



A mathematical model of Ca^{2+} dynamics in rat mesenteric smooth muscle cell: Agonist and NO stimulation

Adam Kapela^a, Anastasios Bezerianos^b, Nikolaos M. Tsoukias^{a,*}

^a Department of Biomedical Engineering, Florida International University, Miami, FL 33199, USA

^b Department of Medical Physics, University of Patras, Patras, Greece

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ABSTRACT

A mathematical model of calcium dynamics in vascular smooth muscle cell (SMC) was developed based on data mostly from rat mesenteric arterioles. The model focuses on (a) the plasma membrane electrophysiology; (b) Ca^{2+} uptake and release from the sarcoplasmic reticulum (SR); (c) cytosolic balance of Ca^{2+} , Na^+ , K^+ , and Cl^- ions; and (d) IP_3 and cGMP formation in response to norepinephrine (NE) and nitric oxide (NO) stimulation. Stimulation with NE induced membrane depolarization and an intracellular Ca^{2+} ($[\text{Ca}^{2+}]_i$) transient followed by a plateau. The plateau concentrations were mostly determined by the activation of voltage-operated Ca^{2+} channels. NE causes a greater increase in $[\text{Ca}^{2+}]_i$ than stimulation with KCl to equivalent depolarization. Model simulations suggest that the effect of $[\text{Na}^+]_i$ accumulation on the $\text{Na}^+/\text{Ca}^{2+}$ exchanger (NCX) can potentially account for this difference. Elevation of $[\text{Ca}^{2+}]_i$ within a concentration window (150–300 nM) by NE or KCl initiated $[\text{Ca}^{2+}]_i$ oscillations with a concentration-dependent period. The oscillations were generated by the nonlinear dynamics of Ca^{2+} release and refilling in the SR. NO repolarized the NE-stimulated SMC and restored low $[\text{Ca}^{2+}]_i$ mainly through its effect on Ca^{2+} -activated K^+ channels. Under certain conditions, Na^+/K^+ -ATPase inhibition can result in the elevation of $[\text{Na}^+]_i$ and the reversal of NCX, increasing resting cytosolic and SR Ca^{2+} content, as well as reactivity to NE. Blockade of the NCX's reverse mode could eliminate these effects. We conclude that the integration of the selected cellular components yields a mathematical model that reproduces, satisfactorily, some of the established features of SMC physiology. Simulations suggest a potential role of intracellular Na^+ in modulating Ca^{2+} dynamics and provide insights into the mechanisms of SMC constriction, relaxation, and the phenomenon of vasomotion. The model will provide the basis for the development of multi-cellular mathematical models that will investigate microcirculatory function in health and disease.

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1. Introduction

The intracellular concentration of free Ca^{2+} , ($[\text{Ca}^{2+}]_i$), in smooth muscle is the main determinant of vascular tone and regional blood flow. An elaborate network of signaling pathways exists that regulates $[\text{Ca}^{2+}]_i$ in smooth muscle cells (SMCs). This network includes intracellular signaling as well as cell-to-cell communication with paracrine factors or diffusion of ionic species through homo- and hetero-cellular gap junctions. This multitude of signaling pathways tightly regulates Ca^{2+} homeostasis. Experimentation has begun to untangle this elaborate network, continuously providing new insights into the physiology of blood

vessels and the mechanisms that regulate tone and blood flow. Mathematical modeling offers a systematic approach for the analysis of these signaling mechanisms and can serve as a tool for data interpretation and for guiding new experimental studies.

With respect to vascular SMCs, only a few mathematical models have been published that describe calcium regulation and cells' electrical behavior. Wong and Klassen (1993) proposed a generic model of calcium regulation in depolarized or agonist-stimulated SMC. Their model included inositol-1,4,5-triphosphate (IP_3)- and Ca^{2+} -sensitive pools, receptor and voltage-operated calcium channels, and active calcium efflux. Later, Wong and Klassen (1996) extended their original model to describe endothelin-induced electrical activity. The major modification was the inclusion of calcium- and voltage-dependent potassium channels in the plasma membrane. A reduction of membrane capacitance resulted in oscillations in membrane potential and in the cytosolic calcium concentration.

* Corresponding author at: Department of Biomedical Engineering, Florida International University, 10555 W. Flagler Street, TEC 2674, Miami, FL 33174, USA. Tel.: +1 305 348 7291; fax: +1 305 348 6954.

E-mail addresses: akapela@fiu.edu (A. Kapela), bezer@upatras.gr (A. Bezerianos), tsoukias@fiu.edu (N.M. Tsoukias).

Parthimos et al. (1999) developed an SMC model to study arterial vasomotion. Their model was based on the two-pool model of Goldbeter et al. (1990) but also included descriptions for voltage-operated Ca^{2+} channels and Ca^{2+} -activated potassium channels. Nonlinear interactions between a membrane oscillator and an intracellular calcium oscillator produced chaotic behavior, similar to the irregular arteriolar contractions seen during vasomotion. The model could simulate a number of experimental findings by modifying selected parameters, corresponding to different pharmacological interventions. Koenigsberger et al. (2004) modified the model of Parthimos et al. (1999) by eliminating the membrane oscillator and incorporating agonist- and calcium-dependent IP_3 formation. The model was then used to build a multi-cellular, 2D model of smooth muscle with electrical, calcium, and IP_3 couplings between cells to study the synchronization of SMCs during vasomotion in rat mesenteric resistance vessels.

Yang et al. (2003) proposed a detailed SMC model for rat cerebrovascular arteries, which included electrochemical and chemomechanical subsystems. The plasma membrane component of the model incorporated transmembrane currents through voltage-operated Ca^{2+} channels, potassium channels (voltage dependent, Ca^{2+} activated, and inwardly rectifying), stretch-sensitive channels, Na^+ – K^+ -ATPase, and Ca^{2+} -ATPase. Significant effort was spent to obtain current descriptions based on electrophysiological recordings from cerebral and other SMCs. The intracellular store included uptake and release compartments. Calcium buffering in the cytosol and balance of intracellular Na^+ and K^+ were also taken into account. The chemomechanical subsystem described passive and active force generation. The model was validated against voltage-clamp data under isotonic and isometric conditions. In a later study, Yang et al. (2005) extended their earlier model by incorporating the nitric oxide (NO)-soluble guanylate cyclase (sGC)-cyclic guanosine monophosphate (cGMP) signaling cascade. The model was able to capture the NO-induced Ca^{2+} concentration reduction and Ca^{2+} desensitization of myosin phosphorylation and force generation.

Jacobsen et al. (2007b) studied possible effects of activation of cGMP-sensitive calcium-dependent chloride channels in rat mesenteric SMC on intracellular calcium oscillations. Unlike the previous SMC studies, which lacked the spatial heterogeneity, their model simulated cytosolic calcium waves induced by calcium release from the stores. A rise in cGMP allowed synchronized calcium release and transition from waves to whole-cell oscillations. Jacobsen et al. (2007a) also used this model to study the synchronization of SMCs in the arterial wall.

Prior modeling efforts have provided significant insights into the physiology of SMC by focusing on the analysis of a specific physiological behavior (i.e., contraction to agonist, vasomotion), incorporating necessary system components and adjusting model parameters to fit the physiological response. However, the amount of experimental data, in particular electrophysiological measurements of membrane currents, is growing rapidly for some vascular beds. This permits to develop tissue-specific models that will incorporate a higher level of detail, integrate new physiological mechanisms, and utilize an increasing number of experimentally estimated parameters. Such an “integrated-system” approach will allow examining interactions between system components and their effects on physiological responses.

This work builds on these previous efforts and aims to develop a more detailed mathematical model of calcium dynamics in vascular SMC by integrating new descriptions for sub-cellular processes and by validating the overall behavior against new experimental data. Still, it is not possible to cover all relevant

signaling pathways of the Ca^{2+} regulation. The study focuses on two opposing signaling pathways that play significant roles in the regulation of Ca^{2+} homeostasis. The model accounts for norepinephrine (NE)-induced elevation of $[\text{Ca}^{2+}]_i$ and NO-dependent Ca^{2+} reduction and incorporates detailed descriptions for the plasma membrane electrophysiology, the Ca^{2+} uptake and release from the sarcoplasmic reticulum (SR), and the cytosolic balance of Ca^{2+} , Na^+ , K^+ , and Cl^- ions, based mostly on data from rat mesenteric resistance arteries (RMA).

NE causes vasoconstriction in most vascular beds by activating α_1 -adrenoceptor present in SMC membrane. Binding of the agonist to this G-protein-coupled receptor activates phospholipase C, resulting in the generation of IP_3 , which leads to the release of Ca^{2+} from the internal stores and to SMC constriction. Additionally, NE may induce vasoconstriction by activation of membrane channels and cell depolarization. NO, the endothelium-derived relaxing factor, is produced by the endothelial cells in response to hemodynamic or agonist stimuli. NO can diffuse across cell membranes to the adjacent smooth muscle and cause relaxation by $[\text{Ca}^{2+}]_i$ reduction. The membrane electrophysiology and balance of the ionic species and IP_3 are important for the NE and NO pathways but also for the intercellular communication through the gap junctions.

Overall, the model is intended to: (a) investigate calcium dynamics emerging from the nonlinear interactions of the incorporated multiple cellular mechanisms, (b) provide a working database of rat mesenteric SMC physiological data, where newly acquired information for SMC electrophysiology or signal transduction pathways can be incorporated, and (c) form the building block for the generation of multi-cellular models of the vascular wall that can be used to investigate intercellular communication and the mechanisms involved in the regulation of arteriolar diameter.

2. Model development

The model can be divided into three components: the plasma membrane, the cytosol, and the intracellular calcium store. A schematic diagram of the model is depicted in Fig. 1. The plasma membrane contains ion channels, pumps, exchangers, and receptors and includes all the major transmembrane currents that have been identified in SMCs of rat mesenteric arterioles. The ion channels included are potassium channels (large conductance Ca^{2+} -activated (BK_{Ca}), voltage-dependent (K_{v}), and an unspecified leak (K_{leak})); Ca^{2+} -activated chloride channels (Cl_{Ca}); non-selective cation channels (NSC); store-operated cation channels (SOC); and voltage-operated Ca^{2+} channels (VOCC). The model also contains mathematical descriptions for the Na^+ – K^+ -ATPase (NaK) and plasma membrane Ca^{2+} -ATPase (PMCA) pumps, the Na^+ – K^+ – Cl^- cotransport (NaKCl), and the Na^+ – Ca^{2+} exchanger (NCX). The intracellular calcium store, mainly representing the sarcoplasmic reticulum, contains sarcoplasmic reticulum Ca^{2+} -ATPase pumps (SERCA), IP_3 receptor Ca^{2+} channels (IP_3R), ryanodine receptor Ca^{2+} channels (RyR), a leak current (leak), and Ca^{2+} buffering by calsequestrin (CSQN). Activation of α_1 -adrenoceptor leading to the formation of IP_3 is incorporated to simulate the effect of NE. The NE-induced formation of IP_3 and diacylglycerol (DAG) affects Ca^{2+} release from the SR and the current through the NSC channel. The vasodilatory action of NO is modeled through a direct effect on BK_{Ca} channels and through the formation of cGMP. In the model, four targets of cGMP are included: the Cl_{Ca} channel, the BK_{Ca} channel, the NCX, and the cotransport. The whole model is integrated by ionic balances for calcium, potassium, sodium, and chloride ions, and includes calcium buffering in the cytoplasm.

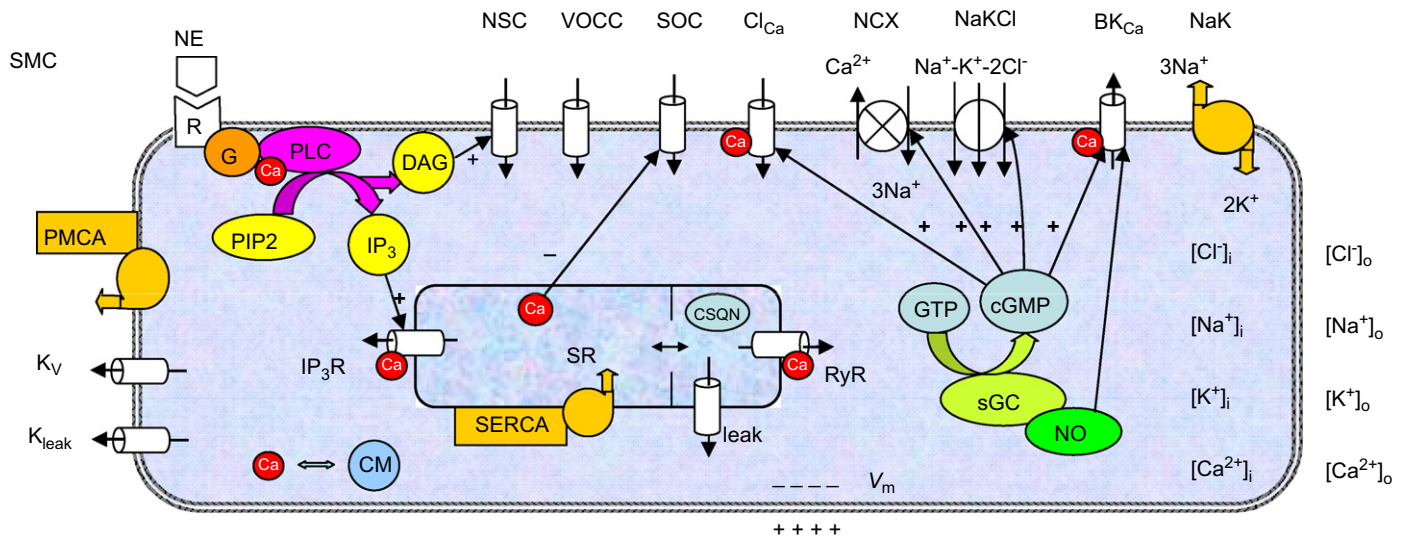


Fig. 1. Schematic diagram of model components and their interactions. BK_{Ca}—large conductance Ca²⁺-activated K⁺ channels, K_{leak}—unspecified K⁺ leak channels, K_v—voltage-dependent K⁺ channels, Cl_{Ca}—Ca²⁺-activated Cl[−] channels, NSC—non-selective cation channels, SOC—store-operated Ca²⁺-permeable non-selective cation channels, VOCC—L-type voltage-operated Ca²⁺ channels, NaK—Na⁺-K⁺-ATPase, PMCA—plasma membrane Ca²⁺-ATPase, NaKCl—Na⁺-K⁺-Cl[−] cotransport, NCX—Na⁺-Ca²⁺ exchange, SR—sarcoplasmic reticulum, IP₃R—IP₃ receptor, RyR—ryanodine receptor, SERCA—SR Ca²⁺-ATPase pumps, CSQN—calsequestrin, CM—calmodulin, R—α₁-adrenoceptor, G—G protein, PLC—phospholipase C, sGC—soluble guanylate cyclase.

2.1. Membrane electrophysiology

The membrane electrophysiology is described with the lumped Hodgkin-Huxley-type formalism, and changes in membrane potential (V_m) are calculated from:

$$\frac{dV_m}{dt} = \frac{-(I_{VOCC} + I_{Kv} + I_{BKCa} + I_{Kleak} + I_{NSC} + I_{SOC} + I_{ClCa} + I_{PMCA} + I_{NaK} + I_{NCX}) + I_{stim}}{C_m} \quad (1)$$

where C_m is the membrane capacitance, I_i ($i = VOCC, K_v, BKCa, K_{leak}, NSC, SOC, ClCa, PMCA, NaK, NCX$) is the transmembrane current through the i component, and I_{stim} is an external stimulation current. Ionic fluxes through the Na⁺-K⁺-Cl[−] cotransport system do not exert any influence on the V_m (i.e., the process is electroneutral) and therefore are not included in Eq. (1). The Na⁺-K⁺-Cl[−] cotransport system is taken into account, however, when determining the balance of ions in the cytosol.

2.1.1. Voltage-operated calcium current (I_{VOCC})

Whole-cell electrophysiological recordings in freshly isolated myocytes from rat small mesenteric arteries indicate the presence of only the L-type, voltage-dependent, calcium current (Cox and Lozinskaya, 1995; Harhun et al., 2000; Lozinskaya and Cox, 1997). This current plays a major role in both resting muscle tone and maintained contractions (Nelson et al., 1990), thus both its steady-state and dynamic characteristics are important. I_{VOCC} is modeled as a Goldman-Hodgkin-Katz (GHK) current with a time- and voltage-dependent open-state probability (Eqs. (A.1)–(A.7)). Parameters for these equations were obtained from Cox et al. (2002), and Lozinskaya and Cox (1997) and from fitting their voltage-clamp data. (See Supplement for detailed explanation of parameter estimation.) Fig. 2a shows the steady-state voltage dependence of I_{VOCC} . Although the magnitude of this steady-state current is rather small, it can significantly affect calcium concentration (Nelson et al., 1990). Equations predict a voltage window for the L-type Ca²⁺ current similar to the experimental data in rat mesenteric myocytes using barium (Harhun et al., 2000). This similarity suggests that the inactivation at positive membrane potentials is independent of intracellular calcium (Ba²⁺ substitutes poorly for Ca²⁺-dependent inactivation). There-

fore, we did not incorporate Ca²⁺-dependent inactivation of L-type channels.

2.1.2. Potassium current through large conductance Ca²⁺-activated K⁺ channels (I_{BKCa})

In rat mesenteric arteriolar SMCs, small and intermediate Ca²⁺-activated K⁺ channels are not present, and the main repolarizing outward current is through large conductance Ca²⁺-activated K⁺ channels (Cox et al., 2001). This channel plays a key role in controlling electrical and mechanical activities of SMCs (Tanaka et al., 2004). The current-voltage (I - V) relationship of the BK_{Ca} channel in physiological K⁺ gradients is well fitted with the GHK equation (Mistry and Garland, 1998) (Eq. (A.8)–(A.16)). Activation time constants and open-state probability are based on patch data from guinea pigs' small mesenteric artery (Benham et al., 1986). The effect of $[Ca^{2+}]_i$ on this current is modeled through an empirical relationship (Eq. (A.13)). The channel density was selected to give a whole-cell current in agreement with data from RMA (Smirnov et al., 1998). Nitric oxide can activate the BK_{Ca} channels in SMCs from rat mesenteric arterioles directly and through the cGMP-dependent pathway without modifying channel conductance (Carrier et al., 1997; Mistry and Garland, 1998; Sampson et al., 2001). We modeled the effects of NO and cGMP by leftward shifts of the V_m -dependent activation curve (Eqs. (A.13)–(A.15)) (Fig. 2b). The maximum NO- and cGMP-induced changes in the half-activation potential agree with data from RMA SMCs (Mistry and Garland, 1998) and mesangial cells (Stockand and Sansom, 1996), respectively. The effects of non-saturating concentrations of NO and cGMP are modeled assuming Michaelis-Menten and sigmoidal behavior (Yang et al., 2005).

