

A computational model predicts sex-specific responses to calcium channel blockers in mesenteric vascular smooth muscle

Reviewed Preprint

Revised by authors after peer review.

About eLife's process

Reviewed preprint version 2
December 20, 2023 (this version)

Reviewed preprint version 1
September 12, 2023

Sent for peer review
July 19, 2023

Posted to preprint server
June 26, 2023

Gonzalo Hernandez-Hernandez, Samantha C. O'Dwyer, Collin Matsumoto, Mindy Tieu, Zhihui Fong, Pei-Chi Yang, Timothy J. Lewis, L. Fernando Santana , Colleen E. Clancy

Department of Physiology & Membrane Biology, University of California School of Medicine, Davis, California, 95616 • Department of Mathematics, University of California, Davis, California, 95616 • Center for Precision Medicine and Data Science, University of California School of Medicine, Davis, California, 95616

 https://en.wikipedia.org/wiki/Open_access

 Copyright information

Abstract

The function of the smooth muscle cells lining the walls of systemic arteries and arterioles is to regulate the diameter of the vessels to control blood flow and blood pressure. Here, we describe an *in-silico* model, which we call the “Hernandez-Hernandez model”, of electrical and Ca^{2+} signaling in arterial myocytes based on new experimental data indicating sex-specific differences in male and female arterial myocytes from resistance arteries. The model suggests the fundamental ionic mechanisms underlying membrane potential and intracellular Ca^{2+} signaling during the development of myogenic tone in arterial blood vessels. Although experimental data suggest that $\text{K}_V1.5$ channel currents have similar amplitudes, kinetics, and voltage dependencies in male and female myocytes, simulations suggest that the $\text{K}_V1.5$ current is the dominant current regulating membrane potential in male myocytes. In female cells, which have larger $\text{K}_V2.1$ channel expression and longer time constants for activation than male myocytes, predictions from simulated female myocytes suggest that $\text{K}_V2.1$ plays a primary role in the control of membrane potential. Over the physiological range of membrane potentials, the gating of a small number of voltage-gated K^+ channels and L-type Ca^{2+} channels are predicted to drive sex-specific differences in intracellular Ca^{2+} and excitability. We also show that in an idealized computational model of a vessel, female arterial smooth muscle exhibits heightened sensitivity to commonly used Ca^{2+} channel blockers compared to male. In summary, we present a new model framework to investigate the potential sex-specific impact of anti-hypertensive drugs.

eLife assessment

The study is of importance for the cardiac modeling field by developing a novel mathematical model with sex difference. The data are **compelling**, and the model is helpful for mechanistic understanding, and thus is also **important** for experimental physiology. The model is based on experimental data and validated against some experimental data.

Introduction

Our primary objective was to develop and implement a novel computational model that comprehensively describes the essential mechanisms underlying electrical activity and Ca^{2+} dynamics in arterial myocytes. We aimed to uncover the key components necessary and sufficient to fully understand the behavior of arterial vascular smooth muscle myocytes and the cellular response to variations in pressure. The model represents the first-ever integration of sex-specific variations in voltage-gated $\text{K}_V2.1$ and $\text{Ca}_V1.2$ channels, enabling the prediction of sex-specific disparities in membrane potential and the regulation of Ca^{2+} signaling in smooth muscle cells from systemic arteries. To further investigate sex-specific responses to antihypertensive medications, we extended our investigation to include a one-dimensional (1D) representation of tissue. This approach enabled us to simulate and forecast the effects of Ca^{2+} channel blockers within the controlled environment of an idealized mesenteric vessel. It is worth noting that this computational framework can be expanded to predict the consequences of antihypertensive drugs and other perturbations, transitioning seamlessly from single-cell to tissue-level simulations.

Previous mathematical models^{1,2,3,4} of vascular smooth muscle myocytes generated to describe the membrane potential and Ca^{2+} signaling in vascular smooth muscle cells have described the activation of G-protein-coupled receptors (GPCRs) by endogenous or pharmacological vasoactive agents activating inositol 1,4,5-trisphosphate (IP_3) and ryanodine (RyR) receptors resulting in the initiation of calcium waves. Earlier models have also provided insights into the contraction activation by agonists and the behavior of vasomotion. In a major step forward, the Karlin model⁵ incorporated new cell structure data and electrophysiology experimental data in a computational model that predicted the essential behavior of membrane potential and Ca^{2+} signaling arising from intracellular domains found in arterial myocytes. One notable limitation of earlier models is that they are based entirely on data from male animals. Furthermore, many data used to parameterize the Karlin model were obtained from smooth muscle from cerebral arteries. While cerebral arteries are important for brain blood flow, they do not control systemic blood pressure. Furthermore, they do not take into consideration the role of $\text{K}_V2.1$ channels in the regulation of smooth muscle cell membrane potential.

The function of the smooth muscle cells that wrap around small arteries is to regulate the diameter of these vessels. Arterial myocytes contract in response to increases in intravascular pressure⁶. Based on work largely done using cerebral arterial smooth muscle, a model has been proposed in which this myogenic response is initiated when membrane stretch activates Na^+ -permeable canonical TRPC6^{7,8} and melastatin-type TRPM4^{9,10}. A recent study in smooth muscle from mesenteric arteries identified two additional TRP channels to the chain of events that link increases in intravascular pressure to arterial myocyte depolarization: TRPP1 (PKD1) and TRPP2 (PKD2) channels^{11,12}. Together, these studies point to an elaborate multiprotein complex that plays a critical role in sensing pressure and initiating the myogenic response by inducing membrane depolarization and activating voltage-gated, dihydropyridine-sensitive L-type $\text{Ca}_V1.2$ Ca^{2+} channels^{13,14}. Ca^{2+} entry via a single or small cluster of $\text{Ca}_V1.2$ channels produces a local increase in intracellular free Ca^{2+} ($[\text{Ca}^{2+}]_i$) called a “ $\text{Ca}_V1.2$ sparklet”^{15–18}. Activation of multiple $\text{Ca}_V1.2$ sparklets produces a global increase in $[\text{Ca}^{2+}]_i$ that activates myosin light chain kinase, which initiates actin-myosin cross-bridge cycling and thus contraction¹⁹.

Negative feedback regulation of membrane depolarization and Ca^{2+} sparklet activation occurs through the activation of large-conductance, Ca^{2+} -activated K^+ (BK_{Ca}) channels as well as voltage-dependent $\text{K}_V2.1$ and $\text{K}_V1.5/1.2$ K^+ channels^{20–23}. BK_{Ca} channels are organized into clusters along the sarcolemma of arterial myocytes²⁴ and are activated by Ca^{2+} sparks resulting from the simultaneous opening of ryanodine receptors type 2 (RyR2) located in a specialized junctional sarcoplasmic reticulum (SR)^{22,25–28}. Because the input resistance of arterial myocytes is

high^{29,30} (about 2-10 GΩ), even relatively small currents (10-30 pA) produced by the activation of a small cluster^{22,31,32} of 6-12 BK_{Ca} channels by a Ca²⁺ spark can transiently hyperpolarize the membrane potential of these cells by 10-30 mV. Accordingly, decreases in BK_{Ca}, K_V1.2, K_V1.5, and/or K_V2.1 channels depolarize arterial myocytes, increasing Ca_V1.2 channel activity, [Ca²⁺]_i, and contraction of arterial smooth muscle^{21,33-36}.

A recent study by O'Dwyer *et al.*²⁰ suggested that K_V2.1 channels have dual conducting and structural roles in mesenteric artery smooth muscle with opposing functional consequences. Conductive K_V2.1 channels oppose vasoconstriction by inducing membrane hyperpolarization. Paradoxically, by promoting the structural clustering of the Ca_V1.2 channel, K_V2.1 enhances Ca²⁺ influx and induces vasoconstriction. Interestingly, K_V2.1 protein is expressed to a larger extent in female than in male arterial smooth muscle. This induced larger Ca_V1.2 clusters and activity in female than in male arterial myocytes.

Here, we describe a new model, which we call the “Hernandez-Hernandez model”, of mesenteric smooth muscle myocytes that incorporates new electrophysiological and Ca²⁺ signaling data suggesting key sex-specific differences in male and female arterial myocytes. The model simulates membrane currents and their impact on membrane potential as well as local and global [Ca²⁺]_i signaling in male and female myocytes. The Hernandez-Hernandez model predicts that K_V2.1 channels play a critical, unexpectedly large role in the control of membrane potential in female myocytes compared to male myocytes. Importantly, our model predicts that clinically used antihypertensive Ca_V1.2 channel blockers cause larger reductions in Ca_V1.2 currents in female than in male arterial myocytes.

Finally, we present a one-dimensional (1D) vessel representation of electrotonically coupled arterial myocytes connected in series. Predictions from the idealized vessel suggest that Ca²⁺ channel blockers are more potent in females resulting in a more substantial [Ca²⁺]_i reduction in female arterial smooth muscle compared to male. The Hernandez-Hernandez model demonstrates the importance of sex-specific differences in Ca_V1.2 and K_V2.1 channels and suggests the fundamental electrophysiological and Ca²⁺ linked mechanisms of the myogenic tone. The model also points to testable hypotheses underlying differential sex-based pathogenesis of hypertension and distinct responses to antihypertensive agents.

Methods

Section 1. Experimental

1.1 Animals

8- to 12-week-old male and female mice C57BL/6J (The Jackson Laboratory, Sacramento, CA) were used in this study. Animals were euthanized with a single lethal dose of sodium pentobarbital (250 mg/kg) intraperitoneally. All experiments were conducted as approved by the Institutional Animal Care and Use Committee.

1.2 Isolation of arterial myocytes from systemic resistance arterioles

Third and fourth-order mesenteric arteries were carefully cleaned of surrounding adipose and connective tissues, dissected, and held in ice-cold dissecting solution (Mg²⁺-PSS; 5 mM KCl, 140 mM NaCl, 2mM MgCl₂, 10 mM glucose, and 10 mM HEPES adjusted to pH 7.4 with NaOH). Arteries were first placed in dissecting solution supplemented with 1.23 mg/ml papain (Worthington Biochemical, Lakewood, NJ) and 1 mg/ml DTT for 14 minutes at 37° C. This was followed by a second 5-minute incubation in dissecting solution supplemented with 1.6 mg/ml collagenase H (Sigma-Aldrich, St. Louis, MO), 0.5 mg/ml elastase (Worthington Biochemical, Lakewood, NJ), and 1

mg/ml trypsin inhibitor from *Glycine max* (Sigma-Aldrich, St. Louis, MO) at 37° C. Arteries were rinsed three times with dissection solution and single cells were obtained by gentle trituration with a wide-bore glass pipette. Myocytes were maintained at 4° C in dissecting solution until used.

1.3 Patch-clamp electrophysiology

All electrophysiological recordings were acquired at room temperature (22–25°C) with an Axopatch 200B amplifier and Digidata 1440 digitizer (Molecular Devices, Sunnyvale, CA). Borosilicate patch pipettes were pulled and polished to resistances of 3–6 MΩ for all experiments using a micropipette puller (model P-97, Sutter Instruments, Novato, CA).

Voltage-gated Ca^{2+} currents (I_{Ca}) were measured using conventional whole-cell voltage-clamp sampled at a frequency of 50 kHz and low-pass filtered at 2 kHz. Arterial myocytes were continuously perfused with 115 mM NaCl, 10 mM TEA-Cl, 0.5 mM MgCl_2 , 5.5 mM glucose, 5 mM CsCl, 20 mM CaCl_2 , and 10 mM HEPES, adjusted to pH 7.4 with CsOH. Micropipettes were filled with an internal solution containing 20 mM CsCl, 87 mM Aspartic acid, 1 mM MgCl_2 , 10 mM HEPES, 5 mM MgATP, and 10 mM EGTA adjusted to pH 7.2 using CsOH. Current-voltage relationships were obtained by exposing cells to a series of 300 ms depolarizing pulses from a holding potential of -70 mV to test potentials ranging from -70 to +60 mV. A voltage error of 9.4 mV due to the liquid junction potential of the recording solutions was corrected offline. Voltage dependence of Ca^{2+} channel activation (G/G_{max}) was obtained from the resultant currents by converting them to conductance via the equation $G = I_{\text{Ca}}/(\text{test potential} - \text{reversal potential of } I_{\text{Ca}})$; normalized G/G_{max} was plotted as a function of test potential. Time constants of activation and inactivation of I_{Ca} were fitted with a single exponential function.

I_{Kv} recordings were performed in the whole-cell configuration with myocytes exposed to an external solution containing 130 mM NaCl, 5 mM KCl, 3 mM MgCl_2 , 10 mM Glucose, and 10 mM HEPES adjusted to 7.4 using NaOH. The internal pipette solution constituted of 87 mM K-Aspartate, 20 mM KCl, 1 mM CaCl_2 , 1 mM MgCl_2 , 5 mM MgATP, 10 mM EGTA, and 10 mM HEPES adjusted to 7.2 by KOH. A resultant liquid junction potential of 12.7 mV from these solutions was corrected offline. To obtain current-voltage relationships cells were subjected to a series of 500 ms test pulses increasing from -70 to +70 mV. To isolate the different K^+ channels attributed to composite I_{K} , cells were first bathed in external I_{K} solution, subsequently exposed to 100 nM Iberitoxin (Alomone, Jerusalem, Israel) to eliminate any BK_{Ca} channel activity and finally immersed in an external solution containing both 100 nM Iberitoxin and 100 nM Stromatoxin (Alomone, Jerusalem, Israel) to block both BK_{Ca} and $\text{K}_{\text{v}2.1}$ activity. Ionic current was converted to conductance via the equation $G = I(V - E_{\text{K}})$. E_{K} was calculated to be -78 mV. Activation time constants for $\text{K}_{\text{v}2.1}$ currents were obtained by fitting the rising phase of these currents with a single exponential function.

BK_{Ca} -mediated spontaneous transient outward currents (STOCs) and membrane potential were recorded using the perforated whole-cell configuration. To measure both, myocytes were continuously exposed to a bath solution consisting of 130 mM NaCl, 5 mM KCl, 2 mM CaCl_2 , 1 mM MgCl_2 , 10 mM glucose, and 10 mM HEPES, pH adjusted to 7.4 with NaOH. Pipettes were filled with an internal solution containing 110 mM K-aspartate, 30 mM KCl, 10 mM NaCl, 1 mM MgCl_2 , 0.5 mM EGTA, and 10 mM HEPES adjusted to a pH of 7.3 with KOH. The internal solution was supplemented with 250 µg/ml amphotericin B (Sigma, St. Louis, MO). STOCs were measured in the voltage-clamp mode and were analyzed with the threshold detection algorithm in Clampfit 10 (Axon Instruments, Inc). Membrane potential was measured using the current clamp mode.

STOCs were recorded using the perforated whole-cell configuration. The composition of the external bath solution consisted of 134 mM NaCl, 6 mM KCl, 1 mM MgCl_2 , 2 mM CaCl_2 , 10 mM glucose, and 10 mM HEPES adjusted to a pH of 7.4 using NaOH. Pipettes were filled with an internal solution of 110 mM K-aspartate, 10 mM NaCl, 30 mM KCl, 1 mM MgCl_2 , 160 µg/ml

amphotericin B, and 10 mM HEPES using NaOH to adjust to pH to 7.2. Myocytes were sustained at a holding potential of -70 mV before being exposed to a 400 ms ramp protocol from -140 to +60 mV. A voltage error of 12.8 mV resulting from the liquid junction potential was corrected for offline. K_{IR} channels were blocked using 100 μ M Ba^{2+} .

1.4 Statistics

Data are expressed as mean $\pm$ SEM. All data sets were tested for normality. Normally distributed data were analyzed using T-tests or one-way analysis of variance (ANOVA). ANOVA analyses were followed by multiple comparison tests (i.e. Tuckey). $P < 0.05$ was considered statistically significant.

Section 2. Computational modeling and simulation

2.1 Cell size and structure

The mean capacitance of the cells was experimentally calculated to be 16 ± 3 pF based on all the male and female WT mesenteric C57BL/6J cells utilized in the experiments ($N=45$). Assuming the cells are roughly cylindrical in shape, the expected radius should be 2.485μ m and a length of 100μ m leading to a surface area of $1.6 \times 10^{-5} \text{ cm}^2$ and a total volume of approximately 1.94×10^{-12} liters. The cell capacitance of excitable membranes is assumed to be $1.0 \times 10^{-6} \text{ F/cm}^2$, with the calculated surface area the estimated total cell capacitance is $C_m = 16$ pF.

Because the total cell volume is roughly 2×10^{-12} liters, it is assumed that 50% of the total cell volume is occupied by organelles. There are three main compartments in vascular myocytes important to the regulation of membrane potential and calcium signaling: the cytosol, sarcoplasmic reticulum (SR), and specialized junctional domains formed by the SR and the plasma membrane. The cytosol occupies approximately 50% of total cell volume ($V_{\text{cyt}} = 1.0 \times 10^{-12} \text{ L}$). The sarcoplasmic reticulum occupies approximately 5% of cell cytosolic volume ($V_{\text{SR}} = 5.0 \times 10^{-14} \text{ L}$), and the junctional domain volume is approximately 1% of the cytosol volume ($V_{\text{Jun}} = 0.5 \times 10^{-14} \text{ L}$).

2.2 Model development

The male and female *in silico* models are single whole-cell models based on the electrophysiology of isolated mesenteric vascular smooth muscle myocytes. A schematic of the proposed model is shown in **Figure 1**. The membrane electrophysiology can be described by the differential equation:

$$\text{Eq. 1} \quad \frac{dV}{dt} = \frac{-I_{\text{ion}}}{C_m}$$

Where V is voltage, t is time, C_m is membrane capacitance I_{ion} is the sum of transmembrane currents. The contribution of each transmembrane current to the total transmembrane ionic current can be described by the following equation:

$$\text{Eq 2.} \quad I_{\text{ion}} = (I_{Kv1.5} + I_{Kv2.1} + I_{BKCa} + I_{K,b} + I_{Cav1.2} + I_{PMCA} + I_{Ca,b} + I_{NCX} + I_{NSC} + I_{NaK} + I_{Na,b})$$

The eleven transmembrane currents are generated by ion channels, pumps, and transporters. Currents from ion channels include the voltage-gated L-type calcium current (I_{Ca}), the nonselective cation current (I_{NSC}), voltage-gated potassium currents ($I_{Kv1.5}$ and $I_{Kv2.1}$), and the large-conductance Ca^{2+} -sensitive potassium current (I_{BKCa}). Additionally, there are three background or leak currents ($I_{K,b}$, $I_{Ca,b}$, and $I_{Na,b}$). Currents from pumps and transporters include the sodium-potassium pump current (I_{NaK}), plasma membrane Ca-ATPase transport current (I_{PMCA}), and sodium-calcium exchanger current (I_{NCX}).

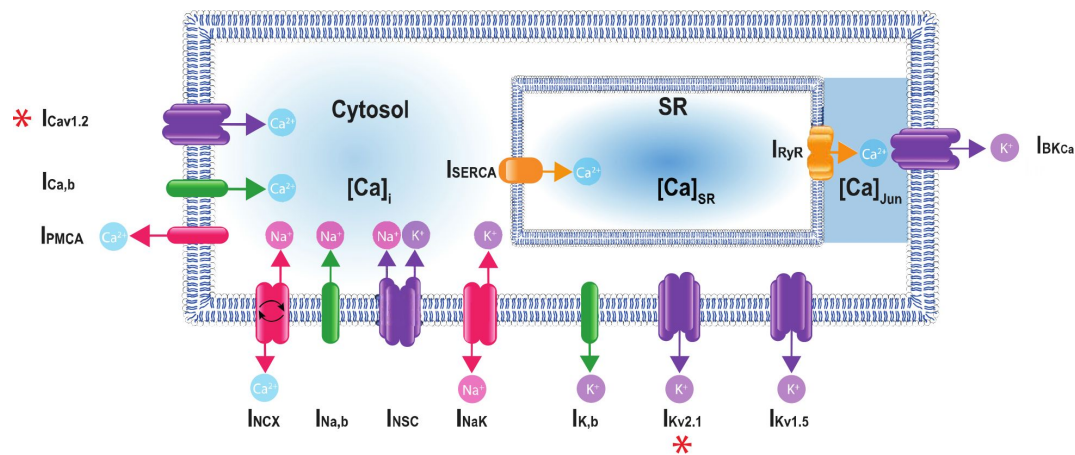


Figure 1.

