A Computational Model of the Ionic Currents, Ca\(^{2+}\) Dynamics and Action Potentials Underlying Contraction of Isolated Uterine Smooth Muscle

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Abstract

Uterine contractions during labor are discretely regulated by rhythmic action potentials (AP) of varying duration and form that serve to determine calcium-dependent force production. We have employed a computational biology approach to develop a fuller understanding of the complexity of excitation-contraction (E-C) coupling of uterine smooth muscle cells (USMC). Our overall aim is to establish a mathematical platform of sufficient biophysical detail to quantitatively describe known uterine E-C coupling parameters and thereby inform future empirical investigations of physiological and pathophysiologial mechanisms governing normal and dysfunctional labors. From published and unpublished data we construct mathematical models for fourteen ionic currents of USMCs: Ca\(^{2+}\) currents (L- and T-type), Na\(^{+}\) current, an hyperpolarization-activated current, three voltage-gated K\(^{+}\) currents, two Ca\(^{2+}\)-activated K\(^{+}\) current, Ca\(^{2+}\)-activated Cl\(^{-}\) current, non-specific cation current, Na\(^{+}\)-Ca\(^{2+}\) exchanger, Na\(^{+}\)-K\(^{+}\) pump and background current. The magnitudes and kinetics of each current system in a spindle shaped single cell with a specified surface area:volume ratio is described by differential equations, in terms of maximal conductances, electrochemical gradient, voltage-dependent activation/ inactivation gating variables and temporal changes in intracellular Ca\(^{2+}\) computed from known Ca\(^{2+}\) fluxes. These quantifications are validated by the reconstruction of the individual experimental ionic currents obtained under voltage-clamp. Phasic contraction is modeled in relation to the time constant of changing [Ca\(^{2+}\)]. This integrated model is validated by its reconstruction of the different USMC AP configurations (spikes, plateau and bursts of spikes), the change from bursting to plateau type AP produced by estradiol and of simultaneous experimental recordings of spontaneous AP, [Ca\(^{2+}\)] and phasic force. In summary, our advanced mathematical model provides a powerful tool to investigate the physiological ionic mechanisms underlying the genesis of uterine electrical E-C coupling of labor and parturition. This will furnish the evolution of descriptive and predictive quantitative models of myometrial electromogenesis at the whole cell and tissue levels.


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Introduction

For over 50 years it has been known that uterine smooth muscle (myometrium) generates spontaneous action potentials (APs) [1–3]. These precede elevations in intracellular Ca\(^{2+}\) that, in turn, facilitate the actomyosin interactions governing myometrial contractions [4,5]. The regulation of electrical activity of myometrial cells therefore plays a crucial role in determining the onset, the duration and the strength of uterine contractions during labor. This is essential for a successful conclusion to pregnancy with the safe delivery of the fetus and placenta. Unfortunately, many pregnancies result in complications of labor that compromise the health of the fetus/newborn. Preterm birth, of which activation of uterine contraction is the major cause, occurs in up to 12% of deliveries and results in a high incidence of mortality and morbidity of the offspring [6]. Prolonged dysfunctional labor at term occurs in 10% of pregnancies and these patients account for 20% of Cesarean sections [7]. An improved understanding of the physiological complexities of myometrial electrical excitability would assist in the task of developing better targeted therapies for these problematic labors.

Modifications of myometrial cell electrophysiological characteristics during pregnancy are evident. The resting membrane potential of myometrial cells becomes progressively more positive towards term [8], gestational-dependent changes in the molecular expressions of ionic channel components occurs [9] and the form of action potentials can change between those of rapid spike-like and tonic plateau-type [10,11]. Electrophysiological recordings have also identified several classes of individual ionic currents in myometrial cells. It is accepted that the major inward depolarizing
current of the AP likely arises from Ca\(^{2+}\) entry via L-type Ca\(^{2+}\) channels [12]. Other myometrial inward currents that have been suggested to be functional, at least in some experimental situations, include those mediated through T-type Ca\(^{2+}\) channels [13], Na\(^+\) channels [14] or Cl\(^-\) channels [13]. Voltage-dependent outward currents, both those that are sensitive or insensitive to 4-aminopyridine (4-AP), have been identified as have calcium-dependent K\(^+\) currents [16–20]. Molecular expression of genes/proteins of electrogenic ion exchangers, the Na\(^+\)-K\(^+\)-ATPase [21] and the Na\(^+\)-Ca\(^{2+}\) exchangers [22], suggest that these too may have a contribution to make to regulating myometrial membrane potential.

There is increasing awareness of the benefits of developing mathematical descriptions of uterine function [23–25] and recent attempts have shown promise regarding the mapping of electrophysiological or contractile data. However, detailed descriptions of the biophysical characteristics of each of the myometrial ionic currents are lacking. In addition, information on how these individual ionic currents are integrated to form the shape and timecourse of APs reflects of those reported for the myometrium is sparse. This severely limits the ability to model simultaneous changes in myometrial membrane potential, [Ca\(^{2+}\)]\(_i\), and force that are the essential elements of electrical E-C coupling. It is important to determine each of these circumstances in order to assess fully the likely physiological relevance to AP genesis of any electrophysiological data that has been recorded in isolation and attributed to a particular ion channel subtype. It is also necessary to consider how these electrical events influence E-C coupling parameters leading to the generation of phasic contractions of uterine smooth muscle as this, after all, determines the success of the parturient effort. Therefore, we had three aims to the present work. First, to develop biophysically detailed quantitative (mathematical) descriptions of all known individual ionic currents of uterine smooth muscle cells pertaining to the near end of pregnancy. Second, to compute these, in alliance with descriptions of dynamic Ca\(^{2+}\) handling parameters, into a mathematical model of myometrial action potential generation. Third, to extend this model to the simulation of concomitant recordings of spontaneous AP, Ca\(^{2+}\) and force in uterine smooth muscle. Moreover, the model is assessed for its ability to simulate published changes in experimental parameters. The development of our quantitative model markedly advances our understanding of the electrophysiological basis of excitation-contraction coupling in uterine smooth muscle. In so doing, it also provides a framework of relevance for exploring the biophysical modeling of individual ionic currents underlying the electrogenic processes in other smooth muscles, tissues and organs.

**Results and Discussion**

The general mathematical formulae used for parameter modeling are given in the Methods (equations 1–9). A glossary of symbols used in the modeling equations is given in Tables S1, S2. Detailed formulations of individual model components are given in Appendix S1 (equations 10–105).

**L-type Calcium current – I\(_{CaL}\)**

Mathematical descriptions of the biophysical characteristics of this current are given in Appendix S1 (equations 10–19).

I\(_{CaL}\) is attributed as the major inward current in myometrial cells [8,14,26–28]. I\(_{CaL}\) first appears at \(V \approx -40\) to \(-30\) mV; the peak of the current-voltage (I-V) relationship arises between \(V = -10\) to \(10\) mV and the reversal potential \(E_{CaL} \approx 45\) to \(60\) mV at \(30\)–\(35\) C with \(1.5\)–\(2.5\) mM [Ca\(^{2+}\)]\(_o\) [12,15,29,30]. L-type calcium channels in other cell types have been reported to be permeable to other cations [31] but there is no data specific to myometrial cells. Thus, the Goldman-Hodgkin-Katz formulation commonly used in other muscle cell models is not used here; instead, \(E_{CaL}\) in the model is fixed at \(45\) mV as suggested by experimental data [12,30,32].

Properties of I\(_{CaL}\) are derived from experimental data at \(30\)–\(35\) C of myometrial cells from late pregnant rat. The equations of I\(_{CaL}\) incorporate an activation gating variable (\(d\)) and fast (\(f_1\)) and slow (\(f_2\)) inactivation gating variables. Different steady-state values for activation and inactivation at \(30\)–\(35\) C have been reported and representatives of the data range are plotted in Figure 1A–B. This may reflect different [Ca\(^{2+}\)]\(_o\) employed between studies or slightly differing residual hormonal influences. Yoshino et al. [33] showed that the half-activation and the I-V relationship were right-shifted by \(\approx 15\) mV when [Ca\(^{2+}\)]\(_o\) was increased from 3 mM to 30 mM; the rather rightward steady-state inactivation values from Amedee et al. [29] were recorded from myometrial cells exposed to 10 mM [Ca\(^{2+}\)]\(_o\). Yamamoto [30] showed that the I\(_{CaL}\) half-inactivation was left-shifted, and the I-V relationship was reduced, in the myometrial cells exposed to estradiol; in rodents, estradiol increases near term. The myometrial cells from late pregnant rats reported by Shmigol et al. [12] were not functional, at least in some experimental situations, and the Goldman-Hodgkin-Katz formulation commonly used in other muscle cell models is not used here; instead, \(E_{CaL}\) in the model is fixed at \(45\) mV as suggested by experimental data [12,30,32].