2.1.3. Voltage-dependent K⁺ channel current (I_{Kv})

K_v channels are important in regulation of vascular tone because of their effects on the resting membrane potential. Three components of the I_{Kv} current (rapidly- and slowly-inactivating transient components and a non-inactivating component) have been reported in cultured rat mesenteric arterial myocytes (Yuan et al., 1993), but only one component was recorded in smooth muscle cells that were freshly isolated from rat mesenteric arterioles (Lu et al., 2001; Smirnov et al., 1998). Since the

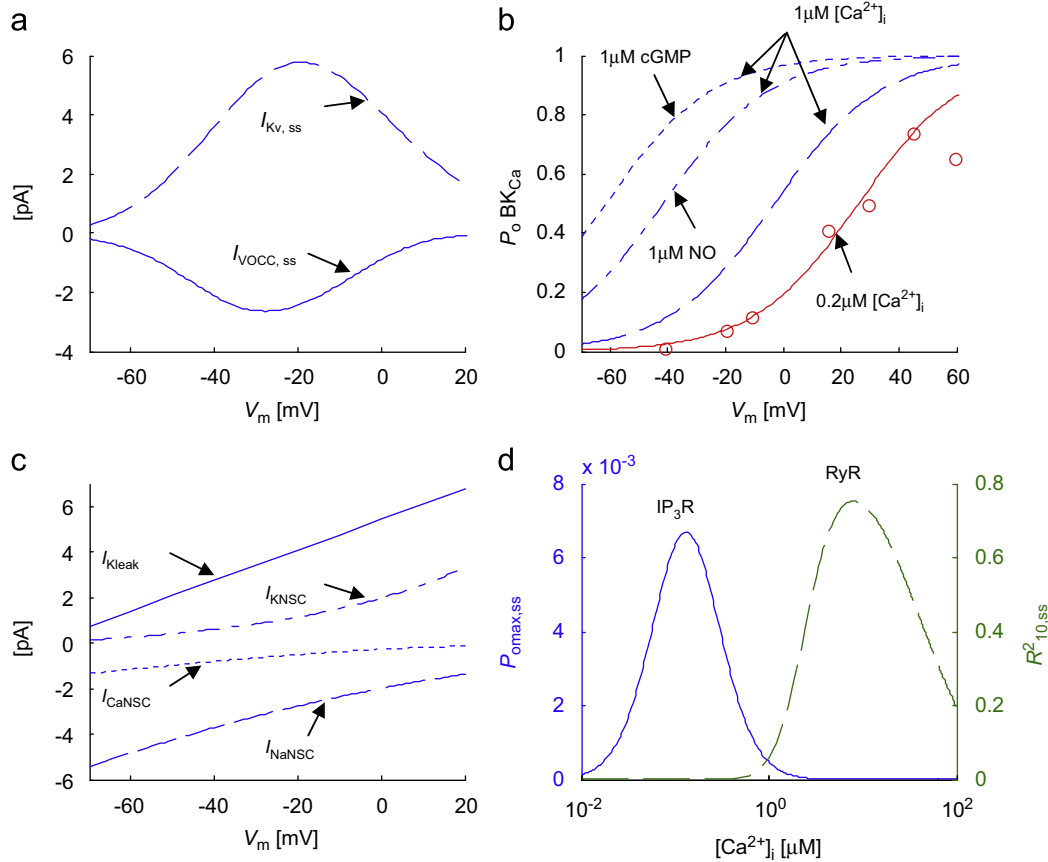


Fig. 2. (a) V_m dependence of steady-state whole-cell currents through VOCC (solid line) and K_v (dashed line) channels. (b) Steady-state open probability of BK_{Ca} channels as a function of V_m for different cytosolic calcium concentrations and in the presence of nearly saturating NO or cGMP. Experimental points from Benham et al. (1986) presented as (o). (c) Whole-cell currents through NSC and K_{leak} channels under basal conditions. (d) Steady-state Ca^{2+} -dependent activation of IP_3R and RyR . $P_{omax,ss} = ([Ca^{2+}]_i / ([Ca^{2+}]_i + K_{act,IP_3}) K_{inh,IP_3} / ([Ca^{2+}]_i + K_{inh,IP_3}))^3 \cdot (R_{10,ss})^2$ vs. $[Ca^{2+}]_i$ was determined by solving corresponding differential equations (Eqs. (A.49)–(A.52)) until steady state was reached for different $[Ca^{2+}]_i$.

electrophysiological properties of arterial SMCs may change significantly in culture (Yuan et al., 1993), the model accounts for only one type of K_v channels (Eq. (A.17)). The steady-state activation and inactivation curves can be described by Boltzmann equations (Lu et al., 2001) (Eqs. (A.18) and (A.21)). Whole-cell conductance and the dependence of the activation time constant on voltage were determined from fitting voltage-clamp data of Lu et al. (2001). The fast- and slow-inactivation time constants are from Lu et al. (2001). The resulting formulation yields significant steady-state current over a voltage window (Fig. 2a) with a maximum value at $V_m = -20$ mV. At $V_m = -40$ mV the current is 3.6 pA, which is close to the measured value of 2.5 pA in myocytes from rabbit cerebral arteries (Nelson and Quayle, 1995).

2.1.4. Unspecified K^+ leak current (I_{Kleak})

K^+ channels inactivated by intracellular ATP have been identified in rat mesenteric arteries (Standen et al., 1989). K_{ATP} channels play a role in regulation of resting membrane potential. Glibenclamide, a K_{ATP} channel blocker, depolarizes SMCs by 5–10 mV in rat small mesenteric arteries (McPherson and Angus, 1991; Nelson and Quayle, 1995). We incorporated this current into an unspecified K^+ leak current (Eq. (A.24)) with linear conductance, g_{Kleak} , that gives a whole-cell current within the experimentally reported values from SMCs in intact rabbit mesenteric arteries (Nelson and Quayle, 1995). Although some studies (Garland and McPherson, 1992; Weidelt et al., 1997) suggest that K_{ATP} channels have a role in NO-mediated responses, other studies did not confirm such an involvement (Carrier et al., 1997; Mistry

and Garland, 1998; Sampson et al., 2001). Thus, NO effects on K_{ATP} channels are considered negligible in the present formulation. Fig. 2c (solid line) depicts whole-cell I_{Kleak} current as a function of V_m .

2.1.5. Current through non-selective cation channels (I_{NSC})

Non-selective cation channels are a key element of agonist-induced sustained calcium elevation in SMCs of rat mesenteric small arteries. According to (Nilsson, 1998; Nilsson et al., 1998), NE activates cation channels that depolarize membrane potential and open VOCC. Inoue et al. (2001) reported that a murine transient receptor potential homologue (mTRP6) expressed in human embryonic kidney cells (HEK293) has biophysical and pharmacological properties very similar to α_1 -adrenoceptor-activated, non-selective cation channel (α_1 -AR-NSCC) identified in rabbit portal vein smooth muscle and proposed this protein as a major component of the vascular α_1 -AR-NSCC. There is evidence that mTRP6 channel is activated by DAG, and HEK293 cells expressing TRP6 exhibit only a small spontaneous current that can be significantly increased by DAG. A similar TRP6 channel was described in A7r5 smooth muscle cells (Jung et al., 2002), and constitutively active non-selective cation channels were recorded in rabbit ear and portal vein myocytes (Albert and Large, 2001; Albert et al., 2003). It has been proposed that these channels contribute to resting membrane potential and basal Ca^{2+} influx. Recently the expression of TRPC1 and TRPC6 and the existence of a TRPC-like non-selective cation current activated by α_1 -adrenoceptor stimulation and DAG analog, OAG, were also demonstrated

in rat mesenteric resistance artery smooth muscle (Hill et al., 2006).

Experimental data of Bartlett et al. (2005) show that IP_3 and DAG accumulations in cells after stimulation of α_{1B} -adrenoceptors closely parallel each other (i.e., suggesting similar release and elimination rates). Based on the above observation, we assume proportional DAG and IP_3 concentrations (Eq. (A.62)). This enables us to express NSC channel activity as a function of the IP_3 concentration. The dependence of this current on DAG (or IP_3) is assumed to follow Michaelis–Menten kinetics (Eqs. (A.26) and (A.27)). Since it was reported that the NSC channels in SMC from RMA contribute only indirectly to agonist-induced calcium influx by membrane depolarization (Nilsson, 1998; Nilsson et al., 1998), we assumed that DAG activates sodium and potassium conductances, while there is only a small background, DAG-independent calcium current (Eqs. (A.25)–(A.28)). Whole-cell Ca^{2+} (dotted line), K^+ (dashed-dotted line) and Na^+ (dashed line) current components through the NSC channels in the absence of DAG are depicted in Fig. 2c as a function of V_m .

2.1.6. Current through store-operated non-selective cation channels (I_{SOC})

Store-operated, Ca^{2+} -permeable, non-selective cation channels are present in vascular smooth muscle cells, including rat mesenteric artery myocytes, but their significance remains unclear (Albert and Large, 2003; Arnon et al., 2000a). In isolated endothelium-denuded rat mesenteric arterioles, depletion of intracellular Ca^{2+} stores with thapsigargin did not affect significantly resting membrane potential and cytosolic calcium (Nilsson et al., 1998). On the other hand, emptying Ca^{2+} stores in cultured rat mesenteric artery myocytes with cyclopiazonic acid and caffeine in Ca^{2+} -free solution caused significant $[Ca^{2+}]_i$ elevation after extracellular Ca^{2+} was restored (Arnon et al., 2000a). This may indicate that the stores must be depleted below physiological $[Ca^{2+}]_i$ for significant activation of I_{SOC} (i.e., removal of extracellular calcium reduces $[Ca^{2+}]_i$ and allows additional store depletion). In SMCs and in physiological extracellular concentrations, the maximum whole-cell I_{SOC} current is between -10 and -5 pA at potentials from -60 to -40 mV, and the I – V relationship is linear (Albert and Large, 2003). In rat mesenteric myocytes, SOC exhibited little inactivation and the Ca^{2+} to Na^+ permeability ratio was $P_{Ca}/P_{Na} = 8.2$ (Arnon et al., 2000a). Based on these data, conductances for the Ca^{2+} (g_{SOCca}) and Na^+ (g_{SOCNa}) components of I_{SOC} were estimated. The resulting current formulation gives a maximum I_{SOC} current of -7.6 pA at -50 mV (Eqs. (A.30)–(A.32)). The open probability of SOC (P_{SOC}) channels is described by a sigmoidal function of calcium concentration in the intracellular store (Parekh and Putney, 2005) with a half-activation constant of 100 nM (Eq. (A.33)).

2.1.7. Ca^{2+} -activated chloride current (I_{ClCa})

cGMP-independent and cGMP-dependent Cl_{Ca} currents have been identified in SMCs from rat mesenteric resistance arteries (Matchkov et al., 2004; Piper and Large, 2004). The cGMP-independent current requires large calcium concentrations, while the cGMP-dependent component can be activated by $[Ca^{2+}]_i < 100$ nM in the presence of saturating cGMP. Hill equations describe I_{ClCa} activation by cGMP and Ca^{2+} (Eq. (A.34)). Parameter values for the cGMP and Ca^{2+} concentrations for half saturation (i.e. EC_{50}) and the Hill coefficients are based on Matchkov et al. (2004), Large and Wang (1996), and Jacobsen et al. (2007b). The maximum conductance g_{ClCa} was determined based on current density data from Matchkov et al. (2004). The cGMP-independent component of I_{ClCa} has been determined from a current density of 0.12 pA/pF in the absence of cGMP (Matchkov et al., 2004).

2.1.8. Plasma membrane Ca^{2+} -ATPase current (I_{PMCA})

PMCA pump of vascular SMCs, including SMCs in rat mesenteric arteries, provides a high-affinity, low-capacity Ca^{2+} extrusion mechanism and maintains a low cytosolic calcium concentration (O'Donnell and Owen, 1994). In the model, it is the major pathway for calcium efflux and a key determinant of intracellular calcium concentration at rest and during stimulation. Michaelis–Menten kinetics describes the dependence of I_{PMCA} on $[Ca^{2+}]_i$ (Eq. (A.35)). A high Ca^{2+} affinity ($K_{m,PMCA} = 170$ nM) is assumed (i.e., due to the presence of saturating calmodulin concentrations) (O'Donnell and Owen, 1994).

2.1.9. Plasma membrane Na^+ - Ca^{2+} exchange current (I_{NCX})

Na^+ - Ca^{2+} exchange activity has been described in isolated cell membranes of rat mesenteric arteries (Matlib et al., 1985). In vascular smooth muscle cells, the activity of $3Na^+:1Ca^{2+}$ exchange is relatively low at rest and most Ca^{2+} extrusion occurs through the PMCA. However, at $[Ca^{2+}]_i > 1$ μ M, the exchanger becomes more significant than the pump (O'Donnell and Owen, 1994). Studies indicate a similarity between the NCX in vascular smooth muscle cells and in cardiac myocytes (O'Donnell and Owen, 1994), although in the latter cell types, the magnitude of the NCX current is significantly larger. We utilized a formulation for I_{NCX} from a rabbit atrial cell model of Lindblad et al. (1996) and reduced the scaling factor g_{NCX} . The effect of cGMP on the exchanger (Eq. (A.36)) is based on data from Furukawa et al. (1991), which indicate that cGMP stimulates NCX activity in cultured rat aortic SMCs.

2.1.10. Sodium pump current (I_{NaK})

The Na^+ - K^+ -ATPase plays an important role in maintaining transmembrane Na^+ and K^+ gradients, as well as it contributes directly to the membrane potential because of its electrogenicity (O'Donnell and Owen, 1994). The pump current depends on the concentration of intracellular sodium, extracellular potassium, and the membrane potential. I_{NaK} has been measured utilizing the perforated patch-clamp technique in SMCs from guinea-pig mesenteric resistance arteries, providing Hill equations that describe the dependence of I_{NaK} on $[K^+]_o$ and $[Na^+]_i$ (Nakamura et al., 1999) (Eq. (A.39)). The voltage dependence of this current was taken from Yang et al. (2003).

2.1.11. Sodium–potassium–chloride cotransport (I_{NaKCl})

Cotransport of sodium, potassium, and chloride ions is present in nearly all types of animal cells. In vascular smooth muscle cells, its physiological role is not fully understood, but the main function is to maintain high intracellular Cl^- concentration above electrochemical equilibrium (Russell, 2000). The cotransporter is electroneutral due to its apparent stoichiometry of 1:1:2, and the coupled fluxes depend only on concentration gradients of the three ions. The expression describing I_{NaKCl} was taken from Strieter et al. (1990) (Eq. (A.40)). Na^+ - K^+ - Cl^- cotransport is regulated by cGMP, which can increase its activity up to 3.5-fold in vascular SMC of rat thoracic aorta (O'Donnell and Owen, 1986, 1994).

2.2. Sarcoplasmic reticulum

The sarcoplasmic reticulum contains ryanodine and IP_3 receptors and occupies over 5% of the volume of the cytoplasm in aortic and pulmonary artery muscles (Miller and Carsten, 1997). Contractile studies in RMA suggest that caffeine and phenylephrine (PE) mobilize a common intracellular calcium pool (Rubio et al., 2002), or at least overlapping calcium pools (Lagaud et al., 1999). There is also evidence that some regions of SR may express

more IP₃ than ryanodine receptors (Laporte et al., 2004). Blaustein et al. (2002), and Golovina and Blaustein (1997) directly visualized SR stores in primary-cultured rat mesenteric artery myocytes with the low-affinity Ca²⁺ indicator, Fura-2 (K_d = 75.6 μM). The SR appeared to be a continuous tubular network but organized into small, separate, and functionally independent compartments. Inhibition of SERCA with cyclopiazonic acid unloaded different, spatially distinct compartments than did caffeine or ryanodine. Serotonin, acting via IP₃, released Ca²⁺ only from the cyclopiazonic acid-sensitive compartments.

In this study, a modified two-compartment model of the SR from the cerebral SMC of Yang et al. (2003) was utilized. It is comprised of an uptake compartment (Eq. (A.46)) with a SERCA pump (Eq. (A.43)) and a release compartment (Eq. (A.47)) with a four-state kinetic model of the ryanodine receptor (Eqs. (A.49)–(A.52)). The effect of heterogeneous distribution of RyR and IP₃R can be investigated by incorporating IP₃R into the uptake compartment. The kinetic description for the IP₃ receptor utilized was originally formulated by Fink et al. (1999) for A7r5 SMC and modified by Bennett et al. (2005) (Eqs. (A.53) and (A.54)). IP₃R and RyR have bell-shaped, steady-state Ca²⁺ dependence with maxima at 130 nM and 8 μM, respectively (Fig. 2d). This ensures large sensitivity of the IP₃R to IP₃ stimulation at resting [Ca²⁺]_i, and significant Ca²⁺-induced Ca²⁺-release (CICR) via RyR only at elevated [Ca²⁺]_i. The Michaelis constant of the SERCA pump, K_{m,up}, was reduced from 80 μM in Yang et al. (2003) to a more physiological value of 1 μM (Miller and Carsten, 1997) with a parallel decrease in the maximum uptake current, $\bar{I}_{\text{SERCA}}$. A calcium leak from the internal stores that is mediated by neither ryanodine nor IP₃ receptors has been described in various cell types, including smooth muscle cells (Camello et al., 2002; Laporte et al., 2004). An important function of the leak is to limit cellular damage by preventing SR calcium overload (Camello et al., 2002). In the model, a calcium leak similar at rest to resting Ca²⁺ release through the ryanodine receptor was also included to prevent excessive Ca²⁺ concentrations in the SR (Eq. (A.45)). This leak corresponds to a decrease rate of 750 μM/min of total calcium in the SR (including buffered calcium), which is close to the 400 μM/min reported for pancreatic acinar cells (Camello et al., 2002). Volpe et al. (1994) reported that aortic smooth muscle cells express calsequestrin, the low-affinity high-capacity Ca²⁺-binding protein found in skeletal- and cardiac-muscle SR. It was also shown that CSQN is not uniformly distributed in SMC SR (Laporte et al., 2004; Meldolesi and Pozzan, 1998; Volpe et al., 1994). In the model, rapid Ca²⁺ buffering was incorporated in the release compartment, similarly to previous cardiac SR models (Jafri et al., 1998).

