/2,Kca}$</td>
<td>Potential of half-maximal activation</td>
<td>$-27 \times 10^{-3}$ $V$</td>
<td>(49)</td>
</tr>
<tr>
<td>$R_{K}$</td>
<td>Slope-factor for potential dependent activation</td>
<td>$12 \times 10^{-3}$ $V$</td>
<td>(49)</td>
</tr>
<tr>
<td>$\tau_{Kca}$</td>
<td>Time constant for calcium-activated potassium current</td>
<td>$0.022$ $s$</td>
<td>(49)</td>
</tr>
</tbody>
</table>
# Table 11

## Background currents

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Definition</th>
<th>Value</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>$g_{\text{back, } Ca^{2+}}$</td>
<td>Background calcium conductance</td>
<td>$1.0 \times 10^{-12} \text{ } S$</td>
<td>Present model</td>
</tr>
<tr>
<td>$g_{\text{back, } Na^+}$</td>
<td>Background sodium conductance</td>
<td>$0.01 \times 10^{-9} \text{ } S$</td>
<td>(69)</td>
</tr>
<tr>
<td>$g_{\text{back, } K^+}$</td>
<td>Background potassium conductance</td>
<td>$0.01 \times 10^{-9} \text{ } S$</td>
<td>(69)</td>
</tr>
<tr>
<td>$g_{\text{back, } Cl^-}$</td>
<td>Background chloride conductance</td>
<td>$0.8 \times 10^{-9} \text{ } S$</td>
<td>Present model</td>
</tr>
</tbody>
</table>
Reference List


9. Bouskela E and Wiederhielm CA. Microvascular myogenic reaction in the wing of the intact unanesthetized bat


56. **Schiff M.** *Arch f Physiol* 523, 1854.


Figure 1. Panel A: Cell model. The compartments considered in the model are the plasma membrane, the cytoplasm and the SR. The picture shows the components related to each of these compartments: 1) Na+/K+-ATPase, 2) Na+/Ca2+-exchanger, 3) Plasma membrane Ca2+-ATPase (PMCA), 4) Sarcoplasmic reticulum Ca2+-ATPase (SERCA), 5) SR calcium release channel, 6) cytoplasmic calcium buffer, 7) SR calcium buffer, 8) cGMP-sensitive calcium-dependent chloride channel, 9) Calcium activated potassium channels and 10) Voltage sensitive calcium channel (L-type calcium channel). Panel B: Isolated spindle shaped vascular smooth muscle cell. For isolation procedure, please see (10).
Figure 2. Properties of the cGMP-sensitive calcium-dependent chloride channel. Panel A: Model: Open probability as a function of cytosolic calcium concentration at different cGMP-concentrations. Panel B (reproduced from The Journal of General Physiology 123: 121-134, 2004 by copyright permission of The Rockefeller University Press): Experimental data: Current density to caffeine application in mesenteric vascular smooth muscle cells. Panel C: Model: Peak current density to caffeine application (peak calcium concentration 600 nM), holding potential -60 mV, ECl= -25mV.
Figure 3. Whole cell cGMP-sensitive calcium-dependent depolarizing current in response to caffeine. Panel A (reproduced from The Journal of General Physiology 123: 121-134, 2004 by copyright permission of The Rockefeller University Press): Experimental data: Holding potential -60 mV. Panel B: Simulated response to caffeine. Holding potential -60 mV, ECl= -25mV. Panel C: Assumed cytoplasmic calcium concentration following caffeine stimulation (29) and underlying the simulated trace of Panel B.
Figure 4. The cytosolic oscillator. Panel A: Calcium oscillations in the cytoplasm. Panel B: Inverse oscillations in the SR from the same place in the cell. Note different scales on y-axis. See text for details.
Figure 5. Panel A: The cytosolic oscillator with lateral diffusion of calcium. Left column of pictures shows a calcium wave sweeping through the cytoplasm of a spindle shaped cell. The wave starts to the left in the cell. Right column of pictures shows the inverse wave in the SR. Time scale shown to the right is linear. Color-code of the calcium concentration shown to the right is in relative units. In the cytoplasm calcium varies within 100 - 350 nM, whereas in the SR calcium oscillates with a low amplitude around 1 mM. Panel B: Diffusion in the generation of cytoplasmic calcium waves. Initially the cell displays a stable wave-pattern (direction indicated by the arrow to the right). At $t = 3$ s the cytoplasmic diffusion coefficient for calcium is set to zero, and the wave pattern immediately breaks up into uncoordinated localized calcium elevations. At $t = 65$ s the diffusion coefficient attains its original value and the waves reemerge.
Figure 6. Wave speed in the plasma membrane free model. Wave speed increases with the fraction of the cell occupied by SR. Each point represents 10 simulations given as mean ± S.E.M.
Figure 7. Calcium waves and whole-cell oscillations in the stimulated cell. Panel A: The ramp increase in [cGMP] applied to the system. Panel B: The cell is in a stimulated state with [IP3] = 1.75 μM. Panel C: As [cGMP] increases, the cell enters a mode of fast whole-cell oscillations and the membrane potential starts to oscillate. Panel D: Small oscillations are seen in [Ca2+]SR during the wave phase. They increase in frequency and amplitude as the cell enters whole-cell oscillations. Panel E: [Ca2+]cyt at two different points in the cytoplasm (grey and black points marked on the insert). The graph shows the traces from these points. In the wave state, calcium oscillates out of phase as waves sweep across the cell from left to right. When the cell enters whole-cell oscillation mode the calcium oscillations synchronize at the two points and frequency increases.
Figure 8. Transition between waves and whole-cell oscillations. Panel A: Ranges where waves and whole-cell oscillations are seen as a function of [cGMP]. At the lowest levels of [cGMP] membrane potential is adjusted to avoid calcium depletion. A short depolarizing pulse (potential clamp for 100 ms at -30 mV) is applied to reset the individual oscillators along the cell and induce whole-cell oscillations. Sustained whole-cell oscillations can be induced only as [cGMP] exceeds 4.7 µM and not as [cGMP] exceeds 6.8 µM. Waves are not seen as [cGMP] exceeds 5.3 µM. In the overlap region, the cell can exist in both states for the same set of parameters. Panel B: Forced transition between waves and whole-cell oscillations, with cytoplasmic [cGMP] = 5 µM in the overlap region of Panel A. At t = 50 s, a 100 ms potential clamp at -30 mV causes transition from waves to whole-cell oscillations. A depolarizing pulse of longer duration (10 s) causes the system to switch back (following calcium flooding of the cytoplasm). A 100 ms hyperpolarizing potential
clamp at -60 mV at t = 150 s once again switches the system to the fast oscillation mode.
Figure 9. Frequency and amplitude of whole cell oscillations. Panel A: Frequency as a function of average membrane potential (see text for details). Relative hyperpolarization causes the system to oscillate with a low frequency (comparable to that of waves). Depolarization causes a substantial acceleration in frequency. Panel B: Same simulations as in panel A. The amplitude (distance between curves) of the cytosolic calcium oscillations is insensitive to oscillation frequency.