There is little information available for voltage-dependent activation time constants of myometrial I\(_{CaL}\), so we proceeded to extract time constants from published I\(_{CaL}\) current tracings. Amedee et al. [29] and Jones et al. [15] had reported I\(_{CaL}\) current tracings at \(30\)–\(35\) C, but in Amedee et al., [29] only at a single voltage step and of poor quality for curve fitting purposes. There are other I\(_{CaL}\) current tracings [14,33–35] at room temperature but we are unaware of published Qo values for myometrial I\(_{CaL}\). The experiments of Jones et al. [15], performed at 35 C, were designed to study I\(_{Ca}^{(Ca)}\) wherein I\(_{CaL}\) was first activated to enable plasmalemmal Ca\(^{2+}\) entry that, subsequently, activated a current taken to be I\(_{Ca}^{(Ca)}\). The initial fast inward current was attributed as I\(_{CaL}\) because it was blocked by nifedipine, was permeable to Ba\(^{2+}\) and was increased by the L-type Ca channel agonist Bay K8644. We presumed that activation of I\(_{Ca}^{(Ca)}\) would be slower than I\(_{CaL}\) and, thus, voltage-dependent activation time constants for I\(_{CaL}\) were obtained by fitting the initial few tens of milliseconds of raw data tracings, i.e. prior to peak current at each voltage step being reached, from Jones et al. [15] (Figure 1C). This assumption is backed up by the activation time constants for I\(_{CaL}\) in other smooth muscles being \(2\)–\(8\) ms whereas that for I\(_{Ca}^{(Ca)}\) has been estimated at \(>50\) ms [36]. The two inactivation time constants, \(f_1\) and \(f_2\), were taken from Amedee et al. [29] (Figure 1D). The fast inactivation \(f_1\) is voltage-independent at \(\approx 12\) ms and the slow inactivation is voltage-dependent with a minimum of \(\approx 55\) ms at \(V = 0\) mV.

Simulated time tracings of I\(_{CaL}\) under voltage-clamp conditions and I\(_{CaL}\) I-V relationships were compared to experimental data in Figure 1E–F. The simulated time tracings closely matched the experimental time data from Jones et al. [15]; I\(_{CaL}\) reached its peak in \(\approx 12\) ms then quickly inactivated. Only the time tracings at voltage steps between \(-40\) to \(-0\) mV from Jones et al. [15] were used for comparison in order to minimize contamination by I\(_{Ca}^{(Ca)}\). The
simulated I–V relationship further shows that $I_{CaL}$ first appears at $V \approx -40$ to $-30$ mV and peaks at $V = 0$ mV, similar to that seen experimentally [12,15,29,30]. Validation of the model is also evinced by the ability to reproduce the effects of estradiol on the $I_{CaL}$ I–V relationships reported by Yamamoto [30]. Herein, the effect on the simulated I–V relationship of experimentally observed estradiol-induced changes in current were examined. The model reproduced the estradiol-mediated leftward shift in inactivation, and the reduction in I–V amplitude, from a $V_h$ of $-40$ mV (Figure S1).

Peak $I_{CaL}$ currents in myometrial cells of late pregnant rat have been reported to be $\approx -6.62 \pm 0.55 \text{ pA pF}^{-1}$ ($V_h = -60$ mV, Jones et al., [15]) and $\approx -5.23 \pm 0.6 \text{ pA pF}^{-1}$ ($V_h = -50$ mV, Okabe et al., [32]) at $30 - 35^\circ C$. This gives a maximal conductance ($g_{CaL}$) of $\approx 0.35 \text{ nS pF}^{-1}$ for modeling the ionic current data.

With $g_{CaL} = 0.35 \text{ nS pF}^{-1}$ in the later development of the USMC action potential simulations, the rate of rise of an AP was $\approx 2.5 \text{ V s}^{-1}$ which was less than the reported experimental range of $5 - 10 \text{ V s}^{-1}$ [37]. Thus, it is necessary to set $g_{CaL}$ at a higher value at $0.6 \text{ nS pF}^{-1}$.

It is possible that the reported $I_{CaL}$ current density may represent the lower limits in late pregnant rat myometrial cells given that (i) the expression of mRNA encoding L-type Ca channel protein subunits increases before labor in rat myometrial cells [38–40] and the protein expression of the pore forming $\alpha_1C$ subunit is regulated by ratio of sex hormones [41]; (ii) the $I_{CaL}$ current density may be underestimated by in vitro experimental conditions: $I_{CaL}$ current density in isolated cells diminishes with time [11,15]. Myometrial $I_{CaL}$ also showed calcium-dependent inactivation [26,29]. This is described by a Hill equation with $K_{Ca} = 1\text{ mM}$ and a Hill coefficient of 4 in the whole USMC cell model.

Mathematical descriptions of the biophysical characteristics of this current are given in Appendix S1 (equations 20–27).
Modeling of \( I_{\text{Na}} \) is accomplished using data from myometrial cells of late pregnant rats or humans recorded at room temperature [14,33,34,42]. \( I_{\text{Na}} \) first appears at \( V \approx -50 \text{ mV} \) and the peak I–V relationship occurs between \( V = -10 \) to \( +10 \text{ mV} \). Raw data current tracings showed that \( I_{\text{Na}} \) reached its peak of activation within \( \approx 1 \text{ ms} \) and almost completely inactivated after \( 10 - 20 \text{ ms} \) [14,33,34,42].

The equation for \( I_{\text{Na}} \) incorporates an activation gating variable (\( m \)) and an inactivation gating variable (\( h \)). Steady-state values for activation and inactivation are shown in Figure 2A. The time constants of activation and inactivation (Figure 2B) were each obtained by fitting the raw data current tracings from the literature [14,33,34,42]. Simulated traces of \( I_{\text{Na}} \) current under voltage-clamp conditions presented in Figure 2C show dynamic profiles similar to the raw data [14,33,34,42]: at voltage steps of \(-40 \text{ mV} \) to \( 0 \text{ mV} \), from a \( V_b \) of \(-90 \text{ mV} \), \( I_{\text{Na}} \) reached its peak in \( \approx 2 \text{ ms} \) then quickly inactivated within \( 10 \text{ ms} \). The reported peak currents for \( I_{\text{Na}} \) range from \(-0.86 \) to \(-3.67 \text{ pA pF}^{-1} \) [33,34,42], which gives a maximal conductance range \( g_{\text{Na}} \) of \( \approx 0.028 - 0.125 \text{ nS pF}^{-1} \). Simulated I–V relationship of \( I_{\text{Na}} \) matched to the experimental data as shown in Figure 2D [14,34,42].

### T-type Calcium current – \( I_{\text{CaT}} \)

Mathematical description of the biophysical characteristics of this current are given in Appendix S1 (equations 28–34). \( I_{\text{CaT}} \) has been reported in human myometrial cells [13,14,28,37,43,44]. Moreover: (i) Ohkubo et al. [40] showed that the expressions of mRNA encoding for the \( z1G \) and \( z1H \) protein subunits of the T-type calcium channel were gestationally regulated in rat myometrial cells; (ii) detailed electrophysiological data of cells expressing \( z1G/Cav3.1 \) are available [43,44]; and (iii) spontaneous contractions in myometrial tissue strips from late pregnant rats were markedly inhibited by the putative T-type calcium channel blockers mibebradil, NNC 55-0396 (a non-hydrolyzable analogue of mibebradil) and Ni\(^{2+} \) [45,46]. Therefore, we developed a model of \( I_{\text{CaT}} \) electrophysiological characteristics from the rat \( z1G/Cav3.1 \) clonal expression cell data recorded at room temperature [43,44] adjusted to the current density of human myometrial cell \( I_{\text{CaT}} \) [13,18,28]. It is noteworthy that the activation and inactivation steady-state values, and the I–V relationships, are similar between these different datasets. \( I_{\text{CaT}} \) first appears at \( V \approx -60 \text{ mV} \), the peak I–V relationship occurs between \(-20 \text{ mV} \) and \(-30 \text{ mV} \), and published raw data current tracings indicate a fast activation but with inactivation temporal profiles varying between \( 7 - 100 \text{ ms} \) [13,18,28,43,44,47].

The equation for \( I_{\text{CaT}} \) incorporates an activation gating variable (\( b \)) and an inactivation gating variable (\( g \)). Steady-state values for activation and inactivation are shown in Figure 3A. A function is shown for activation time constants to fit the time-to-peak experimental data (Figure 3B). The time constant of inactivation is shown in Figure 3C. Simulated \( I_{\text{CaT}} \) tracings under voltage-clamp conditions and I–V relationships are shown in Figure 3D and Figure 3E respectively and are compared to experimental data from Serrano et al. [43] and Hering et al. [44]. In Figure 3E, \( E_{\text{CaT}} \) is fixed at \( 25 \text{ mV} \) to match the experimental values in Serrano et al. [43] and Hering et al. [44]. The reported peak current for \( I_{\text{CaT}} \) is \( \approx -1.5 \text{ pA pF}^{-1} \) at \( V = -30 \text{ mV} \) from a \( V_b \) of \(-80 \text{ mV} \).

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**Figure 2. Myometrial \( I_{\text{Na}} \) model.** Properties of \( I_{\text{Na}} \) are derived from experimental data of myometrial longitudinal cells [14,33,34,42] from late pregnant rats. A, V-dependent steady-states of activation (\( m \)) and inactivation (\( h \)); B, V-dependent time constants of activation (\( t_m \)) and inactivation (\( t_h \)). In both A and B, solid and empty circles are experimental data for activation and inactivation respectively. C, simulated \( I_{\text{Na}} \) at voltage steps of \(-40 \) to \( 0 \text{ mV} \) from a \( V_b \) of \(-90 \text{ mV} \); D, simulated peak I–V relationship of \( I_{\text{Na}} \) at \( V_b = -90 \text{ mV} \) and experimental I–V data. In both C and D, all data are normalized to the peak current value at \( V = 0 \text{ mV} \).