A schematic representation of the Hernandez-Hernandez model.

The components of the model include major ion channel currents shown in purple including the voltage-gated L-type calcium current (I_{Ca}), nonselective cation current (I_{NSC}), voltage-gated potassium currents ($I_{Kv1.5}$ and $I_{Kv2.1}$), and the large-conductance Ca^{2+} -sensitive potassium current (I_{BKCa}). Currents from pumps and transporters are shown in red including the sodium/potassium pump current (I_{NaK}), sodium/calcium exchanger current (I_{NCX}), and plasma membrane ATPase current (I_{PMCA}). Leak currents are indicated in green including the sodium leak current ($I_{Na,b}$), potassium leak current ($I_{K,b}$), and calcium leak current ($I_{Ca,b}$). In addition, two currents in the sarcoplasmic reticulum are shown in orange: the sarcoplasmic reticulum Ca-ATPase current (I_{SERCA}) and ryanodine receptor current (I_{RyR}). Calcium compartments comprise three discrete regions including cytosol ($[Ca]_i$), sarcoplasmic reticulum ($[Ca]_{SR}$), and the junctional region ($[Ca]_{Jun}$). Red stars (*) indicate measured sex-specific differences in ionic currents.

Cytosolic concentrations of sodium and potassium as a function of time are determined by considering the sum of their respective fluxes into the cytosol.

$$\text{Eq. 3} \quad \frac{d[K^+]_{\text{cyt}}}{dt} = - \frac{(I_{Kv2.1} + I_{Kv1.5} + I_{BKCa} + I_{K,b} - 2I_{NaK} + I_{NSC-K})}{Z_K \text{Vol}_{\text{cyt}} F}$$

$$\text{Eq. 4} \quad \frac{d[Na^+]_{\text{cyt}}}{dt} = - \frac{(3I_{NCX} + 3I_{NaK} + I_{Na,b} + I_{NSC-Na})}{Z_{Na} \text{Vol}_{\text{cyt}} F}$$

Where F is the Faraday's constant, Vol_{cyt} is the cytoplasmic volume and Z_K and Z_{Na} are the valence of potassium and sodium ions, respectively.

The calcium dynamics is compartmentalized into three distinct regions: cytosol $[Ca^{2+}]_i$, the sarcoplasmic reticulum $[Ca^{2+}]_{SR}$, and the junctional region $[Ca^{2+}]_{jun}$. The cytosol includes a calcium buffer, which we assume can be described as a first-order dynamics process.

$$\text{Eq. 5} \quad \frac{d[Ca^{2+}]_i}{dt} = - \frac{(I_{NCX} + I_{CaV1.2} + I_{Ca,b} - 2I_{PMCA})}{Z_{Ca} F \text{Vol}_{\text{cyt}}} - J_{SERCA} + J_{jun-cyt} - (k_{BUFon} * C_{a_i} * (BUF_T - BUF_{CAM}) - k_{BUFOff} * BUF_{CAM})$$

Cytosolic calcium region ($[Ca^{2+}]_i$): calcium concentration in this region varies between 100-300 nM¹³ and is mainly influenced by the following fluxes: transmembrane pumps and transporters, the sarcoplasmic reticulum Ca-ATPase (J_{SERCA}), diffusion from the junctional domain region ($J_{jun-cyt}$) and the calcium buffer calmodulin (BUF_{CAM}).

$$\text{Eq. 6} \quad \frac{d[Ca]_{SR}}{dt} = \left[\frac{\text{Vol}_{\text{cyt}}}{\text{Vol}_{SR}} \right] J_{SERCA} - \left[\frac{\text{Vol}_{\text{cyt}}}{\text{Vol}_{SR}} \right] J_{RyR}$$

Sarcoplasmic reticulum region ($[Ca^{2+}]_{SR}$): calcium concentration in this region varies between 100-150 μM ⁴⁰ and it is mainly influenced by the sarcoplasmic reticulum Ca-ATPase (J_{SERCA}) and the flux from the ryanodine receptors (J_{RyR}).

$$\text{Eq. 7} \quad \frac{d[Ca]_{jun}}{dt} = \left[\frac{\text{Vol}_{\text{cyt}}}{\text{Vol}_{jun}} \right] J_{RyR} - \left[\frac{\text{Vol}_{\text{cyt}}}{\text{Vol}_{jun}} \right] J_{jun-cyt}$$

Junctional region ($[Ca^{2+}]_{jun}$): calcium concentration in this region varies between 10-100 μM ^{37,38} and is mainly influenced by the flux from the ryanodine receptors (J_{RyR}), the diffusion from the junctional region to the cytoplasm ($J_{jun-cyt}$)

In the model, the flux of J_{SERCA} was adapted from the Luo-Rudy II model⁴¹ and the flux of J_{RyR} was adapted from previous models of ryanodine receptors activation, originally introduced in the field of cardiac electrophysiology⁴²⁻⁴⁴.

2.3 Parameter optimization and reformulation of the gating ion channel models

The ionic current models of I_{Ca} , $I_{Kv2.1}$, and $I_{Kv1.5}$ were optimized using the approach employed by Kernik *et al.*⁴⁵ (S1-S6). Here, the open probability P_o of each voltage-dependent gating variable “n” was defined by opening- and closing-rate voltage-dependent functions α_n and β_n respectively, and were modeled by simple exponentials of the form:

$$(\text{Eq. 8}) \quad \alpha_n(V) = x_1 e^{\left(\frac{V}{x_2}\right)}$$

$$(\text{Eq. 9}) \quad \beta_n(V) = x_3 e^{\left(\frac{V}{x_4}\right)}$$

$$(\text{Eq. 10}) \quad \tau_n(V) = \frac{1}{\alpha_n(V) + \beta_n(V)} + x_5$$

The steady-state availability remains the same as the classical Hodgkin-Huxley formulations and the time constant values follow a modified version formulation by accommodating an extra parameter x_5 (Eq. 10). (x_1, x_2, x_3, x_4, x_5) are parameters to be optimized using experimental

data. We used the parameter optimization employed by Kernik *et al.*⁴⁵, which minimizes the error between model and experimental data using the Nelder–Mead minimization of the error function. Random small perturbations (<10%) were applied to find local minima, to improve data fit. The parameter fit with the minimal error function value after 1000 to 10000 perturbations was used as the optimal model fit to the data.

2.4 Cellular simulations with noise

The simulations encapsulate the cumulative effect of stochastic ion channel activity on cell voltage dynamics through the fluctuating current term, $\xi(t)$, into the membrane potential (dV/dt) equation⁴⁶, as shown in Eq. 11. Here it is assumed $\xi(t)$ is only a function of time and it is implemented as Gaussian white noise⁴⁷.

$$(Eq. 11) \quad \frac{dV}{dt} = -\frac{I_{total}(V)}{C_m} + \sigma \xi(t)$$

We use the Euler-Maruyama numerical method for updating Eq. 12 as follows.

$$(Eq. 12) \quad V(t + \Delta t) = V(t) - \frac{I(V(t))}{C_m} \Delta t + \sigma * \text{randN} * \sqrt{\Delta t}$$

$$(Eq. 12b) \quad \frac{d[Ca]_{SR}}{dt} = \left[\frac{Vol_{Cyt}}{Vol_{SR}} \right] J_{SERCA} - \left[\frac{Vol_{Cyt}}{Vol_{SR}} \right] J_{RyR} + \sigma * \text{randN} * \sqrt{\Delta t}$$

Where randN is a random number from a normal distribution (N(0,1)) with mean 0 and variance 1. Δt is the time step and σ is the “diffusion coefficient”, which represents the amplitude of the noise. The numerical method for updating the voltage was forward Euler.

2.5 One-dimensional simulations

The idealized one-dimensional representation of a vessel was developed by connecting 400 Hernandez-Hernandez model cells in series via simulated resistances to represent gap junctions. For each cell in the cable, the Hernandez-Hernandez model was used to compute ionic currents and concentration changes. The temporal transmembrane fluxes of the Hernandez-Hernandez model are related to the spatial or current flow by a finite difference approximation of the cable equation^{48–50}.

$$(Eq. 14) \quad \left[C_m \left(\frac{V_i^{(t+1)} - V_i^t}{\Delta t} \right) + I_{ion} + I_{stim} \right] = \frac{a}{4(R_{myo} \frac{R_g}{\Delta x})} \frac{(V_{i-1}^t - 2V_i^t + V_{i+1}^t)}{\Delta x \Delta x}$$

where I_{ion} represents the individual membrane ionic current densities (pA/pF) of the Hernandez-Hernandez model, I_{stim} is the stimulus current density (pA/pF) set to zero in our simulation, a is the radius of the fiber (5 μm), C_m is the membrane capacity (pA/pF), V_i^t is membrane potential at segment i and time t , Δx is the discretization element (100 μm = 0.01 cm). Where R_{myo} is the myoplasmic resistance (R_{myo} =150 Ωcm) and R_g is the gap junction resistance (R_g =71.4 Ωcm^2).

2.6 Sensitivity Analysis

The baseline models in male and female vascular smooth muscle cells were analyzed through a parameter sensitivity assessment using multivariable linear regression, following the methodology introduced by Sobie⁵¹. The scope of the sensitivity analysis encompassed variations in the maximal conductance and maximal ion transport rates of the transmembrane currents, including $I_{Kv1.5}$, $I_{Kv2.1}$, I_{BKCa} , $I_{K,b}$, $I_{Cav1.2}$, I_{PMCA} , $I_{Ca,b}$, I_{NCX} , I_{NSC} , I_{NaK} , and $I_{Na,b}$. All other parameters, notably those defining model kinetics, remained constant at the values established by the foundational model. Scaling factors were randomly selected from a log-normal distribution characterized by a median value of 1 and a standard deviation of 0.1⁴⁵.

2.7 Simulation protocols

Code for simulations and analysis was written in C++ and MATLAB 2018a. The single vascular smooth muscle code was run on an Apple Mac Pro machine with two 2.7 GHz 12-Core Intel Xeon processors and an HP ProLiant DL585 G7 server with a 2.7 GHz 28-core AMD Opteron processor. Vessel simulations were implemented in C++ and parallelized using OpenMP. The C++ code was compiled with the Intel ICC compiler, version 18.0.3. Numerical results were visualized using MATLAB R2018a by The MathWorks, Inc. All codes and detailed model equations are available on GitHub (<https://github.com/ClancyLabUCD/sex-specific-responses-to-calcium-channel-blockers-in-mesenteric-vascular-smooth-muscle>).

Results

In this study, we developed a computational model of the electrical activity of an isolated vascular smooth muscle cell (**Figure 1**). A key goal was to optimize and validate the model with experimental data and then use the model to predict the effects of measured sex-dependent differences in the electrophysiology of smooth muscle myocytes.

In constructing the model, we first set out to measure the kinetics of the voltage-gated L-type $\text{Ca}_v1.2$ currents (I_{Ca}) in male and female myocytes using Ca^{2+} as the charge carrier as shown in **Figure 2**. These data provided information on the kinetics of Ca^{2+} -dependent activation and inactivation of I_{Ca} . I_{Ca} is critical in determining cytosolic concentration $[\text{Ca}^{2+}]_i$ in vascular mesenteric smooth muscle cells and is the predominant pathway for Ca^{2+} entry^{13,15,16,18,28,52}. Experiments using whole-cell patch-clamp were undertaken to measure the time constants of activation and deactivation (**panel 2A**) and inactivation (**panel 2B**) in male and female mesenteric artery smooth muscle cells shown as black and blue symbols, respectively. While the data from male ($n = 10$) and female ($n = 12$) myocytes showed comparable activation time constants, there was an observable trend of faster inactivation in the female cells in the lower voltage range, but the differences were not statistically significant. Steady-state activation and inactivation were also measured as shown in **panel 2C**, with male data in black symbols and female as blue symbols. No observable differences in the gating characteristics of the male and female I_{Ca} were measured. Finally, the current-voltage relationship is shown from measurements in female (blue) and male (black) in **panel 2D**. This analysis suggests that the amplitude of I_{Ca} was larger in female than in male myocytes over a wide range of membrane potentials.

We next used the experimental measurements to build and optimize a Hodgkin-Huxley model based on the data described above. The model includes voltage-dependent activation and inactivation gating variables, dL and dF , respectively. We modeled both gates following the approach by Kernik *et al.*⁴⁵. It is important to note that smooth muscle cells operate within a voltage regime defined by the window current, which ranges between -45 mV and -20 mV. Under these conditions, $[\text{Ca}^{2+}]_i$ remains below 1 μM . Therefore, we did not consider the Ca^{2+} -dependent inactivation gating mode of the channel^{2,53}.

The model of I_{Ca} is described by:

$$(\text{Eq. 15}) \quad I_{\text{Ca}} = P_{\text{Ca}} * dL * dF * \frac{z_{\text{Ca}}^2 F^2 V}{RT} \left(\frac{[\text{Ca}]_i e^{\frac{z_{\text{Ca}} F V}{RT}} - [\text{Ca}]_{\text{out}}}{e^{\frac{z_{\text{Ca}} F V}{RT}} - 1} \right)$$

Where P_{Ca} is the ion permeability, R is the gas constant, F is the Faraday's constant, and z_{Ca} is the valence of the Ca^{2+} ion. Parameters were optimized to male and female experimental data as shown for activation time constants ($T_{\text{activation}}$) and inactivation time constants ($T_{\text{inactivation}}$) as

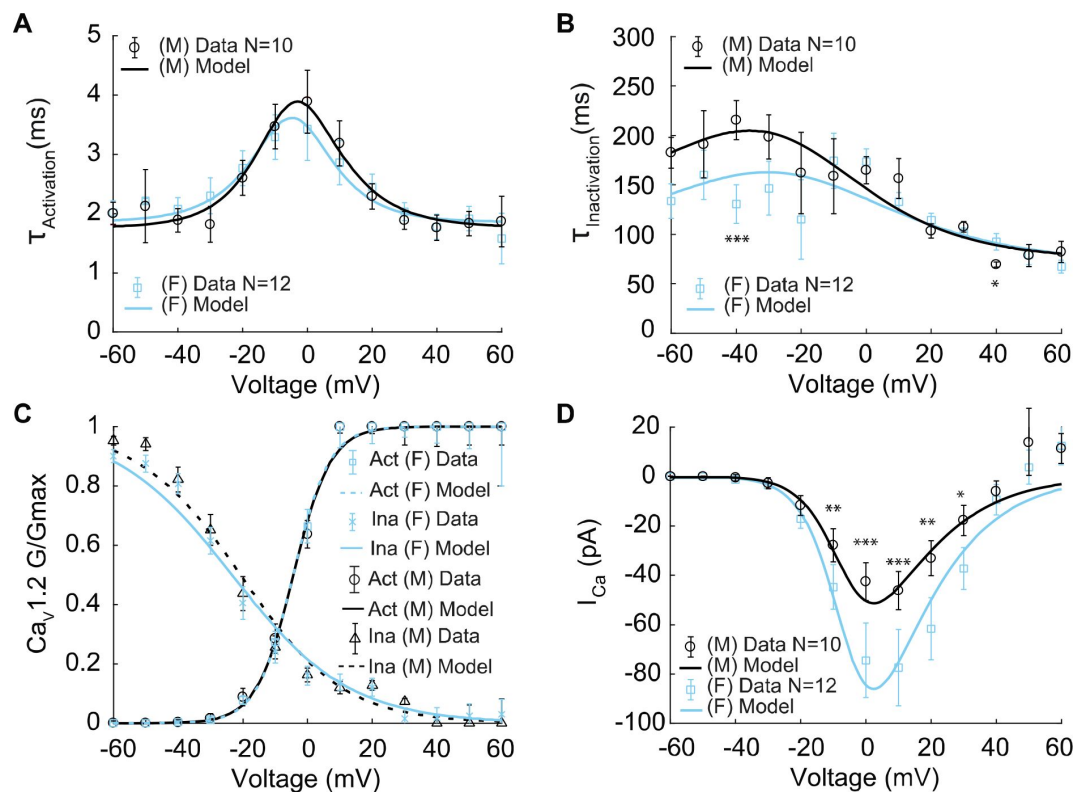


Figure 2.

Experimentally measured and modeled L-type calcium currents (I_{Ca}) from male and female vascular smooth muscle cells.

Properties of I_{Ca} are derived from measurements in male and female vascular smooth muscle (VSM) cells isolated from the mouse mesenteric arteries following voltage-clamp steps from -60 to 60 mV in 10 mV steps from a -80 mV holding potential. Experimental data is shown in black circles for male and blue squares for female. Model fits to experimental data are shown with black solid lines for male and blue solid lines for female. **(A)** Male and female time constants of I_{Ca} activation. **(B)** Male and female time constants of I_{Ca} inactivation. **(C)** Male and female voltage-dependent steady-state activation and inactivation of I_{Ca} . **(D)** Current-voltage (I-V) relationship of I_{Ca} from male and female vascular smooth muscle myocytes. *P < 0.05, **P < 0.01, ***P < 0.001. Error bars indicated mean $\pm$ SEM.

solid lines in **Figure 2A** and **Figure 2B**, respectively. Model optimization to male and female activation and inactivation curves are shown in **Figure 2C**. The model was also optimized to the I_{Ca} current-voltage (I-V) relationships shown as solid lines in **Figure 2D**.

We next set out to determine sex differences in voltage-gated K^+ currents (I_K) in male and female mesenteric smooth muscle cells. I_K is produced by the combined activation of K_V and BK_{Ca} channels. Following the approach previously published by O'Dwyer *et al.*,²⁰ we quantified the contribution of K_V (I_{KV}) and BK_{Ca} (I_{BKCa}) current to I_K . K^+ currents were recorded before and after the application of the channel blocker iberiotoxin (IBTX; 100nM). Once identified the contribution I_{BKCa} current, we isolated the voltage-gated potassium currents (I_{KV}) whose contributors include the voltage-gated potassium channels $K_V1.5$ and $K_V2.1$. The presumed function of $K_V1.5$ and $K_V2.1$ channels on membrane potential is to produce delayed rectifier currents to counterbalance the effect of the inward currents^{19,20}.

Having isolated I_{KV} , $K_V2.1$ currents were identified using the application of the $K_V2.1$ blocker ScTx1 (100 nM). As a result, the remaining ScTx1-insensitive component of the I_{KV} current was attributed to $K_V1.5$ channels. The results are shown in **Figure 3**. Experiments using whole-cell patch-clamp were undertaken to measure the steady-state activation G/G_{max} of the $K_V2.1$ current ($I_{KV2.1}$) as shown in **panel 3A** in female (blue) and male (black) myocytes and no significant differences were observed. Measurements of time constants of activation (**panel 3B**) of $I_{KV2.1}$ in the voltage range of -30 to +40 mV in female (blue, n=10) and male (black, n=7) myocytes exhibited significant differences. Notably, activation time constants in male myocytes were smaller than those in female myocytes, corresponding to a faster activation rate in males. The current-voltage relationship of $I_{KV2.1}$ is shown from measurements in female (blue, n=20) and male (black, n=10) myocytes in **panel 3C**. Significant differences were observed in $I_{KV2.1}$ at various voltages. In **panel 3C**, data points without asterisks are not considered significant. Similarly, we measured the steady-state activation of the $K_V1.5$ current ($I_{KV1.5}$) as shown in **panel 3D** where male and female experimental data in myocytes are shown with blue and black symbols. Properties of $I_{KV1.5}$ steady-state activation G/G_{max} show minimal sex-specific differences. The current-voltage relationship of $I_{KV1.5}$ is shown from measurements in female (blue, n=10) and male (black, n=7) myocytes in **panel 3E**. Finally, the current-voltage relationship of the contribution from $I_{KV1.5}$ and $I_{KV2.1}$ to the total voltage-gated current (I_{KVTOT}) is shown in **panel 3F** with male and female data shown with black and blue symbols, respectively. Data points in **panel D**, **panel E**, and **panel F** without asterisks are not significant. The table in **panel 3H** summarizes the sex-dependent maximal conductance and the current response at specific voltages of -50 mV, -40 mV, -30 mV, and -20 mV for both $I_{KV1.5}$ and $I_{KV2.1}$.