2.3. α_1 -Adrenoceptor activation and IP₃ formation

A detailed mathematical model of IP₃ formation following α_1 -adrenoceptor activation in vascular smooth muscle cells is given in Bennett et al. (2005). Under the assumption of fast ligand binding, the kinetic scheme for receptor activation can be described by two coupled differential equations (Eqs. (A.55) and (A.56)). The G-protein is activated by unphosphorylated ligand-bound receptor, LR, and to a smaller degree by unbound receptor, R, accounting for background activity (Eqs. (A.57) and (A.58)). The activated G-protein activates phospholipase C, which hydrolyzes membrane-bound PIP₂ to IP₃ and DAG (Eqs. (A.59)–(A.62)). IP₃ then activates IP₃ receptors in the SR, and DAG opens non-selective cation channels in the plasma membrane.

2.4. sGC activation and cGMP formation

The sGC activation by NO has been described previously as a two-step kinetic mechanism by Zhao et al. (1999) and modified by

Ballou et al. (2002) and Condorelli and George (2001). The pseudo steady-state approximation employed by Condorelli and George (2001) provides a description of the relative cGMP formation rate ($V_{\text{cGMP}}/V_{\text{cGMP,max}}$) at steady state as a function of NO concentration (Eq. (A.63)). The exact value of NO concentration for the half-maximum cGMP formation rate, K_{m,cGMP}, has not been established, and estimates range from few nM to few hundred nM. The assumed kinetic mechanism in this study yields K_{m,cGMP} = 66 nM. The sGC activation half-life is NO-dependent and on the order of a few seconds, while inactivation half-lives from 10 to 100 min in vitro and up to 2 min in vivo have been reported. We have previously suggested (Tsoukias et al., 2004) that the slow deactivation of sGC may improve the efficacy of transient NO release in controlling vascular tone and allow under certain conditions frequency-dependent rather than amplitude-dependent regulation of cGMP production. A mono-exponential relationship describes the transient nature of sGC activation and inactivation (Eq. (A.66)) (Tsoukias et al., 2004). The balance equation for cGMP (Eq. (A.67)) is from Yang et al. (2005).

2.5. Ionic balances

The model includes balances for the four major ionic species in the cytosol (i.e., calcium (Eqs. (A.71) and (A.72)), sodium (Eqs. (A.73) and (A.74)), potassium (Eqs. (A.75) and (A.76)), and chloride (Eqs. (A.77) and (A.78)). It also includes the calcium balance in the uptake and release compartments of the SR (Eqs. (A.46) and (A.47)). Calcium balances account for buffering (i.e., by calmodulin and other buffers in the cytosol and by calsequestrin in the store) using the fast buffering approximation (Jafri et al., 1998).

2.6. Parameter values

The majority of parameters, including those having a decisive effect on model performance (see sensitivity analysis results), were assigned values based on primary biophysical data (such as patch-clamp and whole-cell studies) as described above. Models formulated from such primary data have potential for independent predictions, and this approach was favored in the current study. However, a number of parameter values have not been determined in RMA and had to be estimated or modified based on secondary data to ensure correct model behavior. The scaling factor of Na⁺–K⁺–Cl[–] cotransport (L_{NaKCl}) and the maximum rates of Na⁺–K⁺–ATPase ($\bar{I}_{\text{NaK}}$) and of PMCA ($\bar{I}_{\text{PMCA}}$) were assigned values that balance the transmembrane fluxes and produce stable physiological resting membrane potential and intracellular ionic concentrations. The unknown DAG concentration for half-activation of NSC channels (K_{NSC}) was chosen to obtain NE concentration of 3 μM for near maximal response as reported in isolated SMC from RMA (Baro and Eisner, 1995). The range of NCX activity (g_{NCX}) used in this study ensures that at rest most of calcium extrusion is due to PMCA and that the exchanger becomes significant only during the calcium transient or reduced Na⁺ gradient. The presence and absence of SMC desensitization to NE, both reported in the literature, were taken into account by modifying the phosphorylation rate of the adrenoceptor (k_{pG}). The ability to generate oscillations was introduced into the model by modifying the leak parameter (R_{leak}) and the maximum rate of SERCA ($\bar{I}_{\text{SERCA}}$). The maximum cGMP production rate (V_{cGMP,max}) and the Michaelis–Menten constant (K_{m,pde}) in the equation for the cGMP balance (Eq. (A.67)) were adjusted based on the sensitivity of isolated RMA to NO (Diederich et al., 1990). Table A1 summarizes parameter values used in the model.

2.7. Sensitivity analysis

For a systematic sensitivity analysis of the overall model, the method of Latin hypercube sampling (LHS) (McKay et al., 1979) was applied as described by Bui et al. (1998) in a model of bronchial circulation. LHS was preferred as it requires fewer model runs for adequate analysis when large numbers of parameters are involved. Twenty-five parameters were selected that span all system components and include parameters with different degrees of uncertainty as well as parameters that may be accurately estimated but can vary in different experimental setups (i.e., extracellular concentrations). To reduce the subjectivity in the selection of uncertainty ranges, we assumed a range of $\pm 20\%$ for parameter values estimated from RMA data, $\pm 40\%$ for values taken from other models or not from RMA, and $\pm 80\%$ if no data were available. The 25 parameters were assigned 50 different values equally spaced within the corresponding uncertainty range. Fifty simulation runs were performed after selecting parameter values randomly and without replacement. For the sensitivity analysis, the model was stimulated with NE and NO. The phosphorylation rate of the adrenoceptor was set to zero ($k_{PG} = 0$). Resting V_m , NE-induced depolarization, resting $[Ca^{2+}]_i$, NE-induced peak and plateau $[Ca^{2+}]_i$, and NO-dependent $[Ca^{2+}]_i$ were selected as model outputs. The selected outputs characterize all major aspects of the model's function: resting conditions, membrane electrophysiology, transient and maintained calcium responses to agonist, and NO. The sensitivity index β_{ij} of output i to parameter j was determined from linear least-squares multiple regression applied to the following relationship:

$$Y_i^k = \beta_{i,0} + \beta_{i,1}X_1^k + \beta_{i,2}X_2^k + \dots + \beta_{i,25}X_{25}^k \quad (2)$$

where Y_i^k is the value of the i th output at k th run, and X_j^k is a change in % of the central value of the j th parameter. Confidence intervals for the sensitivity indexes were calculated from an 'elliptically shaped' joint $100(1-\alpha)\%$ confidence region for all the sensitivity indexes to determine whether they are statistically different from zero using the F -statistic (Draper and Smith, 1981) (Eq. (A.79)).

2.8. Numerical methods

The model was implemented in Fortran 90, and the 26 differential equations were solved with the Gear's backward differentiation formulas (BDF) method for stiff systems using a routine from the IMSL Numerical Library. The maximum time step was 4 ms, and tolerance for error control was 0.0005.

3. Results

3.1. Resting conditions

The resting V_m is determined mostly by the balance between depolarizing currents through NSC channels and hyperpolarizing currents through K_v and K_{leak} channels, and by NaK and PMCA pumps (see also sensitivity analysis results). It is also affected indirectly by extracellular Na^+ and K^+ concentrations through their effects on the Nernst potentials. In the model, resting V_m was fine-tuned to around -60 mV by adjusting the conductance of K_{leak} channels within physiological range. Total resting membrane resistance estimated with ± 1 pA current stimulations (I_{stim}) was 3 G Ω , falling within the 2 – 3 G Ω range reported for SMC of rat mesenteric arteries (Yuan et al., 1993). Membrane resistance was not considered in the previous SMC models, yet it is an important quantity that may determine behavior of electrically coupled,

multi-cellular models. It provides a rough validation that the magnitudes of the model's resting currents are correct. The resting $[Ca^{2+}]_i$ is 68 nM and depends mainly on the balance between I_{CaNSC} , I_{VOCC} , and I_{PMCA} currents. Steady-state calcium concentrations, including the resting state, are essentially independent of calcium release from the stores, since the release is balanced by Ca^{2+} sequestration through the SERCA pump and I_{SOC} is small.

3.2. Agonist stimulation

Fig. 3 shows selected model responses to $1 \mu M$ NE (solid lines). In some figures model responses to $0.3 \mu M$ NE are also shown (dashed lines). Fig. 3a presents the evolution of intracellular IP_3 concentration. Following the initial release of IP_3 upon NE binding, calcium release from the internal stores accelerates IP_3 formation. Thus, NE-induced, Ca^{2+} -dependent IP_3 formation exhibits a pronounced initial spike. The amplitude and time delay of the peak depend on NE concentration (compare traces for 1 and $0.3 \mu M$ NE concentrations). The IP_3 peak is followed by a slowly decaying plateau. The decay is caused by α_1 -adrenoceptor desensitization and is faster at larger NE concentrations but can be compensated by increasing cytosolic calcium through the Ca^{2+} dependence of IP_3 formation (plateau slopes -4.7 and 0.17 nM/s for 1 and $0.3 \mu M$ of NE, respectively). At the same time, activation of non-selective cation channels by DAG results in large inward sodium current (Fig. 3b) that depolarizes plasma membrane from resting -59 mV to around -40 mV (Fig. 3c). The depolarization increases calcium influx through voltage-operated L-type Ca^{2+} channels (Fig. 3d). Increased Ca^{2+} influx through VOCC channels of the plasma membrane and the IP_3R channels of the SR raises $[Ca^{2+}]_i$ and opens ryanodine receptors (Fig. 3e), which leads to further elevation of $[Ca^{2+}]_i$ (Fig. 3f). The resulting positive feedback loop through the Ca^{2+} -induced Ca^{2+} -release mechanism generates a fast large-amplitude calcium spike (Fig. 3f) terminated by the depletion of the release compartment (Fig. 3g). Decreasing agonist concentration decreases the amplitude of the calcium transients (from 900 to 400 nM) and increases time to peak (from 4.5 to 12.6 s), for 1 and $0.3 \mu M$ NE, respectively (Fig. 3f). The agonist-induced membrane depolarization is limited by opening of voltage-dependent K_v and calcium-activated BK_{Ca} potassium channels (Fig. 3i). They provide large repolarizing currents to counteract the inward Na^+ current through the NSC channels. These large Na^+ and K^+ fluxes are only partially compensated in the model by an increase in the rate of NaK (Fig. 3h) and cause changes in $[Na^+]_i$ and $[K^+]_i$.

3.3. Cytosolic calcium dependence on membrane potential

Fig. 4 depicts model predictions (solid lines) of $[Ca^{2+}]_i$ as a function of V_m next to experimental data (dotted lines) from Nilsson et al. (1994). Membrane potential and $[Ca^{2+}]_i$ were measured simultaneously in rat mesenteric arterioles during cumulative additions of NE (solid circles) and $[K^+]_o$ (solid squares) in the range 0.6 – $10 \mu M$ and 6 – 125 mM, respectively. The experimental protocol was simulated in silico with step changes of NE and $[K^+]_o$ every 400 s. During KCl stimulation, $[Na^+]_o$ was reduced on an equimolar basis. Extracellular concentrations were modified to match the experimental values in Nilsson et al. (1994): $[Ca^{2+}]_o = 2.5$ mM, $[Na^+]_o = 144$ mM, $[Cl^-]_o = 124$ mM, $[K^+]_o = 4.7$ mM. Desensitization of α_1 -AR was not observed in this study, and thus we set the phosphorylation rate k_{PG} to zero. The scaling factor of Na^+ – Ca^{2+} exchanger (g_{NCX} in Eq. (A.36)) was also set to the low value from Table A1 to prevent excessive Ca^{2+} influx caused by intracellular Na^+ accumulation during prolonged NE stimulation. Simulation results were in good

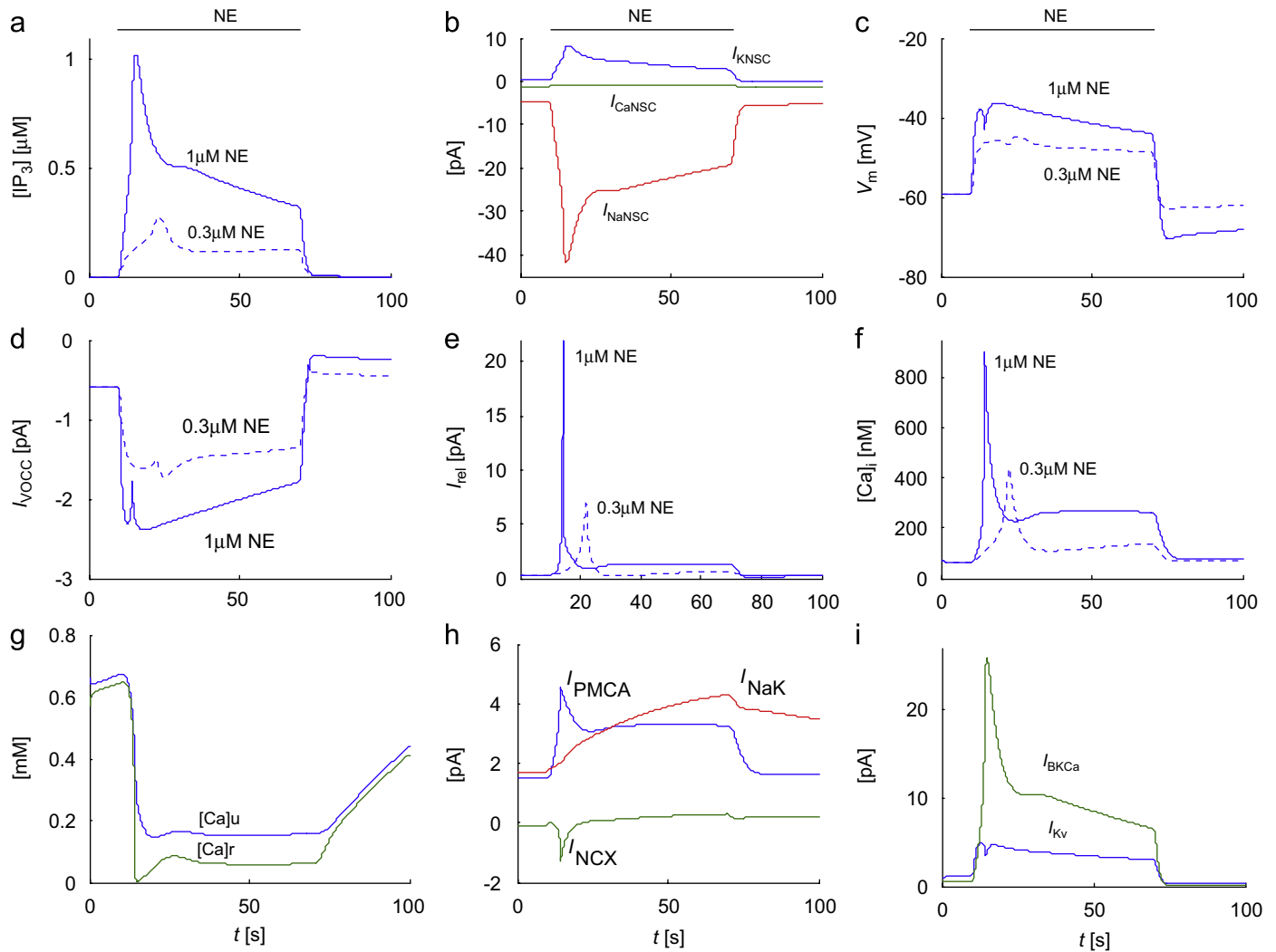


Fig. 3. Model responses to 0.3 and 1 μM NE (dotted and solid lines, respectively). (a) NE-induced Ca^{2+} -dependent IP_3 transients. The adrenoceptor desensitization causes slow IP_3 decay. Currents through NSC channels (b) activated by DAG depolarize plasma membrane (c). Calcium current through VOCC (d) and calcium release from the intracellular stores through the RyR (e) produce large-amplitude cytosolic calcium spike followed by a plateau (f). The spike is terminated by depletion of the stores (g). (h) Currents through PMCA, NaK and NCX. (i) Opening of K_v and BK_{Ca} channels limiting the agonist-induced membrane depolarization.

agreement with the data within the physiological range of V_m . Experimental data and simulation results suggest a larger $[\text{Ca}^{2+}]_i$ at the same V_m during stimulation with NE. In the model, this extra Ca^{2+} enters through the NCX working in the reverse mode following the Na^+ influx through receptor-operated NSC channels.

3.4. Calcium oscillations

In simulations using control parameter values (Table A1), no oscillations were observed. However, it is well established that stimulated vascular SMCs often generate calcium oscillations that are likely to play an important physiological role. Oscillations may originate from the internal stores, and the release and uptake currents in SR are thought to play a key role. Therefore, we investigated oscillatory behavior after modifying parameters affecting uptake and release currents from the SR. Fig. 5a shows the bifurcation diagram with non-oscillatory (I) and oscillatory (II) domains for constant NE = 300 nM and R_{leak} and $\bar{I}_{\text{SERCA}}$ as the bifurcation parameters. Oscillations appear when $\bar{I}_{\text{SERCA}}$ is increased more than 1.4-fold relative to the assumed control value. The 2-fold increase of $\bar{I}_{\text{SERCA}}$ generates oscillations with period $T = 25$ s and amplitude of 460 nM. Increasing $\bar{I}_{\text{SERCA}}$ results in an

increase in SR Ca^{2+} content, in oscillation period, and in oscillation amplitude. A parallel increase in the leak current prevents the store overload and reduces period and amplitude. Oscillation periods for specific combinations of the bifurcation parameters are given in Fig. 5a (circles), with the filled circle corresponding to $\bar{I}_{\text{SERCA}}$ and R_{leak} values used in Figs. 5b–d and 8.

Fig. 5b shows a bifurcation diagram for the NE stimulation scenario with $\bar{I}_{\text{SERCA}}$ and R_{leak} increased 3- and 5-fold, respectively. The diagram reveals the presence of Hopf bifurcation and presents the Ca^{2+} and NE concentration window (158–292 and 222–392 nM, respectively) for oscillations. The window for oscillations depends on $\bar{I}_{\text{SERCA}}$ and R_{leak} . Fig. 5b shows also that the oscillation period depends on agonist concentration and varies from 30 to 15 s for the assumed $\bar{I}_{\text{SERCA}}$ and R_{leak} .