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in human myometrial cells [13], which gives a maximal conductance $g_{CaT} \approx 0.055 \text{nS pF}^{-1}$. For incorporation of the $I_{CaT}$ model in the later development of the USMC AP simulations, $E_{CaT} = 42 \text{mV}$ so as to mimic that of Blanks et al., [13].

Hyperpolarization-activated current – $I_h$

Mathematical description of the biophysical characteristics of this current are given in Appendix S1 (equations 35–39).

$I_h$ has been reported in myometrial cells of pregnant rats [48,49]. Activated by hyperpolarization beyond resting membrane potential, $I_h$ first appears at $V \approx -70 \text{mV}$ from a $V_h$ of $-50 \text{mV}$. In the voltage-clamp experiments, activation of $I_h$ is slow, taking $\geq 1 \text{s}$, and it does not inactivate. It is more permeable to $K^+$ ions than $Na^+$ ions, is blocked by $Cs^+$, and has a reversal potential ($E_h$) of $\approx -20 \text{mV}$.

$I_h$ was modeled at room temperature to 30°C using myometrial cells of pregnant rats [48,49]. Our model of $I_h$ biophysical characteristics was first developed with the data of [49] with an activation gating variable ($y$) and $E_h$ approximated by the Goldman-Hodgkin-Katz (GHK) equation with a permeability ratio $P_{Na}/P_{K} = 0.35$. The half-activation was adjusted and the activation time constant was corrected with the reported $Q_{10} = 3.5$ [49] in order to match the experimental I–V relationship of Satoh [48] (Figure 4). The current density was 1.03 pA pF$^{-1}$ at $V = -120 \text{mV}$ from a $V_h$ of $-50 \text{mV}$ [48], which gives a maximum conductance of $g_h = 0.0542 \text{nS pF}^{-1}$.

Potassium Currents

We have considered the electrophysiological data of several major types of potassium currents described from myometrial cells of rat and human myometrium: (two) voltage-gated potassium currents ($I_K$ and $I_{K2}$), A-type transient potassium current ($I_{K1}$), and Ca$^{2+}$-activated potassium currents ($I_{K(Ca)}$). The kinetics of individual potassium currents are described in detail below; their current densities are discussed in the later section concerned with total potassium current.

Voltage-dependent potassium currents – $I_{K1}$ and $I_{K2}$

Mathematical descriptions of the biophysical characteristics of these currents are given in Appendix S1 (equations 40–58).

Myometrial potassium currents have been roughly categorized by their inactivation properties and sensitivity to pharmacological blockers of varying channel subtype specificity [17,19]. At least two different types of potassium currents with rectifying properties were found in myometrial cells of late pregnant rats [17] and humans [19]; their dynamics were very slow compared to other membrane currents in myometrial cells. These potassium currents were separated as $C1$ and $C2$ components of the total potassium current in Wang et al., [17] and as $I_{K1}$ and $I_{K2}$ in Knock et al., [19].

$C1$ and $I_{K1}$, and $C2$ and $I_{K2}$ have similar voltage-dependent kinetics. Both $C1$ and $I_{K1}$ first appear at $V \approx -50$ to $-40 \text{mV}$ and with half-inactivation ($V_{0.5,inact}$) between $-62 \text{mV}$ to $-68 \text{mV}$. Both $C2$ and $I_{K2}$ first appear at $V \approx -40$ to $-30 \text{mV}$ and with $V_{0.5,inact}$ between $-30 \text{mV}$ to $-20 \text{mV}$. Wang et al., [17] distinguished between $C1$ and $C2$ by their activation thresholds and inactivation properties whereas Knock et al., [19] separated $I_{K1}$ and $I_{K2}$ by these properties and current sensitivities to 4-aminoypyridine (4-AP) and TEA. As such, we developed mathe-

![Figure 3. Myometrial $I_{CaT}$ model. Properties of $I_{CaT}$ are derived primarily from experimental data of Serrano et al., [43] and Hering et al., [44]. A, Voltage-dependent steady-states of activation ($b_v$) and inactivation ($a_v$); experimental data in brackets were extrapolated from the published I–V relationships and normalized to the maximum value. B, superimposed simulated and experimental time-to-peak of $I_{CaT}$ at different $V$ stepped from $V_h$ of $-100 \text{mV}$; a function for the $V$-dependent activation time constant is chosen so that the simulated time-to-peak (empty circles) matched the experimental data (solid circle). C, Voltage-dependent inactivation time constant ($\tau_i$). D, simulated $I_{CaT}$ at voltage steps of $-60$ to $20 \text{mV}$ from a $V_h$ of $-80 \text{mV}$; E, simulated peak I–V relationship of $I_{CaT}$ and experimental I–V data. In both D and E, all data are normalized to the peak current value at $V \approx -25 \text{mV}$. DOI:10.1371/journal.pone.0018685.g003](figure3.png)
matical models predominantly based upon the more abundant information of electrophysiological characteristics of human myometrial I(K1) and I(K2) and complemented these with data on rat myometrial C1 and C2 of Wang et al., [17] at room temperature.

The equations of I(K1) [not to be confused with the myocardial inward rectifying potassium current commonly designated also as I(K1) [50]] and I(K2) each incorporate three gating variables: an activation gating variable (q for I(K1); p for I(K2)), a fast inactivation gating variable (τ1 for I(K1); k1 for I(K2)) and a slow inactivation gating variable (τ2 for I(K1); k2 for I(K2)). The activation and inactivation steady-state values were used as reported from Wang et al., [17] with the assumption that both currents were completely inactivated (Figure 5A, 6A, see below). For I(K1), voltage-dependent steady-state of inactivation (τq) is formulated with the reported half-inactivation of −63 mV and slope factor of 6.3 mV and, for I(K2), voltage-dependent steady-state of inactivation (τk) is assessed with the reported half-inactivation of −21.2 mV and slope factor of 5.7 mV reported by Wang et al., [17].

Activation time constants of I(K1) and I(K2) currents were from Knock et al., [19] (Figure 5B, 6B) for I(K1) and I(K2) respectively. However, Knock et al., [19] reported the activation time constants of I(K1) and I(K2) currents elicited at only one voltage step (Vh of −80 mV stepped to +10 mV): inactivation of I(K1) was described as a double exponential and a constant whereas inactivation of I(K2) was described as a monoexponential and a constant. Their inclusion of constant values was due to the currents not inactivating during the course of the 10 sec voltage pulse. However, using these values it was impossible to simulate the published raw current tracings of the voltage-clamp protocols for I(K1) and I(K2) (Figure 4 in Knock et al., [19]). We therefore sought to extract a more complete set of inactivation time constants that encompassed currents elicited at each voltage step of the protocols listed in Knock et al., [19]. This was accomplished by examining the raw data tracings kindly supplied by Drs Greg Knock and Phil Aaronson (Kings College London). The I(K1) or I(K2) currents in each of these datasets were produced in 10 mV steps between −50 mV and 10 mV from a Vh of −80 mV. Averaging the I(K1) (5 cells, Figure S4) or I(K2) (4 cells, Figure S5) at each step enabled a calculation of the voltage-dependent inactivation time constants (Figure 5C and 6C for I(K1) and I(K2) respectively). The inactivations of I(K1) and I(K2) were described by a fast and a slow time constants. Moreover, we removed the need for a constant value used by Knock et al., [19] by assuming that each current was completely inactivated. This, in fact, was reported to be the case by Knock et al., [19] when they extended the experimental voltage pulses beyond 10 seconds. Satisfactory simulation of the published I–V curves and raw current data was now possible. Simulated I–V relationships of I(K1) and I(K2) (Figure 5D, 6D) stepping from two different Vh, −80 mV and −40 mV, showed that while I(K1) was mostly inactivated with Vh = −40 mV, I(K2) remained available. From the simulated current tracings (Figure 5E, 6E) both I(K1) and I(K2) took more than 10 s to inactivate but I(K2) was inactivated faster than I(K1). Current densities of I(K1) and I(K2) are discussed in the section of total potassium current.

A-type transient potassium current – I(Ka)

Mathematical descriptions of the biophysical characteristics of this current are given in Appendix S1 (equations 59–65).

I(Ka) is a 4-AP sensitive, TEA-insensitive potassium current with very fast activation and inactivation kinetics. It is found in myometrial cells of both rat and human [27,51].

I(Ka) is first evident at V ≈ −40 mV and raw data tracings show I(Ka) peak activation within ≈10 ms and almost completely inactivated within 50 ms [27,51]. In human myometrial cells, I(Ka) has a half-inactivation of ≈−70 mV and a slope factor of

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**Figure 4. Myometrial I(K) model.** Properties of I(K) are derived from experimental data of Okabe et al., [49] in rat circular myometrial cells and adjusted to experimental data of longitudinal cells [48]. A, V-dependent activation steady-state (yq); B, V-dependent activation time constant (τq). C, simulated voltage-clamp I(K) at voltage steps of −130 to −70 mV from a holding potential of −30 mV. D, simulated I–V relationship of I(K) and experimental I–V data Satoh [48]. In both C and D, all data are normalized to the current value at V = 110 mV.
These characteristics are very similar to the transient potassium current in myometrial cells isolated from immature rats [52] which were inhibited by 1 mM of 4-AP and were measured within 3–6 ms of the voltage step; it has a half-inactivation of \( \approx -48 \text{ mV} \) and a slope factor of 8.7 mV.