To understand the contribution of each K^+ current to the total voltage-gated current (I_{KVTOT}) in mesenteric vascular smooth muscle cells we built and optimized a Hodgkin-Huxley model to the data described above. First, we developed a model to describe the $K_V2.1$ current. The optimized model to $K_V2.1$ experimental data contains only a voltage-dependent activation gating variable ($X_{2.1act}$). Since inactivation time is slow and is well estimated by steady-state³, we did not consider its effects in our model. The model of $I_{KV2.1}$ is described by:

$$(Eq. 16) \quad I_{KV2.1} = G_{KV2.1} * X_{KV2.1act} * (V - E_K)$$

Where $G_{KV2.1}$ is the maximal conductance of $K_V2.1$ channels and E_K is the Nernst potential for potassium. Parameters were optimized to male and female experimental data as shown for activation curves in **Figure 3A**. Model optimization to male and female time constants of activation ($K_V2.1 \tau_{Activation}$) are shown as solid lines in **Figure 3B**. The model was also optimized to the $I_{KV2.1}$ current-voltage (I-V) relationships shown as solid lines in **Figure 3C**.

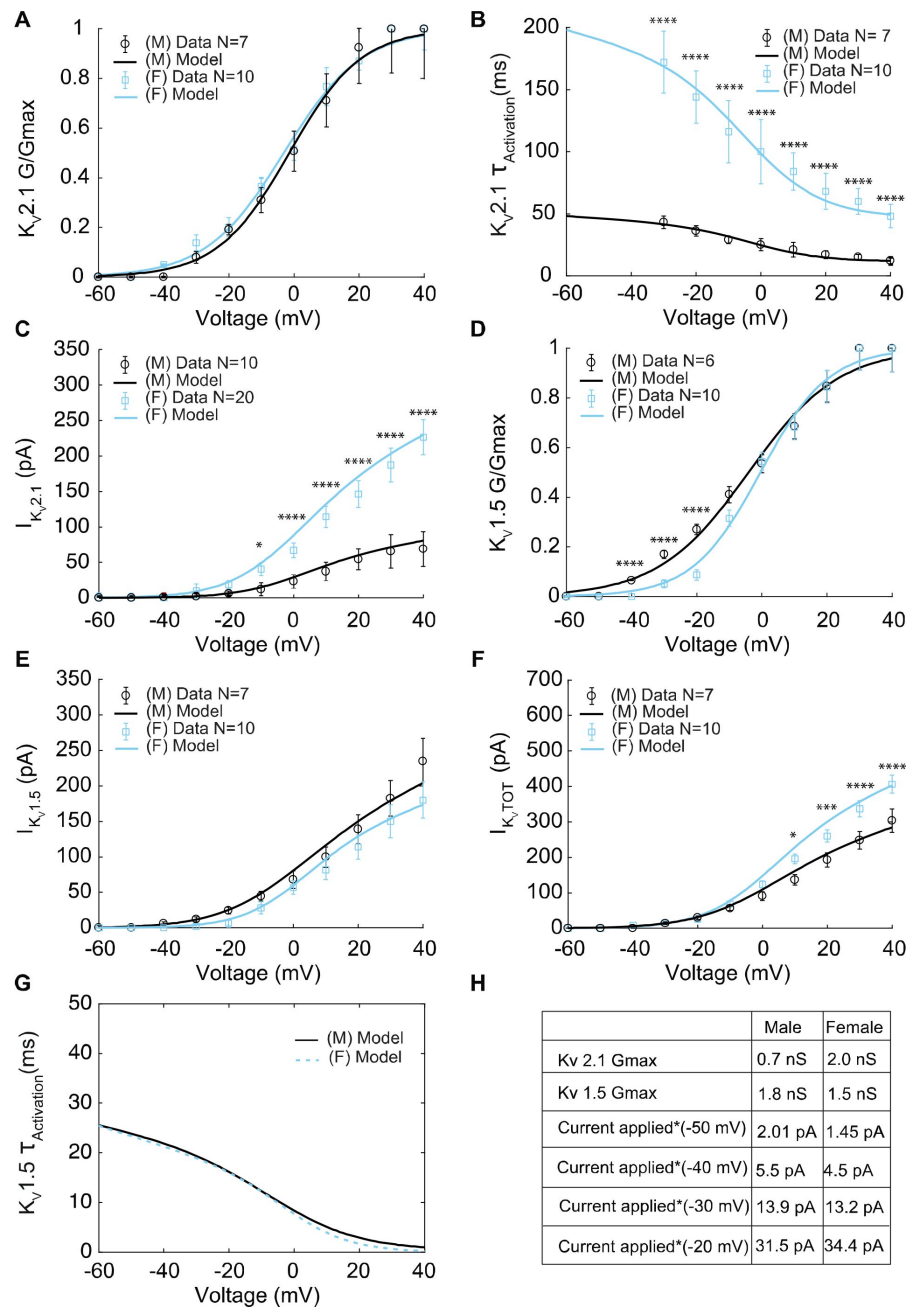


Figure 3.

Experimentally measured and modeled potassium currents (I_{KvTOT}) from male and female vascular smooth muscle cells.

The properties of $I_{Kv1.5}$ and $I_{Kv2.1}$ from experimental measurements in male and female vascular smooth muscle cells isolated from the mouse mesenteric arteries were recorded in response to voltage-clamp from -60 to 40 mV in 10 mV steps (holding potential -80mV). Experimental data is shown as black circles for male and blue squares for female. Model fits to experimental data are shown with black solid lines for male and blue solid lines for female. (A) Male and female voltage-dependent steady-state activation of $I_{Kv2.1}$. (B) Male and female time constants of $I_{Kv2.1}$ activation. (C) Current-voltage (I-V) relationship of $I_{Kv2.1}$ from male and female myocytes. (D) Male and female voltage-dependent steady-state activation of $I_{Kv1.5}$. (E) Current-voltage (I-V) relationship of $I_{Kv1.5}$ from male and female myocytes. (F) Male and female total voltage-gated potassium current $I_{KvTOT} = I_{Kv1.5} + I_{Kv2.1}$. (G) Predicted male and female time constants of the $I_{Kv1.5}$ activation gate. (H) Table showing sex-specific differences in conductance and steady-state total potassium current-voltage dependence. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$. Data points without asterisks are not significant. Error bars indicated mean $\pm$ SEM.

Similarly, we developed a model for $K_{V1.5}$. The model was optimized to the $K_{V1.5}$ experimental data and contains only a voltage-dependent activation gating variable ($X_{K_{V1.5}act}$). The model of $I_{K_{V1.5}}$ is described by:

$$(Eq. 17) \quad I_{K_{V1.5}} = G_{K_{V1.5}} * X_{K_{V1.5}act} * (V - E_K)$$

$G_{K_{V1.5}}$ is the maximal conductance of $K_{V1.5}$ channels and E_K is the Nernst potential for potassium. Parameters were optimized to male and female experimental data as shown for activation curves in **Figure 3D**. The model was also optimized to the $I_{K_{V1.5}}$ current-voltage (I-V) relationships shown as solid lines in **Figure 3E**. From experiments, we optimized the model to reproduce the overall time traces of K_V currents. The model predicted that male and female myocytes have comparable time constants of activation in $I_{K_{V1.5}}$ as shown in **Figure 3G**. Finally, the optimized model of the total voltage-gated current ($I_{K_{V}TOT}$) is shown in **Figure 3F**. The total voltage-gated K^+ current ($I_{K_{V}TOT}$) is the sum of $I_{K_{V1.5}}$ and $I_{K_{V2.1}}$ mathematically described as:

$$(Eq. 18) \quad I_{K_{V}TOT} = I_{K_{V2.1}} + I_{K_{V1.5}}$$

Notably, the main specific sex-specific differences observed in the total voltage-gated K^+ current ($I_{K_{V}TOT}$) is attributable to the sex-specific differences in the current produced by $K_{V2.1}$ channels.

We next analyzed the contribution of large-conductance calcium-activated potassium (BK_{Ca}) channels to vascular smooth muscle cell electrophysiology. BK_{Ca} channels are activated by membrane depolarization or increased $[Ca^{2+}]_i$ and are expressed in the membrane of vascular smooth muscle cells with α and $\beta 1$ subunits^{22,54,55}. In smooth muscle cells, Ca^{2+} sparks are the physiological activators of BK_{Ca} channels. We relied on the assumption by Tong *et al.*⁵⁶ that BK_{Ca} currents ($I_{BK_{Ca}}$) are produced by two current subtypes, one consisting of α subunits ($I_{BK_{\alpha}}$) and the other consisting of α and $\beta 1$ subunits ($I_{BK_{\alpha\beta 1}}$). Experimental evidence indicates that BK_{Ca} channels with $\alpha\beta 1$ subunits form clusters in the plasma membrane in specialized junctional domains formed by the sarcoplasmic reticulum and the sarcolemma. BK_{Ca} channels with $\alpha\beta 1$ subunits colocalize with ryanodine receptors (RyRs) to in the junctional domains. During a Ca^{2+} spark, $[Ca^{2+}]_i$ elevations ranging from 10 to 100 μM activate BK_{Ca} channels^{38,39,52,57,58}. In our model, Ca^{2+} sparks are the physiological activators of BK_{Ca} channels.

The mathematical formulation of the BK_{Ca} with $\alpha\beta 1$ current ($I_{BK_{\alpha\beta 1}}$) was optimized to fit the experimental whole-cell electrophysiological data from Bao and Cox⁵⁴ obtained at room temperature with a BK_{Ca} channel α subunit clone from mSlo-mbr5 and a $\beta 1$ subunit clone from bovine expressed in *Xenopus laevis* oocytes⁵⁴. Experimental data for steady-state activation and time constants of activation are shown in **Panel 4A** and **Panel 4B** respectively. The activation gating variable (X_{ab}) depends on both voltage and junctional calcium ($[Ca^{2+}]_{jun}$). The activation gate was adapted from the Tong-Taggart model⁵⁶. The model of $I_{BK_{\alpha\beta 1}}$ is described by:

$$(Eq. 19) \quad I_{BK_{\alpha\beta 1}} = P_{BK_{Ca}} * X_{ab}(V, [Ca]_{jun}) * \frac{z_K^2 F^2 V}{RT} \left(\frac{[K]_{in} e^{\frac{z_K F V}{RT}} - [K]_{out}}{e^{\frac{z_K F V}{RT}} - 1} \right)$$

Where $P_{BK_{Ca}}$ is the BK_{Ca} ion permeability, R is the gas constant, F is Faraday's constant, and z_K is the valance of the potassium ions. Model optimization to activation curves are shown with solid lines in **Figure 4A** at three different $[Ca^{2+}]_{jun}$ concentrations 1 μM , 10 μM , and 100 μM . The results from the steady-state activation measurements at 10 μM are also in agreement with the experimental data in vascular myocytes in *bufo marinus*⁵⁸ (green symbols) which suggests that BK_{Ca} channels are exposed to a mean junctional Ca^{2+} concentration ($[Ca^{2+}]_{jun}$) of 10 μM . Time constants of activation were measured experimentally at $[Ca^{2+}]_{jun} = 0.003 \mu M$, our model was optimized and fit under the same conditions shown in **Figure 4B** as solid lines. Notably when the model was run under predicted $[Ca^{2+}]_{jun} = 10 \mu M$ conditions as shown in **Figure 4B** dashed lines, there was no effect of the change in $[Ca^{2+}]_{jun}$ on the time constant. The predicted current-voltage (I-V) relationships of $I_{BK_{\alpha\beta 1}}$ are shown in **Figure 4C** using three different $[Ca^{2+}]_{jun}$ concentrations; 1 μM , 10 μM , and 100 μM . We observed that the I-V curves are similar at $[Ca^{2+}]_{jun}$

concentrations of 10 μM (black trace) and 100 μM (orange trace) but markedly reduced when $[\text{Ca}^{2+}]_{\text{Jun}} = 1 \mu\text{M}$ (blue trace). As expected, the amplitude of the current shown in the I-V curves in **Figure 4D** is sensitively dependent on the number of BK_{Ca} channels as shown, we set $[\text{Ca}^{2+}]_{\text{Jun}} = 10 \mu\text{M}$ and simulated the I-V curves using a BK_{Ca} cluster size of 4, 6, 8 and 10 channels.

In vascular smooth muscle cells, the membrane potential over the physiological range of intravascular pressures is less negative than the equilibrium potential of potassium ($E_{\text{K}} = -84 \text{ mV}$), suggesting active participation of inward currents regulated by sodium conductance. It has been postulated that basally activating TRP channels generate nonselective cations currents (I_{NSC}) that depolarize the membrane potential. We built a model for I_{NSC} as linear and time-independent cation current permeable to K^+ and Na^+ with permeability ratios $P_{\text{Na}}: P_{\text{K}} = 0.9:1.3$ adapted from Tong-Taggart model with a reversal potential (E_{NSC}) described by:

$$(\text{Eq. 20}) \quad E_{\text{NSC}} = \left(R * \frac{T}{F} \right) * \log \left(\frac{P_{\text{K}} * K_{\text{out}} + P_{\text{Na}} * Na_{\text{out}}}{P_{\text{K}} * K_{\text{in}} + P_{\text{Na}} * Na_{\text{in}}} \right);$$

where R is the gas constant, F is the Faraday's constant, T is the temperature, Na_{in} and K_{in} are the intracellular sodium and potassium intracellular concentrations. Similarly, Na_{out} and K_{out} are the extracellular sodium and potassium concentrations. The model of I_{NSC} is described by

$$(\text{Eq. 21}) \quad I_{\text{NSC}} = I_{\text{NaNSC}} + I_{\text{KNSC}}$$

$$(\text{Eq. 22}) \quad I_{\text{NaNSC}} = G_{\text{NaNSC}} * (V - E_{\text{NSC}})$$

$$(\text{Eq. 23}) \quad I_{\text{KNSC}} = G_{\text{KNSC}} * (V - E_{\text{NSC}})$$

Where I_{NaNSC} represents sodium current contribution, I_{KNSC} represents potassium current contribution, and G_{NaNSC} and G_{KNSC} are the maximal conductances of the contributing sodium and potassium currents. In addition, we also included models for leak currents of ion i calculated as

$$(\text{Eq. 24}) \quad I_{i,b} = G_{i,b} (V - E_i)$$

Where the Nernst potential of ion i with valance z_i is given by:

$$(\text{Eq. 25}) \quad E_i = \frac{RT}{z_i F} \ln \left(\frac{[i]_{\text{out}}}{[i]_{\text{in}}} \right), \quad i = \text{Na}^+, \text{K}^+ \text{ and } \text{Ca}^{2+}$$

Where R is the gas constant, F is the Faraday's constant, T is the temperature and $[i]_{\text{out}}$ denotes the extracellular concentration of ion i . The final parameters are detailed in **S9**.

The remaining ionic currents, pumps, and transporters were optimized to data available in the experimental literature and/or taken from computational models of vascular smooth muscle and cardiac cells. The sodium-potassium pump (I_{NaK}) current was modeled using data from smooth muscle cells from mesenteric resistance arteries of the guinea pig and the voltage dependency was adapted from the Luo-Rudy II model. The sodium-calcium exchanger current

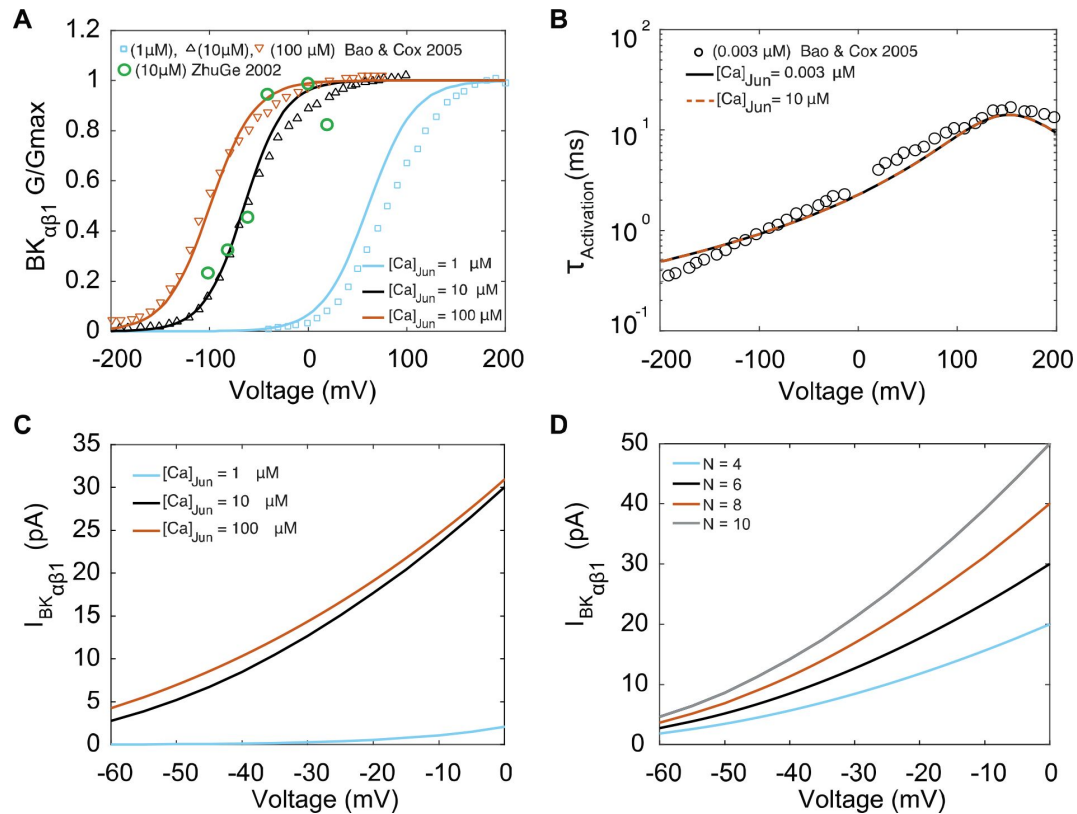


Figure 4.

Experimentally measured and modeled large-conductance Ca^{2+} -activated K^+ currents ($I_{\text{BK}\alpha\beta 1}$).

The model was optimized to data from Bao and Cox (Bao & Cox, 2005). (A) Voltage-dependent activation of $I_{\text{BK}\alpha\beta 1}$ from experiments performed with three different $[\text{Ca}]_{\text{Jun}}$ concentrations (1 μM , 10 μM , 100 μM) shown in green circles is the data from (ZhuGe *et al.*, 2002) (B) Voltage-dependent activation time constants with $[\text{Ca}]_{\text{Jun}} = 0.003 \mu\text{M}$ and simulations $[\text{Ca}]_{\text{Jun}} = 10 \mu\text{M}$. (C) Simulated I-V curve at different peak levels of $[\text{Ca}]_{\text{Jun}}$ levels. (D) Simulated I-V curve with different BK_{Ca} average cluster sizes ($N = 4, 6, 8, \text{ and } 10$).