Figs. 5c and d depict model simulations during stimulation with increasing $[\text{K}^+]_o$ with $\bar{I}_{\text{SERCA}}$ and R_{leak} increased 3- and 5-fold, respectively, as in Fig. 5b. At high $[\text{K}^+]_o$, oscillations appear both in V_m and $[\text{Ca}^{2+}]_i$. The theoretical $[\text{K}^+]_o$ threshold is 25 mM and corresponds to an average $[\text{Ca}^{2+}]_i$ of 168 nM. For a given $[\text{K}^+]_o$, the average $[\text{Ca}^{2+}]_i$ during oscillations (Fig. 5c; dashed line) is equal to $[\text{Ca}^{2+}]_i$ after inhibition of the intracellular oscillator. The oscillatory periods vary from 27 to 22 s for $[\text{K}^+]_o$ from 25 to 40 mM, respectively. Small V_m oscillations are secondary to the Ca^{2+}

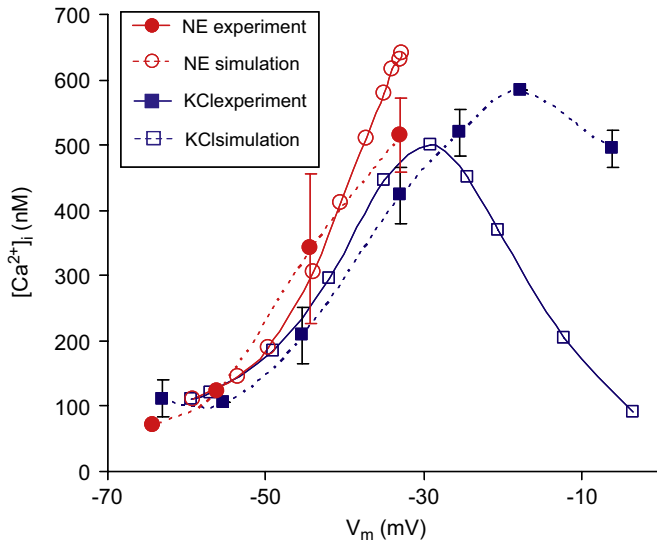


Fig. 4. V_m dependence of $[Ca^{2+}]_i$. Cumulative NE and KCl stimulation was simulated by step-changes of NE (0, 0.1, 0.2, 0.5, 1, 2, 4, 6, 8 and 10 μ M) and $[K^+]_o$ (4.7, 5.9, 12, 20, 30, 40, 50, 60, 85 and 125 mM) every 400 s. 'Steady-state' $[Ca^{2+}]_i$ was measured at the end of every 400 s interval. During KCl stimulation, $[Na^+]_o$ was reduced on equimolar basis. Resting extracellular concentrations, in mM: $[Ca^{2+}]_o = 2.5$, $[Na^+]_o = 144$, $[Cl^-]_o = 124$, $[K^+]_o = 4.7$.

oscillations, and they are produced through the action of BK_{Ca} and Cl_{Ca} channels.

3.5. NO-induced relaxation

Fig. 6 depicts model-generated responses to NO. Stimulation of the cell with 3 μ M NE causes sustained membrane depolarization to about -40 mV and $[Ca^{2+}]_i$ elevation to around 590 nM (Fig. 6a and b; dashed lines). Application of saturating 1 μ M NO to the prestimulated SMC induces instantaneous repolarization to -50 mV due to direct activation of BK_{Ca} channels by NO, followed by a further repolarization to -65 mV after activation of sGC and cGMP production (Fig. 6a; solid line). NO reduced $[Ca^{2+}]_i$ by 83%. After inhibition of sGC, 1 μ M NO repolarized the cell to -50 mV and reduced calcium by 60% (Fig. 6a and b; dashed-dotted lines). Application of 1 μ M NO hyperpolarizes the unstimulated SMC from -60 to -77 mV (Fig. 6a; dotted line) but did not elicit any significant change in $[Ca^{2+}]_i$ (Fig. 6b; dotted line). NO-induced calcium reduction could be mimicked by a voltage clamp to equivalent repolarization (data not shown).

Fig. 7a shows steady-state NO- V_{cGMP} (Eqs. (A.63) and (A.66)) and NO-cGMP relationships (Eq. (A.67)). The NO concentrations for half-maximal cGMP production rate $K_{m,VcGMP}$ and cGMP concentration $K_{m,cGMP}$ were 92 and 66 nM, respectively, which is within ranges reported in other models (Tsoukias et al., 2004; Yang et al., 2005). In mesenteric arteries physiological cGMP concentrations were estimated to be 2–4 μ M (Matchkov et al., 2004). Figs. 7b and c depict model predictions for the effect of NO concentration on V_m and $[Ca^{2+}]_i$ reduction (i.e., $\Delta[Ca^{2+}]_i$), respectively. NO effects are examined following precontraction with 3 μ M NE similarly to Fig. 6. NO concentrations for half-maximum repolarization were 80 nM (i.e. $IC_{50} = 7.1$) for control and 244 nM ($IC_{50} = 6.6$) after sGC block, while the NO concentrations for half-maximum $\Delta[Ca^{2+}]_i$ were 32 nM ($IC_{50} = 7.9$) and 124 nM ($IC_{50} = 6.9$), respectively. The inhibition of sGC reduced maximal repolarization by 13 mV and maximal $\Delta[Ca^{2+}]_i$ by 20%. Fig. 7d summarizes data for the dependence of $[Ca^{2+}]_i$ on V_m . In the model, the reduction of Ca^{2+} is determined mainly by the membrane repolarization, regardless of the stimulus utilized to

alter V_m , e.g., NO or voltage clamp. $[Ca^{2+}]_i$ loses sensitivity to V_m as the cell becomes more hyperpolarized.

3.6. Effects of $Na^+ - K^+$ -ATPase inhibition

Fig. 8 shows the effect of 50% reduction of $\bar{I}_{NaK}$ and 100% inhibition of NCX's reverse mode (i.e., when electrochemical gradients favor Ca^{2+} entry) on model response to 1 μ M NE. In the simulations, g_{NCX} value of 0.487 pS was utilized, while the $\bar{I}_{SERCA}$ and R_{leak} were increased 3- and 5-fold, respectively, relative to the control values. In Fig. 8a, changes in the intracellular Na^+ concentration are presented. Reduction of $\bar{I}_{NaK}$ caused elevation of intracellular Na^+ that was further increased with NE stimulation. Blockade of NCX had minimal effect on Na^+ accumulation. Fig. 8b depicts the I_{NCX} current. Positive currents are generated when the exchanger works in the reverse (i.e., Ca^{2+} entry) mode. Under control conditions (solid line), the exchanger extrudes Ca^{2+} at rest (i.e., negative current). Following NE stimulation, there is a transient activation of this current followed by reversal of the exchanger. For the parameter values assumed, oscillations appear in the plateau phase of NE stimulation. The NaK inhibition reverses NCX at rest (dashed line). Transient Ca^{2+} extrusion also appears during NE stimulation that is followed by a more pronounced reversal relative to control. After blockade of NCX, the channel is inactive at rest, and only a reduced transient appears following NE application (dash-dot line). The NaK inhibition (Fig. 8c; dashed line) results in an increase of resting $[Ca^{2+}]_i$ relative to control from 68.4 nM to 93 nM (Fig. 8c inset). This calcium increase was entirely due to NCX working now in the reverse mode. The increase was not due to activation of the L-type channels, since the membrane depolarization was insignificant, agreeing with literature data (Weston et al., 2002). The 17.2 nM $[Ca^{2+}]_i$ elevation increased calcium sequestration into the sarcoplasmic reticulum by 15.3 μ mol/(l cytoplasm). This corresponds to 890-fold amplification, compared to the 2500 fold amplification predicted in Blaustein (1993). Transient Ca^{2+} elevation and average plateau levels are also significantly enhanced. Blockade of the NCX reverse mode (dash-dot line) nearly eliminated NaK inhibition-induced resting cytosolic Ca^{2+} elevation and decreased the transient response to NE. The average plateau Ca^{2+} was reduced back to the control levels.

3.7. Sensitivity analysis

Fig. 9 depicts 50 different model responses using different combinations of parameter values within the ranges reported in Table S1. For the assumed uncertainty ranges of parameter values, membrane potential shows significant variability although it remains within physiological ranges (Fig. 9a). V_m levels after NE and NO stimulation are correlated with the initial resting membrane potential. Fig. 9b presents Ca^{2+} responses. $[Ca^{2+}]_i$ changes several fold under some parameter value scenarios. In some cases, model predictions fall outside physiological ranges. The initial Ca^{2+} peak may either be absent or may increase several folds while the model fails to stabilize at a new Ca^{2+} plateau following NE stimulation in some cases. Sensitivity analysis results using LHS are summarized in Table S1 in Supplement.

At rest, V_m has statistically significant (at significance level $\alpha = 0.05$) positive sensitivity coefficients for P_{NaNSC} and P_{CaNSC} (i.e., for the parameters that determine depolarizing currents via NSC channels). Negative coefficients are for g_{Kleak} , g_K , $\bar{I}_{NaK}$, and $\bar{I}_{PMCA}$, and each of them affects hyperpolarizing currents. $[Na^+]_o$ and $[K^+]_o$ have statistically positive coefficients, a result of their effects on Na^+ and K^+ Nernst potentials. The NE-induced membrane depolarization peak is sensitive to seven parameters. Statistically

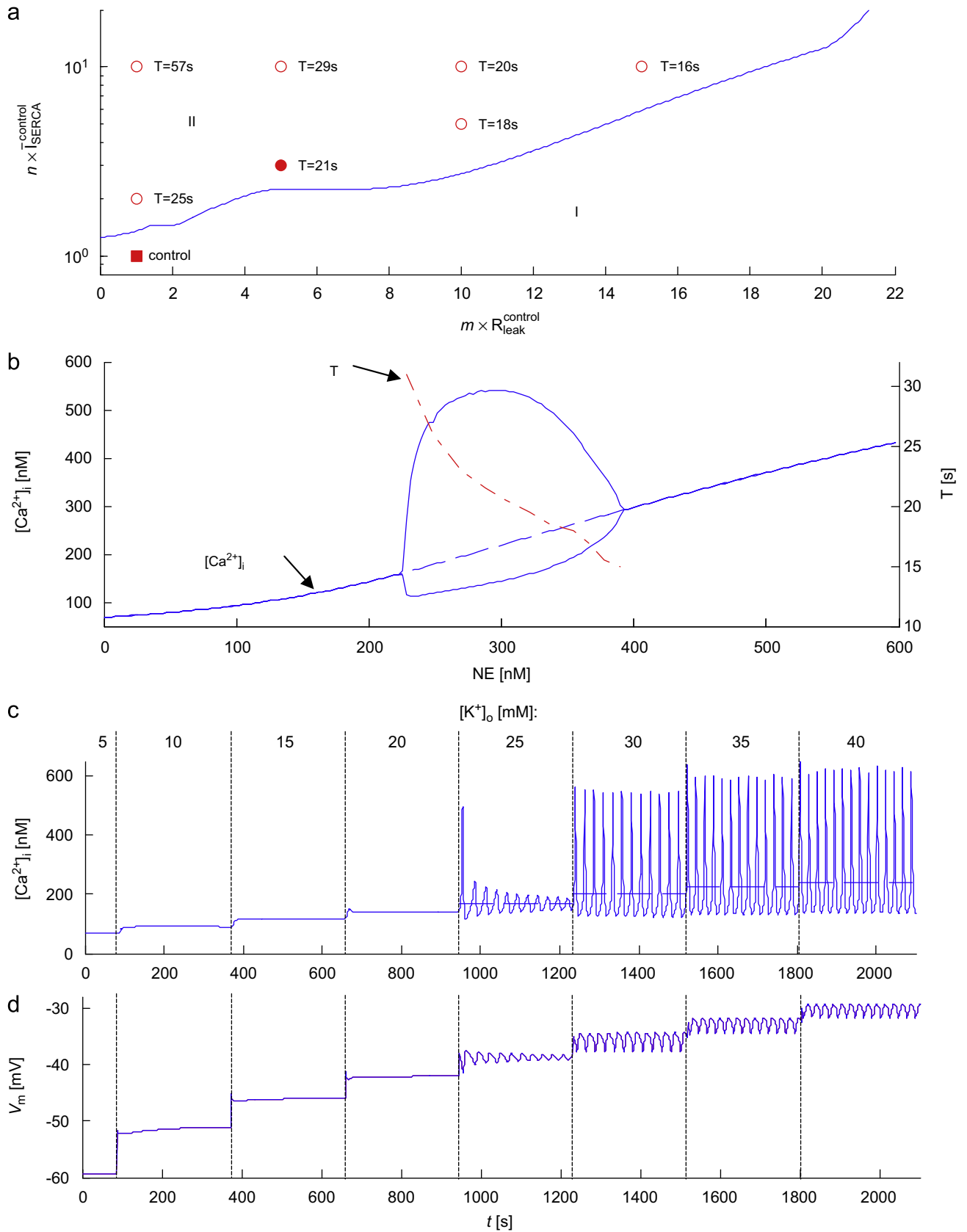


Fig. 5. (a) Two-parameter bifurcation diagram with non-oscillatory (I) and oscillatory (II) domains for constant NE = 300 nM. The oscillation periods (T) correspond to combinations of the bifurcation parameters I_{SERCA} and R_{leak} denoted by circles. (b) Bifurcation diagram and oscillation period for the NE stimulation scenario with I_{SERCA} and R_{leak} increased 3- and 5-fold, respectively. (c) KCl-induced calcium oscillations, and (d) membrane potential depolarization.

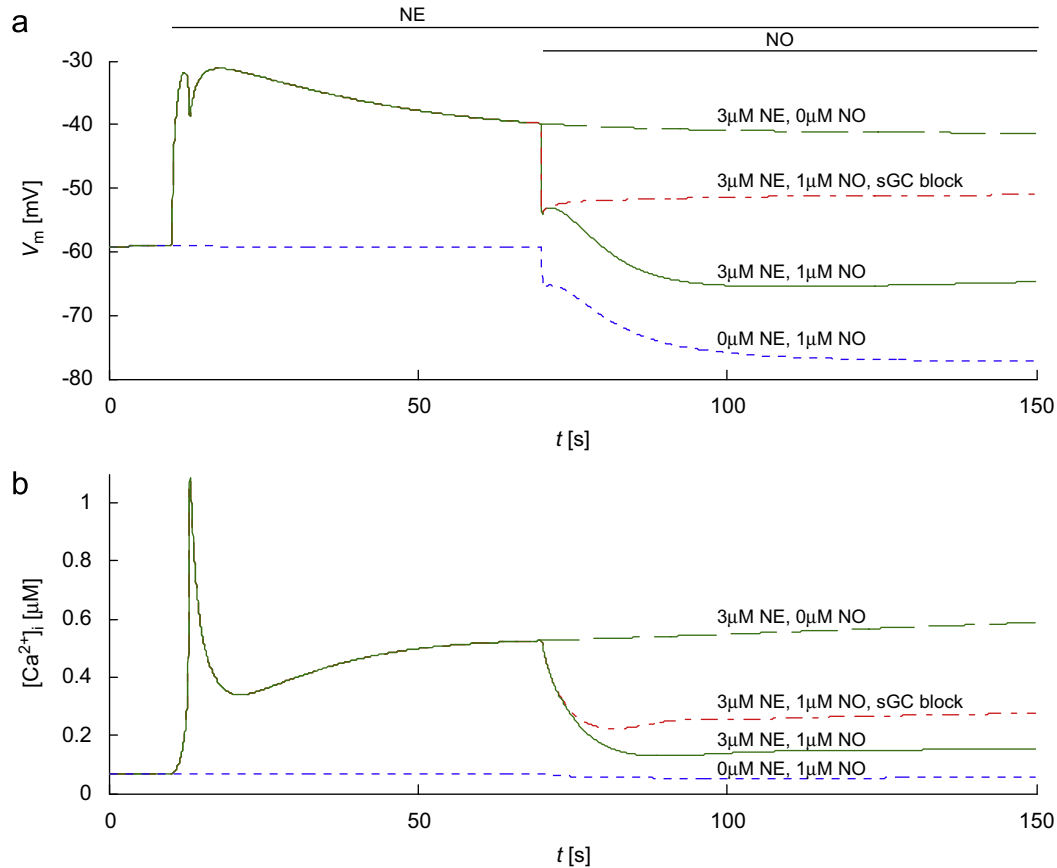


Fig. 6. Effect of 1 μM NO on V_m (a) and $[\text{Ca}^{2+}]_i$ (b) at rest (dotted lines) and following NE stimulation (solid lines). Dashed lines represent control responses in the absence of NO stimulation. The instantaneous component of the membrane response to NO is due to its direct effect on BK_{Ca} channels. The delayed response is due to activation of sGC and accumulation of cGMP. Dash-dot lines represent effect of NO after complete sGC inhibition.

positive sensitivity coefficients appear for $[\text{Na}^+]_o$, $[\text{K}^+]_o$, and P_{NaNSC} , while K_{NSC} , P_{KNSC} , g_{K} , and N_{BKCa} have negative coefficients. Thus, sensitivity analysis suggests that BK_{Ca} channels are not as important at rest due to low $[\text{Ca}^{2+}]_i$, but they become significant during stimulation. On the contrary, I_{CaNSC} , I_{Kleak} , I_{NaK} , and I_{PMCA} are less important during depolarization compared to large amplitude I_{NaNSC} , I_{KNSC} , I_{Kw} , and I_{BKCa} (Fig. 3). Resting $[\text{Ca}^{2+}]_i$ is sensitive to the L-type channel conductance P_{VOCC} and the maximum rate of PMCA $\bar{I}_{\text{PMCA}}$, but it is not sensitive to P_{CaNSC} despite the fact that I_{VOCC} and I_{CaNSC} currents have similar amplitudes (Figs. 2a and c). This is caused by the compensatory effect of membrane depolarization on increased Ca^{2+} permeability of NSC channels (Fig. 2c; dotted line). The peak Ca^{2+} was most sensitive to P_{VOCC} (i.e., Ca^{2+} influx through VOCC initiates and regulates the magnitude of CICR) and to $\bar{I}_{\text{PMCA}}$. Peak Ca^{2+} also exhibits high sensitivity to the concentrations of buffers that attenuate $[\text{Ca}^{2+}]_i$ transients and increase Ca^{2+} availability in the stores. Increasing the conductivity for Ca^{2+} efflux from the SR (i.e., lowering τ_{rel}) has a negative effect on the Ca^{2+} peak because of SR depletion.

The NE-induced Ca^{2+} plateau was most sensitive to the maximum PMCA rate, but it also had a statistically significant positive sensitivity index for the scaling factor of NCX (g_{NCX}). This is because Na^+ accumulation during NE stimulation reverses the exchanger's mode, leading to Ca^{2+} influx and a positive correlation between the plateau Ca^{2+} and the conductance of NCX. This effect of the exchanger depends on the value assumed for the maximum rate of NaK, $\bar{I}_{\text{NaK}}$. The NO-induced $[\text{Ca}^{2+}]_i$ reduction was most sensitive to $\bar{I}_{\text{NaK}}$. NaK maintains a steep transmembrane K^+ gradient that is necessary for NO-induced hyperpolarization/

repolarization. NaK also prevents a decrease in Na^+ gradient that can lead to a NO-insensitive Ca^{2+} influx through the NCX. Overall, $\bar{I}_{\text{NaK}}$ and $\bar{I}_{\text{PMCA}}$ are the parameters that have a significant impact on model predictions, and their values remain largely unidentified. Their sensitivity indexes were statistically significant for three- and four-model outputs, respectively, indicating their central role in determining model's behavior.