\( I_{K_a} \) is modeled from data of myometrial cells from pregnant rats and humans recorded at room temperature. The model of \( I_{K_a} \) incorporates one activation gating variable (\( s \)) and an inactivation gating variable (\( x \)). Steady-state values for activation and inactivation are shown in Figure 7A. Voltage-dependent steady-states of activation (\( q_{s,s} \)) and inactivation (\( r_{s,s} \)), B, V-dependent activation time constants (\( t_{q_1} \)), C, V-dependent fast (\( t_{r_1} \)) and slow (\( t_{r_2} \)) inactivation time constants. The experimental fast (solid circles) and slow (empty circles) inactivation time constants were extracted by fitting voltage-clamp time tracings averaged from five cells (1 published and 4 unpublished with the average values labeled as ‘Knock et al 1999+unpublished (Knock & Aaronson)’ in the figure). D, simulated I–V relationship of \( I_{K_1} \) from holding potentials of \(-80 \text{ mV}\) and \(-40 \text{ mV}\) with \([K^-]_o=5 \text{ mM}\) and \([K^-]_i=110 \text{ mM}\); all values are normalized to the peak current at \( V=-60 \text{ mV}\) from \( V_h=-80 \text{ mV}\). E, simulated time tracings and averaged raw data of \( I_{K_1} \) at voltage steps of \(-40\) to \(-10 \text{ mV}\) from \( V_h=-80 \text{ mV}\); both simulated and experimental currents are normalized to the peak current at \( 10 \text{ mV}\); F, enlarged E showing activation of \( I_{K_1} \) during the first few hundred milliseconds.

\( I_{K_1} \) is a calcium-activated potassium current. Mathematical descriptions of the biophysical characteristics of this current are given in Appendix S1 (equations 66–78).

Calcium-activation potassium current – \( I_{K(Ca)} \)

Mathematical descriptions of the biophysical characteristics of this current are given in Appendix S1 (equations 66–78).

Calcium-activated potassium currents \( I_{K(Ca)} \) have been suggested to play important roles in suppressing the excitability of smooth muscle cells especially those in the vasculature. In
myometrial cells $I_{K(Ca)}$ is under complex gestational-mediated regulation: the large conductance Ca$^{2+}$-activated K$^{+}$ channels (termed BKCa channel) subunit compositions and current density are diminished near to term. As such, although BKCa channels have been a focus of much interest in the myometrium [16,17,19,53–62], detailed biophysical information on whole cell current is rather restricted. When detected in myometrial whole cell recordings, $I_{K(Ca)}$ was distinctly noisy and its activation was almost instantaneous [17,27]. From the reported recordings of $I_{K(Ca)}$ in myometrial cells by Khan et al., [16,61,62], Wang et al., [17] and Noble et al., [20] many of the biophysical parameters required to model complete ion current characteristics are absent. Therefore, a biophysical quantification of the $I_{K(Ca)}$ current is developed from experimental whole cell electrophysiological data obtained at room temperature from cloned mammalian smooth muscle α (pore-forming) and β1 (regulatory) subunits of BKCa subsequently expressed in Xenopus laevis oocytes [63,64]. The current densities of $I_{K(Ca)}$ in the model are adjusted to replicate published human myometrial cell data [65,66].

We assumed that the transmembrane β1 subunits were separately regulated from the pore-forming α subunits and, therefore, two subtypes of $I_{K(Ca)}$ were developed: one where $I_{α}$ reflects an $I_{K(Ca)}$ consisting of α subunits; another where $I_{αβ1}$ represents an $I_{K(Ca)}$ consisting of α and β1 subunits; the total $I_{K(Ca)}$ is then taken as the sum of $I_{α}$ and $I_{αβ1}$. This also enabled investigation of the effects of changing voltage- and calcium-sensitivities of $I_{K(Ca)}$.

The conductances of $I_{α}$ and $I_{αβ1}$ are each modeled by an activation gating variable ($x_α$ for $I_{α}$; $x_{αβ1}$ for $I_{αβ1}$). The half-
activation and the corresponding gating charge were functions of \([Ca^{2+}]_\text{i}\), (Figure 8A); the simulated activation steady-states in comparison to the experimental values at different \([Ca^{2+}]_\text{i}\) [63,64] are shown in Figure 8B and the activation time constants in Figure 8C. A ratio of 70% \(I_{K}\) to 30% \(I_{K1}\) was found to produce the best fit of myometrial cell experimental I–V relationships [65,66]. Using estimates of resting and peak global \([Ca^{2+}]_\text{i}\), in myometrial cells of 100 nM and 800 nM respectively [67], the simulated I–V curves showed that high \([Ca^{2+}]_\text{i}\) increased \(I_{K(Ca)}\) at positive membrane potentials (Figure 8D). Current density of \(I_{K(Ca)}\) is discussed in the section of total potassium current.

**Background potassium current – \(I_{K}\)**

Mathematical description of the biophysical characteristics of this current are given in Appendix S1 (equation 79).

We have described so far the biophysical properties of the major myometrial \(K^+\) currents for which there is sufficient detailed electrophysiological information \(I_{K1}, I_{K2}, I_{K}\) and \(I_{K(Ca)}\). Other, less biophysically detailed electrophysiological information, together with evolving molecular and pharmacological data, suggests the possible existence of other myometrial \(K^+\) current sub-types including small-conductance \(Ca^{2+}\)-activated \(K^+\) channels (termed \(SK_{Ca}\)) and voltage-dependent \(Kv7\) \([KCNQ]\) channels [20,68–71]. Therefore, \(I_{K}\), a linear background potassium current is added and it collectively represents the remaining \(K^+\) currents.

**Whole cell total potassium current – \(I_{K}\)**

In order to model the whole cell \(I_{K}\) it is necessary to combine the current densities of each of the potassium current components.

The current densities of voltage-gated potassium currents \(I_{K1}\) and \(I_{K2}\) reported in myometrial cells show considerable variability. The total voltage-gated potassium current at the voltage step of 60 mV from \(V_h\) between –80 mV and –100 mV in myometrial cells studied by Knock et al. [19,51] varied between \(\approx 8 – 12\ \text{pA}\ \text{pF}^{-1}\). Interestingly, the majority of human myometrial cells consisted of either \(I_{K1}\) (24/42 cells) or \(I_{K2}\) (18/42 cells) as the dominant potassium current [19] with only a very small number of myometrial cells reported to exhibit both \(I_{K1}\) and \(I_{K2}\) [51]. In contrast, Wang et al., [17] reported a voltage-gated potassium current density of 40.1 \(\text{pA}\ \text{pF}^{-1}\) at 70 mV from \(V_h\) of –80 mV. The potassium current was a mixture of 67% \(CI\) (corresponding to \(I_{K1}\) in Knock et al. [19]) and 23% \(C2\) (corresponding to \(I_{K2}\) in Knock et al. [19]) and, together, they accounted for almost 90% of total potassium current during a 10 s voltage step; the remaining 10% were sustained currents consisting of mostly \(I_{K(Ca)}\) with an activation threshold of \(V \geq 0\) mV.

The reported peak current for \(I_{K}\) ranges between \(\approx 2 – 4\ \text{pA}\ \text{pF}^{-1}\) in human myometrial cells [51] and \(\approx 18\ \text{pA}\ \text{pF}^{-1}\) in rat myometrial cells [27] at voltage steps of 40–60 mV from \(V_h\) of –80 mV. However, from the raw time tracing [27,51], the ratio of the peak \(I_{K}\) (occurring at \(\approx 10\) ms) with respect to the peak total potassium current (occurring at \(\approx 50\) ms) was consistent at \(\approx 0.34 – 0.44\) over a range of voltage steps from –10 mV to +40 mV. Therefore, the maximal conductance of \(I_{K}\) was chosen so that the peak of \(I_{K}\) corresponds to 40% of the peak total potassium current (Figure 9A).

We have chosen the maximal conductances of \(I_{K1}, I_{K2}, I_{K(Ca)}\) and \(I_{K}\) such that, together, the simulated total potassium current under different voltage-clamp protocols fits the profiles of experimental voltage-clamp results in Miyoshi et al., [27] and Wang et al., [17] (Figure 9).

In the later development of the USMC AP simulations, the total potassium current density was scaled to match the experimental
data of whole cell potassium current in Okabe et al. [25, 32];
4pA F\{1\} at \(V\sim0\) mV from a \(V_h\) of \{50 mV\}. Other membrane currents
A non-selective cation current (\(I_{\text{NSCC}}\)) and a calcium-activated
chloride current (\(I_{\text{Cl(Ca)}}\)) have been reported for myometrial cells
from late pregnant rats. We also formulated electrogenic
currents for the \(\text{Na}^+\)-\(\text{K}^+\)ATPase and \(\text{Na}^+\)-\(\text{Ca}^{2+}\) exchangers,
\(I_{\text{NaK}}\) and \(I_{\text{NaCa}}\) respectively, by extrapolating data from other
cell systems. \(I_{\text{NaCa}}\) will be discussed with \(\frac{1}{2}\) \(\text{Ca}^{2+}/\text{Cl}^{-}\)
dynamics in a later section.