(I_{NCX}) was adapted from the formulation in the ten Tusscher model⁶² and the Luo-Rudy II model⁴¹. Finally, the sarcolemma calcium pump (I_{PMCA}) current was adapted from the Kargacin model⁶³. The final parameters are detailed in **S10**.

$$(Eq. 26) \quad N_{pow} = \left(Q10^{\frac{T-309.2}{10}} \right);$$

$$(Eq. 27) \quad N_1 = \frac{(K_{out}^{1.1})}{K_{out}^{1.1} + KmNaK_K^{1.1}};$$

$$(Eq. 28) \quad N_2 = \frac{(Na_{in}^{1.7})}{Na_{in}^{1.7} + KmNaK_K^{1.7}};$$

$$(Eq. 29) \quad N_0 = \frac{1.0}{1 + \left(0.1245 \cdot \exp(-0.1 \cdot V \cdot \frac{F}{R \cdot T}) \right) + \left(2.19e-3 \cdot \left(e^{\left(\frac{Na_{out}}{49.71} \right)} \cdot e^{\left(-1.9 \cdot V \cdot \frac{F}{R \cdot T} \right)} \right) \right)};$$

$$(Eq. 30) \quad I_{NaK} = I_{NaK_{max}} * N_1 * N_2 * N_0 * N_{pow}$$

$$(Eq. 31) \quad phi_F = \exp\left(gammax * V * \frac{F}{R \cdot T}\right)$$

$$(Eq. 32) \quad phi_R = \exp\left((gammax - 1) * V * \frac{F}{R \cdot T}\right)$$

$$(Eq. 33) \quad X_{NCX} = \frac{(Na_{in}^3) * Ca_{out} * phi_F - (Na_{out}^3) * Ca_{in} * phi_R}{1 + 0.0003 * ((Na_{out}^3) * Ca_{in} + (Na_{in}^3) * Ca_{out})};$$

$$(Eq. 34) \quad I_{NCX} = P_{NCX} * X_{NCX}$$

$$(Eq. 35) \quad I_{PMCA} = I_{PMCAbar} * \frac{Ca_i^2}{Ca_i^2 + K_{mPMCA}^2}$$

We next set out to connect the ionic models and models of Ca^{2+} handling to make predictions in the whole cell. In **Figure 5**, experimental data indicate that the electrical activity of isolated mesenteric smooth muscle cells in male and female myocytes recorded in current-clamp mode, is characterized by an oscillating membrane potential under physiological conditions. The membrane potential is marked by repetitive spontaneous transient hyperpolarization (TH), a ubiquitous feature of vascular smooth muscle cells^{57,64-66} as shown in **panel 5A**. Both male (black trace) and female (blue trace) myocytes exhibited membrane hyperpolarizing transients in the potential range of -50 to -20 mV. Notably, we observed that female myocytes always maintained a higher depolarizing state between the hyperpolarization events compared to male myocytes.

We assessed the predictive capacity of our *in silico* model by comparing it to experimental data. We first compared the morphology of the membrane potential in experiments **panel 5A** versus simulations **panel 5B** in male and female myocytes. Upon comparative analysis between male and female experimental data and simulations, we noted that the baseline membrane potential for male myocytes was around -40 mV, while female myocytes exhibited a slightly more depolarized membrane potential at approximately -30 mV. Despite these variations in baseline membrane potential, both male and female myocytes presented similar peak hyperpolarization values of approximately 10-15 mV, ranging from -50 mV to -30 mV. Similarly, the frequency of THs from multiple myocytes was calculated to be 1 to 2.8 Hz in the range of -50 mV to -30 mV which is identical to the simulated frequency.

In the physiological range in which smooth muscle cells operate (-50 to -20 mV), ionic currents are small and produced by the activation of a small number of ion channels. Local fluctuations in the function of ion channels lead to noisy macroscopic signals that are important to the variability of vascular smooth muscle cells¹⁹. In addition, smooth muscle cells are subject to high input resistance where small perturbations can lead to large changes in the membrane potential^{19,29}. To approximate the physiological realities, we applied two sources of noise to

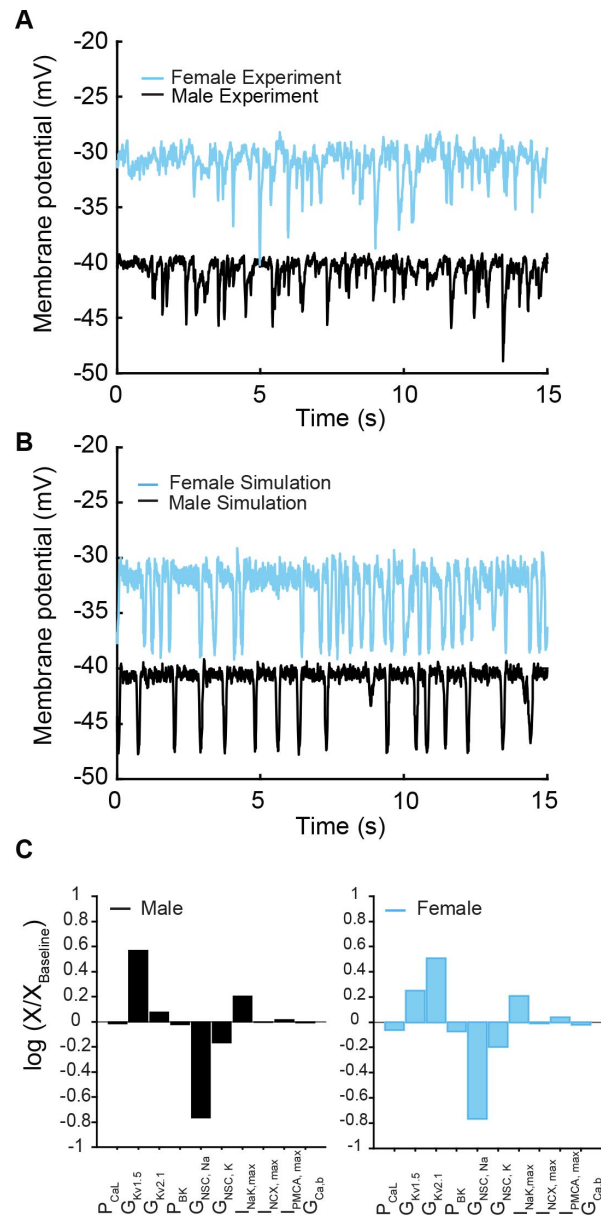


Figure 5.

Membrane potential from experiments and simulations in male and female vascular smooth muscle myocytes.

A) Whole-cell membrane potential recordings in male and female myocytes showing spontaneous repeat transient hyperpolarization of the membrane potential. **B)** Simulated whole-cell membrane potential with physiological noise. **C)** Comparison of sensitivity analysis performed around the baseline membrane potential in male and female models using multivariable regression.

our deterministic *in silico* model to simulate the stochastic fluctuations. The first source of the noise was introduced by adding a fluctuating current term to the differential equations describing changes in membrane potential (dV/dt) which represents the combined effect of the stochastic activity of ion channels in the plasma membrane⁴⁶. Second, we introduced noise into the $[Ca]_{SR}$ to replicate the physiological responses consistent with those observed in experimental studies⁴⁰. Simulated whole-cell membrane potential with physiological noise is shown in **Figure 5B** in male (black trace) and female (blue trace) myocytes.

We conducted a sensitivity analysis to determine which model parameters could underlie the sex-specific differences observed in the experimental data. It is important to note that we have experimental data indicating the amplitude and kinetics for a variety of currents in male and female myocytes. For this reason, those model components were fit to the data, fixed, and were not subject to sensitivity analysis. Our analysis, which focused solely on variations in maximal conductance and maximal ion transport rates of the transmembrane currents, indicated that the non-selective cation currents (I_{NSC}) and delayed rectifier currents ($I_{KVTOT} = I_{Kv2.1} + I_{Kv1.5}$) interact to regulate the baseline membrane potential in both male and female vascular smooth muscle myocytes (**Figure 5C**). Given that I_{KVTOT} responds to depolarization, the primary stimulus that triggers depolarization was determined to be attributable solely to the non-selective cation currents (I_{NSC}). Indeed, when we adjusted the conductance of the non-selective cation currents and implemented an increase in the conductance of I_{NSC} in the female model, we readily reproduced the sex-specific baseline membrane potential observed experimentally (**Figure 5A**).

Next, using the whole-cell vascular smooth muscle myocyte computational model, we investigated the sex-specific differences in the contribution to total voltage-gated current (I_{KVTOT}) in mesenteric vascular smooth muscle cells. An interesting prediction from the *in silico* simulations is that at different depolarizing states (-45, -40, and -35 mV) induced by changing the conductance of nonselective cationic leak currents (I_{NSC}), the contribution of $I_{Kv2.1}$ and $I_{Kv1.5}$ to I_{KVTOT} is different based on sex. In male vascular myocytes, the contribution to total voltage-gated current (I_{KVTOT}) is largely attributable to the current produced by $K_v1.5$ channels as shown in the lower panel in **Figure 6A**. Our results are consistent with previous studies^{35,67,68} in animal rodent male models showing the characteristic behavior of $I_{Kv1.5}$ to control membrane potential. However, the model predicts that in female myocytes, the contribution to total voltage-gated current (I_{KVTOT}) is largely provided by the current produced by $K_v2.1$ channels as shown in the upper panel in **Figure 6B**. To illustrate this point quantitatively, at a membrane potential of -40 mV, the contribution of I_{KVTOT} from $I_{Kv1.5}$ and $I_{Kv2.1}$ is 86% and 14%, respectively, in male myocytes compared to female myocytes in which the contribution from $I_{Kv1.5}$ and $I_{Kv2.1}$ is 23% and 77%, respectively. Regardless of the depolarization state at -45, -40, or -35 mV, the profiles for male and female myocytes remain essentially the same as shown in **Figures 6C, 6D**, and **6E**. The *in silico* simulations suggest a distinctive sex-based function of $K_v1.5$ and $K_v2.1$ channels that produce the delayed rectifier currents to counterbalance the effect of inward currents causing graded membrane potential depolarizations.

Having explored the regulation of graded membrane potential by the activation of I_{KVTOT} to counterbalance the nonselective cations currents (I_{NSC}), we next explored the effects of steady membrane depolarization in the *in silico* vascular smooth muscle cell myocyte model on I_{Ca} in male and female myocytes. We predicted I_{Ca} in our male and female simulations at steady-state membrane depolarization after simulation for 500 seconds. We observed that as the membrane depolarizes from -55 to -35 mV, I_{Ca} in male myocytes increased from 0 to 1.0 pA while in female myocytes I_{Ca} increased from 0 to 1.5 pA as shown in **Figure 7A**, suggesting that I_{Ca} is larger in female compared to those of male myocytes. We recorded the predicted $[Ca^{2+}]_i$ and observed that I_{Ca} led to a higher calcium influx in female compared to male simulations as shown in **Figure 7B**. To illustrate in detail, we show in **Figure 7C-D**, time traces of *in silico* predictions of membrane voltage at -40 mV (*top panel*), I_{Ca} (*middle panel*), and $[Ca^{2+}]_i$ (*lower panel*) corresponding to the male and female data points indicated by black and blue arrows respectively

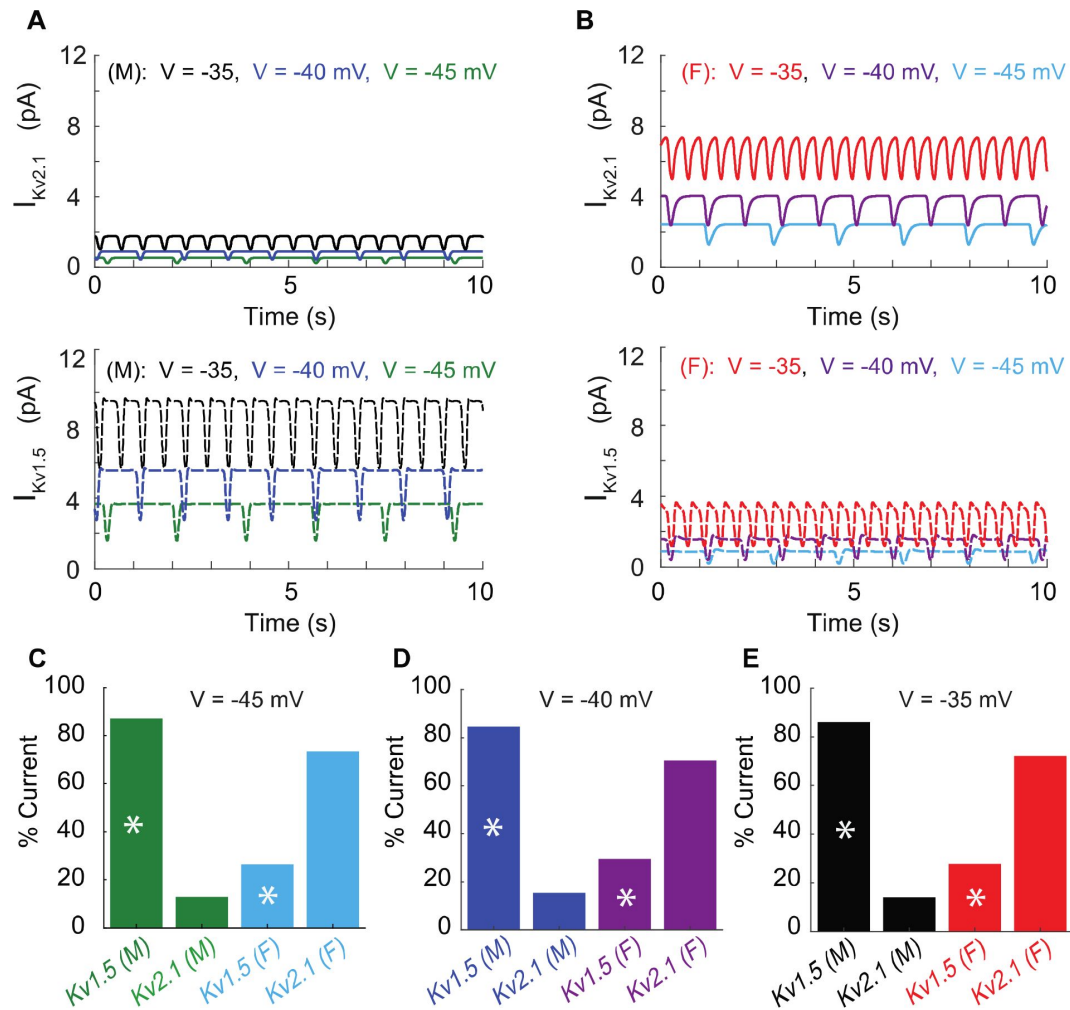


Figure 6.

Differential effects of voltage-gated potassium current (I_{KVTOT}) block in male and female myocytes.

A) Simulated time course of male $I_{Kv2.1}$ (top panel, solid traces) and $I_{Kv1.5}$ (lower panel, dashed traces) at three different baseline membrane potentials (-45 mV green, -40 mV blue, and -35 mV black). **B**) Simulated time course of female $I_{Kv2.1}$ (top panel solid traces) and $I_{Kv1.5}$ (lower panel, dashed traces) at three different baseline membrane potentials (-45 mV light blue, -40 mV purple, and -35 mV red). Current contribution to I_{KVTOT} from $Kv1.5$ (indicated by asterisks) and $Kv2.1$ in male and female myocytes at a baseline membrane potential of -45 mV (**C**), -40 mV (**D**), and -35 mV (**E**).

shown in **Figure 7A-B**. In the male case (**Figure 7C**), at a steady membrane potential of -40 mV, L-type calcium $\text{Ca}_v1.2$ channels produced a current of 0.5 pA. However, in female simulations (**Figure 7D**), we observed that at a steady membrane potential of -40 mV, L-type calcium $\text{Ca}_v1.2$ channels produced a current of 0.65 pA. We calculated that at -40 mV, two $\text{Ca}_v1.2$ channels are needed to sustain 0.5 pA of current in male myocytes while three $\text{Ca}_v1.2$ channels are needed to sustain 0.65 pA of current in female myocytes. Although the sex-specific differences in male and female simulations at -40 mV are small, a 15 nM difference in the overall response of $[\text{Ca}^{2+}]_i$ can have a profound effect on the constriction state of the myocytes. The predictions from the Hernandez-Hernandez model provide a comprehensive picture of physiological conditions and support the idea that a small number of $\text{Ca}_v1.2$ channels supply the steady Ca^{2+} influx needed to support a maintained constricted state in small arteries and arterioles^{53,69}. The differences between males and females are notable in the context of observations indicating varied sex-based responses to antihypertensive agents that target the Ca^{2+} handling system in vascular smooth muscle cells.

Next, we simulated the effects of calcium channel blocker nifedipine on I_{Ca} at a steady membrane potential of -40 mV in male and female simulations. Briefly, previous studies⁷⁰ have shown that at the therapeutic dose of nifedipine (i.e., about 0.1 μM) L-type $\text{Ca}_v1.2$ channel currents are reduced by about 70%. Accordingly, we decreased I_{Ca} in our mathematical simulations by the same extent. In **Figure 7C-D**, we show the predicted male (gray) and female (pink) time course of membrane voltage at -40 mV (*top panel*), I_{Ca} (*middle panel*), and $[\text{Ca}^{2+}]_i$ (*lower panel*). First, we observed that in both male and females 0.1 μM nifedipine modifies the frequency of oscillation in the membrane potential, by causing a reduction in oscillation frequency. Second, both male and female simulations (middle panels) show that 0.1 μM nifedipine caused a reduction of I_{Ca} to levels that are very similar in male and female myocytes following treatment. Consequently, the reduction of I_{Ca} causes both male and female simulations to reach a very similar baseline $[\text{Ca}^{2+}]_i$ of about 85 nM (lower panels). As a result, simulations provide evidence supporting the idea that $\text{Ca}_v1.2$ channels are the predominant regulators of intracellular $[\text{Ca}^{2+}]$ entry in the physiological range from -40 mV to -20 mV. Importantly, these predictions also suggest that clinically relevant concentrations of nifedipine cause larger overall reductions in Ca^{2+} influx in female than in male arterial myocytes.

Thus far, we have shown the development and application of models of vascular smooth muscle myocytes incorporating measured sex-specific differences in currents from male and female isolated cells. Given that hypertension is essentially a consequence of the spatial organization and function of smooth muscle cells^{71,72}, we next expanded our study to include a one-dimensional (1D) tissue representation of electrotonically coupled tissue by connecting arterial myocytes in series.

A well-known phenomenon in excitable systems is that electrotonic coupling between cells results in the minimization of individual cellular differences, thereby producing a smoothing effect across the tissue⁷³⁻⁷⁵. We simulated 400 female or 400 male vascular smooth muscle myocytes and set the gap junctional conductivity to zero to uncouple the simulated cells. As expected, the uncoupled cells in both male and female cases demonstrated the characteristic behavior of arterial myocytes, exhibiting spontaneous hyperpolarization. Of the 400 cells, we show the simulated representative traces of Cell 1, Cell 50, and Cell 100 for female (**Figure 8A**) and male (**Figure 8B**).