3.8. Dynamic vs. time-independent RyR

Changes in model's responses after incorporation of the equivalent time-independent description for RyR were examined. At steady state the kinetic four-state mechanism (Eqs. (A.49)–(A.52)) reduces to four linear algebraic equations that can be solved for R_{10} :

$$R_{10} = \frac{[\text{Ca}]_i^2}{[\text{Ca}]_i^2 + (K_{\text{act,RyR}})^2} \frac{1}{1 + [\text{Ca}]_i/K_{\text{inh,RyR}}} \quad (3)$$

where $K_{\text{act,RyR}} = 1.74 \mu\text{M}$ and $K_{\text{inh,RyR}} = 80 \mu\text{M}$ are the activation and inactivation parameters.

For the stimulation protocol from Fig. 3, no significant changes were observed. The calcium peak increased by 2% and was reached 0.5 s faster, consistent with approximately 0.5 s activation/inactivation time constant of RyR. However, the oscillations from Fig. 5 had an increased amplitude by more than 13% and a reduced period by 7%. This indicates that the delayed activation/inactivation of RyR is not essential for the model's ability to generate oscillations but can significantly modulate them, especially if the oscillations have higher frequency.

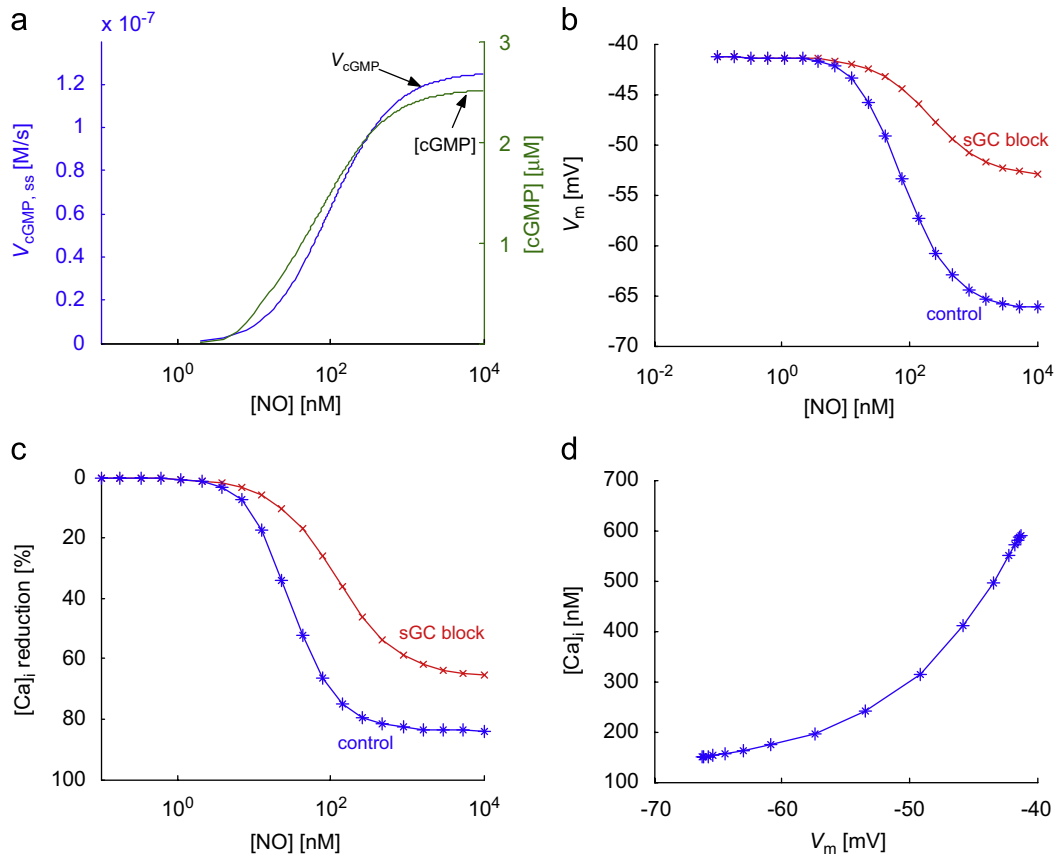


Fig. 7. (a) Steady-state cGMP formation rate $V_{cGMP,ss}$ and cGMP concentration as a function of NO. V_m repolarization (b), and $[Ca^{2+}]_i$ reduction (c) at different NO concentrations in 3μ M NE prestimulated cell under control conditions (*) and with complete blockade of sGC ($\times$). (d) Relationship between V_m and $[Ca^{2+}]_i$ in the presence of 3μ M NE.

4. Discussion

The mesenteric microcirculation is a region of highest resistance to blood flow. In addition, due to its accessibility, it is one of the most thoroughly studied vascular beds, and a vast amount of experimental data is available. This enabled us to identify values for the majority of the model's parameters based on independent experimental studies. A limited number of parameter values, however, were not available, and these had to be selected from other tissues/species or by fitting. It is relatively easy to reproduce almost any particular experimental trace by adjusting model parameters, but the acceptable parameter-value space is significantly restricted when one attempts to fit several independent experimental data. The estimation of parameter values is complicated by significantly different experimental information for some model components and outputs that cannot be attributed to biological variability. Overall model's behavior is determined mainly by model structure and parameters derived from primary experimental data, while a few parameters (K_{NSC} , $\bar{I}_{PMCA}$, g_{NCX} , $\bar{I}_{NaK}$, L_{NaKCl} , $\bar{I}_{SERCA}$, R_{leak} , $V_{cGMP,max}$, and k_{pG}) were adjusted based on whole-cell/vessel behavior or were assigned a wide range of values.

4.1. Sarcoplasmic reticulum

The spatial organization and dynamics of the intracellular Ca^{2+} store (i.e., the arrangement of IP_3R and RyR , the mechanisms of store Ca^{2+} release and replenishment, the buffer concentration distribution) have not been completely elucidated (Golovina and

Blaustein, 1997; Lagaud et al., 1999; Laporte et al., 2004; Meldolesi and Pozzan, 1998; Rubio et al., 2002; Volpe et al., 1994). Different mathematical descriptions for the SR have been previously presented that utilize a number of different assumptions. The two-pool model of Goldbeter et al. (1990) assumes that the IP_3 -sensitive store is rapidly replenished by external Ca^{2+} , while a separate Ca^{2+} -sensitive pool is replenished by cytosolic Ca^{2+} through active Ca^{2+} transport. Wong and Klassen (1993, 1996) assumed that the Ca^{2+} -sensitive store is replenished by external Ca^{2+} and has a fluid connection with the IP_3 -sensitive store. In the SMC model of Yang et al. (2003) and in models of cardiac myocytes (Jafri et al., 1998; Lindblad et al., 1996; Luo and Rudy, 1994), the store consists of an uptake compartment with SERCA pump and a release compartment with $RyRs$. The store is refilled by cytosolic Ca^{2+} through SERCA.

Since the more recent data suggest that the SR is not replenished directly by external Ca^{2+} (Albert and Large, 2003), and there is evidence for a common, spatially heterogeneous intracellular Ca^{2+} store (Laporte et al., 2004; Meldolesi and Pozzan, 1998; Rubio et al., 2002; Volpe et al., 1994), we based our formulation on the two-compartment model of (Yang et al., 2003). Increasing the Ca^{2+} permeability between the uptake and release compartments (Eq. (A.44)) will decrease spatial heterogeneity in SR, and, for high permeability values, an effective single homogeneous store is created. Although the proposed arrangement of the SR stems from previous modeling efforts, it is not unique and requires more experimental evidence (e.g., morphological evidence or evidence from staining of the RyR , IP_3R , and SERCA). It provides, however, a first attempt to account for the effect of store heterogeneity on Ca^{2+} dynamics.

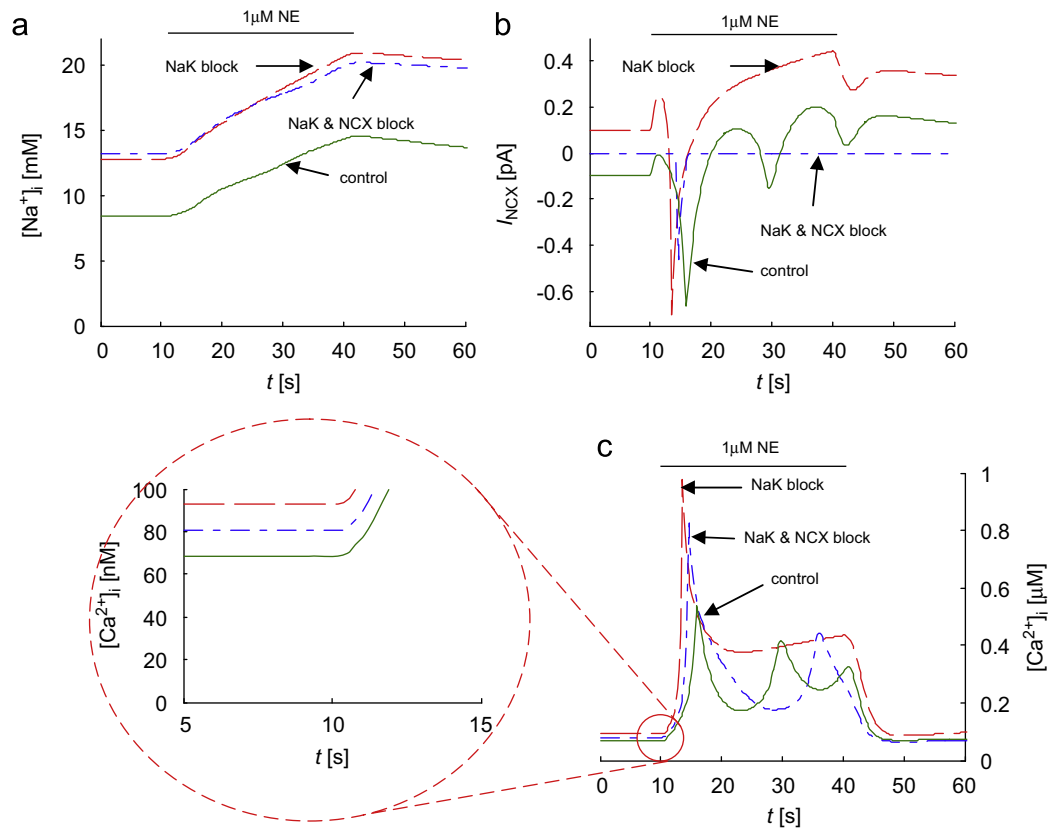


Fig. 8. Effects of 50% reduction of the maximum rate of NaK and total block of NCX reverse mode on $[Ca^{2+}]_i$ at rest and during stimulations with $1 \mu M$ NE. (a) Cytosolic sodium concentration, (b) current through NCX, and (c) cytosolic calcium concentration. Responses presented under control conditions (solid line) and in the presence of the NaK inhibition with (dash-dot line) and without (dashed line) the block of NCX reverse mode. Positive currents in (b) correspond to the reverse mode, i.e., Ca^{2+} influx through NCX. NaK inhibition significantly enhanced amplitude of Ca^{2+} release and plateau levels. Block of NCX reverse mode nearly eliminated effects of NaK inhibition on $[Ca^{2+}]_i$.

4.2. Agonist stimulation

NE induces a Ca^{2+} transient with an initial, large-amplitude peak followed by a plateau (Fig. 3). The peak amplitude depends on NE concentration and the Ca^{2+} content of the store. In experimental data, this peak is usually less pronounced or even absent probably due to the averaging effect of fluorescent microscopy (Mauban et al., 2001). NE concentration affects not only the magnitude of the response but also its morphology, with lower concentrations producing peaks at a later time. This might explain the diversity in reported Ca^{2+} transients. The plateau depends on the Ca^{2+} fluxes through the plasma membrane and the rate of phosphorylation/desensitization of α_1 -adrenoceptor. Experimental data in endothelium-denuded rat mesenteric arterial strips show a similar decrease in Ca^{2+} plateau during maintained exposure to $1.5 \mu M$ PE (Lambole et al., 2003).

In isolated SMCs from rat mesenteric arterioles, NE (1 – $10 \mu M$) produced only transient Ca^{2+} increase with 11 s delay (in the model 2.6–18 s for 10 and $0.2 \mu M$ NE, respectively), suggesting that the main Ca^{2+} source was the SR (Baro and Eisner, 1995). If the extracellular Ca^{2+} concentration was elevated from 1 to 3 mM, however, a small maintained Ca^{2+} response was also present. In isolated rat small mesenteric arteries, NE typically induces maintained Ca^{2+} response in SMCs that is sensitive to L-type Ca^{2+} channel inhibitors (Nilsson et al., 1998). The model-generated Ca^{2+} responses shown in Fig. 3 include both transient Ca^{2+} elevations from store release, as well as maintained elevation through transmembrane Ca^{2+} entry.

The sensitivity of V_m and $[Ca^{2+}]_i$ to NE depends to a large extent on the unknown parameter K_{NSC} (Table A1). We selected its value

so that near maximum responses are elicited by $3 \mu M$ NE, in agreement with experimental data on isolated SMC from RMA (Baro and Eisner, 1995). The maximum membrane depolarization to NE brings V_m up to -28 mV and depends on the conductivity of the NSC channel (Table A1). The maximum depolarization agrees with experimental data in Weston et al. (2002), which showed that $10 \mu M$ PE depolarized SMCs in endothelium-denuded RMA from a resting value of -58.8 mV to around -30 mV. This indicates adequate magnitudes for the model's NSC currents, although they were not formulated from RMA data. In the model, the bulk of Ca^{2+} release from SR is through CICR via RyR, and the IP_3 receptor plays only a modulatory role. This is consistent with data from RMA where inhibition of CICR with $10 \mu M$ ryanodine prevented the transient response to NE (Lagaud et al., 1999).

4.3. Calcium dependence on membrane potential

Vascular tone is regulated mainly by the intracellular Ca^{2+} concentration and the Ca^{2+} sensitivity of the contractile machinery in SMCs. The majority of vasoconstrictors that produce sustained Ca^{2+} elevation act by depolarizing membrane potential, which in turn opens voltage-dependent Ca^{2+} channels. On the other hand, many vasodilators act primarily by hyperpolarizing cell membranes. Therefore, the steady-state dependence of $[Ca^{2+}]_i$ on V_m is a major characteristic of a SMC's functionality. Fig. 4 depicts the relationship between $[Ca^{2+}]_i$ and V_m as predicted by the model next to experimental data from Nilsson et al. (1994). The important finding is that different stimulations may produce different $[Ca^{2+}]_i$ - V_m relationships. In particular, NE elevates $[Ca^{2+}]_i$

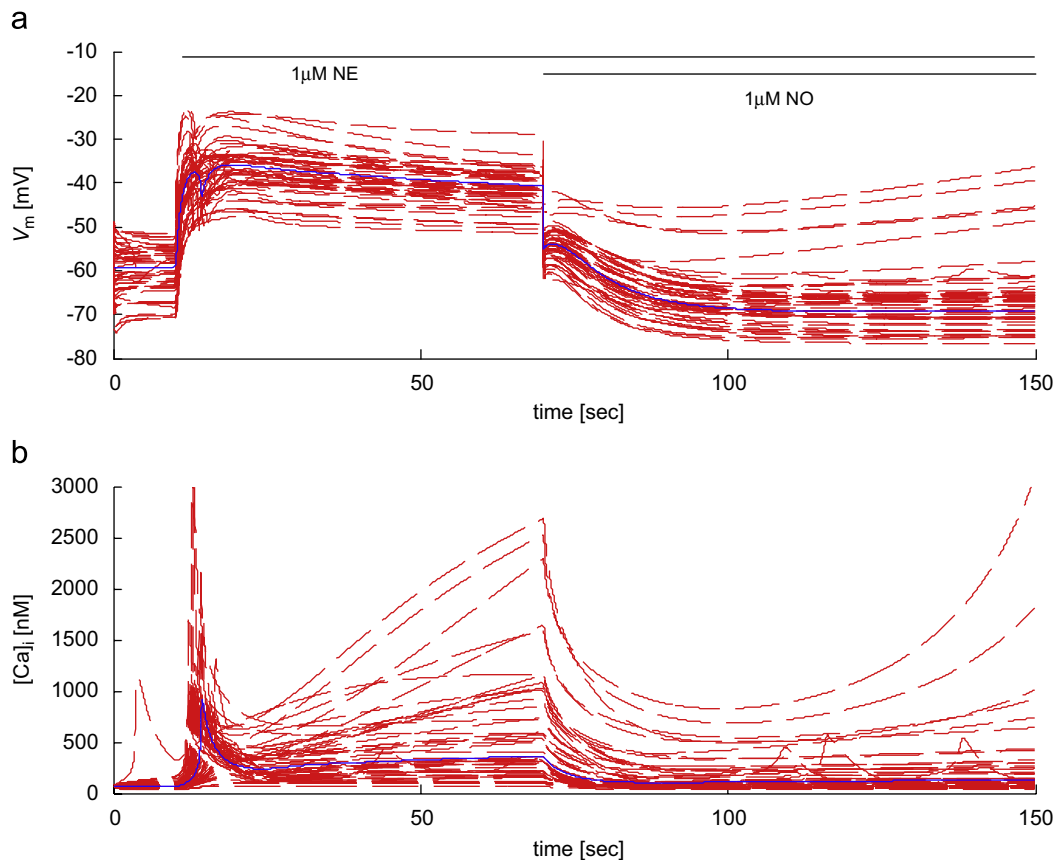


Fig. 9. Sensitivity analysis using the Latin Hypercube Sampling method. Membrane potential (a) and cytosolic calcium concentration (b) changes during stimulations with $1 \mu\text{M}$ NE and $1 \mu\text{M}$ NO for different values of parameters from Table S1.

more than KCl does for the same V_m depolarization, and this difference was ascribed to store release with a possible contribution by the modulation of Ca^{2+} channel conductance (Nilsson et al., 1994). In the model, however, this difference is caused by Ca^{2+} entry through the reverse mode of NCX, following Na^+ accumulation during prolonged NE stimulation. This prediction depends on the assumed conductance for NCX. It may be also altered if other isoforms of NaK or spatial gradients and cytosolic subcompartments are incorporated into the model. The apparent discrepancy between model results and experimental data for stimulation with high concentrations of KCl (i.e., high V_m) is a consequence of the assumed VOCC activation and inactivation parameters, for which different values have been reported by different groups.