Calcium-activated chloride current – \(I_{\text{Cl(Ca)}}\)
Mathematical descriptions of the biophysical characteristics of this current
are given in Appendix S1 (equations 80–86).

The presence of channels permeable to chloride in myometrial
cells was first reported by Coleman & Parkington [72]. Subsequently, there have been several reports of calcium-activated
chloride current in myometrial cells, albeit the biophysical
characteristics have not been as thoroughly explored as in other
smooth muscles and tissues [15, 17, 73, 74]. In addition, Clca
isoforms 3 and 4, suggested to encode for channel proteins
responsible for \(I_{\text{Cl(Ca)}}\), have been found in the uterus and the
induced expression of Clca4 in mammalian cells elicited a
calcium-dependent chloride current [75, 76].

The only serious single cell electrophysiological assessment of
\(I_{\text{Cl(Ca)}}\) in myometrial cells (rat, 35°C) is from Jones et al., [15] and
therefore, this is the experimental data used for our modeling
purposes. They used two different voltage-clamp protocols: a
single step voltage-clamp and a two-step voltage-clamp (illustrated

Figure 8. Myometrial \(I_{\text{Cl(Ca)}}\) model. The calcium- \{\([\text{Ca}^{2+}]_i\)\}, voltage- \(V\) and time-dependent kinetics for the two types of \(I_{\text{Cl(Ca)}}\) currents, \(I_a\) and
\(I_{ab}\), are developed with experimental data from cloned mammalian myometrial and smooth muscle MaxiK \(\alpha\) and \(\beta_1\) subunits expressed in Xenopus
laevis oocytes [63,64]; the current density and proportion of \(I_a : I_{ab}\) are adjusted with \(I-V\) relationships from different mammalian myometrial cells
[17,65,66]. In A and C, solid and empty circles are experimental data for \(I_a\) and \(I_{ab}\) respectively. A, \([\text{Ca}^{2+}]_i\)-dependent half-activation \(V_{0.5}\) and
activation gating charge. B, simulated activation steady-states for \(I_a\) and \(I_{ab}\) at different \([\text{Ca}^{2+}]_i\); solid and empty circles are experimental data from
Orio et al., [64] and Bao & Cox [63] respectively, C, V-dependent activation time constants for \(I_a\) and \(I_{ab}\). D, simulated \(I-V\) relationships of \(I_{\text{Cl(Ca)}}\) at
anticipated myometrial resting and peak \([\text{Ca}^{2+}]_i\) levels, with the proportion of \(I_a : I_{ab}\) = 0.7 : 0.3. Both \(I-V\) relationships are normalized to \(I_{\text{Cl(Ca)}}\) at
\(V=60\) mV at peak \([\text{Ca}^{2+}]_i\) level.
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Other membrane currents
A non-selective cation current (\(I_{\text{NSCC}}\)) and a calcium-activated
chloride current (\(I_{\text{Cl(Ca)}}\)) have been reported for myometrial cells
from late pregnant rats. We also formulated electrogenic
currents for the \(\text{Na}^+\)-\(\text{K}^+\) ATPase and \(\text{Na}^+\)-\(\text{Ca}^{2+}\) exchangers,
\(I_{\text{NaK}}\) and \(I_{\text{NaCa}}\) respectively, by extrapolating data from other
cell systems. \(I_{\text{NaCa}}\) will be discussed with \(\frac{1}{2}\) \(\text{Ca}^{2+}/\text{Cl}^{-}\)
dynamics in a later section.

Calcium-activated chloride current – \(I_{\text{Cl(Ca)}}\)
Mathematical descriptions of the biophysical characteristics of this current
are given in Appendix S1 (equations 80–86).
in Figures 1 and 2, respectively, of Jones et al., [15]. Both protocols relied on the activation of \( I_{\text{CaL}} \) to raise \( \frac{1}{2} \text{Ca}^{2+}/C_{i} \), which, in turn, was proposed to activate \( I_{\text{Cl(Ca)}} \). \( \frac{1}{2} \text{Ca}^{2+}/C_{i} \), however, was not clamped in Jones et al., [15] and so, for modeling purposes, it was not possible to determine the steady-state values nor the activation kinetics. However, such information is available from the data of Arreola et al., [77] for \( I_{\text{Cl(Ca)}} \) in rat parotid acinar cells whereupon \( \text{Ca}^{2+} \) buffers were introduced intracellularly to control \( \frac{1}{2} \text{Ca}^{2+}/C_{i} \). This enabled the recording and modeling of calcium- and voltage-dependencies of \( I_{\text{Cl(Ca)}} \). In addition, the Arreola et al., [77] model could reproduce the calcium- and voltage-dependencies of \( I_{\text{Cl(Ca)}} \) in pulmonary vascular smooth muscle cells [78]. As such, we applied the model of Arreola et al., [77] to simulate the myometrial data of Jones et al., [15]. Utilizing the values for the calcium-dependent time constant of activation from Arreola et al., [77], or even changing them substantially, failed to provide a suitable fit to the Jones et al., [15] \( I_{\text{Cl(Ca)}} \) dynamics. If one assumed only a voltage-dependency to the activation time constant then the raw data time tracings of Jones et al., [15] could be fitted by the Arreola et al., [77] model (Figure 10). Thus we include \( I_{\text{Cl(Ca)}} \) in our later

**Figure 9. Myometrial total \( I_{k} \) model.** Potassium currents including \( I_{\text{K1}}, I_{\text{K2}}, I_{\text{Ka}}, I_{\text{K(Ca)}} \) and \( I_{b} \) were combined to simulate the whole cell \( I_{k} \) data of Miyoshi et al., [27] and Wang et al., [17]. A, simulated effects of 10 mM TEA (left), which blocks \( I_{\text{K1}}, I_{\text{K2}} \) and \( I_{\text{K(Ca)}} \) but not \( I_{\text{Ka}} \), at a voltage step of 40 mV from a holding potential \( (V_{h}) \) of −80 mV; corresponding experimental results [27] (right). B, simulated whole cell potassium currents (left) and corresponding experimental results [17] (right) at voltage steps from −40 mV to 70 mV from a \( V_{h} \) of −80 mV; and C, from a \( V_{h} \) of −40 mV. D, simulated inactivation of whole cell potassium currents with the same two-step protocol in Wang et al., [17]: \( V_{h} = −80 \text{ mV} \), followed with a 10 s conditional step ranging from −140 mV to 0 mV, then a final test step at 70 mV for 180 ms. The peak current during the the test steps is normalized to test step at −140 mV; E, the I–V relationships at peak and at the end of the voltage step in B and C. In B and C, simulated currents are normalized to the peak current at \( V = 70 \text{ mV} \) from \( V_{h} = −80 \text{ mV} \).
model of USMC AP form with the caveat that the activation kinetics are different from that described in other cells [77,78].

**Non-selective cation current – $I_{NSCC}$**

Mathematical descriptions of the biophysical characteristics of this current are given in Appendix S1 (equations 87–92).

Miyoshi et al., [79] had identified a non-specific cation current in late pregnant rat myometrial cells. $I_{NSCC}$ is a linear, time-independent cation current. It is permeable to $K^+$, $Na^+$, $Cs^+$ and $Ca^{2+}$, with relative permeability ratios of $P_K : P_{Cs} : P_{Na} : P_{Ca} = 1.3 : 1 : 0.9 : 0.89$. The conductance of $I_{NSCC}$ depends on extracellular concentrations of permeable cations and it was inhibited by extracellular $Mg^{2+}$, $La^{3+}$ and $Gd^{3+}$. The reported reversal potential and current density under standard conditions in Miyoshi et al., [79], with $0.1\text{mM } [\text{Mg}^{2+}]_o$ and utilizing a voltage ramp protocol, were, respectively, $\approx -5\text{mV}$ and $0.6 \pm 0.46 \text{pA pF}^{-1}$.

$I_{NSCC}$ is modeled with data from late pregnant rat myometrial cells recorded at room temperature. The reversal potential of $I_{NSCC}$ ($E_{NSCC}$) is approximated by the Goldman-Hodgkin-Katz (GHK) equation [80] with the reported permeability ratio [79]. Intracellular and extracellular concentrations of $Cs^+$ and NMDG with $P_{NMDG} : P_{Cs} = 0.2$ were included in the calculation of $E_{NSCC}$ while fitting experimental data in Miyoshi et al., [79]; these parameters for $Cs^+$ and NMDG were excluded in the later development of the USMC whole cell model.

The conductances of $I_{NSCC}$ for different cations from the voltage ramp $I$–$V$ relationships have a ratio of $Ca^{2+} : Na^+ :$...
K⁺ : Ca⁺ = 0.5 : 1 : 1.19 : 1.6 [79]. Similar to $\mathcal{I}_{NSCC}$ in guinea-pig endocardial endothelial cells [81], conductance of myometrial $\mathcal{I}_{NSCC}$ was reduced with decreasing [Na⁺]o. With reference to Manabe et al. [81], this relationship was described by a Hill equation with a half-saturating concentration of 150 mM and a Hill coefficient of 2. We have normalized the Hill equation with the Na⁺ conductance at 125 mM [Na⁺]o and we assumed the same relationship held for other permeable cations; for Ca⁺ ions, the Hill equation is normalized to the Ca⁺ conductance observed at 20 mM [Ca⁺]o. Inhibition by [Mg⁺]o is described by a Hill equation with a half-saturating concentration of 0.28 mM and a Hill coefficient of 1.5 [79]. $\mathcal{I}_{NSCC}$ is also permeable to other cations [79] and, therefore, a small leak component (gL) in its conductance is needed to match the experimental voltage-clamp data. Under physiological conditions with 0.1 mM [Mg⁺]o, the simulated $\mathcal{I}_{NSCC}$ consists of mostly Na⁺ and leak components.