Next, we modeled 400 cells but with electrotonic coupling by setting the simulated gap junctional resistance to $71.4 \Omega\text{cm}^2$ ⁷⁶. In this case, we observed that the spontaneous hyperpolarizations, previously observed in the uncoupled cells, diminished when cells were coupled. The overall smoothing effect observed in **Figure 8C** is attributed to the electrotonic coupling and consequential influence of neighboring cells. The electrical response is consistent across the spatial domain for both male (**Figure 8C**; *black trace*) and female (**Figure 8C**; *blue trace*) one-

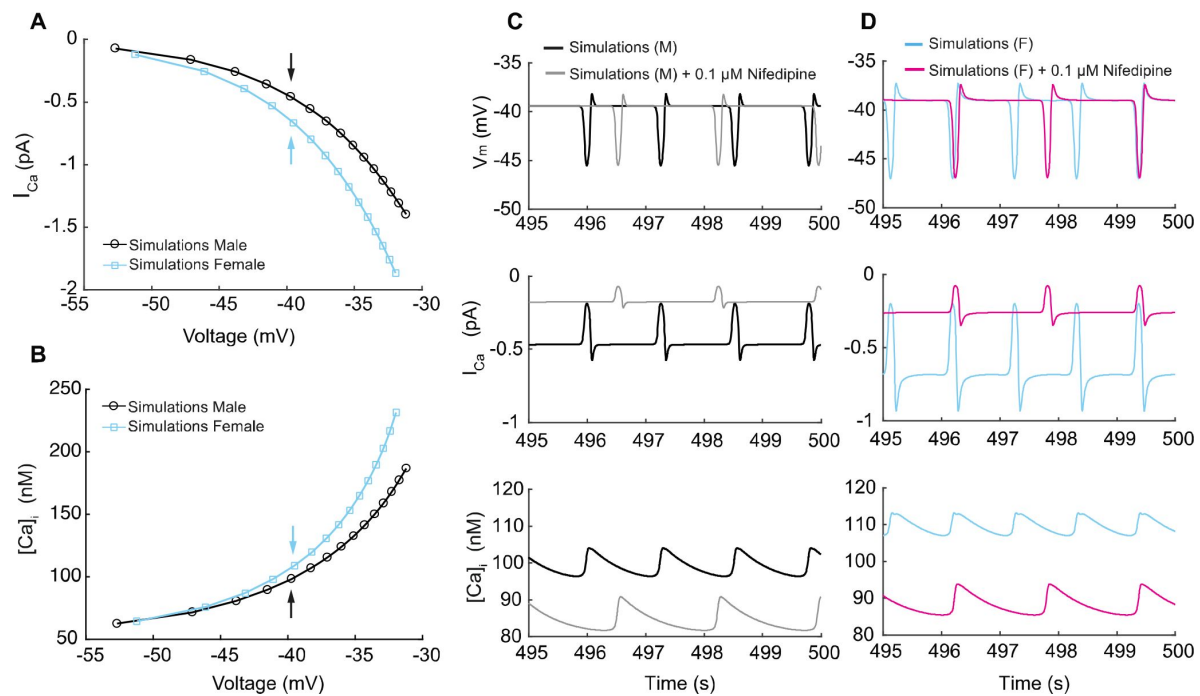


Figure 7.

Simulated L-type calcium currents (I_{Ca}) and calcium influx in male and female vascular smooth muscle cells.

A) Male and female whole-cell I_{Ca} membrane potential relationship. **(B)** Male and female intracellular calcium concentration in the cytosolic compartment at indicated membrane potential. **(C)** Time course of membrane potential in male vascular smooth muscle cells before (black) and after (gray) simulated nifedipine application (*top panel*). Corresponding time course of L-type calcium current I_{Ca} before (black) and after (gray) simulated nifedipine application (*middle panel*) and intracellular calcium $[Ca^{2+}]_i$ concentration before (black) and after (gray) simulated nifedipine application (*lower panel*). **(D)** Time course of membrane potential in female vascular smooth muscle cells before (blue) and after (pink) simulated nifedipine application (*top panel*). Corresponding time course of L-type calcium current I_{Ca} before (blue) and after (pink) simulated nifedipine application (*middle panel*) and intracellular calcium $[Ca^{2+}]_i$ concentration before (blue) and after (pink) simulated nifedipine application (*lower panel*).

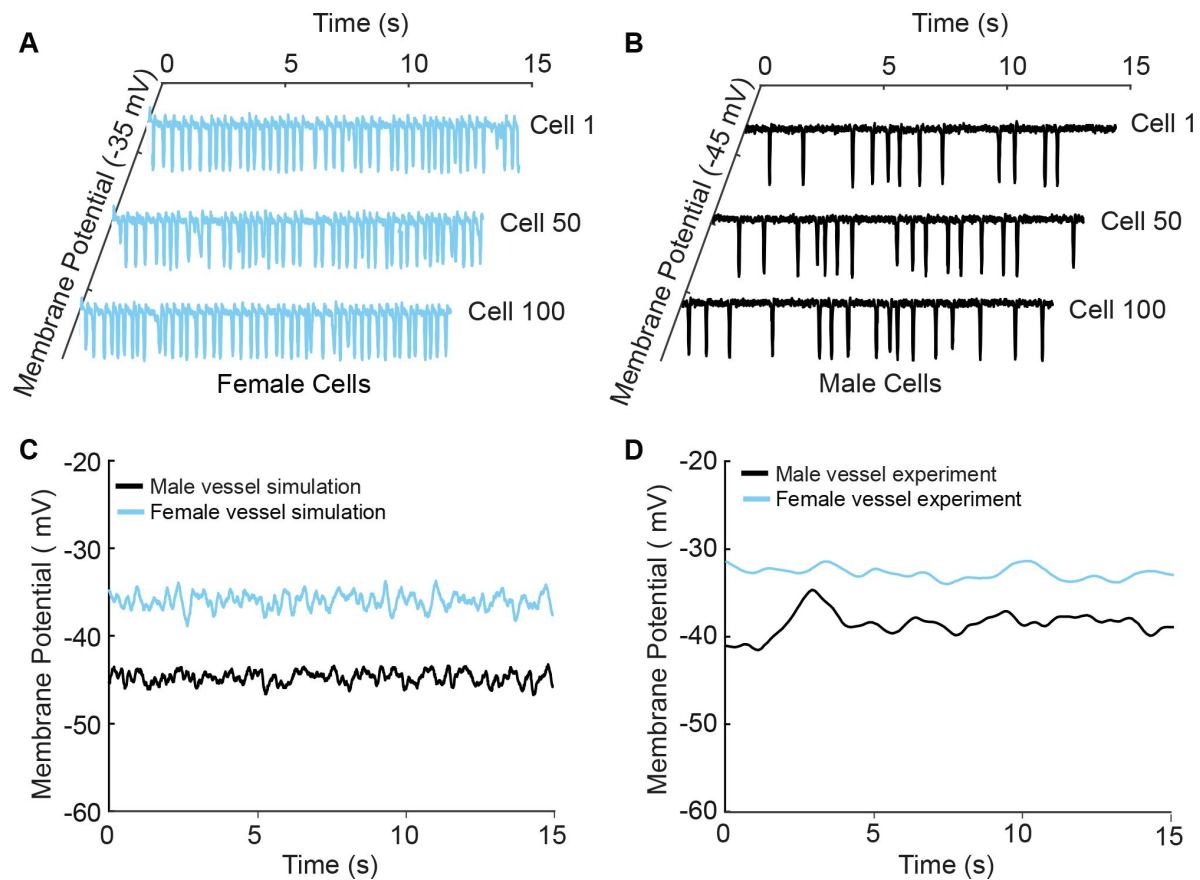


Figure 8.

A one-dimensional tissue model representation of vascular smooth muscle cells connected in series.

A) Uncoupled female vessel simulation showing cell 1, cell 50, and cell 100 at a baseline membrane potential of -35 mV. **B)** Uncoupled male vessel simulations showing cell 1, cell 50, and cell 100 at a baseline membrane potential of -45 mV. **C)** Composite female (blue trace) and male (black trace) membrane potential of 400 coupled smooth muscle cells connected with gap junctional resistance of $71.4 \Omega \text{cm}^2$ in a one-dimensional tissue representation. **(D)** Sharp-electrode records of the membrane potential of smooth muscle in pressurized (80-mmHg) female and male arteries from O'Dwyer *et al.*, 2020.

dimensional tissue representations. Notably, the model predicts a more depolarized female membrane potential in the one-dimensional tissue representations consistent with experimental measurements as shown in **Figure 8D** [↗](#).

Having developed an idealized model of a vessel, we set out to validate the model predictions of variable $[Ca^{2+}]_i$ between males and females by comparing the computed calcium signaling in vascular smooth muscle with experimental recordings O'Dwyer *et al.*²⁰ [↗](#). Given that membrane potential predominantly governs calcium influx in vascular smooth muscle¹⁴ [↗](#), we varied the conductance of the nonselective cation currents (I_{NSCC}) in our simulations. Tuning of I_{NSCC} was performed to replicate the effects of pressure-induced membrane depolarization, which results in activation of the voltage-gated L-type Ca^{2+} channels and increases $[Ca^{2+}]_i$.

Our simulations (lines) are well validated by experimental recordings (symbols) in **Figure 9A** [↗](#). A distinctive feature from the model prediction, which was validated by experimental recordings is the observation that female (**Figure 9A** [↗](#), blue trace and symbols) vessels accommodate more $[Ca^{2+}]_i$ compared to male (**Figure 9A** [↗](#), black trace and symbols) vessels. Intriguingly, the mechanism of different $[Ca^{2+}]_i$ in male and female vessels was revealed in single-cell simulations, which showed attributable sex-based differences in L-type Ca^{2+} currents.

Finally, in our simulations, we computed the effects of $[Ca^{2+}]_i$ after the application of clinically relevant calcium channel blocker nifedipine. We observed a substantial reduction of $[Ca^{2+}]_i$ in both male (**Figure 9A** [↗](#), dashed black line) and female (**Figure 9A** [↗](#), dashed blue line). Significant differences were found in the physiological range of intravascular pressure from 40 to 120 mmHg. In the summary data (**Figure 9B** [↗](#)), we quantified the relative change of $[Ca^{2+}]_i$ in male (black) and female (blue) after the application of 0.1 mM L-type Ca^{2+} channel blocker nifedipine at 80 mmHg and 120 mmHg. Our results show that nifedipine, when applied to male vessels, decreases $[Ca^{2+}]_i$ by 22% and 25% at 80 mmHg and 120 mmHg, respectively. However, the same dose of nifedipine when applied to female vessels decreases $[Ca^{2+}]_i$ by 38% and 45% at 80 mmHg and 120 mmHg. The results suggest that female arterial smooth muscle is more sensitive to clinically used Ca^{2+} channel blockers than male smooth muscle.

Discussion

Here, we describe the development, validation, and application of an *in-silico* model to simulate and understand the mechanisms of electrical activity and Ca^{2+} dynamics in a single mesenteric vascular smooth muscle cell. The Hernandez-Hernandez model is the first model to incorporate sex-specific differences in voltage-gated $K_{V2.1}$ and $Ca_{V1.2}$ channels and predicts sex-specific differences in membrane potential and Ca^{2+} signaling regulation in the smooth muscle of both sexes from systemic arteries. In the pursuit of stratifying sex-specific responses to antihypertensive drugs, we expanded our exploration to encompass a one-dimensional (1D) tissue representation. Such an approach allowed us to simulate and predict the impact of Ca^{2+} channel blockers within a mesenteric vessel. Notably, the computational framework can be expanded to forecast the impact of antihypertensives and other perturbations from single-cell to tissue-level simulations.

To specifically investigate the impact of sex-specific differences measured from ion channel experiments and their impact on membrane potential and $[Ca^{2+}]_i$, we focused on the isolated myocyte in the absence of complex signaling pathways. We first explored the effects of $Ca_{V1.2}$ and $K_{V2.1}$ channels on membrane potential as experimental data suggest key sex-specific differences in channel expression and kinetics. Notably, the peak of the current-voltage (I-V) relationship of L-type $Ca_{V1.2}$ current is 40% smaller in male compared to female myocytes (**Figure 2D** [↗](#)).

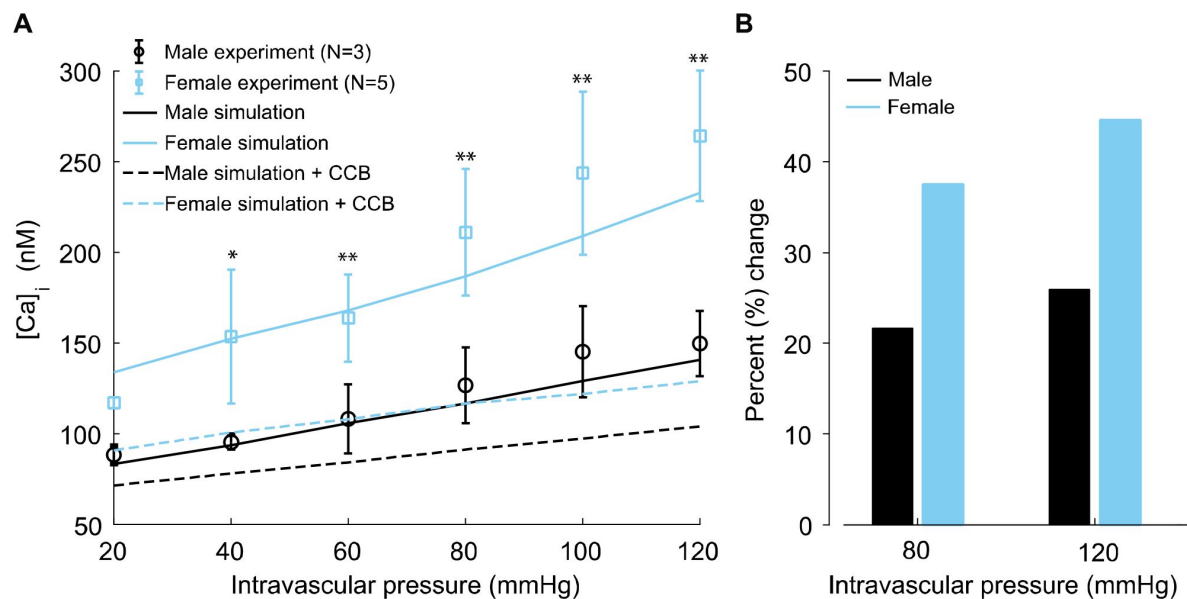


Figure 9.

Experimentally measured and modeled intracellular calcium [Ca]_i in male and female vessels and response to clinically used L-type Ca²⁺ channel blocker.

A) Intracellular calcium [Ca]_i in female (blue symbols) and male (black symbols) arteries at intravascular pressures ranging from 20 to 120 mmHg. Simulations showing [Ca]_i in the idealized female and male vessels are shown with blue and black solid lines, respectively. Simulated [Ca]_i after the application of clinically used L-type Ca²⁺ channel blocker nifedipine is shown with dashed lines for male (black) and female (blue). **B)** Comparison of the percentage change of [Ca]_i in male (black) and female (blue) after the application L-type Ca²⁺ channel blocker nifedipine at 80 mmHg and 120 mmHg. *P < 0.05, **P < 0.01. Error bars indicated mean ± SEM.

Similarly, the peak current-voltage (I-V) relationship of the voltage-gated $K_{V2.1}$ current ($I_{K_{V2.1}}$) is 70% smaller in male compared to female myocytes at +40 mV (**Figure 3C**). O'Dwyer and coauthors²⁰ showed sex-dependent expression of $K_{V2.1}$ in the plasma membrane, where male arterial myocytes have a total of about 75,000 channels compared to 183,000 channels in female myocytes. Notably, less than 0.01% of channels are conducting in male and female myocytes. In the computational model, we found that to reproduce the experimentally measured amplitude of the $K_{V2.1}$ I-V curve (**Figure 3C**), a maximum of ~44 male $K_{V2.1}$ channels was sufficient to reproduce the peak current (68.8 pA at 40 mV). In contrast, ~143 channels were predicted to be needed in female myocytes to reproduce the experimentally measured peak current (226.42 pA at +40 mV) of the $K_{V2.1}$ I-V relationship. Modeling and simulation led to the prediction that in male arterial myocytes, $I_{K_{V_{TOT}}}$ is largely dictated by $K_{V1.5}$ channels. In contrast, in female arterial myocytes, $K_{V2.1}$ channels dominate $I_{K_{V_{TOT}}}$ (**Figure 3F**).

An important aspect of the Hernandez-Hernandez model is that it includes Ca^{2+} -mediated signaling between RyRs in the junctional SR and BK_{Ca} channel clusters in the nearby sarcolemma membrane. This section of the model is similar to the one included in the Karlin model⁵ with some modifications. The Karlin model described how subcellular junctional spaces influence membrane potential and $[Ca^{2+}]_i$ in response to intravascular pressure, vasoconstrictors, and vasodilators. In this study, we reduced the complexity of the model representation of subcellular Ca^{2+} signaling spaces to include just three compartments: the cytosol, SR, and the SR-sarcolemma junction. Our model represents on average, the behavior of a single junctional SR unit that is functional in a cell at a time. The model uses a deterministic approach but mimics the process of production of Ca^{2+} sparks that activate BK_{Ca} channel clusters²⁹. We represented the activity of the RyRs in the junctional domain deterministically in the model so that Ca^{2+} spark- BK_{Ca} currents occur at a frequency of about 1 Hz at -40 mV in a space equivalent to 1% of the total cell surface area of the plasma membrane²².

Based on experimental observations, the Hernandez-Hernandez computational model employs three key assumptions: First, Ca^{2+} sparks in the junctional domain are initiated by activation of RyRs, where RyR gating opening probability is correlated with SR load. Second, Ca^{2+} sparks lead to a $[Ca^{2+}]_{jun}$ increase between 10-20 mM to match the amplitude measured in experiments (**Figure 4A**).^{54,58} Third, activation of BK_{Ca} channels and the resultant current amplitude derives from the experimentally observed spontaneous outward currents (STOCs) in both amplitude and morphology.

Notably, model simulations revealed important mechanisms that may underlie experimental observations in measurements of membrane potential (**Figure 5A**). The model predicts that the mechanism of intrinsic oscillatory behavior in the vascular myocytes results from a delicate balance of currents. Activation of non-selective cation currents (I_{NSC}) likely causes membrane depolarization, but the delayed rectifier currents ($I_{K_{V_{TOT}}}$) oppose them, resulting in membrane potential baseline in the physiological range of -45 to -20 mV. Interestingly, the voltage-gated L-type $Ca_{V1.2}$ currents activation threshold sits within this range at ~-45 mV. Therefore, small increases in I_{NSC} can overwhelm $I_{K_{V_{TOT}}}$ below -20 mV and result in sufficient depolarization to bring the membrane potential to the threshold for activation of I_{Ca} . It is important to note that $I_{K_{V_{TOT}}}$ increases sharply upon depolarization from -45 to -20 mV, resulting in tight control of membrane potential and prevention of large transient depolarization resulting from I_{NSC} . Activation of L-type Ca^{2+} channels upon depolarization and subsequent Ca^{2+} release within the small volume junction then activates the BK_{Ca} channels, which results in hyperpolarization. Hyperpolarization reignites the oscillatory cascade as an intrinsic resetting mechanism. Since vascular myocytes are subject to substantial noise from the stochastic opening of ion channels in the plasma membrane, and fluctuations in the local junctional domain components, such as the SR load, RyR opening, and BK_{Ca} channel activity, we included noise in the simulation. To simulate the physiological noise in the vascular smooth muscle cell (**Figure 5B**), we added Gaussian noise to the dV/dt equations and $[Ca]_{SR}$.

Female mesenteric artery myocytes are more depolarized than male myocytes at physiological intravascular pressures²⁰. Our model suggests that female myocytes are more depolarized than male myocytes due to larger non-selective cation currents in female compared to male myocytes, most likely due to the activation of Na⁺-permeable TRP channels. To our knowledge, the only TRP channels found to regulate the membrane potential of mesenteric artery smooth muscle are TRPP1 and TRPP2^{11,12}. Future work will have to determine if TRPC6⁸ and TRPM4^{9,10}, which have been shown to mediate the myogenic response of cerebral artery smooth muscle, and/or other non-selective cation channels also depolarize mesenteric artery smooth muscle⁷⁷.

The Hernandez-Hernandez model predicts that very few channels (based on total current amplitude) are likely to control the baseline fluctuations in membrane potential in the physiological range of -60 to -20 mV. The intrinsic oscillatory properties of the vascular myocyte operating in the low voltage regime under conditions of high resistance membrane are similar to other types of oscillatory electrical cells including cardiac pacemaker cells.