4.4. Extracellular ionic changes

An important feature of the current model is the consistent formulation of transmembrane fluxes as a function of intracellular and extracellular ionic concentrations, which allows examining effects of changing ionic composition. The majority of earlier models include a balance for cytosolic calcium only and assume constant reversal potentials for Na^+ , K^+ , and Ca^{2+} currents (Koenigsberger et al., 2004; Parthimos et al., 1999; Wong and Klassen, 1993, 1996). The most detailed description of an SMC (Yang et al., 2003) incorporates balances for intracellular Na^+ , K^+ , and Ca^{2+} , but not for Cl^- , and assumes a constant reversal potential for the critical VOCC. Since cytosolic concentrations of ions change during stimulations and different interventions, reversal potentials change even with fixed extracellular ionic concentrations.

An increase in extracellular K^+ concentration typically produces depolarization by changing the K^+ Nernst potential and results in $[\text{Ca}^{2+}]_i$ elevation in SMCs. However, there are reports on hyperpolarizing and vasodilatory effects of K^+ in RMA, attributed to an SMC inward rectifier current and/or to $\text{Na}^+-\text{K}^+-\text{ATPase}$ isoforms containing α_2 - and/or α_3 -subunits that are sensitive to small increases in $[\text{K}^+]_o$ (Weston et al., 2002). This model includes only the housekeeping isoform of the Na^+ pump, which is nearly fully activated by normal $[\text{K}^+]_o = 5 \text{ mM}$ ($[\text{K}^+]$ for half maximum activity, $K_{d\text{KO}} = 1.6 \text{ mM}$). It does not include other isoforms due to the lack of detailed electrophysiological data, even though RT-PCR, Western blotting, and immunohistochemical techniques have demonstrated the presence of α_1 -, α_2 -, and α_3 -subunits in rat mesenteric myocytes (Weston et al., 2002). Based on findings that the inwardly rectifying potassium channels are not functionally present in rat mesenteric SMCs (Crane et al., 2003), this current was not incorporated into the model. Consequently, the potential ability of K^+ to induce hyperpolarization is not captured by the model, but this may not be a serious limitation since its role as an EDHF in RMA remains controversial and has not been established (Doughty et al., 2000; Lacy et al., 2000).

4.5. Calcium oscillations

NE- or KCl-induced $[\text{Ca}^{2+}]_i$ elevation to about 160 nM led to Hopf bifurcation and stable oscillations (Fig. 5). The rising phase of the cytosolic calcium oscillations is generated by the CICR through the RyRs and is terminated by depletion of the release compartment ($[\text{Ca}^{2+}]_r$ drops from maximum $560 \mu\text{M}$ to minimum $20 \mu\text{M}$). The uptake compartment is not significantly depleted and calcium concentration, $[\text{Ca}^{2+}]_u$, oscillates only within 450 – $760 \mu\text{M}$

range. In the model this over 10-fold concentration gradient is possible due to relatively slow calcium diffusion between the two compartments. $[Ca^{2+}]_i$ is restored to its equilibrium value by PMCA. As the RyRs close and SERCA refills the SR, there is a net calcium uptake by the store that produces further decreases in $[Ca^{2+}]_i$. When the store is refilled, $[Ca^{2+}]_i$ increases again, activating RyRs and the process repeats itself. The agonist concentration window for oscillations and the frequency of the resulting oscillations were surprisingly close to those observed experimentally during vasomotion, taking into account the relatively few parameter adjustments in the model that simulates a variety of other experimental data. The model suggests possible importance of a heterogeneous distribution of RyRs and SERCA and emptying of a small release compartment as a termination mechanism. However, the physiological relevance of this mechanism remains to be determined, and it does not exclude other well known models of calcium oscillations, e.g. based on inhibition of IP_3 receptors (Aalkjaer and Nilsson, 2005).

Significant calcium oscillations (~ 2 to 3 min^{-1}) in most regions of the SR were observed in intact rat astrocytes stimulated with glutamate, compare Fig. 4e in Golovina and Blaustein (1997). Vasomotion has been observed in rat mesenteric small arteries induced by $0.3\text{--}0.5 \mu\text{M}$ NE with a period of 6 s (Schuster et al., 2004), $0.6 \mu\text{M}$ PE with a period of 33 s (Lamboley et al., 2003) and $3\text{--}10 \mu\text{M}$ NE with a period of 13 s (Sell et al., 2002). KCl stimulation of isolated RMA induced vasomotion with a 25 s period and a $[K^+]_o$ threshold of 20 mM (Schuster et al., 2004). As shown in Fig. 5a, for physiologically relevant values of R_{leak} and $\bar{I}_{SERCA}$, the oscillation periods and amplitudes are close to those observed experimentally. Oscillations cease at low SERCA activity in agreement with experimental data where SERCA blockade inhibits the oscillations (Aalkjaer and Nilsson, 2005). NE-induced tension oscillations in RMA were also inhibited by elevated K^+ (Gustafsson and Nilsson, 1994). In the model, moderate elevation of $[K^+]_o$ either before or after NE stimulation could inhibit the NE-induced oscillations by moving the resultant $[Ca^{2+}]_i$ levels above the oscillatory domain, but this theoretical explanation requires experimental confirmation. Slight differences between NE- and KCl-induced oscillations are due to different mechanisms of membrane depolarization and activation of IP_3 receptors. The agonist-dependent increase of oscillation frequency (Fig. 5b) has been observed experimentally, in a different system, and it has been proposed that the oscillatory frequency may determine SMC contraction (Perez and Sanderson, 2005a,b). Fig. 5c is very similar to Fig. 3 in Schuster et al. (2004), and the predicted dependence on V_m in Fig. 5d agrees with some but not all experimental data. Also the bifurcation diagram in Fig. 5b is consistent with some experimental data and other SMC models (e.g. Koenigsberger et al., 2004). However, it results from the particular choices and simplifications made in the model and it may not be representative for a real cell or vessel. For example, the type of bifurcation may change on addition of mitochondria or after intercellular coupling, and some experimental data suggest that calcium oscillations are all-or-none reactions with the infinite period bifurcation (Koenigsberger et al., 2004; Schuster and Marhl, 2001).

4.6. NO-dependent relaxation

Nitric oxide is the major vascular SMC relaxant, which reduces $[Ca^{2+}]_i$ and/or desensitizes the actin-myosin contractile system to Ca^{2+} . NO has multiple molecular targets, and the predominant mechanism of $[Ca^{2+}]_i$ reduction may differ between vascular beds and species. For example, NO inhibits L-type Ca^{2+} channels in the A7r5 cell line (Blatter and Wier, 1994). In rabbit and mouse thoracic aorta, NO enhances refilling of intracellular stores by

SERCA, causing inhibition of the major store-operated Ca^{2+} influx pathway (Cohen et al., 1999). In RMA SMCs, electrophysiological studies indicate that the main target for the NO/cGMP pathway are the BK_{Ca} channels, although K_{ATP} channels have been also suggested (Garland and McPherson, 1992; Weidelt et al., 1997). Activation of the K^+ channels repolarizes/hyperpolarizes the plasma membrane and closes VOCC channels. In the present study, the formulation of the NO/cGMP pathway is based on Yang's model (Yang et al., 2005) and is extended by cGMP-dependent activation of Cl_{Ca} channels, NCX, and NaKCl. It has been suggested that the cGMP-dependent Cl_{Ca} channels play a role in synchronization of RMA SMCs (Matchkov et al., 2004). A multi-cellular extension of the present model will allow us to test this hypothesis. In Yang et al. (2005), SMC precontraction (i.e., prior to NO-dependent relaxation) was simulated by increasing the permeability of VOCC channels. In the present study, NE prestimulation depolarizes SMC by NSC channels and indirectly activates VOCC channels. In spite of the large depolarizing Na^+ current, NO-induced opening of BK_{Ca} channels was sufficient to repolarize maximally stimulated SMC and reduce $[Ca^{2+}]_i$ by as much as 83% without the need for activation of K_{ATP} channels. The model also predicts equivalent relaxation by an external hyperpolarizing current (data not shown).

Since, in rat mesentery resistance arteries, the relationship between $[Ca^{2+}]_i$ and contraction is not affected significantly by cGMP and is roughly linear for $[Ca^{2+}]_i < 1 \mu\text{M}$ (Andriantsitohaina et al., 1995), it is possible to compare model predictions for $[Ca^{2+}]_i$ with relaxation studies. In isolated rat mesenteric resistance arteries with intact endothelium and contracted with $3 \mu\text{M}$ NE, exogenous NO-induced transient relaxations with $IC_{50} = 6.5 \pm 0.1$ (316 nM) up to $88 \pm 3\%$ of initial contraction. Sodium nitroprusside (SNP), a NO donor, relaxed the vessels with $IC_{50} = 5.6$ and maximum response of $84 \pm 2\%$. In the same study, endothelium removal increased sensitivity to SNP 124-fold (Diederich et al., 1990). In the model cell prestimulated with $3 \mu\text{M}$ NE, the maximum NO-induced $[Ca^{2+}]_i$ reduction of 83% (Fig. 7c) compares well with these data. The order of magnitude difference between the theoretical half-maximal NO concentration of 32 nM and the experimental value of 316 nM may be attributed, at least in part, to the desensitizing effect of endothelium. In similar in vitro studies on endothelium-intact RMA precontracted with $3 \mu\text{M}$ PE, both SNP and S-nitroso-N-acetylpenicillamine (SNAP) produced maximally $\sim 80\%$ relaxation. Inhibition of cGMP-dependent protein kinase PKG with KT-5823 reduced the magnitude of relaxation by $\sim 50\%$ (Carrier et al., 1997). DEA NONOate, another NO donor, produced sustained relaxation ($> 90\%$) of endothelium-denuded RMA contracted with $3 \mu\text{M}$ PE, and ODQ, a selective inhibitor of sGC, significantly attenuated the relaxation (Sampson et al., 2001). Also, SNP mimicked endothelium-dependent hyperpolarizing current in RMA with damaged endothelium without need for sGC activation (Weidelt et al., 1997). In the model, inhibition of the cGMP pathway also reduced NO-dependent hyperpolarization and relaxation by 13 mV and 20%, respectively. In endothelium-denuded rabbit aorta, half-maximum relaxation was achieved by 100 nM NO (Adachi et al., 2004), while in endothelium-intact rabbit and mouse aorta, the NO concentration was 10–30 nM (Cohen et al., 1999). In rat superior mesenteric artery contracted with $1 \mu\text{M}$ NE, 378 ± 129 nM of exogenous NO were necessary for half-maximal relaxation, but acetylcholine produced only up to a 21 ± 6 nM increase in endothelium-dependent NO (Simonsen et al., 1999). On the other hand, in rat mesenteric resistance arteries, the maximal NO release after stimulation of NO synthase with a Ca^{2+} ionophore was 380 ± 30 nM (Tschudi et al., 1996). The above data indicate that the sensitivity of smooth muscle to NO may differ significantly between different vascular beds and species, and the sensitivity

of the model SMC to NO is within the physiological range for RMA.

4.7. Effects of Na^+ – K^+ -ATPase inhibition

Vascular smooth muscle responsiveness to vasoconstrictors and the arteriolar tone are affected by cardiotonic steroids such as ouabain, which selectively blocks NaK. It has been proposed that in some pathological conditions, ouabain induces inhibition of the NaK, elevates cytosolic Na^+ concentration and reduces or reverses Na^+ gradient-dependent Na^+ – Ca^{2+} exchange, leading to an increase in cytosolic Ca^{2+} . The blockade of NCX may thus restore SMC Ca^{2+} and contractility in the presence of ouabain (Blaustein, 1977, 1993; Iwamoto et al., 2004, 2005; Rossoni et al., 1999). We investigated this hypothesis with the model. Model predictions suggest that such a scenario is feasible in RMA SMC. Simulations in Fig. 8 suggest that resting and plateau phases of the NE-induced $[\text{Ca}^{2+}]_i$ profile can be affected by the presence of ouabain and that $[\text{Ca}^{2+}]_i$ levels can be restored by NCX inhibition. The magnitude of the ouabain effect, however, will depend on the balance between the NCX current and the NaK pump in the whole cell or in local subdomains of the plasmalemma. Thus, more accurate descriptions for these two components and their spatial organization should be incorporated in the model.

Representative model simulations, using certain parameter values, corroborate Blaustein's prediction (1993) for the potential role of SR as a Ca^{2+} amplifier in the presence of ouabain. This amplification is demonstrated in Fig. 8 as an enhanced transient response to NE. Importantly, the effects of ouabain are now examined in a detailed SMC model. Model simulations in Fig. 8, however, use parameter values for $\bar{I}_{\text{SERCA}}$ and R_{leak} at the high end of the proposed range in Table A1 (solid circle in Fig. 5a). If the SERCA, $\bar{I}_{\text{SERCA}}$, and leak, R_{leak} , parameters were reduced to the low end of this range (data not shown), the amplification effect could be prevented. For certain values of $\bar{I}_{\text{SERCA}}$ and R_{leak} , elevated resting $[\text{Ca}^{2+}]_i$ increases Ca^{2+} release via RyRs to a level higher than Ca^{2+} uptake by SERCA; this depletes SR and prevents Ca^{2+} amplification. Thus, the role of NaK inhibition in SMC reactivity depends also on the SR dynamics and may differ between vascular beds.

Iwamoto et al. (2004) reported that SEA0400, a specific blocker of Ca^{2+} entry mode of NCX type 1, reverses ouabain-induced cytosolic Ca^{2+} elevation and contraction in mouse small mesenteric arteries. In the model, blockade of the NCX's reverse mode nearly eliminated resting $[\text{Ca}^{2+}]_i$ elevation and hyperresponsiveness to NE induced by NaK inhibition. The model predicts also that agents blocking both forward and reverse modes of NCX will be less effective at reducing a potential ouabain-induced hyperreactivity (data not shown). The forward mode can limit the initial large Ca^{2+} spike (Fig. 8) or extrude Ca^{2+} at rest during NaK pump inhibition that does not reverse the Na^+ – Ca^{2+} exchanger.

4.8. Sodium balance

While Na^+ accumulation after Na^+ – K^+ -ATPase inhibition seems plausible and discussed in many papers (Blaustein, 1993; Blaustein et al., 2006; Lagaud et al., 1999), Na^+ balance under normal conditions has not been well characterized. It has been suggested that the activation of TRPC6 channels by α_1 -adrenoceptor may contribute to vasoconstriction as a Ca^{2+} entry pathway per se, via depolarization, or via modulation of Na^+ – Ca^{2+} exchange through increased Na^+ entry (Hill et al., 2006). Na^+ entry, local increase in intracellular Na^+ concentration, and modulation of colocalized NCX has been described recently as a key mechanism of TRPC signaling (Eder et al., 2005). It has been shown that, in the rabbit abdominal aorta, NE causes ^{22}Na retention and intracellular

Na^+ -dependent increase in ^{45}Ca influx, the latter possibly due to increased Na^+ – Ca^{2+} exchange (Khoiyi et al., 1991, 1993). The ^{22}Na content of the tissues was increased 50–70% after stimulation with 10 μM NE for 2–12 min under control conditions in PSS with 65 mM NaCl (compare Figs. 5–7 in Khoiyi et al., 1993). In cultured rat aortic SMCs, purinergic stimulation promotes DAG-activated Na^+ entry via TRPC6 channels, local and global $[\text{Na}^+]_i$ elevation, and Ca^{2+} entry via NCX reversal (Lemos et al., 2007; Poburko et al., 2007; Sytyong et al., 2007). However, caution must be taken when extrapolating the results from cultured to intact arterial SMCs (Blaustein and Wier, 2007), and some studies on RMA suggest that the Na^+ balance and NCX may be important only under extreme conditions (Lagaud et al., 1999; Mulvany et al., 1984).

Simulation results (i.e., prolonged NE stimulation, Fig. 4; inhibition of NaK, Fig. 8) suggest that the Na^+ accumulation is possible under normal and pathological situations and it can have a significant impact on SM calcium dynamics. This study is the first to include important components of the Na^+ regulation, (i.e., $[\text{Na}^+]_i$ -dependent NCX, NaK), and agonist-induced membrane depolarization via Na^+ influx. Na^+ accumulation was significant during large-amplitude and long-lasting NE stimulations, but it was less important upon weak or short stimulations. However, a more detailed model is required for quantitative validation with experimental data and the predicted $[\text{Na}^+]_i$ changes are probably larger than physiological. Activation of NSC channels causes a large sodium influx, depolarizing the plasma membrane, but it is not accompanied by sufficient increase in Na^+ pump rate. The large increase in $[\text{Na}^+]_i$ impairs cell responses by changing the Na^+ Nernst potential and causing excessive Ca^{2+} influx through the Na^+ – Ca^{2+} exchanger working in the reverse mode. Thus, this model suggests that isozymes of NaK containing α_3 -subunits are necessary for proper cell function. The α_3 isoform has lower affinity for Na^+ than the native housekeeping α_1 isoform, and it may only be active after an increase in intracellular Na^+ concentration. It was proposed that, in repeatedly stimulated rat neurons, the high affinity isoforms are working at saturation, while the α_3 isoform will function as an auxiliary pump to restore the dissipated Na^+ and K^+ gradients (Blanco and Mercer, 1998). The presence of α_3 subunits in rat mesenteric arteries has been documented (Weston et al., 2002).

4.9. Model limitations

The model uses some parameter values from cells other than RMA SMC, and a few parameters were adjusted based on secondary data. This limits the ability of the model to predict and/or describe certain features characteristic for RMA SMC and stems from the absence of appropriate experimental data. Sensitivity analysis and a large range of values examined for some parameters address part of this limitation. Sensitivity analysis can be also utilized for model validation. For example, the high sensitivity of resting $[\text{Ca}^{2+}]_i$ to the L-type channel conductance is somewhat contrary to experiments with calcium channel antagonists. Additional experimentation and better data (e.g. small steady-state membrane currents at resting V_m and $[\text{Ca}^{2+}]_i$) are required to improve the model's predictive ability by better characterizing model components, particularly ones with significant effect on the model. According to the sensitivity analysis with the LHS method, $\bar{I}_{\text{NaK}}$ and $\bar{I}_{\text{PMCA}}$ are the parameters likely to introduce the largest uncertainty on the model's outputs.

The model is run at room temperature, but certain parameters are based on 37 °C data. Because the model is highly nonlinear and some cellular components have steep temperature dependence,

this introduces significant uncertainty into the model's behavior. Different model responses could arise after incorporation of other possible signaling pathways. For example, NE was shown to be able to activate directly L-type calcium channels from rabbit mesenteric arteries (Nelson et al., 1988). However, currently no corresponding data are available for RMA. We chose, therefore, to follow a conceptual model of agonist-induced activation of rat mesenteric small arteries proposed in Nilsson (1998) where such pathway is not incorporated.