**Sodium potassium pump current — $\mathcal{I}_{NaK}$**

Mathematical descriptions of the biophysical characteristics of this current are given in Appendix S1 (equations 93–96).

Evidence of Na⁺-K⁺ pump activity has been reported in myometrial cells of late pregnant rats [82–84] and human [8]. mRNA and protein expression corresponding to z and $\beta$ subunits of the Na⁺-K⁺ ATPase have been reported in rodent and human myometrium with isoform-specific changes associated with advancing gestation and/or estrogen treatment [21,84–86]. In sodium-rich myometrial tissues of late pregnant rats [82,83] and human [8], changes of the membrane potential were sensitive to ouabain, the absence of external potassium or intracellular sodium and to low temperature, results that are suggestive of an electrogenic $\mathcal{I}_{NaK}$. Despite this molecular and biophysical data supporting a role of the Na⁺-K⁺ pump in regulating myometrial activity, there is little information about the biophysical properties of $\mathcal{I}_{NaK}$ current in myometrial cells. Therefore, we adopted the formulation of an electrogenic $\mathcal{I}_{NaK}$ from rodent myometrial cells [87], which was dependent on membrane voltage, [K⁺]o, [Na⁺]o and [Ca⁺]o. The parameter values of voltage, [K⁺]o, and [Na⁺]o dependencies, as well as current densities, are then fitted with the experimental data from rodent vascular smooth muscle cells [88] at 36°C. A Q10 value of 1.87 for 10°C change between 26–36°C is reported for vascular smooth muscle cells [88]. We assumed the same [Na⁺]o dependency with $\mathcal{I}_{NaK}$ in smooth muscle cells as in the myometrial cells.

**Calcium fluxes**

Mathematical descriptions of the plasmalemmal Ca⁺⁺ fluxes are given in the Methods (equation 7) and Appendix S1 (equations 97–103).

In myometrial cells from near-term pregnant rats, intracellular Ca⁺⁺ ions are removed from the cytoplasm principally by the plasmalemmal Ca⁺⁺-ATPase (PMCA) and Na⁺⁺-Ca⁺⁺ exchanger [12,67,89,90]. From the decay rate constants, $\approx 60–70\%$ of cytoplasmic Ca⁺⁺ removal was estimated to be via the Na⁺⁺-Ca⁺⁺ exchanger and sequestration into intracellular stores, and 30% via PMCA when the cell was stimulated by ten short depolarization pulses between $\pm 80\text{mV}$ and 0 mV [67].

We modified a myometrial intracellular calcium model [24] for inclusion in the development of the USMC AP simulations by incorporating time-dependent kinetics from membrane calcium currents. We also modified the formulation of the Na⁺⁺-Ca⁺⁺ exchanger to overcome its limits in fitting published Ca⁺⁺ decay tracings. For example, we found that the calcium decay tracings in Shmigol et al. [67] and Shmigol et al. [12] could only be fitted by the procedure described in Bursztyn et al. [24] with [Na⁺]o = 16.55 mM. However, no sodium ions were included in the pipette (intracellular) solution used by Shmigol et al. [67]. The resultant reversal potential of the Na⁺⁺-Ca⁺⁺ exchanger was predicted at $\approx -90\text{mV}$ which would mean the Na⁺⁺-Ca⁺⁺ exchanger bringing in extracellular calcium at resting membrane potentials of $\approx 50\text{mV}$ to $\approx 80\text{mV}$ which is incorrect. Our use of the well-described formula of Weber et al. [91] obviated this and enabled us to fit the Ca⁺⁺ fluxes with the same tonic concentrations used in Shmigol et al. [67] and Shmigol et al. [12]. With [Na⁺]o = 0 mM, the resultant reversal potential was in the positive membrane potential range and, thus, the Na⁺⁺-Ca⁺⁺ exchanger was predicted to extrude intracellular Ca⁺⁺ in the physiological range of resting membrane potentials.

We have modeled three major plasmalemmal calcium fluxes: the voltage-dependent membrane channels permeable to Ca⁺⁺ ($J_{Ca,mem}$), the Na⁺⁺-Ca⁺⁺ exchanger ($J_{NaCa}$); and the PMCA ($J_{PMCA}$).

The parameters for $J_{NaCa}$ and $J_{PMCA}$ are refitted with experimental results of calcium decay in late pregnant rat myometrial cells recorded at 35°C from Shmigol et al. [12,67]; the modified calcium sub-system is further validated with experimental data (Figure S7A). Details of individual fluxes are described below.

**Membrane Ca⁺⁺ channels — $J_{Ca,mem}$**

$J_{Ca,mem}$, which includes all the membrane ion channel calcium currents: $I_{Ca-L}$, $I_{Ca-T}$ and the calcium component of $\mathcal{I}_{NSCC}$ ($\mathcal{I}_{NSCC,Ca}$), was calculated from the total membrane calcium current as described in the Methods (equation 7).

**Sodium-calcium exchanger — $J_{NaCa}$**

The Na⁺⁺-Ca⁺⁺ exchanger has been suggested to be involved in calcium translocation in myometrial cells from pregnant rats [12,67,89,90]. However, it is unknown whether the myometrial Na⁺⁺-Ca⁺⁺ exchanger is electrogenic although the earliest studies of the effects of changing [Na⁺]o and [Ca⁺⁺]o on the rat myometrial cell membrane properties suggested so [92].

There are three Na⁺⁺-Ca⁺⁺ exchanger isoforms (NCX1, NCX2, NCX3) and NCX mRNA and protein has been reported in myometrium [93,94]. NCX2 is the predominantly expressed isoform in smooth muscle tissues, including the uterus, but its stoichiometry and electrogenic properties are unknown. Cloning of NCX2 [95] shows that it shares $\approx 80\%$ similarity in amino acid sequences with NCX1, the predominant isoform in heart tissues, and they were functionally similar with respect to their I–V relationship and voltage-dependency [96,97]. Compared to NCX1, NCX2 has a higher dissociation rate [Kd] for Ca⁺⁺ at Kd,Cal = 1.5 μM and a lower [Na⁺]o affinity at Kd,Na = 28 μM. As the Na⁺⁺-Ca⁺⁺ exchangers in cardiac myocytes [98] and aortic smooth muscle cells are electrogenic [99] and the properties of NCX1 and NCX2 isoforms are similar, we presumed the myometrial sodium calcium exchanger would also be electrogenic.

We used an electrogenic Na⁺⁺-Ca⁺⁺ exchanger equation for cardiac cells from Weber et al., [91] that describes current dependencies on membrane potential, intra- and extra-cellular calcium and sodium concentrations and has a stoichiometry of Na⁺⁺ : Ca⁺⁺ = 3 : 1. Dissociation constants for [Ca⁺⁺]i and [Na⁺]o were set as $K_{d,Cal} = 1.5–7\text{μM}$ and $K_{d,Na} = 28\text{μM}$, respectively [22,95]. Dissociation constants for [Ca⁺⁺]i and [Na⁺]o were assumed the same as Weber et al., [91]. The maximum calcium flux via $J_{NaCa}$ and parameters for [Ca⁺⁺]i, allosteric activation were refitted with experimental results of calcium decay in late pregnant rat myometrial cells [12,67]. Membrane current from the Na⁺⁺-Ca⁺⁺ exchanger, $\mathcal{I}_{NaCa}$, is converted from the fitted calcium fluxes $J_{NaCa}$. 
Plasma membrane Ca$^{2+}$ ATPase – $J_{\text{PMCA}}$

PMCA activity in rat myometrial cells has been characterized in fractionated plasma membranous vesicles with a reported ATP-dependent uptake with half saturation at 0.4–0.5 μM [Ca$^{2+}$]$_i$ and a Hill coefficient of 1.3–1.7 [22,100–102]. PMCA is described by a Hill equation with a half saturation at 0.5 mM [Ca$^{2+}$]$_i$ and a Hill coefficient of 2.

Cell and tissue modeling: simulations of APs, [Ca$^{2+}$]$_i$ and force

Our ability to integrate the information obtained from the above biophysically detailed models of individual ionic fluxes into simulations of APs and the ensuing changes in [Ca$^{2+}$]$_i$ and force at a cellular/tissue level were assessed by the following validations.

Model validation 1: simulation of different myometrial action potential configurations

Myometrial cells can produce different forms of APs including those consisting of a single spike, a burst of spikes or a plateau-type. A first task of validation was to assess if integration of our individual ionic current models and [Ca$^{2+}$]$_i$ fluxes could simulate these different AP forms.