As shown in (**Figure 6**), the model predicts that, at -40 mV, the amplitude of steady-state K_v2.1 currents is about 0.8 pA in male and 3.3 pA in female arterial myocytes, indicating that the contribution of K_v2.1 and K_v1.5 channels to membrane potential is different in males and females. At -30 mV, it is 2.34 pA and 9.2 pA in male and female myocytes respectively. Assuming a single channel current at -40 and -30 mV of 0.7 pA, we calculated that, on average, in male myocytes a single channel is open at -40 mV and 3 channels are open at any particular time at -30 mV. In female myocytes, 6 channels are predicted to be open at -40 mV, while 13 are predicted to be active at -30 mV.

The Hernandez-Hernandez model also allowed us to calculate the number of Ca_v1.2 channels needed to sustain the steady-state concentration of [Ca²⁺]_i in the physiological range from -60 to -20 mV (**Figure 7**). The model predicts that at -40 mV in mouse male myocytes, 2 channels were required to generate 0.5 pA of steady-state Ca_v1.2 current. On the other hand, we found that in female myocytes, 3 channels were sufficient to generate 0.65 pA of Ca_v1.2 current. These data are consistent with the work of Rubart et al.⁶⁹, which suggested that steady-state Ca²⁺ currents at -40 mV were likely produced by the opening of 2 Ca_v1.2 channels in rat cerebral artery smooth muscle cells.

The observation that a very small number of the conducting K_v2.1 and Ca_v1.2 channels are involved in the regulation of membrane potential and Ca²⁺ influx in male and female arterial myocytes at physiological membrane potentials is important for several reasons. *First*, the analysis suggests that small differences in the number of K_v2.1 and Ca_v1.2 channels can translate into large, functionally important differences in membrane potential and [Ca²⁺]_i and hence affect and control myogenic tone under physiological and pathological conditions. *Second*, the small number of K_v2.1 and Ca_v1.2 channels gating between -40 and -30 mV likely makes smooth muscle cells more susceptible to stochastic fluctuations in the number and open probabilities of these channels than in cells where a large number of channels regulate membrane excitability and Ca²⁺ influx (e.g., adult ventricular myocyte⁷⁸). This, at least in part, likely contributes to Ca²⁺ signaling heterogeneity in vascular smooth muscle.

Hypertension fundamentally manifests through the spatial organization of cellular components, particularly evident in the context of the tunica media, the middle layer of vessels is predominantly constituted of smooth muscle cells which play a pivotal role in vessel contraction and relaxation^{71,72}. Such intricate biological machinery is imperative in orchestrating the regulation of blood flow and blood pressure. Our approach began with a process of distillation, aiming to shed light on cellular mechanisms within isolated vascular myocytes from small systemic vessels and arterioles, which control blood pressure, of both male and female mice.

Earlier research has confirmed that in mesenteric arteries, the pathogenesis leading to hypertension is largely determined by the downregulation of $K_V2.1$ ³⁶ and/or $K_V1.5$ ^{67,79} and a concurrent increase in the activity of $Ca_V1.2$ ⁸⁰ channels. Building upon this knowledge, we broadened our study to encompass a one-dimensional (1D) tissue model of electrotonically linked tissue, achieved by connecting arterial myocytes in series. The 1D cable model has anatomical relevance because the structure of third and fourth-order mesenteric arteries have a singular layer of vascular myocytes encircling the lumen in a cylindrical arrangement. The cable structure is analogous to an “unrolled” or lateral arrangement of the vessel. Such an approach allowed a conceptual framework to bridge the gap between understanding the combined effects of membrane potential and $[Ca^{2+}]_i$ in isolated cells and in the wider context of small vessels.

For instance, previous studies have proposed that gap junctions enable vessels to function in a way that is analogous to a large capacitor^{57,81}. The gap junctions actively filter and transform single-cell electrical activity into sustained responses across the tissue⁸¹. Recent studies add to this understanding by demonstrating that Connexin 37 (Cx37), a component of these gap junctions, seems to be expressed in the mesenteric arteries⁸². In our simulations, we showed (**Figure 8A-B**) that indeed uncoupled cells exhibit a spontaneous oscillatory behavior which studies have confirmed is not an artifact due to isolation from the vessel but rather an intrinsic behavior required to sustain electrical signals. When the cells are connected (**Figure 8C**) the spontaneous hyperpolarization previously observed in the uncoupled cells diminished, the effect is attributed to the electrotonic coupling and consequential influence of neighboring cells. In addition, in our simulations, we found that it is required to have stochastic fluctuations to allow the system to average the membrane potential behavior that dictates the amount of $[Ca^{2+}]_i$ in the vessels.

Regarding, $Ca_V1.2$ channels, simulations forecast the clinically relevant concentrations (0.1 μM) at which common Ca^{2+} channel blockers (e.g., nifedipine) effectively block $Ca_V1.2$ channels in both male and female smooth muscle (**Figure 9**). Our simulations in isolated arterial myocytes and in the one-dimensional (1D) tissue model suggest heightened sensitivity to calcium channel blockers in the female compared to male.

The model predictions are aligned with documented sex-specific differences in antihypertensive drug responses^{83,84}. Previous studies, notably by Kloner et al., have illustrated this point quantitatively, highlighting a more pronounced diastolic BP response in women (91.4%) compared to men (83%) when treated with dihydropyridine-type channel blockers, such as amlodipine. Importantly, this distinction persisted even after adjusting for confounding factors such as baseline BP, age, weight, and dosage per kilogram⁸⁴. Another interesting observation from Kajiwarra et al. emphasizes that vasodilation-related adverse symptoms occur more frequently in younger women (<50 years) compared to their male counterparts, again suggesting a heightened sensitivity to dihydropyridine-type calcium channel blockers⁸⁵.

Limitations

The model presented here describes the necessary and sufficient ion channels, pumps, and transporters to describe the electrical activity and Ca^{2+} signaling of an isolated mesenteric smooth muscle cell in the absence of complex signaling pathways. Such an approach enabled us to perform a component dissection to analyze the sex-specific differences observed in the fundamental electrophysiology of male and female myocytes. However, it is well known that vascular smooth muscle cells are subject to a plethora of stimuli from endothelial cells, neurotransmitters, endocrine, and paracrine signals⁵. The next phase of the project includes an expansion of the model to incorporate receptor mediated signaling pathways that are essential for blood pressure control.

Excitation-contraction coupling refers to an electrical stimulus that drives the release of calcium from the sarcoplasmic reticulum and results in the physical translocation of fibers that underlies muscle contraction. In the present model, we did not explicitly consider the mechanical description of muscle contraction. Nevertheless, we can imply contractile effects by tracking membrane potential and the elevation of $[Ca^{2+}]_i$ as a proxy.

To conclude, we developed and present the Hernandez-Hernandez model of male and female isolated mesenteric vascular myocytes. An additional limitation of our study is the reliance on predominantly murine data. Although mouse arteries do present numerous parallels with human arteries—including analogous intravascular pressure-myogenic tone relationships, resting membrane potentials, and the expression of typical ionic channels like $Ca_v1.2$, BKCa channels, and RyRs^{86–88}. Future research should assess the direct applicability and implication of our findings in human subjects.

Conclusions

The Hernandez-Hernandez model of the isolated mesenteric vascular myocyte was informed and validated with experimental data from male and female vascular myocytes. We then used the model to reveal sex-specific mechanisms of $K_v2.1$ and $Ca_v1.2$ channels in controlling membrane potential and Ca^{2+} dynamics. In doing so, we predicted that very few channels are needed to contribute to and sustain the oscillatory behavior of the membrane potential and calcium signaling. We expanded our computational framework to include a one-dimensional (1D) tissue representation, providing a basis for simulating the effect of drug effects within a vessel. The model predictions suggested differences in the response of male and female myocytes to drugs and the underlying mechanisms for those differences. These predictions may constitute the first step towards better hypertensive therapy for males and females.

Acknowledgements

This work was supported by National Institutes of Health Common Fund Grant OT2OD026580 (to C.E.C., L.F.S.), National Heart, Lung, and Blood Institute grants R01HL128537 (C.E.C, L.F.S.), R01HL152681 (to C.E.C., L.F.S.). UC Davis Department of Physiology and Membrane Biology Research Partnership Fund (to C.E.C.) as well as UC Davis T32 Predoctoral and Postdoctoral Training in Basic and Translational Cardiovascular Medicine fellowship supported in part by NHLBI Institutional Training Grant T32HL086350 (to C.M., G.H.H.)

References

1. Jacobsen J. C. B., et al. (2007) **Activation of a cGMP-sensitive calcium-dependent chloride channel may cause transition from calcium waves to whole cell oscillations in smooth muscle cells** *Am. J. Physiol. Heart Circ. Physiol* **293**:H215–228
2. Kapela A., Bezerianos A., Tsoukias N. M. (2008) **A mathematical model of Ca²⁺ dynamics in rat mesenteric smooth muscle cell: agonist and NO stimulation** *J. Theor. Biol* **253**:238–260
3. Yang J., Clark J. W., Bryan R. M., Robertson C. (2003) **The myogenic response in isolated rat cerebrovascular arteries: smooth muscle cell model** *Med. Eng. Phys* **25**:691–709
4. Parthimos D., Edwards D. H., Griffith T. M. (1999) **Minimal model of arterial chaos generated by coupled intracellular and membrane Ca²⁺ oscillators** *Am. J. Physiol* **277**:H1119–1144
5. Karlin A. (2015) **Membrane potential and Ca²⁺ concentration dependence on pressure and vasoactive agents in arterial smooth muscle: A model** *J. Gen. Physiol* **146**:79–96
6. Bayliss W. M. (1902) **On the local reactions of the arterial wall to changes of internal pressure** *J. Physiol* **28**:220–231
7. Welsh D. G., Morielli A. D., Nelson M. T., Brayden J. E. (2002) **Transient Receptor Potential Channels Regulate Myogenic Tone of Resistance Arteries** *Circ. Res* **90**:248–250
8. Spassova M. A., Hewavitharana T., Xu W., Soboloff J., Gill D. L. (2006) **A common mechanism underlies stretch activation and receptor activation of TRPC6 channels** *Proc. Natl. Acad. Sci. U. S. A* **103**:16586–16591
9. Earley S., Waldron B. J., Brayden J. E. (2004) **Critical Role for Transient Receptor Potential Channel TRPM4 in Myogenic Constriction of Cerebral Arteries** *Circ. Res* **95**:922–929
10. Earley S., Straub S. V., Brayden J. E. (2007) **Protein kinase C regulates vascular myogenic tone through activation of TRPM4** *Am. J. Physiol. Heart Circ. Physiol* **292**:H2613–2622
11. Sharif-Naeini R., et al. (2009) **Polycystin-1 and -2 Dosage Regulates Pressure Sensing** *Cell* **139**:587–596
12. Bulley S., et al. (2018) **Arterial smooth muscle cell PKD2 (TRPP1) channels regulate systemic blood pressure** *eLife* **7**
13. Moosmang S., et al. (2003) **Dominant role of smooth muscle L-type calcium channel Cav1.2 for blood pressure regulation** *EMBO J* **22**:6027–6034
14. Knot H. J., Nelson M. T. (1998) **Regulation of arterial diameter and wall [Ca²⁺] in cerebral arteries of rat by membrane potential and intravascular pressure** *J. Physiol* **508**:199–209
15. Navedo M. F., Santana L. F. (2013) **CaV1.2 sparklets in heart and vascular smooth muscle** *J. Mol. Cell. Cardiol* **58**:67–76
16. Navedo M. F., Amberg G. C., Votaw V. S., Santana L. F. (2005) **Constitutively active L-type Ca²⁺ channels** *Proc. Natl. Acad. Sci* **102**:11112–11117

17. Navedo M. F., Amberg G. C., Nieves M., Molkentin J. D., Santana L. F. (2006) **Mechanisms Underlying Heterogeneous Ca²⁺ Sparklet Activity in Arterial Smooth Muscle** *J. Gen. Physiol* **127**:611–622
18. Amberg G. C., Navedo M. F., Nieves-Cintrón M., Molkentin J. D., Santana L. F. (2007) **Calcium sparklets regulate local and global calcium in murine arterial smooth muscle** *J. Physiol* **579**:187–201
19. Nelson M. T., Patlak J. B., Worley J. F., Standen N. B. (1990) **Calcium channels, potassium channels, and voltage dependence of arterial smooth muscle tone** *Am. J. Physiol* **259**:C3–18
20. O'Dwyer S. C., et al. (2020) **Kv2.1 channels play opposing roles in regulating membrane potential, Ca²⁺ channel function, and myogenic tone in arterial smooth muscle** *Proc. Natl. Acad. Sci. U. S. A* **117**:3858–3866
21. Amberg G. C., Santana L. F. (2006) **Kv2 channels oppose myogenic constriction of rat cerebral arteries** *Am. J. Physiol. Cell Physiol* **291**:C348–356
22. Nelson M. T., et al. (1995) **Relaxation of arterial smooth muscle by calcium sparks** *Science* **270**:633–637
23. Plane F., et al. (2005) **Heteromultimeric Kv1 channels contribute to myogenic control of arterial diameter** *Circ. Res* **96**:216–224
24. Sato D., et al. (2019) **A stochastic model of ion channel cluster formation in the plasma membrane** *J. Gen. Physiol* **151**:1116–1134
25. Ledoux J., Werner M. E., Brayden J. E., Nelson M. T. (2006) **Calcium-Activated Potassium Channels and the Regulation of Vascular Tone** *Physiology* **21**:69–78
26. Brayden J. E., Nelson M. T. (1992) **Regulation of Arterial Tone by Activation of Calcium-Dependent Potassium Channels** *Science* **256**:532–535
27. Jaggar J. H., Stevenson A. S., Nelson M. T. (1998) **Voltage dependence of Ca²⁺ sparks in intact cerebral arteries** *Am. J. Physiol.-Cell Physiol* **274**:C1755–C1761
28. Knot H. J., Standen N. B., Nelson M. T. (1998) **Ryanodine receptors regulate arterial diameter and wall [Ca²⁺] in cerebral arteries of rat via Ca²⁺-dependent K⁺ channels** *J. Physiol* **508**:211–221
29. Pucovsky V., Bolton T. B. (2006) **Localisation, function and composition of primary Ca(2+) spark discharge region in isolated smooth muscle cells from guinea-pig mesenteric arteries** *Cell Calcium* **39**:113–129
30. Yuan X. J., Goldman W. F., Tod M. L., Rubin L. J., Blaustein M. P. (1993) **Ionic currents in rat pulmonary and mesenteric arterial myocytes in primary culture and subculture** *Am. J. Physiol* **264**:L107–115
31. Jaggar, et al. (1998) **Ca²⁺ channels, ryanodine receptors and Ca²⁺-activated K⁺ channels: a functional unit for regulating arterial tone: Ca²⁺ sparks in vascular smooth muscle** *Acta Physiol. Scand* **164**:577–587
32. Wang S.-Q., et al. (2004) **Imaging Microdomain Ca²⁺ in Muscle Cells** *Circ. Res* **94**:1011–1022

33. Zhong X. Z., et al. (2010) **Stromatoxin-sensitive, heteromultimeric Kv2.1/Kv9.3 channels contribute to myogenic control of cerebral arterial diameter** *J. Physiol* **588**:4519–4537
34. Archer S. L., et al. (1998) **Molecular identification of the role of voltage-gated K⁺ channels, Kv1.5 and Kv2.1, in hypoxic pulmonary vasoconstriction and control of resting membrane potential in rat pulmonary artery myocytes** *J. Clin. Invest* **101**:2319–2330
35. Lu Y., Hanna S. T., Tang G., Wang R. (2002) **Contributions of Kv1.2, Kv1.5 and Kv2.1 subunits to the native delayed rectifier K⁺ current in rat mesenteric artery smooth muscle cells** *Life Sci* **71**:1465–1473
36. Amberg G. C., Rossow C. F., Navedo M. F., Santana L. F. (2004) **NFATc3 regulates Kv2.1 expression in arterial smooth muscle** *J. Biol. Chem* **279**:47326–47334
37. Pérez G. J., Bonev A. D., Nelson M. T. (2001) **Micromolar Ca²⁺ from sparks activates Ca²⁺-sensitive K⁺ channels in rat cerebral artery smooth muscle** *Am. J. Physiol.-Cell Physiol* **281**:C1769–C1775
38. Pérez G. J., Bonev A. D., Patlak J. B., Nelson M. T. (1999) **Functional coupling of ryanodine receptors to K_{Ca} channels in smooth muscle cells from rat cerebral arteries** *J. Gen. Physiol* **113**:229–238
39. Kaßmann M., et al. (2019) **Role of Ryanodine Type 2 Receptors in Elementary Ca²⁺ Signaling in Arteries and Vascular Adaptive Responses** *J. Am. Heart Assoc* **8**
40. ZhuGe R., et al. (1999) **The Influence of Sarcoplasmic Reticulum Ca²⁺ Concentration on Ca²⁺ Sparks and Spontaneous Transient Outward Currents in Single Smooth Muscle Cells** *J. Gen. Physiol* **113**:215–228
41. Luo C. H., Rudy Y. (1994) **A dynamic model of the cardiac ventricular action potential. I. Simulations of ionic currents and concentration changes** *Circ. Res* **74**:1071–1096
42. Hernandez-Hernandez G., Alvarez-Lacalle E., Shiferaw Y. (2015) **Role of connectivity and fluctuations in the nucleation of calcium waves in cardiac cells** *Phys. Rev. E* **92**
43. Greene D., Shiferaw Y. (2021) **Mechanistic link between CaM-RyR2 interactions and the genesis of cardiac arrhythmia** *Biophys. J* **120**:1469–1482
44. Shiferaw Y., Watanabe M. A., Garfinkel A., Weiss J. N., Karma A. (2003) **Model of Intracellular Calcium Cycling in Ventricular Myocytes** *Biophys. J* **85**:3666–3686
45. Kernik D. C., et al. (2019) **A computational model of induced pluripotent stem-cell derived cardiomyocytes incorporating experimental variability from multiple data sources** *J. Physiol* **597**:4533–4564
46. Goldwyn J. H., Shea-Brown E. (2011) **The What and Where of Adding Channel Noise to the Hodgkin-Huxley Equations** *PLOS Comput. Biol* **7**
47. Tanskanen A. J., Alvarez L. H. R. (2007) **Voltage noise influences action potential duration in cardiac myocytes** *Math. Biosci* **208**:125–146
48. Shaw R. M., Rudy Y. (1997) **Ionic Mechanisms of Propagation in Cardiac Tissue** *Circ. Res* **81**:727–741