Major limitations of this model, as well as most of the previous SMC models, are the assumption of spatial homogeneity and the absence of many other cellular components. There is evidence for spatial heterogeneity in the cytosol that can affect physiological responses. Using confocal microscopy, for example, it was demonstrated that calcium oscillations in SMC may have the form of Ca^{2+} waves originating from intracellular stores (Aalkjaer and Nilsson, 2005). Low level adrenergic stimulation of RMA SMC elicited propagated Ca^{2+} transients, although higher stimulations induced vasomotion associated with spatially uniform oscillations of intracellular calcium (Mauban et al., 2001). Other data indicate that low concentrations of ouabain increase sodium retention only in subplasma membrane microdomains adjacent to SR, rather than in the bulk cytosol (Arnon et al., 2000b). NCX co-localization with $\text{NaK } \alpha_2$ and α_3 isoforms in these microdomains can then raise Ca^{2+} in SR and cytosol.

The RyRs in the vicinity of IP_3R or calcium channels may be exposed to calcium levels much higher than the average cytoplasmic calcium concentration. In the current model, the RyR responds to the bulk calcium concentration thus it is never fully activated. However, this low activation can be interpreted as an average response, since in reality only small fraction of RyRs is likely to be exposed to very high calcium levels simultaneously. Mitochondria, the Golgi apparatus, diffusible buffers, the presence of nucleus and other components have decisive effect on the calcium microdomains and affect activity of plasma membrane channels (McCarron et al., 2006). Thus, the model could be significantly improved by incorporating various subdomains, new cellular components, and spatial heterogeneity.

5. Summary

We developed a detailed biophysical model that can simulate several aspects of calcium regulation in vascular smooth muscle by integrating independent formulations of cellular components with minimal parameter adjustments. The model is tissue-specific and includes a detailed description of plasma membrane electrophysiology, an intracellular Ca^{2+} store, balances for the four major ionic species, and an extended NO/cGMP signaling pathway. Simulation results suggest a possible modulatory role of sodium dynamics in calcium regulation and provide insights for the mechanisms of SMC constriction, relaxation, and vasomotion. The model's detailed description of membrane conductivity, the balance of the ionic species in the cytosol, and the IP_3 and NO signaling pathways will allow us to construct integrated multi-cellular models of the vascular wall where smooth muscle and endothelial cells (Silva et al., 2007) will be coupled through ionic exchange and the diffusion of secondary messengers. Subsequent studies need to evaluate a number of choices in model development and modify components according to the scope that this model will be utilized for. Studies can utilize this model, for example, to examine specific cellular behaviors and provide reduced versions that will incorporate only the necessary components and level of detail. On the other hand, one can build on the current formulations and include more detailed descriptions for the components and additional signaling mechanisms.

Acknowledgments

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Appendix A

A.1. Standard model parameter values

$$\begin{aligned} z_K = z_{\text{Na}} = 1, \quad z_{\text{Ca}} = 2, \quad z_{\text{Cl}} = -1, \quad N_{\text{Av}} = 6.022 \times 10^{23}, \\ R = 8341.0 \text{ J/mol K}, \quad F = 96487.0 \text{ C/mol}, \\ T = 293.0 \text{ K}, \quad C_m = 25 \text{ pF}, \quad A_m = C_m/10^6 \text{ cm}^2, \\ [\text{Ca}]_o = 2.0 \text{ mM}, \quad [\text{Na}]_o = 140.0 \text{ mM}, \\ [\text{Cl}]_o = 129.0 \text{ mM}, \quad [\text{K}]_o = 5.0 \text{ mM} \end{aligned}$$

A.2. Model equations

A.2.1. Membrane electrophysiology

A.2.1.1. L-type voltage-operated Ca^{2+} channels.

$$\begin{aligned} I_{\text{VOCC}} = 10^6 A_m P_{\text{VOCC}} d_L f_L V_m \frac{(z_{\text{Ca}} F)^2}{RT} \\ \times \frac{[\text{Ca}]_o - [\text{Ca}]_i \exp(V_m z_{\text{Ca}} F/RT)}{1 - \exp(V_m z_{\text{Ca}} F/RT)} \text{ [pA]} \end{aligned} \quad (\text{A.1})$$

$$\begin{aligned} \frac{dd_L}{dt} = \frac{\bar{d}_L - d_L}{\tau_{d_L}}, \quad \bar{d}_L = \left(1 + \exp\left(-\frac{V_m}{8.3 \text{ mV}}\right)\right)^{-1}, \\ \tau_{d_L} = 2.5 \exp\left(-\left(\frac{V_m + 40 \text{ mV}}{30 \text{ mV}}\right)^2\right) + 1.15 \text{ [ms]} \end{aligned} \quad (\text{A.2} - 4)$$

$$\begin{aligned} \frac{df_L}{dt} = \frac{\bar{f}_L - f_L}{\tau_{f_L}}, \quad \bar{f}_L = \left(1 + \exp\left(\frac{V_m + 42.0 \text{ mV}}{9.1 \text{ mV}}\right)\right)^{-1}, \\ \tau_{f_L} = 65 \exp\left(-\left(\frac{V_m + 35 \text{ mV}}{25 \text{ mV}}\right)^2\right) + 45 \text{ [ms]} \end{aligned} \quad (\text{A.5} - 7)$$

A.2.1.2. Large conductance Ca^{2+} -activated K^+ channels.

$$I_{\text{BKCa}} = A_m N_{\text{BKCa}} P_{\text{KCa}} i_{\text{KCa}} \text{ [pA]} \quad (\text{A.8})$$

$$\begin{aligned} P_{\text{KCa}} = 0.17 p_f + 0.83 p_s, \quad \frac{dp_f}{dt} = \frac{\bar{p}_o - p_f}{\tau_{p_f}}, \\ \frac{dp_s}{dt} = \frac{\bar{p}_o - p_s}{\tau_{p_s}} \end{aligned} \quad (\text{A.9} - 11)$$

$$\bar{p}_o = \left(1 + \exp\left(-\frac{V_m - V_{1/2\text{KCa}}}{18.25 \text{ mV}}\right)\right)^{-1} \quad (\text{A.12})$$

$$\begin{aligned} V_{1/2\text{KCa}} = -41.7 \log_{10}([\text{Ca}]_i) - 128.2 - dV_{1/2\text{KCaNO}} R_{\text{NO}} \\ - dV_{1/2\text{KCaGMP}} R_{\text{cGMP}} \end{aligned} \quad (\text{A.13})$$

$$R_{\text{NO}} = \frac{[\text{NO}]}{[\text{NO}] + 200 \text{ nM}}, \quad R_{\text{cGMP}} = \frac{[\text{cGMP}]^2}{[\text{cGMP}]^2 + (1.5 \text{ } \mu\text{M})^2} \quad (\text{A.14, A.15})$$

$$i_{\text{KCa}} = 10^6 P_{\text{BKCa}} V_m \frac{F^2}{RT} \frac{[\text{K}]_o - [\text{K}]_i \exp(V_m F/RT)}{1 - \exp(V_m F/RT)} \text{ [pA]} \quad (\text{A.16})$$

A.2.1.3. Voltage-dependent K^+ channels.

$$I_{\text{KV}} = g_{\text{KV}} p_K (0.45 q_1 + 0.55 q_2) (V_m - E_K) \text{ [pA]} \quad (\text{A.17})$$

$$\bar{p}_K = \frac{1}{1 + \exp(-(V_m + 11.0 \text{ mV})/15.0 \text{ mV})};$$

$$\tau_{p_K} = 61.5 \exp(-0.027 V_m) \text{ [ms]}; \quad \frac{dp_K}{dt} = \frac{\bar{p}_K - p_K}{\tau_{p_K}} \quad (\text{A.18 – 20})$$

$$\bar{q} = \frac{1}{1 + \exp((V_m + 40.0 \text{ mV})/14.0 \text{ mV})},$$

$$\frac{dq_1}{dt} = \frac{\bar{q} - q_1}{\tau_{q1}}, \quad \frac{dq_2}{dt} = \frac{\bar{q} - q_2}{\tau_{q2}} \quad (\text{A.21 – 23})$$

A.2.1.4. Unspecified K^+ leak channels.

$$I_{K\text{leak}} = g_{K\text{leak}}(V_m - E_K) \quad (\text{A.24})$$

A.2.1.5. Non-selective cation channels.

$$I_{\text{NSC}} = I_{\text{NaNSC}} + I_{\text{KNSC}} + I_{\text{CaNSC}} \text{ [pA]} \quad (\text{A.25})$$

$$I_{\text{NaNSC}} = 10^6 A_m \left(\frac{[\text{DAG}]}{[\text{DAG}] + K_{\text{NSC}}} + d_{\text{NSCmin}} \right) P_{\text{oNSC}} P_{\text{NaNSC}}$$

$$\times V_m \frac{F^2}{RT} \frac{[\text{Na}]_o - [\text{Na}]_i \exp(V_m F/RT)}{1 - \exp(V_m F/RT)} \quad (\text{A.26})$$

$$I_{\text{KNSC}} = 10^6 A_m \left(\frac{[\text{DAG}]}{[\text{DAG}] + K_{\text{NSC}}} + d_{\text{NSCmin}} \right) P_{\text{oNSC}} P_{\text{KNSC}}$$

$$\times V_m \frac{F^2}{RT} \frac{[\text{K}]_o - [\text{K}]_i \exp(V_m F/RT)}{1 - \exp(V_m F/RT)} \quad (\text{A.27})$$

$$I_{\text{CaNSC}} = 10^6 A_m d_{\text{NSCmin}} P_{\text{oNSC}} P_{\text{CaNSC}} V_m \frac{(z_{\text{Ca}} F)^2}{RT}$$

$$\times \frac{[\text{Ca}]_o - [\text{Ca}]_i \exp(z_{\text{Ca}} V_m F/RT)}{1 - \exp(z_{\text{Ca}} V_m F/RT)} \quad (\text{A.28})$$

$$P_{\text{oNSC}} = 0.43 + \frac{0.57}{1 + \exp(-(V_m - 47.12 \text{ mV})/24.24 \text{ mV})} \quad (\text{A.29})$$

A.2.1.6. Store-operated non-selective cation channels.

$$I_{\text{SOC}} = I_{\text{SOCCa}} + I_{\text{SOCNa}} \text{ [pA]} \quad (\text{A.30})$$

$$I_{\text{SOCCa}} = g_{\text{SOCCa}} P_{\text{SOC}}(V_m - E_{\text{Ca}}),$$

$$I_{\text{SOCNa}} = g_{\text{SOCNa}} P_{\text{SOC}}(V_m - E_{\text{Na}}) \quad (\text{A.31, A.32})$$

$$P_{\text{SOC}} = \left(1 + \frac{[\text{Ca}]_u}{100 \text{ nM}} \right)^{-1} \quad (\text{A.33})$$

A.2.1.7. Calcium-activated chloride channels.

$$I_{\text{ClCa}} = C_m g_{\text{ClCa}} P_{\text{Cl}}(V_m - E_{\text{Cl}}) \text{ [pA]} \quad (\text{A.34})$$

$$P_{\text{Cl}} = 0.0132 \frac{([\text{Ca}]_i)^{n_{\text{ClCa}}}}{([\text{Ca}]_i)^{n_{\text{ClCa}}} + (K_{\text{ClCa}})^{n_{\text{ClCa}}}}$$

$$+ \alpha_{\text{Cl}} \frac{([\text{Ca}]_i)^{n_{\text{ClCa}}}}{([\text{Ca}]_i)^{n_{\text{ClCa}}} + (K_{\text{ClCa,cGMP}})^{n_{\text{ClCa}}}}$$

$$\alpha_{\text{Cl}} = \frac{([\text{cGMP}])^{n_{\text{ClcGMP}}}}{([\text{cGMP}])^{n_{\text{ClcGMP}}} + (K_{\text{ClcGMP}})^{n_{\text{ClcGMP}}}}$$

$$K_{\text{ClCa,cGMP}} = (1 - 0.9\alpha_{\text{Cl}})400 \text{ nM}$$

A.2.1.8. Plasma membrane Ca^{2+} pump.

$$I_{\text{PMCA}} = \bar{I}_{\text{PMCA}} \frac{[\text{Ca}]_i}{[\text{Ca}]_i + K_{\text{m,PMCA}}} \quad (\text{A.35})$$

A.2.1.9. Plasma membrane $\text{Na}^+ - \text{Ca}^{2+}$ exchange.

$$I_{\text{NCX}} = g_{\text{NCX}} R_{\text{NCX,cGMP}}$$

$$\times \frac{[\text{Na}]_i^3 [\text{Ca}]_o \Phi_F - [\text{Na}]_o^3 [\text{Ca}]_i \Phi_R}{1 + d_{\text{NCX}}([\text{Na}]_o^3 [\text{Ca}]_i + [\text{Na}]_i^3 [\text{Ca}]_o)} [\text{pA}] \quad (\text{A.36})$$

$$R_{\text{NCX,cGMP}} = 1 + 0.55 \frac{[\text{cGMP}]}{[\text{cGMP}] + 45 \mu\text{M}};$$

$$\Phi_F = \exp\left(\frac{\gamma V_m F}{RT}\right), \quad \Phi_R = \exp\left(\frac{(\gamma - 1)V_m F}{RT}\right),$$

$$\gamma = 0.45, \quad d_{\text{NCX}} = 0.0003 \quad (\text{A.37, A.38})$$

A.2.1.10. Sodium-potassium pump.

$$I_{\text{NaK}} = C_m \bar{I}_{\text{NaK}} Q \frac{([\text{K}]_o)^{n_{\text{HKo}}}}{([\text{K}]_o)^{n_{\text{HKo}}} + (K_{\text{dKo}})^{n_{\text{HKo}}}}$$

$$\times \frac{([\text{Na}]_i)^{n_{\text{HNai}}}}{([\text{Na}]_i)^{n_{\text{HNai}}} + (N_{\text{dNai}})^{n_{\text{HNai}}}} \frac{V_m + 150 \text{ mV}}{V_m + 200 \text{ mV}} \quad (\text{A.39})$$

$$Q = Q_{10}^{(T-309.15 \text{ K}/10)}, \quad Q_{10} = 1.87$$

A.2.1.11. Sodium-potassium-chloride cotransport.

$$I_{\text{NaKCl}}^{\text{Cl}} = -R_{\text{NaKCl,cGMP}} z_{\text{Cl}} A_m L_{\text{NaKCl}} R F T$$

$$\times \log_e \left(\frac{[\text{Na}]_o [\text{K}]_o ([\text{Cl}]_o)^2}{[\text{Na}]_i [\text{K}]_i ([\text{Cl}]_i)^2} \right) \quad (\text{A.40})$$

$$R_{\text{NaKCl,cGMP}} = 1 + 3.5 \frac{[\text{cGMP}]}{[\text{cGMP}] + 6.4 \mu\text{M}}$$

$$I_{\text{NaKCl}}^{\text{Na}} = I_{\text{NaKCl}}^{\text{K}} = -0.5 I_{\text{NaKCl}}^{\text{Cl}} \quad (\text{A.41})$$

Reversal potentials

$$E_A = \frac{RT}{z_A F} \ln \left(\frac{[A]_o}{[A]_i} \right),$$

where A denotes K, Na, Ca or Cl. (A.42)

A.2.2. Sarcoplasmic reticulum

$$I_{\text{SERCA}} = \bar{I}_{\text{SERCA}} \frac{[\text{Ca}]_i}{[\text{Ca}]_i + K_{\text{m,up}}} \quad (\text{A.43})$$

$$I_{\text{tr}} = ([\text{Ca}]_u - [\text{Ca}]_r) z_{\text{Ca}} F \text{ vol}_u / \tau_{\text{tr}} \quad (\text{A.44})$$

$$I_{\text{rel}} = (R_{10}^2 + R_{\text{leak}})([\text{Ca}]_r - [\text{Ca}]_i) z_{\text{Ca}} F \text{ vol}_r / \tau_{\text{rel}} \quad (\text{A.45})$$

$$\frac{d[\text{Ca}]_u}{dt} = \frac{I_{\text{SERCA}} - I_{\text{tr}} - I_{\text{IP3}}}{z_{\text{Ca}} F \text{ vol}_u} \quad (\text{A.46})$$

$$\frac{d[\text{Ca}]_r}{dt} = \frac{I_{\text{tr}} - I_{\text{rel}}}{z_{\text{Ca}} F \text{ vol}_r} \left(1 + \frac{[\text{CSQN}] K_{\text{CSQN}}}{(K_{\text{CSQN}} + [\text{Ca}]_r)^2} \right)^{-1}$$

$$\tau_{\text{tr}} = 1000 \text{ ms}; \quad \tau_{\text{rel}} = 0.033 \text{ ms} \quad (\text{A.47})$$

A.2.2.1. Ryanodine receptor.

$$R_{00} = 1 - R_{01} - R_{10} - R_{11} \quad (\text{A.49})$$

$$\frac{dR_{10}}{dt} = K_{r1}[Ca]_i^2 R_{00} - (K_{-r1} + K_{r2}[Ca]_i)R_{10} + K_{-r2}R_{11} \quad (\text{A.50})$$

$$\frac{dR_{11}}{dt} = K_{r2}[Ca]_i R_{10} - (K_{-r1} + K_{-r2})R_{11} + K_{r1}[Ca]_i^2 R_{01} \quad (\text{A.51})$$

$$\frac{dR_{01}}{dt} = K_{r2}[Ca]_i R_{00} + K_{-r1}R_{11} - (K_{-r2} + K_{r1}[Ca]_i^2)R_{01} \quad (\text{A.52})$$

$$K_{r1} = 2500 \text{ mM}^{-2} \text{ ms}^{-1}, K_{r2} = 1.05 \text{ mM}^{-1} \text{ ms}^{-1}, \\ K_{-r1} = 0.0076 \text{ ms}^{-1}, K_{-r2} = 0.084 \text{ ms}^{-1}.$$

A.2.2.2. IP₃ receptor.

$$I_{IP3} = \bar{I}_{IP3} z_{Ca} \text{vol}_{Ca} F \left(\frac{[IP_3]}{[IP_3] + K_{IP3}} \frac{[Ca]_i}{[Ca]_i + K_{act,IP3}} h_{IP3} \right)^3 \\ \times ([Ca]_u - [Ca]_i) \quad (\text{A.53})$$

$$\frac{dh_{IP3}}{dt} = k_{on,IP3}(K_{inh,IP3} - ([Ca]_i + K_{inh,IP3})h_{IP3}) \quad (\text{A.54})$$