We began to assemble a model of AP configuration that incorporated all of the currents and ion fluxes described above. However, under physiological conditions of ionic concentrations [32], this model configuration produced a resting membrane potential (RMP) that was too depolarized (−19 mV) and a basal [Ca$^{2+}$]$_i$ that was too high (610 nM). Many of the ionic currents described above were found in only a subset of the studied myometrial smooth muscle cells. In particular, $I_{Na}$ was reported in only 2/30 myometrial cells in Miyoshi et al., [27]. Removing $I_{Na}$ from the model, therefore, produced an RMP of −54 mV with a resting [Ca$^{2+}$]$_i$ of 116 nM. When some $I_{Na}$ is included ($g_{Na} < 0.078 nS/pF$), the USMC model became more excitable with lower voltage threshold (≈−42 mV) and current threshold (≈−0.48 pA/pF, 20 ms stimulus). The parameters and initial conditions of the USMC model configuration are given in Table S3, S4.

The USMC model is excitable and responds to a brief stimulus with an all-or-none AP. The voltage threshold is ≈−35 mV; the corresponding current threshold is ≈−1.05 pA/pF by a 20 ms stimulus. The simulated AP usually overshoots 0 mV with a maximum rate of rise (dV/dt) up to ≈4.8 V/s and the AP duration (APD) measured at −20 mV ranges between 40–45 ms, similar to the experimental values of dV/dt [37] and APD [100] for rodent myometrium.

The range of AP shapes reported for the pregnant rat myometrium at 30–35 C – repetitive spike [10], repetitive spike AP upon a depolarized basal membrane potential [101], repetitive spike AP leading to plateau [102] and a plateau-like AP [10] – are reconstructed in Figure 11. The variety of action potential shapes can be produced by this model with small variations in parameter sets and initial conditions. Of the four AP configurations illustrated in Figure 11: a bursting type AP was simulated with a current clamp of −0.3 pA/pF$^{-1}$ and with the conductance of $I_{Na}$ at $g_{Na} = 0.12 nS/pF$; a bursting type AP upon a depolarized V was simulated with a current clamp of −0.4 pA/pF$^{-1}$ and with a slope factor of 5.5 mV for the $I_{CaL}$ inactivation steady-state; a mixed bursting-plateau type AP was simulated with [K$^{+}$]$_e$ stepped from 6 to 10 mM; a plateau type AP was simulated with a current clamp of −1.2 pA/pF$^{-1}$. Thus the integrated model can accommodate a variety of APs seen in uterine in smooth muscle cells.

Model validation 2: simulation of the experimental changes induced by estradiol on myometrial AP and [Ca$^{2+}$]$_i$ configurations

The cell model is validated with voltage-clamp and current-clamp experimental data from pregnant rat myometrial cells at 30–35 C [11,30,32,49] under control conditions and upon exposure to estradiol (Figure 12). Estradiol has been reported to reduce peak $I_{CaL}$. Estradiol has also been reported to reduce whole cell potassium currents [30,32,49] and change the USMC AP configuration from a bursting type AP upon a depolarized V to a plateau type AP [11]. The model was able to simulate this change in AP form by adjusting the appropriate current parameters: left-shifting the half-inactivation of $I_{CaL}$ to −45 mV and alters its slope factor to 10 mV, and reducing total potassium conductance by 40% (Figure S1).

Model validation 3: simulation of simultaneous recordings of membrane potential, [Ca$^{2+}$]$_i$ and force

The extraction of the mathematical descriptions for modeling calcium-dependent force changes is denoted in Figure S6 and the resultant equations listed in the Methods (equations 3 and 8–9) and Appendix S1 (equations 104–105).

A final step in our validation of the model was to establish if it was able to accommodate the integration of uterine smooth muscle electrical, Ca$^{2+}$ and contractile events necessary for excitation-contraction coupling. In this regard two broad scenarios of E-C coupling were again considered whereupon contractile events arose from either repetitive spike APs or from plateau-type APs. Figure 13 shows the results of simulations of $AP_{Na}$, [Ca$^{2+}$]$_i$, and force compared to published experimental measurements of these variables from rat myometrial tissue at 30 C [103,104]. Of note, we chose to reproduce the repetitive spike AP data with four separate consequent stimuli for two reasons. First, the present USMC model, when induced by a current clamp, exhibited a lower limit for bursting frequency at ≈0.7 Hz which was faster than that of the experimental recordings. Second, the experimental measurements of relative membrane potential changes from Burdyga et al., [103,104] were averaged from bundles of myometrial muscle strips. Thus, the low bursting frequency of spikes observed from these data may be a result of the extra electro-potential load from the multicellular environment. Alternatively, one cannot completely rule out the possibility that the four consecutive APs spikes were separate events resulting re-entrant excitation waves.

The model could also reproduce several additional published E-C coupling datasets of $V(t)$, [Ca$^{2+}$]$_i(t)$ and force recorded from pregnant rats at 30–35 C [103–106] Figure S7, S8.

Limitations and Conclusions

Our approach has resulted in a number of advances for our understanding of uterine smooth muscle E-C coupling. The model encompasses the most comprehensive biophysical description of ion channel and exchanger electric currents applied to the myometrium with 14 separate electrogenic components, summarized in Figure 14, used to simulate published myometrial AP forms and their alteration by specific experimental manoeuvres. Using 105 mathematical equations, it is the first model to integrate these electrogenic components with descriptions of Ca$^{2+}$ dynamics and phasic force production, the three essential components of electrical E-C coupling, and replicate published myometrial experimental recordings of simultaneous membrane potential, Ca$^{2+}$ and force.

As with any mathematical model of biological phenomena there are limitations. The 14 electrogenic currents are likely to be an
This highlights a lack of sufficient biophysical detail on other currents. In biophysical modeling of cells and tissues, it is often the case that some published electrophysiological information on particular currents is of insufficient detail to furnish biophysical modeling of all its steady-state and dynamic characteristics. Therefore, data from different resources with close cell types, or the same type of cells from different species, are used. This is the same case for the present model. The model is a hybrid containing information primarily from rat myometrium but also from human myometrium and cells expressing smooth muscle ion channel subunits, and this data has been obtained from experiments using different in vitro solutions and at different temperatures. Although this neglects any species-specific quantitative differences in uterine electrogenesis and E-C coupling, it presently is unavoidable. It is also common in biophysical modeling approaches when it is rare that all information is available for one cell type from one species. However, an advantage of the comprehensive assembly of this mathematical model is that it enables identification of gaps in our knowledge of myometrial electrogenesis. This will inform future empirical work in several ways.

First, the putative contribution of many ion channel subtypes to myometrial function has often been extrapolated from molecular data (mRNA or protein) which is incomplete (not all isotypes of channel or exchanger sub-families have been examined) or pharmacological data utilizing compounds of weak specificity (e.g. there are many putative pharmacological blockers/openers \( \text{K}^+ \) channel sub-family that have not yet been examined on uterine function). In addition, electrophysiological isolation of currents is often lacking. Clearly, identification of the molecular expression patterns of all ion channel and ion exchanger subtypes in myometrial cells of the uterus is essential (e.g. [107]), and marrying such data to precise electrophysiological, pharmacological and simulated profiles (even if initially this is in clonal cells), is required to furnish a complete biophysical characterization of normal uterine function. This should be accomplished for rodent and human myometrium to enable one to move from the present

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**Figure 11. Varieties of action potentials.** The USMC model can produce a range of myometrial action potentials (APs) using different initial conditions and parameters values. Four examples are shown (left); all four simulated APs were induced by a 10 s stimulus applied at \( t = 1 \) s. Representative experimental APs from published recordings ([10,101,102]) are shown for comparison (right). A, bursting type AP with afterpotentials at resting membrane potential (RMP); B, bursting type AP with depolarized afterpotentials; C, a mixed bursting-plateau type AP with initial repetitive spikes that gradually become a flat plateau at \( \approx -20 \text{ mV} \). D, plateau type AP.

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hybrid model to species-specific formulations. Procedures outlined in the development of this mathematical model indicate how this can assist in improving our understanding of uterine E-C coupling. Second, from the present information, it is clear that isolated myometrial cells exhibit heterogeneity in ion channel electrophysiology and Ca\(^{2+}\) handling characteristics (for example, the proportion of examined cells exhibiting \(I_{Na}\) or particular \(I_K\) currents). It will be important as one moves forward to consider spatiotemporal aspects of E-C coupling that we establish the implications of this for tissue level electrogenesis [5,108].

Third, the model serves as a useful tool in the design and assessment of agents that act as putative channel/exchanger blockers or activators. Refinement of the model with continued empirical/theoretical iterations will serve to increase its predictive capacity for use in the \textit{in silico} assessment of new uterotonic agents especially as species-specific models are developed. For example, if electrophysiological data of sufficient detail for biophysical modeling is known for the actions of a new agonist/antagonist of a particular uterine ion channel then one can develop predictions of the likely action of this drug on uterine E-C coupling for that species. These will serve as hypotheses to be tested in \textit{ex vivo} or \textit{in vivo} experimentation. In the longer term, this should bring attendant benefits to developing drugs for the treatment of aberrant uterine activity such as preterm labor, whether experimentally induced in rodents [109] or arising spontaneously in humans, prolonged dysfunctional labor or poorly contracting uterus post-partum.