49. Jack J., Noble D., Tsien R. (1975) **Electric Current Flow In Excitable Cells**
50. Hodgkin A. L., Rushton W. A. H., Adrian E. D. (1997) **The electrical constants of a crustacean nerve fibre** *Proc. R. Soc. Lond. Ser. B - Biol. Sci* **133**:444–479
51. Sobie E. A. (2009) **Parameter Sensitivity Analysis in Electrophysiological Models Using Multivariable Regression** *Biophys. J* **96**:1264–1274
52. Hill-Eubanks D. C., Werner M. E., Heppner T. J., Nelson M. T. (2011) **Calcium signaling in smooth muscle** *Cold Spring Harb. Perspect. Biol* **3**
53. Fleischmann B. K., Murray R. K., Kotlikoff M. I. (1994) **Voltage window for sustained elevation of cytosolic calcium in smooth muscle cells** *Proc. Natl. Acad. Sci. U. S. A* **91**:11914–11918
54. Bao L., Cox D. H. (2005) **Gating and ionic currents reveal how the BKCa channel's Ca²⁺ sensitivity is enhanced by its beta1 subunit** *J. Gen. Physiol* **126**:393–412
55. Brenner R., et al. (2000) **Vasoregulation by the β 1 subunit of the calcium-activated potassium channel** *Nature* **407**:870–876
56. Tong W.-C., et al. (2011) **A computational model of the ionic currents, Ca²⁺ dynamics and action potentials underlying contraction of isolated uterine smooth muscle** *PloS One* **6**
57. Jaggar J. H., Porter V. A., Lederer W. J., Nelson M. T. (2000) **Calcium sparks in smooth muscle** *Am. J. Physiol. Cell Physiol* **278**:C235–256
58. Zhuge R., Fogarty K. E., Tuft R. A., Walsh J. V. (2002) **Spontaneous transient outward currents arise from microdomains where BK channels are exposed to a mean Ca(2+) concentration on the order of 10 microM during a Ca(2+) spark** *J. Gen. Physiol* **120**:15–27
59. Dwyer L., et al. (2011) **Basally activated nonselective cation currents regulate the resting membrane potential in human and monkey colonic smooth muscle** *Am. J. Physiol. Gastrointest. Liver Physiol* **301**:G287–296
60. Setoguchi M., Ohya Y., Abe I., Fujishima M. (1997) **Stretch-activated whole-cell currents in smooth muscle cells from mesenteric resistance artery of guinea-pig** *J. Physiol* **501**:343–353
61. Nakamura Y., Ohya Y., Abe I., Fujishima M. (1999) **Sodium-potassium pump current in smooth muscle cells from mesenteric resistance arteries of the guinea-pig** *J. Physiol* **519**:203–212
62. ten Tusscher K. H. W. J., Noble D., Noble P. J., Panfilov A. V. (2004) **A model for human ventricular tissue** *Am. J. Physiol. Heart Circ. Physiol* **286**:H1573–1589
63. Kargacin G. J. (1994) **Calcium signaling in restricted diffusion spaces** *Biophys. J* **67**:262–272
64. Désilets M., Driska S. P., Baumgarten C. M. (1989) **Current fluctuations and oscillations in smooth muscle cells from hog carotid artery. Role of the sarcoplasmic reticulum** *Circ. Res* **65**:708–722
65. Bychkov R., Gollasch M., Ried C., Luft F. C., Haller H. (1997) **Regulation of spontaneous transient outward potassium currents in human coronary arteries** *Circulation* **95**:503–510

66. Bae Y. M., Park M. K., Lee S. H., Ho W. K., Earm Y. E. (1999) **Contribution of Ca²⁺-activated K⁺ channels and non-selective cation channels to membrane potential of pulmonary arterial smooth muscle cells of the rabbit** *J. Physiol* **514**:747–758
67. Sung D. J., et al. (2013) **Serotonin contracts the rat mesenteric artery by inhibiting 4-aminopyridine-sensitive Kv channels via the 5-HT_{2A} receptor and Src tyrosine kinase** *Exp. Mol. Med* **45**
68. Bratz I. N., Swafford A. N., Kanagy N. L., Dick G. M. (2005) **Reduced functional expression of K(+) channels in vascular smooth muscle cells from rats made hypertensive with N{omega}-nitro-L-arginine** *Am. J. Physiol. Heart Circ. Physiol* **289**:H1284–1290
69. Rubart M., Patlak J. B., Nelson M. T. (1996) **Ca²⁺ currents in cerebral artery smooth muscle cells of rat at physiological Ca²⁺ concentrations** *J. Gen. Physiol* **107**:459–472
70. Davis M. J., Meininger G. A., Zawieja D. C. (1992) **Stretch-induced increases in intracellular calcium of isolated vascular smooth muscle cells** *Am. J. Physiol.-Heart Circ. Physiol* **263**:H1292–H1299
71. Mulvany M. J., Aalkjaer C. (1990) **Structure and function of small arteries** *Physiol. Rev* **70**:921–961
72. Martinez-Lemus L. A. (2012) **The Dynamic Structure of Arterioles** *Basic Clin. Pharmacol. Toxicol* **110**:5–11
73. Heppner D. B., Plonsey R. (1970) **Simulation of electrical interaction of cardiac cells** *Biophys. J* **10**:1057–1075
74. Rudy Y., Quan W. L. (1987) **A model study of the effects of the discrete cellular structure on electrical propagation in cardiac tissue** *Circ. Res* **61**:815–823
75. Henriquez C. S., Plonsey R. (1987) **Effect of resistive discontinuities on waveshape and velocity in a single cardiac fibre** *Med. Biol. Eng. Comput* **25**:428–438
76. Yamamoto Y., Klemm M. F., Edwards F. R., Suzuki H. (2001) **Intercellular electrical communication among smooth muscle and endothelial cells in guinea-pig mesenteric arterioles** *J. Physiol* **535**:181–195
77. Earley S., Brayden J. E. (2015) **Transient Receptor Potential Channels in the Vasculature** *Physiol. Rev* **95**:645–690
78. Guarina L., et al. (2022) **Biological noise is a key determinant of the reproducibility and adaptability of cardiac pacemaking and EC coupling** *J. Gen. Physiol* **154**
79. Bae Y. M., et al. (2006) **Serotonin depolarizes the membrane potential in rat mesenteric artery myocytes by decreasing voltage-gated K⁺ currents** *Biochem. Biophys. Res. Commun* **347**:468–476
80. Navedo M. F., et al. (2010) **Increased Coupled Gating of L-Type Ca²⁺ Channels During Hypertension and Timothy Syndrome** *Circ. Res* **106**:748–756
81. Diep H. K., Vigmond E. J., Segal S. S., Welsh D. G. (2005) **Defining electrical communication in skeletal muscle resistance arteries: a computational approach** *J. Physiol* **568**:267–281

82. Earley S., Resta T. C., Walker B. R. (2004) **Disruption of smooth muscle gap junctions attenuates myogenic vasoconstriction of mesenteric resistance arteries** *Am. J. Physiol.-Heart Circ. Physiol* **287**:H2677–H2686
83. Ueno K., Sato H. (2012) **Sex-related differences in pharmacokinetics and pharmacodynamics of anti-hypertensive drugs** *Hypertens. Res. Off. J. Jpn. Soc. Hypertens* **35**:245–250
84. Kloner R. A., Sowers J. R., DiBona G. F., Gaffney M., Wein M. (1996) **Sex- and age-related antihypertensive effects of amlodipine. The Amlodipine Cardiovascular Community Trial Study Group** *Am. J. Cardiol* **77**:713–722
85. Kajiwar A., et al. (2014) **Younger females are at greater risk of vasodilation-related adverse symptoms caused by dihydropyridine calcium channel blockers: results of a study of 11,918 Japanese patients** *Clin. Drug Investig* **34**:431–435
86. Yang Y., et al. (2013) **Function of BKCa channels is reduced in human vascular smooth muscle cells from Han Chinese patients with hypertension** *Hypertens. Dallas Tex* **1979** **61**:519–525
87. Nystoriak M. A., et al. (2017) **Ser1928 phosphorylation by PKA stimulates the L-type Ca²⁺ channel CaV1.2 and vasoconstriction during acute hyperglycemia and diabetes** *Sci. Signal* **10**
88. Nieves-Cintrón M., et al. (2017) **Impaired BKCa channel function in native vascular smooth muscle from humans with type 2 diabetes** *Sci. Rep* **7**

Article and author information

Gonzalo Hernandez-Hernandez

Department of Physiology & Membrane Biology, University of California School of Medicine, Davis, California, 95616
ORCID iD: [0000-0001-6940-9005](https://orcid.org/0000-0001-6940-9005)

Samantha C. O'Dwyer

Department of Physiology & Membrane Biology, University of California School of Medicine, Davis, California, 95616
ORCID iD: [0000-0001-5334-9984](https://orcid.org/0000-0001-5334-9984)

Collin Matsumoto

Department of Physiology & Membrane Biology, University of California School of Medicine, Davis, California, 95616
ORCID iD: [0000-0002-5370-1156](https://orcid.org/0000-0002-5370-1156)

Mindy Tieu

Department of Physiology & Membrane Biology, University of California School of Medicine, Davis, California, 95616

Zhihui Fong

Department of Physiology & Membrane Biology, University of California School of Medicine, Davis, California, 95616
ORCID iD: [0000-0002-0521-0088](https://orcid.org/0000-0002-0521-0088)

Pei-Chi Yang

Department of Physiology & Membrane Biology, University of California School of Medicine, Davis, California, 95616

ORCID iD: [0000-0002-5753-1131](https://orcid.org/0000-0002-5753-1131)

Timothy J. Lewis

Department of Physiology & Membrane Biology, University of California School of Medicine, Davis, California, 95616, Department of Mathematics, University of California, Davis, California, 95616

L. Fernando Santana

Department of Physiology & Membrane Biology, University of California School of Medicine, Davis, California, 95616

For correspondence: lsantana@ucdavis.edu

ORCID iD: [0000-0002-4297-8029](https://orcid.org/0000-0002-4297-8029)

Colleen E. Clancy

Department of Physiology & Membrane Biology, University of California School of Medicine, Davis, California, 95616, Center for Precision Medicine and Data Science, University of California School of Medicine, Davis, California, 95616

ORCID iD: [0000-0001-6849-4885](https://orcid.org/0000-0001-6849-4885)

Copyright

© 2023, Hernandez-Hernandez et al.

This article is distributed under the terms of the [Creative Commons Attribution License](https://creativecommons.org/licenses/by/4.0/), which permits unrestricted use and redistribution provided that the original author and source are credited.

Editors

Reviewing Editor

Olujimi Ajijola

University of California, Los Angeles, Los Angeles, United States of America

Senior Editor

Olujimi Ajijola

University of California, Los Angeles, Los Angeles, United States of America

Reviewer #1 (Public Review):

The authors developed computational models that capture the electrical and Ca^{2+} signaling behavior in mesenteric arterial cells from male and female mice. Sex-specific differences in the L-type calcium channel and two voltage-gated potassium channels were carefully tuned based on experimental measurements. To incorporate the stochasticity of ion channel openings seen in smooth muscle cells under physiological conditions, noise was added to the membrane potential and the sarcoplasmic Ca^{2+} concentration equations. Finally, the models were assembled into 1D vessel representations and used to investigate the tissue-level electrical response to an L-type calcium channel blocker. This comprehensive computational framework helped provide nuanced insight into arterial myocyte function difficult to achieve through traditional experimental methods and can be further expanded into tissue-level studies that incorporate signaling pathways for blood pressure control.

Throughout the paper, model behavior was both validated by experimental recordings and well supported by previously published data. The main findings from the models suggested that sex-specific differences in membrane potential regulation and Ca^{2+} handling are attributable to variability in the gating of a small number of voltage-gated potassium channels and L-type calcium channels. This variability contributes to a higher Ca^{2+} channel blocker sensitivity in female arterial vessels. Overall, the study successfully presented novel sex-specific computational models of mesenteric arterial myocytes and demonstrated their use in drug-testing applications.

- <https://doi.org/10.7554/eLife.90604.2.sa2>

Reviewer #2 (Public Review):

In this study, Hernandez-Hernandez et al developed a gender-dependent mathematical model of arterial myocytes based on a previous model and new experimental data. The ionic currents of the model and its sex difference were formulated based on patch clamp experimental data, and the model properties were compared with single cell and tissue scale experimental results. This is a study that is of importance for the modeling field as well as for experimental physiology.

- <https://doi.org/10.7554/eLife.90604.2.sa1>

Reviewer #3 (Public Review):

Summary:

This hybrid experimental/computational study by Hernandez-Hernandez sheds new light on sex-specific differences between male and female arterial myocytes from resistance arteries. The authors conduct careful experiments in isolated myocytes from male and female mice to obtain the data needed to parameterized sex-specific models of two important ionic currents (i.e., those mediated by $\text{CaV}1.2$ and $\text{KV}2.1$). Available experimental data suggest that $\text{KV}1.5$ channel currents from male and female myocytes are similar, but simulations conducted in the novel Hernandez-Hernandez sex-specific models provide a more nuanced view. This gives rise to the first of the authors' three key scientific claims: (1) In males, $\text{KV}1.5$ is the dominant current regulating membrane potential; whereas, in females, $\text{KV}2.1$ plays a primary role in voltage regulation. They further show that this (2) the latter distinction drives sex-specific differences in intracellular Ca^{2+} and cellular excitability. Finally, working with one-dimensional models comprising several copies of the male/female myocyte models linked by resistive junctions, they use simulations to (3) predict that sensitivity of arterial smooth muscle to Ca^{2+} channel-blocking drugs commonly used to treat hypertension is heightened in female compared to male cells.

In my opinion, the following strengths of the work are particularly notable:

- The Methodology is described in exquisite detail in straightforward language that will be easy to understand for most if not all peer groups working in computational physiology. The authors have deployed standard protocols (e.g., parameter fitting as described by Kernik et al., sensitivity analysis as described by Sobie et al.) and appropriate brief explanations of these techniques are provided. The manoeuvre used to represent stochastic effects on voltage dynamics is particularly clever and something I have not personally encountered before. Collectively, these strengthen the credibility of the model and greatly enrich the manuscript.
- The Results section describes findings that robustly support the three key scientific claims outlined in my Summary. It is evident these experiments were carefully designed and carried out with care and intentionality. Several figures show experimental data side-by-side with

outputs from the corresponding models. These are an excellent illustration of the power of the authors' novel sex-specific computational simulation platform.

- <https://doi.org/10.7554/eLife.90604.2.sa0>

Author Response

The following is the authors' response to the original reviews.

Public Reviews:

Reviewer #1 (Public Review):

Summary:

The authors developed computational models that capture the electrical and Ca^{2+} signaling behavior in mesenteric arterial cells from male and female mice. A baseline model was first formulated with eleven transmembrane currents and three calcium compartments. Sex-specific differences in the L-type calcium channel and two voltage-gated potassium channels were then tuned based on experimental measurements. To incorporate the stochastic ion channel openings seen in smooth muscle cells under physiological conditions, noise was added to the membrane potential and the sarcoplasmic Ca^{2+} concentration equations. Finally, the models were assembled into 1D vessel representations and used to investigate the tissue-level electrical response to an L-type calcium channel blocker.

Strengths:

A major strength of the paper is that the modeling studies were performed on three different scales: individual ionic currents, whole-cell, and 1D tissue. This comprehensive computational framework can help provide mechanistic insight into arterial myocyte function that might be difficult to achieve through traditional experimental methods.

The authors aimed to develop sex-specific computational models of mesenteric arterial myocytes and demonstrate their use in drug-testing applications. Throughout the paper, model behavior was both validated by experimental recordings and supported by previously published data. The main findings from the models suggested that sex-specific differences in membrane potential and Ca^{2+} handling are attributable to variability in the gating of a small number of voltage-gated potassium channels and L-type calcium channels. This variability contributes to a higher Ca^{2+} channel blocker sensitivity in female arterial vessels. Overall, the study successfully met the aims of the paper.

Thank you for your insightful review and for recognizing the strengths of our study. We appreciate your encouraging comment regarding our multi-scale approach. Indeed, we believe that by systematically connecting these scales—individual ionic currents, whole-cell, and 1D tissue—we can integrate and reconcile experimental and clinical data. We anticipate that this approach will not only provide mechanistic insights into arterial myocyte function that may not be easy to glean from traditional experimental methods but will also facilitate the translation of this information into the development of therapeutic interventions.

Weaknesses:

A main weakness of the paper, as addressed by the authors, is the simplicity of the 1D vessel model; it does not take into account various signaling pathways or interactions with other cell types which could impact smooth muscle electrophysiology.

Thank you for highlighting areas for improvement in our study. The strength of computational modeling lies in its iterative nature, allowing us to introduce and examine variables in a systematic manner. While our current model is simplified and does not contain all details, the modular nature of the build will allow continuous expansion to add the important elements described by the reviewer. We are enthusiastic about progressively enriching the model in subsequent studies, introducing signaling pathways in a step-by-step manner, and ensuring their validation with rigorous experimental data.

Another potential shortcoming is the use of mouse data for optimizing the model, as there could be discrepancies in signaling behavior that limit the translatability to human myocyte predictions.

We appreciate this important comment. Our model was parametrized using data from mouse mesenteric artery smooth muscle cells as initial proof of concept. Mouse arteries are a good representation of human arteries, as they have similar intravascular pressure-myogenic tone relationships, resting membrane potentials, and express similar ionic channels (e.g., CaV1.2, BK channels, RyRs, etc) (PMID: 28119464, PMID: 29070899, PMID: 23232643). In response to the reviewer, we have modified the discussion section of the manuscript to specifically note the mouse is not identical to the human but does share some common important features that make mice a good approximate model.

Reviewer #2 (Public Review):

In this study, Hernandez-Hernandez et al developed a gender-dependent mathematical model of arterial myocytes based on a previous model and new experimental data. The ionic currents of the model and its sex difference were formulated based on patch-clamp experimental data, and the model properties were compared with single-cell and tissue scale experimental results. This is a study that is of importance for the modeling field as well as for experimental physiology.

Thank you for the comment. In fact, we developed a model that incorporates sex-dependent differences that allowed for male and female models. It's an important distinction as sex is a biological variable and gender is a self-ascribed characteristic.

Reviewer #3 (Public Review):

Summary:

This hybrid experimental/computational study by Hernandez-Hernandez sheds new light on sex-specific differences between male and female arterial myocytes from resistance arteries. The authors conduct careful experiments in isolated myocytes from male and female mice to obtain the data needed to parameterize sex-specific models of two important ionic currents (i.e., those mediated by CaV1.2 and KV2.1). Available experimental data suggest that KV1.5 channel currents from male and female myocytes are similar, but simulations conducted in the novel Hernandez-Hernandez sex-specific models provide a more nuanced view. This gives rise to the first of the authors' three key scientific claims: (1) In males, KV1.5 is the dominant current regulating membrane potential; whereas, in females, KV2.1 plays a primary role in voltage regulation. They further show that this (2) the latter distinction drives sex-specific differences in intracellular Ca²⁺ and cellular excitability. Finally, working with one-dimensional models comprising several copies of the male/female myocyte models linked by resistive junctions, they use simulations to (3) predict that the sensitivity of arterial smooth muscle to Ca²⁺ channel-blocking drugs commonly used to treat hypertension is heightened in female compared to male cells.

Strengths:

The Methodology is described in exquisite detail in straightforward language that will be easy to understand for most if not all peer groups working in computational physiology. The authors have deployed standard protocols (e.g., parameter fitting as described by Kernik et al., sensitivity analysis as described by Sobie et al.) and appropriate brief explanations of these techniques are provided. The manoeuvre used to represent stochastic effects on voltage dynamics is particularly clever and something I have not personally encountered before. Collectively, these strengthen the credibility of the model and greatly enrich the manuscript.

We appreciate your comment highlighting the robustness of our methodology. Your acknowledgment of our approach to represent stochastic effects on voltage dynamics is especially encouraging. Indeed, noise is a fundamental component of physiological systems, including in vascular myocytes

Broadly speaking, the Results section describes findings that robustly support the three key scientific claims outlined in my summary. While there is certainly room for further discussion of some nuanced points as outlined below, it is evident these experiments were carefully designed and carried out with care and intentionality. In the present version of the manuscript, there are a few figures in which experimental data is shown side-by-side with outputs from the corresponding models. These are an excellent illustration of the power of the authors' novel sex-specific computational simulation platform. I think these figures will benefit from some modest additional quantitative analysis to substantiate the similarities between experimental and computational data, but there is already clear evidence of a good match.

We sincerely appreciate your constructive feedback on the Results section. We have included additional quantitative analysis to substantiate the similarities between experimental and computational data. We agree with the reviewer that the suggestion on the potential value of a more quantitative assessment. As such we have updated the figure to include an in-depth analysis that provides greater insights and solidifies the power of our simulation predictions when compared to experimental results. A detailed analysis of the male and female data as well as the male and female simulations are summarized in the text as follows:

Baseline membrane potential is -40 mV in male myocytes compared to -30 mV. The frequency of hyperpolarization transients (THs) is 1 Hz in male and 2.5 Hz in female cells for the specific baseline membrane potential shown in Figure 5 A-B. In the range of membrane potentials from -50 mV to -30 mV the frequency increases from 1-2.8Hz which is identical to the experimental frequency range.