A.2.3. α_1 -Adrenoceptor activation and IP₃ formation

$$\frac{d[R_G^S]}{dt} = k_{r,G}\xi_G[R_{T,G}] - \left(k_{r,G} + \frac{k_{p,G}[NE]}{K_{1,G} + [NE]} \right) [R_G^S] \\ - k_{r,G}[R_{p,G}^S] \quad (\text{A.55})$$

$$\frac{d[R_{p,G}^S]}{dt} = [NE] \left(\frac{k_{p,G}[R_G^S]}{K_{1,G} + [NE]} - \frac{k_{e,G}[R_{p,G}^S]}{K_{2,G} + [NE]} \right) \quad (\text{A.56})$$

$$\rho_{r,G} = \frac{[NE][R_G^S]}{\xi_G[R_{T,G}](K_{1,G} + [NE])} \quad (\text{A.57})$$

$$\frac{d[G]}{dt} = k_{a,G}(\delta_G + \rho_{r,G})([G_{T,G}] - [G]) - k_{d,G}[G] \quad (\text{A.58})$$

$$r_{h,G} = \alpha_G \frac{[Ca]_i}{[Ca]_i + K_{c,G}} [G] \quad (\text{A.59})$$

$$\frac{d[IP_3]}{dt} = \frac{r_{h,G}}{\gamma_G} [PIP_2] - k_{deg,G}[IP_3] \quad (\text{A.60})$$

$$\frac{d[PIP_2]}{dt} = -(r_{h,G} + r_{r,G})[PIP_2] - r_{r,G}\gamma_G[IP_3] + r_{r,G}[PIP_{2,T}] \quad (\text{A.61})$$

$$[R_{T,G}] = 2 \times 10^4, \quad K_{1,G} = k_1^-/k_1^+ = 0.01 \text{ mM} \\ K_{2,G} = k_2^-/k_2^+ = 0.2 \text{ mM}, \quad k_{r,G} = 1.75 \times 10^{-7} \text{ ms}^{-1} \\ k_{e,G} = 6 \times 10^{-6} \text{ ms}^{-1}, \quad \xi_G = 0.85, \quad [G_{T,G}] = 1 \times 10^5 \\ k_{deg,G} = 1.25 \times 10^{-3} \text{ ms}^{-1}, \quad k_{a,G} = 0.17 \times 10^{-3} \text{ ms}^{-1} \\ k_{d,G} = 1.5 \times 10^{-3} \text{ ms}^{-1}, \quad [PIP_{2,T}] = 5 \times 10^7 \\ r_{r,G} = 0.015 \times 10^{-3} \text{ ms}^{-1}, \quad K_{c,G} = 0.4 \times 10^{-3} \text{ mM} \\ \alpha_G = 2.781 \times 10^{-8} \text{ ms}^{-1}, \quad \gamma_G = 10^{-15} N_{AV} \text{ vol}_i$$

$$[DAG] = [IP_3] \quad (\text{A.62})$$

A.2.4. sGC activation and cGMP formation

$$\bar{V}_{cGMP} = V_{cGMP,max} \frac{B5_{sGC}[NO] + [NO]^2}{A0_{sGC} + A1_{sGC}[NO] + [NO]^2} \quad (\text{A.63})$$

$$\tau_{sGC} = \begin{cases} \tau_{sGC}^a & \text{if } (\bar{V}_{cGMP} - V_{cGMP}) \geq 0 \\ \tau_{sGC}^d & \text{otherwise} \end{cases} \quad (\text{A.64, A.65})$$

$$\frac{dV_{cGMP}}{dt} = \frac{\bar{V}_{cGMP} - V_{cGMP}}{\tau_{sGC}} \quad (\text{A.66})$$

$$\frac{d[cGMP]}{dt} = V_{cGMP} - k_{pde,cGMP} \frac{[cGMP]^2}{[cGMP] + K_{m,pde}} \quad (\text{A.67})$$

$$k_{1,sGC} = 2 \times 10^3 \text{ mM}^{-1} \text{ ms}^{-1}, \quad k_{-1,sGC} = 15 \times 10^{-3} \text{ ms}^{-1}, \\ k_{2,sGC} = 0.64 \times 10^{-5} \text{ ms}^{-1}, \quad k_{-2,sGC} = 0.1 \times 10^{-6} \text{ ms}^{-1}, \\ k_{3,sGC} = 4.2 \text{ mM}^{-1} \text{ ms}^{-1}, \quad k_{D,sGC} = 0.4 \times 10^{-3} \text{ ms}^{-1}, \\ k_{Dr,sGC} = 0.1 \times 10^{-3} \text{ ms}^{-1}, \quad B5_{sGC} = k_{2,sGC}/k_{3,sGC} \quad (\text{A.68})$$

$$A0_{sGC} = \frac{(k_{-1,sGC} + k_{2,sGC})k_{D,sGC} + k_{-1,sGC}k_{-2,sGC}}{k_{1,sGC}k_{3,sGC}} \quad (\text{A.69})$$

$$A1_{sGC} = \frac{(k_{1,sGC} + k_{3,sGC})k_{D,sGC} + (k_{2,sGC} + k_{-2,sGC})k_{1,sGC}}{k_{1,sGC}k_{3,sGC}} \\ k_{pde,cGMP} = 0.0695 \times 10^{-3} \text{ ms}^{-1} \quad (\text{A.70})$$

A.2.5. Ionic balances

$$I_{Catotm} = I_{SOCCa} + I_{VOCC} - 2I_{NCX} + I_{PMCA} + I_{CaNSC} \quad (\text{A.71})$$

$$\frac{d[Ca]_i}{dt} = - \frac{I_{Catotm} + I_{SERCA} - I_{rel} - I_{IP3}}{z_{Ca}F \text{vol}_{Ca}} \\ \times \left(1 + \frac{[\bar{S}_{CM}]K_d}{(K_d + [Ca]_i)^2} + \frac{[\bar{B}_F]K_{dB}}{(K_{dB} + [Ca]_i)^2} \right)^{-1} \quad (\text{A.72})$$

$$I_{Natotm} = I_{NaKCl}^{Na} + I_{SOCNa} + 3I_{NaK} + 3I_{NCX} + I_{NaNSC}, \\ \frac{d[Na]_i}{dt} = - \frac{I_{Natotm}}{F \text{vol}_i} \quad (\text{A.73, A.74})$$

$$I_{Ktotm} = I_{NaKCl}^K + I_{Kv} + I_{BKCa} + I_{KNSC} + I_{Kleak} - 2I_{NaK}, \\ \frac{d[K]_i}{dt} = - \frac{I_{Ktotm}}{F \text{vol}_i} \quad (\text{A.75, A.76})$$

$$I_{Cltotm} = I_{NaKCl}^{Cl} + I_{ClCa}, \quad \frac{d[Cl]_i}{dt} = - \frac{I_{Cltotm}}{z_{Cl}F \text{vol}_i} \quad (\text{A.77, A.78})$$

A.2.6. Sensitivity analysis

$$(\hat{\beta} - \hat{\beta})^T X^T X (\hat{\beta} - \hat{\beta}) \leq p s^2 F(p, v, 1 - \alpha) \quad (\text{A.79})$$

$\hat{\beta}$ is the vector of least squares estimates of β , $F(p, v, 1 - \alpha)$ is 1- α point of the $F(p, v)$ distribution, $\alpha = 0.05$; $p = 26$; $v = 50 - p$; $s^2 = (\text{sum of squares of residuals})/v$.

A.3. Initial conditions

$$V_m = -59.4 \text{ mV}; \quad [Ca]_i = 68 \text{ nM}; \quad [Ca]_u = 0.66 \text{ mM}; \quad [Ca]_r = 0.57 \text{ mM}; \\ [Na]_i = 8.4 \text{ mM}; \quad [K]_i = 140 \text{ mM}; \quad [Cl]_i = 59.4 \text{ mM}; \\ [IP_3] = [DAG] = [cGMP] = 0; \quad V_{cGMP} = 0. \\ I_{VOCC}: d_L = 1/(1 + \exp(-V_m/8.3)); \quad f_L = 1/(1 + \exp((V_m + 42.0)/9.1)) \\ I_{BKCa}: V_{1/2KCa} = -41.7 \log_{10}([Ca]_i) - 128.2; \quad p_r = p_s = 1/(1 + \exp \\ (-V_m - V_{1/2KCa})/18.25)).$$

Table A1
Parameter values

Parameter	Value	Meaning	Source and cell type
<i>L-type voltage-operated Ca^{2+} channels</i>			
P_{VOCC}	$1.88 \times 10^{-5} \text{ cm/s}$	Whole-cell permeability	Fit to voltage-clamp data (Lozinskaya and Cox, 1997); RMA SMC
τ_{dL}, τ_{fL}	V_m dependent	Activation and inactivation time constants	
$\bar{d}_L, \bar{f}_L$	V_m dependent	Steady-state activation and inactivation gates	Modified from Lozinskaya and Cox (1997) for GHK expression; RMA SMC
<i>Large conductance Ca^{2+}-activated potassium channels</i>			
P_{BKCa}	$3.9 \times 10^{-13} \text{ cm}^3/\text{s}$	Single channel permeability	Mistry and Garland (1998); RMA SMC
$dV_{1/2\text{KCaNO}}$	46.3 mV	Max. NO-induced $V_{1/2\text{KCa}}$ shift	
N_{BKCa}	$6.6 \times 10^6 \text{ cm}^{-2}$	Channel density	Estimated from whole-cell current in RMA SMC (Smirnov et al., 1998)
$\bar{p}_o$	V_m dependent	Steady-state open probability	Fits to isolated patch data and distribution of open times (Benham et al., 1986); guinea-pig small mesenteric arteries SMC
$V_{1/2\text{KCa}}$	Ca^{2+} -, cGMP- & NO-dependent	Half-activation potential	
τ_{pf}, τ_{ps}	0.84 ms, 35.9 ms	Fast and slow activation time constants	
$R_{\text{NO}}, R_{\text{cGMP}}$	NO-, cGMP-dep.	Regulatory functions	Yang et al. (2005); vascular SMC model
$dV_{1/2\text{KCa cGMP}}$	76 mV	Max. cGMP-induced $V_{1/2\text{KCa}}$ shift	Based on mesangial cell data (Stockand and Sansom, 1996)
<i>Voltage-dependent K^+ channels</i>			
g_{Kv}	1.35 nS	Max. whole-cell conductance	Fit to voltage clamp data (Lu et al., 2001); RMA SMC
τ_{PK}	V_m dependent	Activation time constant	
$\bar{p}_K, \bar{q}$	V_m dependent	Steady-state activation gates	Lu et al. (2001); RMA SMC
τ_{q1}, τ_{q2}	371 ms, 2884 ms	Fast and slow inactivation time constants	
<i>ATP-sensitive K^+ channels</i>			
g_{Kleak}	0.067 nS	Whole-cell conductance	Based on Nelson and Quayle (1995); cerebral artery SMC
<i>Nonselective cation channels</i>			
K_{NSC}	300 nM	DAG concentration for half-maximum activation	Based on sensitivity of isolated RMA SMC to NE (Baro and Eisner, 1995)
P_{NaNSC}	$5.11 \times 10^{-7} \text{ cm/s}$	Whole-cell Na^+ , K^+ and Ca^{2+} permeabilities	Estimated from max. and spontaneous current densities through murine TRP6 channels expressed in HEK293 cells and their open probabilities (Inoue et al., 2001)
P_{KNSC}	$1.06 \times P_{\text{NaNSC}}$		
P_{CaNSC}	$4.54 \times P_{\text{NaNSC}}$		
d_{NSCmin}	0.0244	Constituent activation	
P_{oNSC}	V_m dependent	Voltage inhibition	
<i>Store-operated nonselective cation channels</i>			
g_{SOCCa}	0.0083 nM	Whole-cell conductances to Ca^{2+} and Na^+	Based on Albert and Large (2003) and Arnon et al. (2000a); mouse myocytes and RMA SMC
g_{SOCNa}	0.0575 nM		
K_{SOC}	100 nM	Ca^{2+} concentration in SR for half-activation	Assumed based on Arnon et al. (2000a), Nilsson et al. (1998); RMA SMC
<i>Calcium-activated chloride channels</i>			
g_{ClCa}	0.23 nS/pF	Maximum conductance, cGMP-indep. component,	Estimated from whole-cell current densities (Matchkov et al., 2004); RMA SMC
$R_{\text{Cl cGMPmin}}$	0.0132	Hill coefficient	
n_{ClCa}	2		
K_{ClCa}	365 nM	EC_{50} for Ca^{2+} -dependent activation	Large and Wang (1996); rat portal vein SMC
$K_{\text{ClCa cGMP}}$	cGMP-dep.		Jacobsen et al. (2007b); RMA SMC
$n_{\text{Cl cGMP}}$	3.3	Hill coefficient, EC_{50} for cGMP-dependent activation	Matchkov et al. (2004), RMA SMC
$K_{\text{Cl cGMP}}$	6.4 μM		
<i>Plasma membrane Ca^{2+}-ATPase</i>			
I_{PMCA}	5.37 pA	Max. current	Estimated from resting Ca^{2+} balance
$K_{\text{m PMCA}}$	170 nM	Michaelis constant	O'Donnell and Owen (1994); bovine aorta SM
<i>Plasma membrane Na^+–Ca^{2+} exchange</i>			
g_{NCX}	0.0487–0.487 pS	Scaling factor	Control-0.487pS, adjusted relative to PMCA (O'Donnell and Owen, 1994); vascular SMCs
$R_{\text{NCX cGMP}}$	cGMP dependent	cGMP-dependent regulation	Furukawa et al. (1991); rat aortic SMC
<i>Sodium–potassium pump</i>			
I_{NaK}	2.31 pA/pF	Max. current density	Estimated from resting K^+ and Na^+ balance and V_m
$K_{\text{dKo}}, K_{\text{dNai}}$	1.6 mM, 22 mM	Binding constants and Hill coefficients for K_o^+ and Na_i^+	Nakamura et al. (1999); guinea-pig mesenteric resistance arteries SMC
$n_{\text{HKO}}, n_{\text{HNai}}$	1.1, 1.7		
f_{NaK}	V_m dependent	V_m -dependence parameter	Yang et al. (2003); vascular SMC model
<i>Sodium–potassium–chloride cotransport</i>			
L_{NaKCl}	$1.79 \times 10^{-8} \text{ nmol}^2/(\text{J} \times \text{s} \times \text{cm}^2)$	Cotransport coefficient	Estimated from resting Cl^- balance
$R_{\text{NaKCl cGMP}}$	cGMP dependent	cGMP-dependent regulation	O'Donnell and Owen (1986, 1994); rat thoracic aorta SMC

Table A1 (continued)

Parameter	Value	Meaning	Source and cell type
Sarcoplasmic reticulum (parameters as in Yang et al. (2003) except:)			
$K_{m,up}$	1 μ M	Michaelis constant	Miller and Carsten (1997); vascular SMCs
I_{SERCA}	6.68–20 pA	Max. uptake current	Control value 6.68pA, modified from Yang et al. (2003); vascular SMC model
R_{leak}	1.07×10^{-5} – 5.35×10^{-5}	Leakage parameter	Control value 1.07×10^{-5} based on Camello et al. (2002)
K_{CSQN}	0.8 mM	Binding affinity of calsequestrin	Volpe et al. (1994); rat aorta SMC
$[CSQN]$	15 mM	Concentration of calsequestrin in the release compartment	Jafri et al. (1998); ventricular myocytes model
I_{IP3}	2880×10^{-6} mM/ms	Rate constant of Ca^{2+} release by IP_3R ;	Fink et al. (1999); A7r5 SMC model
$K_{act,IP3}$	170 nM	dissociation constants for Ca^{2+}	
$K_{inh,IP3}$	100 nM	activation and inhibitory sites	
K_{IP3}	120 nM	Dissociation constant for IP_3 binding to	Bennett et al. (2005); vascular SMC model
$k_{on,IP3}$	$1.4 \text{ mM}^{-1} \text{ ms}^{-1}$	IP_3R ; rate of Ca^{2+} binding to the inhibitory site	
α_1-Adrenoceptor activation and IP_3 formation (parameters as in Bennett et al., 2005, except:)			
$k_{p,G}$	0–0.1 s^{-1}	Receptor phosphorylation rate	0.1 s^{-1} (Bennett et al., 2005) at Fig. 3, otherwise 0 to eliminate desensitization to NE; RMA
sGC activation and cGMP formation			
$\bar{V}_{cGMP}$	NO dependent	Steady-state cGMP production	Condorelli and George (2001)
$\tau_{sGC}^a, \tau_{sGC}^d$	0.23 s, 10 s	sGC activation and inactivation time constants	From analytical approx. of Condorelli and George (2001) for 1 μ M NO and $k_{D,sGC} = 0.1 \text{ s}^{-1}$
$V_{cGMP,max}$	1.26×10^{-7} mM/ms	Max. cGMP formation rate	Based on sensitivity of isolated RMA to NO (Diederich et al., 1990)
$K_{m,pde}$	10^{-3} mM	Michaelis-Menten constant	
Ca^{2+} buffering in the cytosol			
$[S_{CM}], [B_F]$	0.1 mM, 0.1 mM	Concentration of calmodulin and other buffers and their dissociation constants	Based on Fink et al. (1999), Wang (1985), Yang et al. (2003); vascular SMC models
K_d, K_{dB}	260 nM, 530 nM		
Volumes			
$vol_i, vol_{Ca}, vol_u, vol_r$	1.0 pL, 0.7 pL 0.07 pL, 0.007 pL	Volumes: cytosolic, available to free Ca^{2+} , uptake and release compartments	Miller and Carsten (1997), Yang et al. (2003); vascular SMCs

$$I_{KV}: p_K = 1/(1+\exp(-(V_m+11)/15)); q_1 = q_2 = 1.0/(1+\exp((V_m+40)/14)).$$

$$RyR: R_{01} = 0.995; R_{10} = 0.0033; R_{11} = 4.0 \times 10^{-6}.$$

$$IP_3R: h_{IP3} = K_{inh,IP3}/([Ca]_i + K_{inh,IP3}).$$

$$\alpha_1\text{-adrenoceptor: } [R_G^S] = [R_{T,G}]\xi_G; [R_{P,G}^S] = 0; [PIP_2] = [PIP_{2,T}] - (1 + k_{deg,G}/r_{r,G})\gamma_G[IP_3];$$

$$r_{h,G} = k_{deg,G}/G[IP_3]/[PIP_2]; G = r_{h,G} (K_{c,G} + [Ca]_i)/(\alpha_G[Ca]_i); \delta_G = k_{d,G}G/(G_{a,G}([G_{T,G}] - G)).$$

Appendix B. Supplementary materials

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.jtbi.2008.03.004.

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