**Methods**

**Overview**

A mathematical model of uterine smooth muscle cell (USMC) function at late pregnancy was developed from the integration of data of individual ionic currents, calcium dynamics and contraction. A glossary of symbols used in the equations is given in Table S1. The USMC model is a system of first-order ordinary differential equations,

\[
\frac{dV}{dt} = - \sum I_{\text{tot}}/C_m \quad (1)
\]

\[
\frac{d[Ca^{2+}]_i}{dt} = - \sum J \quad (2)
\]

\[
\text{dForce} = f([Ca^{2+}]_i) \quad (3)
\]

where \(C_m\) is the specific membrane capacitance. Eq. 1 describes the electrophysiological activities of myometrial membrane potential (\(V\)), which is proportional to the sum of membrane ionic currents (\(I_{\text{tot}}\)). Eq. 2 describes the corresponding intracellular calcium (\([Ca^{2+}]_i\)) dynamics, which is proportional to the sum of calcium fluxes (\(J\)). Eq. 3 describes the rate of change of force as a function of \([Ca^{2+}]_i\).

**Electrophysiology**

The individual membrane current components that were modeled were (i) four inward currents: L-type and T-type Ca\(^{2+}\) currents (\(I_{CaL}\), \(I_{CaT}\)); a fast inward Na\(^{+}\) current (\(I_{Na}\)) and a hyperpolarization-activated current (\(I_{h}\)); (ii) five outward currents: two voltage-gated K\(^{+}\) currents (\(I_{K1}\), \(I_{K2}\)); an A-type transient K\(^{+}\) current (\(I_{Ka}\)) and two Ca\(^{2+}\)-activated K\(^{+}\) currents (\(I_{Ca}\)\(I_{Ca,ab}\)); (iii) a non-specific cation current (\(I_{NSCC}\)); (iv) a Ca\(^{2+}\)-activated Cl\(^{-}\) current (\(I_{Cl(Ca)}\)); (v) a small background potassium current (\(I_b\)); and (vi) an electrogenic Na\(^{+}\)-K\(^{+}\) pump (\(I_{NaK}\) and a

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**Figure 12. Simulating estradiol effects on simultaneous recordings of \(V\) and [Ca\(^{2+}\)]\(_i\).** Action potentials (\(V(t)\)) and corresponding calcium transients ([Ca\(^{2+}\)]\(_i(t)\)) during a 2 s depolarizing current clamp (\(I_{st}\)) under, A, control conditions and, B, the effects of estradiol. In both cases, the initial conditions of the cell model were at their corresponding equilibrium. Action potentials in rat longitudinal myometrial single cells under similar experimental conditions [11,49] are shown for comparison (insets).

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Na⁺-Ca²⁺ exchanger (I_{NaCa}). Properties of these currents are developed based on published voltage- and current-clamp experimental data of, wherever possible, late pregnant rat myometrial cells and tissues in the literature; where rat myometrial data is not available, but complementary data is available, e.g., from human USMC, or clonal cells expressing rat-derived proteins, then this has been mentioned.

Most of the membrane currents were modeled with Hodgkin-Huxley type formulation in the following form:

\[ I = g_m(V - E_{rev}) \]  

(4)

\[ E_{rev} = (R/\Delta t) \ln \left( \left[ X_{i0} / X_i \right] \right) \]  

(5)

\[ dy/dt = (y_x - y) / \tau_y \]  

(6)

where \( g \) is maximum conductance, \( E_{rev} \) is the reversal potential, \( R \) is the universal gas constant, \( T \) is absolute temperature and \( X_{i0} \) and \( X_i \) are the extracellular and intracellular concentrations of ion \( X \). The dimensionless gating variable \( y \) describes the time-dependent activation or inactivation profile of the channel conductance where \( y_x \) is the steady-state value, and \( \tau_y \) is the time constant, functions of voltage and/or ionic concentrations. For the electrogenic \( I_{NaK} \) and \( I_{NaCa} \), we adopted the formulations used in the description of cardiac ventricular cells from Nakao & Gadsby [87] and Weber et al., [91] respectively. The nomenclature for the dynamic gating variables of individual membrane currents is listed in Table S2. Experimental data at body temperature, or a reported \( Q_{10} \) for an individual current, was available for \( I_{CaL} \), \( I_h \), \( I_{Cl(Ca)} \), \( I_{NaK} \), and \( I_{NaCa} \). For other currents, we had to assume the simplest case whereby the dynamics were similar at both room and body temperature.

**Calcium dynamics**

Bursztyn et al., [24] modeled \([Ca^{2+}]_{i}\) dynamics with three major calcium fluxes in myometrial cells: membrane calcium channels (\( J_{Ca,\text{mem}} \)), \( Na^+\)-\( Ca^{2+} \) exchanger (\( J_{NaCa} \)) and plasma membrane \( Ca^{2+} \) ATPase (\( J_{PMCA} \)) assuming \( J_{Ca,\text{mem}} \) was at its equilibrium, i.e., time-independent. Herein we have included the temporal dynamics of membrane current currents in \( J_{Ca,\text{mem}} \), and adopted the Weber et al., [91] formula for \( Na^+\)-\( Ca^{2+} \) exchanger.

\[ J_{Ca,\text{mem}} = C_m A_c \beta (I_{CaL} + I_{CaT} + I_{NSCC,Ca}) / (z_{Ca} F V_c) \]  

(7)

where \( C_m \) is the specific membrane capacitance; \( F \) is the Faraday constant; \( z_{Ca} \) is the valency of \( Ca^{2+} \) ions; \( A_c \) is the cell membrane surface area; \( V_c \) is cell volume; and \( \beta \) is the proportion of free intracellular \( Ca^{2+} \) ions.

The geometry of a uterine smooth muscle cell is assumed to be two cone shapes joined end-to-end at their bases [110]. Reported cell sizes for late pregnant myometrial cells are 100–300 \( \mu \)m in length with a radius of 10–20 \( \mu \)m [29,33]. As these dimensions cover a wide range, we represented the cell geometry with a single parameter, \( A_c / V_c \), as the surface area to volume ratio. We did not model cytoplasmic \( Ca^{2+} \) buffering proteins or intracellular calcium stores because such information for myometrial cells is too scant. Instead we assumed simply a tiny fraction of the membrane calcium influx to be free ions and the quantity is represented by the parameter \( \beta \) [111].

**Contractile mechanism**

Force development during uterine contraction was modeled with a simple first-order ordinary differential equation:

\[ \text{Force} = \maxForce \omega \]  

(8)
Model simulations

Action potentials were induced in the whole cell model by applying an external stimulus current (I_h), either as brief square pulses for single spike AP or with a current clamp for bursting or plateau AP.

The initial values of the dynamical variables \([v, [Ca^{2+}]_i]\), membrane current gating variables, and \(\omega\) are listed in Table S3. The parameter values, which remain constant during simulations, are listed in Table S4. All the equations are given in Appendix S1.

Simulations were computed with a fixed time step of 0.02 ms, using XPPAUT [113] with either the fourth-order Runge-Kutta numerical integration method or the Euler Method, in a IBM laptop PC with an Intel(R) Pentium(R) M 1.5 GHz single processor. The Runge-Kutta was the method of choice for developing individual components and short simulations of the whole cell model whereas the Euler method was mainly used for simulations requiring longer integration times. Solutions of the whole USMC model using both integration methods are almost identical.

A copy of the model source code written in the C programming language is included in Appendix S2.

Annotation of Figures

Within the body of some Figures there are textual annotations that mention the source references for the data plotted in those diagrams. Those references without parenthesis indicate published values that we have reproduced in the diagram. The references mentioned within parentheses reflect data that we have extracted from published raw tracings and refitted as displayed in the figures. Data referred to as ‘unpublished’ is remarked upon in the main text.

Supporting Information

Supporting Information

Figure S1 Simulating the effect of estradiol on the inactivation of myometrial \(I_{CaL}\).

Figure S2 Different inactivation kinetics of myometrial \(I_{CaT}\).

Figure S3 Divalent ion concentration versus \(I_{CaT}\) inactivation time constant of rat myometrial \(I_{CaT}\).

References


Figure S4 Experimental current tracings of \(I_{K1}\) from five cells and an example of extrapolated \(I_{K1}\) at the voltage step (\(V_T\)) +10 mV averaged from these five cells.

Figure S5 Experimental current tracings of \(I_{K2}\) from four cells and an example of extrapolated \(I_{K2}\) at the voltage step (\(V_T\)) +10 mV averaged from these four cells.

Figure S6 Modeling the dynamics of \([Ca^{2+}]_i\)-dependent active force.

Figure S7 Modeling simultaneous changes in V and \([Ca^{2+}]_i\) or \([Ca^{2+}]_i\) and force development.

Figure S8 Modeling force output consequent to spike APs or plateau-like APs.

Table S1 Definitions of the equation symbols.

Table S2 Constant parameter values used in model simulations.

Table S3 Initial values of the dynamics variables used in model simulations.

Table S4 Initial values of the dynamics variables used in model simulations.

Appendix S1 Equations used in the model simulations.

Appendix S2 Model source code.

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Author Contributions

Conceived and designed the experiments: MJT HZ AVH. Performed the experiments: WCT CYC SK. Analyzed the data: MJT HZ AVH WCT. Contributed reagents/materials/analysis tools: MJT HZ AVH WCT. Wrote the paper: MJT HZ WCT.