Areas for Improvement:

The authors used experimental data from a prior publication to calibrate their model of the BKCa current. As indicated in the manuscript, these data are for channel activity measured in a heterologous expression system (Xenopus oocytes). A similar principle applies to other major ion channels/pumps/etc. Is it possible there might be relevant sex-specific differences in these players as well? In the context of the present work, this feels like an important potential caveat to highlight, in case male/female differences in the activity of BKCa or other currents might influence model-predicted differences (e.g., the relative importance of KV1.5 and KV2.1). This should be discussed, and, if possible, related to the elegant sensitivity analysis presented in Fig. 5C (which shows, for example, that the models are relatively insensitive to variation in GBK).

We fully agree with the reviewer - an important caveat to highlight is the unknown sex-specific differences in all the other players regulating membrane potential and calcium signaling. While our initial assessments indicated that the contribution of BKCa channels to the total voltage-gated K⁺ current (IKvTOT) was small within the physiological range of -50 mV to -30 mV, further analysis of spontaneous transient outward currents revealed sex-specific variations. We have investigations underway to explore if BKCa channel expression and organization may be also sex-dependent.

The authors state that their model can be expanded to 2D/3D applications, "transitioning seamlessly from single-cell to tissue-level simulations". I would like to see more discussion of this. For example, given the modest complexity of the cell-scale model, how considerable would the computational burden be to implement a large network model of a subset of the human female or male arterial system? Are there sex-specific differences in vessel and/or network macro-structure that would need to be considered? How would this influence feasibility? Rather than a 1D cable as implemented here, I imagine a multi-scale implementation would involve the representation of myocytes wrapped around vessels. How would the behavior of such a system differ from the authors' presented work using a 1D representation of 100 myocytes coupled end-to-end? Could these differences partially explain why the traces in Fig. 8D are smoother than those in Fig. 8C? From my standpoint, discussing these points would enrich the paper.

We appreciate the reviewer's thoughtful and forward-looking ideas! Indeed, we are very interested to extend the model to incorporate a number of these important items.

Our choice for the 1D cable model was driven by its anatomical relevance to the structure of third and fourth-order mesenteric arteries. These arteries possess a singular layer of vascular myocytes encircling the lumen in a cylindrical arrangement. When we conceptualize this structure as unrolled or viewed laterally, it aligns with a flat, rectangular form, closely paralleling our 1D cable implementation. One option is to expand this into a 2D representation by connecting multiple 1D cables together. Another option would be to connect the 1D cable end-to-end to create a ring to represent a cross section. While these approaches would appear to be different geometries, in either case, the dynamics will remain consistent because the cells comprising the tissue are the same. There is no propagating impulse (for example – although even then in a 2D homogenous tissue, a planar wave is identical in 1D), and the only effect will be an increase in electrotonic load (sink) from neighboring cells, which can readily be approximated in 1D by increasing coupling or modification of the boundary conditions.

We totally agree that future investigation should include exploration into the potential sex-specific differences in vessel and/or network macro-structure, as these factors may critically impact predictions and indeed the difference in traces observed between Fig. 8D and Fig. 8C may well involve “insulating” effects of vessel layers and interaction between various cell types and other structural factors. In particular, the contribution of endothelial cells in modulating membrane potential in vascular myocytes might be one such influential factor. In future studies, we are also keen to investigate blood flow regulation where a 3D configuration might become necessary.

The nifedipine data presented in Fig. 9 are quite compelling, and a nice demonstration of the potential power of the new models. How does this relate to what is known about the clinical male/female responses to nifedipine? Are there sex differences in drug efficacy?

Thank you for your comment regarding Fig. 9.

It is well known that sex-specific differences in pharmacokinetics and pharmacodynamics influence antihypertensive drug responses [PMID: 8651122., PMID: 22089536]. Previous studies, notably by Kloner et al., have illustrated this point quantitatively, highlighting a more pronounced diastolic BP response in women (91.4%) compared to men (83%) when treated with dihydropyridine-type channel blockers, such as amlodipine/nifedipine. Importantly, this distinction persisted even after adjusting for confounding factors such as baseline BP, age, weight, and dosage per kilogram [PMID: 8651122]. An interesting observation from Kajiwarra et al. emphasizes that vasodilation-related adverse symptoms occur significantly more frequently in younger women (<50 years) compared to their male counterparts, suggesting a heightened sensitivity to dihydropyridine-type calcium channel blockers [PMID: 24728902].

While our findings resonate with clinical observations, a word of caution is in order. Our data suggest that, in the mouse model, nifedipine elicits distinct sex-specific effects. Importantly, future research should test the direct translatability and implications of these observations in human subjects.

Reviewer #1 (Recommendations For The Authors):

1. *Cellular simulations with noise: It might be useful to also include in this section how noise was introduced specifically into the [Ca]SR equations.*

We agree. The manuscript now includes an expanded explanation of how noise was incorporated into the model. This includes the addition of Equation 6 into section 2.4 "Cellular simulations with noise" to describe how noise was specifically integrated into the [Ca]SR equations. Please see LINE 355.

1. *For equation 14, the description might be confusing. RCG and Ri are not explicitly included.*

Thank you – this has been corrected.

1. *In the paragraph starting with, "Having explored the regulation of graded membrane potential..." , the references to Figure 7C-D do not seem to match the content of the text. Namely, the figures show female versus male responses to nifedipine, which is not introduced until the next paragraph. Additionally, the graphs in 7C-D do not have the panels titled and the y-axes labeled.*

We apologize for the error. We have modified the text and figures to address these issues.

1. *Perhaps give more detail on how the effects of nifedipine were mathematically simulated at the ionic current level.*

Good suggestion. Briefly, previous studies [PMID: 1329564] have shown that at the therapeutic dose of nifedipine (i.e., about 0.1 μ M) L-type Cav1.2 channel currents are reduced by about 70%. Accordingly, we decreased ICaL in our mathematical simulations by the same extent. It is known that dihydropyridine-type channel blockers exhibit a voltage-dependent behavior, predominantly binding to the inactivated state. In smooth muscle cells, these blockers initiate inhibition quickly within a voltage range of -60 to -40 mV. This range aligns with the membrane potential baseline of vascular muscle cells (PMID: 8388295), ensuring the blockers are effective without the need of inducing significant depolarization. Therefore, the voltage dependency of dihydropyridine-type channel blockers can be neglected.

1. For the simulations with 400 uncoupled myocytes, the methods stated that the "gap junctional resistance [was set] to zero". Did the authors mean to use "conductivity" or am I misunderstanding?

Thank you for bringing up this issue with the term "gap junctional resistance." We now state that the "gap junctional conductivity" was set to zero to indicate no electrical communication/coupling.

1. Address whether there are differences-such as in cell geometry, degree of sex-based ionic current changes, and frequency of spontaneous hyperpolarization-between mice and human smooth muscle myocytes that could limit the predictive capability of the model.

Excellent point. Our model was parametrized using data from mouse mesenteric artery smooth muscle cells as initial proof of concept. In general terms, mouse arteries are a good animal model for human arteries, as they have similar intravascular pressure-myogenic tone relationships, resting membrane potentials, and express similar ionic channel (e.g., CaV1.2, BK channels, RyRs, etc) (PMID: 28119464, PMID: 29070899). Unfortunately, these studies have largely been done in male arteries and myocytes. Thus, while we recognize that the physiological distinctions between mice and humans could introduce variances in the model's outcomes. Our model offers valuable insights into the sex-specific mechanisms of KV2.1 and CaV1.2 channels in controlling membrane potential and Ca²⁺ dynamics in mice. It has been shown that sex-specific differences in pharmacokinetics and pharmacodynamics influence antihypertensive drug responses [PMID: 8651122., PMID: 22089536]. Previous studies, notably by Kloner et al., have illustrated this point quantitatively, highlighting a more pronounced diastolic BP response in women (91.4%) compared to men (83%) when treated with dihydropyridine-type channel blockers, such as amlodipine/nifedipine. Importantly, this distinction persisted even after adjusting for confounding factors such as baseline BP, age, weight, and dosage per kilogram [PMID: 8651122]. An interesting observation from Kajiwarra et al. emphasizes that vasodilation-related adverse symptoms occur significantly more frequently in younger women (<50 years) compared to their male counterparts, suggesting a heightened sensitivity to dihydropyridine-type calcium channel blockers [PMID: 24728902].

While our findings resonate with clinical observations, a word of caution is in order. Our data suggest that, in the mouse model, nifedipine elicits distinct sex-specific effects. Importantly, future research should test the direct translatability and implications of these observations in human subjects.

1. "A virtual drug-screening system that can model drug-channel interactions" (pg 32) sounds very novel.

Thank you for highlighting this. We recognize the typo in our manuscript and have made the necessary corrections to ensure clarity and accuracy.

Reviewer #2 (Recommendations For The Authors):

The manuscript is well written. I only have some minor comments:

1. *In the patch clamp experiments, there is no information on the recovery of the ionic currents. Is recovery important or not in arterial myocytes? This question is related to the results shown in Figs 5-7. In Fig.5, is the oscillation caused by noise alone or a spontaneous oscillation (such as the oscillation in Fig.6-7) modulated by noise? In general, recovery is an important parameter for the frequency of spontaneous oscillations. It seems to me that the spontaneous oscillations in Fig.8 are mainly noise-driven since they disappear after the cells are coupled through gap junctions.*

One important aspect of the oscillatory behavior of the smooth muscle cells is the very long timescales, with fluctuations occurring on the order of seconds. But the majority of ion channels are operating and recovering on the order of milliseconds, so a reasonable approximation is that most ion channels in the cell are operating at steady state at low voltages.

Oscillations in Fig.5: Both the intrinsic oscillations and the noise play key roles in shaping in the oscillations.

The intrinsic deterministic dynamics of the model cells are oscillatory (as seen in Figures 6-7), but the noise can trigger sparks early or delay them, which leads to substantial fluctuations in the inter-spark intervals. Therefore, the spontaneous oscillations are technically modulated by the noise rather than driven by the noise. Nevertheless, in both cases, recovery dynamics play an essential role in shaping the oscillations and determining their frequency

Note however that, when an excitable system is around the bifurcation for oscillations and noise is included, the "firing" statistics in the oscillatory state and the non-oscillatory state are indistinguishable for moderate to high levels of noise.

Noise Exclusion in Figures 6-7: To offer a clear and undistracted interpretation of the results, noise was intentionally omitted from Figures 6-7. This was done to ensure that the primary phenomena under investigation were not obscured. While we recognize the significance of incorporating all elements, including noise, in simulating biological systems, in this case we prioritized a clear point to be made in this context.

Oscillations in Fig.8: Your observation regarding Fig.8 is insightful. Here, uncoupled cells indeed display a spontaneous oscillatory behavior. As documented in previous research, this behavior is not an artifact resulting from cell isolation from the vessel but represents an intrinsic characteristic vital for maintaining electrical signals. The noise in the cells leads to substantial fluctuations in the inter-spike intervals. Because the noise in each cell is uncorrelated, it acts to desynchronize the activity of the cells. Therefore, instead of synchronizing the activity of the cells, the gap junction coupling quenches the large-scale oscillations (the spikes), creating lower amplitude irregular oscillations.

1. *The calcium level is much higher in women than in men as shown in Figs.7 and 9. Do women have higher arterial pressure than men?*

We thank the reviewer for the observation regarding the calcium levels in Figs.7 and 9. All data presented comes from both male and female C57BL/6J animal models, forming the foundation of our experimental framework.

From earlier studies by the Santana lab (PMID: 32015129), distinct sex-specific differences were found between male and female vascular mesenteric vessels. When the endothelium was removed from small arteriole segments and these segments were subsequently pressurized within a range of 20–120 mmHg, the female arterioles exhibited a pronounced

myogenic response in comparison to the male ones. This brings to the forefront the marked sex-based differences, especially in the context of vascular smooth muscle activity.

Yet, when examining the behavior of whole, intact vessels, a different picture emerges. Despite clear sex-specific differences in conditions with the endothelium removed, these distinctions become less pronounced in whole, intact vessels. In essence, both male and female mice exhibit analogous arterial pressure patterns. This suggests possible compensatory mechanisms related to the caliber and structure of the small vessels.

To address the core issue: Despite our data showing higher calcium levels in female samples, it doesn't necessarily imply females consistently exhibit higher arterial pressure across all physiological scenarios.

1. In Fig.9, where is the intravascular pressure (a variable or a parameter) in the mathematical model?

In our model, the intravascular pressure effects are implicitly introduced by modulating the conductance of the non-selective cation currents (INSCC). Specifically, the increase in INSCC is our way of simulating the effects of pressure-induced membrane depolarization. This approach allows us to capture the physiological response to intravascular pressure changes without explicitly introducing it as a separate parameter in the model. We have modified the manuscript to ensure that this rationale is clarified.

1. In Eq.14, the given units of R_{myo} (Ohmcm) and R_g (Ohmcmcm) are different, but Eq.14 implies they should have the same unit.

We sincerely appreciate the reviewer's meticulous observation regarding the units discrepancy in Eq.14. We have revised the manuscript to correct the error.

Reviewer #3 (Recommendations For The Authors):

Suggestions for improved or additional experiments, data, or analyses:

Fig. 5 A-B: This is a beautiful qualitative comparison between experimental and simulation data! I think it would be even more impactful if the authors carried out some quantitative analysis of the similarity between male/female experimental/simulation data. For example, the "resting" V_m levels (approx. -30 mV and -40 mV for females and males, respectively) and the peak levels of V_m hyperpolarization could be compared, as well as the frequency of transient hyperpolarization events. It seems like the female model is much more prone to intervals of relative quiescence (i.e., absence of transient hyperpolarization events - e.g., from ~5-6.5 s). Is this consistent with the duration of such ranges in the experimental data (e.g., from 0 to 2.5 s in Fig. 5A).

Thank you for your positive remarks concerning the qualitative comparison in Fig. 5 A-B. We are indeed enthusiastic about the parallels we've identified between experimental and simulation outcomes. We agree with the reviewer that the suggestion on the potential value of a more quantitative assessment. As such we have updated the figure to include an in-depth analysis that provides greater insights and solidifies the power of our simulation predictions when compared to experimental results. A detailed analysis of the male and female data as well as the male and female simulations are summarized in the text as follows:

Baseline membrane potential is -40 mV in male myocytes compared to -30 mV. The frequency of hyperpolarization transients (THs) is 1 Hz in male and 2.5 Hz in female cells for the specific baseline membrane potential shown in Figure 5 A-B. In the range of membrane potentials

from -50 mV to -30 mV the frequency increases from 1-2.8Hz which is identical to the experimental frequency range.

• Fig. 7 C-D: Likewise, it would be helpful to quantitatively characterize male/female differences in the model's response to simulated Ca channel blockade (e.g., rate of transient hyperpolarization events, relative levels of I_{Ca} and [Ca]_i).

Thank you for the constructive feedback on Fig. 7 C-D. We appreciate the emphasis on a quantitative approach to solidify our understanding and have modified the results as follows:

Next, we simulated the effects of calcium channel blocker nifedipine on I_{Ca} at a steady membrane potential of -40 mV in male and female simulations. Briefly, previous studies⁷⁰ have shown that at the therapeutic dose of nifedipine (i.e., about 0.1 μM) L-type Cav1.2 channel currents are reduced by about 70%. Accordingly, we decreased I_{Ca} in our mathematical simulations by the same extent. In Figure 7C-D, we show the predicted male (gray) and female (pink) time course of membrane voltage at -40 mV (top panel), I_{Ca} (middle panel), and [Ca²⁺]_i (lower panel). First, we observed that in both male and females 0.1 μM nifedipine modifies the frequency of oscillation in the membrane potential, by causing a reduction in oscillation frequency. Second, both male and female simulations (middle panels) show that 0.1 μM nifedipine caused a reduction of I_{Ca} to levels that are very similar in male and female myocytes following treatment. Consequently, the reduction of I_{Ca} causes both male and female simulations to reach a very similar baseline [Ca²⁺]_i of about 85 nM (lower panels). As a result, simulations provide evidence supporting the idea that Cav1.2 channels are the predominant regulators of intracellular [Ca²⁺] entry in the physiological range from -40 mV to -20 mV. Importantly, these predictions also suggest that clinically relevant concentrations of nifedipine cause larger overall reductions in Ca²⁺ influx in female than in male arterial myocytes.

Recommendations for improving the writing and presentation:

When I accessed the GitHub repository linked in section 2.7 (Aug 17, 13:30 PT) it only contained a LICENSE file and none of the described codes and model equations appeared to be publicly available. I would like to access and examine these files. Based on the Clancy lab's excellent track record for making their work publicly available, I have no doubt that the published files will be complete, thoroughly documented, and ready for implementation in studies to reproduce or extend the work described in this manuscript.

<https://github.com/ClancyLabUCD/sex-specific-responses-to-calcium-channel-blockers-in-mesenteric-vascular-smooth-muscle>

We sincerely apologize for the omission regarding the GitHub repository. It was never our intention to omit the crucial files that should accompany our manuscript. We deeply regret any inconvenience this may have caused in your review process.

We deeply value transparency and the importance of making our work accessible to fellow researchers and the wider community. As you rightly pointed out, the Clancy lab has always been committed to ensuring that our work is available publicly, and this instance is no exception. Please find all codes and documentation here:

Minor corrections to the text and figures:

The introduction is somewhat lengthy, and some of the material contained therein might be more suitable to be merged into the Discussion instead (e.g., paragraphs on negative feedback regulation and the recent study by O'Dwyer et al.).

Thank you – we have updated the introduction but left some foundational work descriptions intact.

- Page 6, section 1.1: There is a missing word (mice?) in the first sentence.
- Page 11, under Eqn. 7: Luo is misspelled as Lou. (Also twice on Page 20.)

Thank you – these have been corrected.

Figs. 2-3: As a colorblind person, it was somewhat challenging for me to differentiate between the red and black lines. Choosing a higher-contrast colour pairing would be beneficial. For some reason, this is not so much of an issue for other figures that use the red/black scheme later in the manuscript (e.g., Figs. 5, 7-8).

We truly appreciate your feedback on the color contrast used in our figures. Accessibility and clarity are crucial to us, and we regret any difficulty you encountered due to the color choices. Based on your valuable feedback, we have included different color pairings in our visual representations to ensure they are comprehensible to all readers, including those who are colorblind.

*Fig. 2-3: I am also confused about the use of symbols to indicate significant differences in these plots. In Fig. 2, ** is defined in the legend but not used in the figure. In both figures, the symbols are placed above/below specific sets of points, but it is unclear whether large differences for other x-axis values are statistically significant (e.g., -20 mV in Fig. 3B, +40 mV in Fig. 2C, etc.) This should be clarified.*

Thank you – we now have included all the significant differences in the data discussed in the manuscript.

Page 22: The authors state that they "introduced noise into the [Ca]SR..." but the specifics of this approach are not described. As with other aspects of the Methods section, it would be suitable to provide a brief description of the technique used in ref. 40, perhaps added to section 2.4.

Thank you – it has been corrected.

Fig.7 C-D: Axis labels and units are missing. Even though the labels and units will be inferred by most readers, it would be helpful to include them here (at least in C).

Thank you for pointing out the inconsistency between the textual references and Figure 7C-D. We have added the corrected figure.

Page 32: "...the first step toward the development of a virtual drug-screaming system..." I think the authors mean drug-screening. As a side note, this is immediately in the running for the best typo I've ever seen as a peer reviewer.

Thank you for pointing out this error, and we sincerely appreciate your sense of humor about it. You are indeed correct; the intended word is "drug-screening." We have corrected this typo in the manuscript. We're grateful for your thorough review and the light-hearted way you brought this to our attention.