Computational Study of Action Potential Generation in Urethral Smooth Muscle Cell

Chitaranjan Mahapatra(✉)

Cardiovascular Research Institute,
University of California San Francisco, San Francisco 94158, USA
chitaranjan.mahapatra@ucsf.edu

Abstract. Stress urinary incontinence is defined by the involuntary loss of urine during the sneezing and coughing. The urethral smooth muscle cell contributes to stress urinary incontinence by generating spontaneous mechanical and electrical activities. It generates spontaneous electrical events in the terms of membrane depolarization and action potentials. Therefore, a complete understanding of the urethral smooth muscle cell's spontaneous action potential biophysics will help in identifying novel pharmacological targets for the stress urinary incontinence. The action potential is evoked by the activation of various ion channels across the cell membrane. This study aims in establishing a computational model of the single urethral smooth muscle cell to simulate the action potential after incorporating all-important ion channels. The ion channels are designed with Hodgkin- Huxley formalism, where the internal kinetics are expressed in terms of the ordinary differential equations. This computational model generates experimental spontaneous action potential and the underlying ionic currents in urethral smooth muscle cell successfully. In summary, this mathematical model contributes an elemental tool to investigate the physiological ionic mechanisms underlying the spikes in the urethral smooth muscle cell, which in turn can shed light on the genesis of stress urinary incontinence.

Keywords: Stress urinary incontinence · Urethral smooth muscle cell · Action potential · Ion channels · Computational modeling

1 Introduction

The International Continence Society has defined urinary incontinence (UI) as a condition in which involuntary loss of urine is objectively demonstrable and is a social or hygiene problem [1]. Among different types of UI, stress urinary incontinence (SUI) is one, which is a common syndrome in women that is typically associated with advanced age, obesity, diabetes mellitus, and fertility [2]. Stress urinary incontinence, defined as a "complaint of involuntary loss of urine on effort or physical exertion or on sneezing or coughing" by the International Continence Society [3, 4]. The smooth muscles from the urinary bladder and urethra display spontaneous contractility patterns, which are associated with UI and SUI. The mammalian urethra is known to exhibit spontaneous tonic contraction activity during the urine-storage phase [5]. Although the factors regulating

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the SUI are not still precisely identified, it is also widely demonstrated that the abnormal urethral smooth muscle (USM) cell contraction phenomena play an important role in regulating these activities [6–8]. The isolated USM cell from various species shows slow waves, spontaneous depolarization (SD), and spontaneous action potentials (sAPs) as its' intracellular electrical activity [7, 9, 10]. The sAPs trigger spontaneous contractions by permitting extracellular calcium (Ca^{2+}) via the voltage gated Ca^{2+} channels across the membrane and releasing stored Ca^{2+} from the sarcoplasmic reticulum (SR) in the intracellular compartment [5, 10, 11]. The resting membrane potential (RMP) values of the USM cell are in the range from – 35 mV to – 45 mV [12–14]. The sAPs can be fired spontaneously or evoked by the external stimulation [13]. The array of ion channels located across the USM cell membrane play a crucial role in regulating both RMP and sAP formation and therefore the overall function of the urethra [15]. Therefore, a better understanding of the ion channel kinetics in forming the USM cell sAP would shed light on developing improved therapies for the SUI.

The biophysical constrained computational models always provide a virtual experimental set up to investigate the underlying ionic mechanisms for the cell's electrical activities. Over the past decades, several computational models have been developed for the neuronal and cardiac cells to investigate individual ion channels' contribution in generating the action potential. However, there are a few numbers of computational models are developed for smooth muscle electrophysiology. To address this gap, recently, we have developed a biophysically constrained computational model for the detrusor smooth muscle (DSM) AP by incorporating nine ion channels [16–19]. As both DSM and USM contractions are related to UI and SUI, this paper presents the first biophysically based model of USM AP which integrates some ionic currents underlying the electrogenic processes in the urethra. This single-cell USM model can be subsequently coupled to other active ionic currents and a syncytium model to examine hypotheses concerning the generation of SUI.

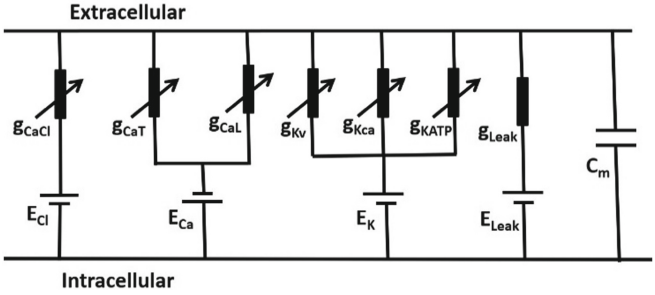

Fig. 1. A USM cell parallel conductance model. It describes all membrane currents and transmembrane potential.

2 Methods

The first step in developing this computational model is to form a conceptual model expressed by the mathematical equations. The classical Hodgkin-Huxley (HH) approach

is implemented to form this conceptual model. According to the HH formalism, the cell membrane can be interpreted into an equivalent parallel conductance circuit consisting of membrane capacitance and several variable conductances representing all ion channels. The USM cell model simulation is performed in "NEURON" [20] software environment. The "NEURON" simulation platform is designed to investigate electrophysiological properties in biological excitable cells at different spatiotemporal levels. For USM cell geometry, a cylindrical morphology is considered with length and diameter of 200 μm and 6 μm respectively. The membrane capacitance (C_m), membrane resistance (R_m), and axial resistance (R_a) are basic electrical properties of the excitable cell membrane. For this model, the C_m, R_m, and R_a are taken as 1 μF/cm^2, 138 MΩ– cm^2, and 181 Ω-cm respectively. Figure 1 illustrates the USM cell model as a parallel conductance model. The membrane capacitance (C_m) is shunted by an array of ion channel conductances g_{ion} with respective Nernst potentials E_{ion}. The ion channels in the USM cell model are Ca^{2+} activated Cl^- channel (g_{CaCl}, E_{Cl}), voltage-gated Ca^{2+} channel (g_{CaL}, g_{CaT}, E_{Ca}), voltage-gated K^+ channel (g_{Kv}, E_K), Ca^{2+} activated K^+ channel(g_{Kca}, E_K), ATP-dependent K^+ channel (g_{KATP}, E_K) and leakage currents (g_{Leak}, E_{Leak}). The leakage current is considered as a constant value. Applying Kirchhoff's current law, we will get the following differential equation describing changes in transmembrane potential V_m. The time dependence of the membrane potential is governed by the following differential equation

$$\frac{dV_m}{dt} = -\frac{I_{ion(t)}}{C_m} \tag{1}$$

where both V_m, and I_{ion} represent the transmembrane potential and sum of the ionic currents across the cell membrane. The units of both V_m and I_{ion} are in mV and pA respectively.

$$\frac{dV_m}{dt} = -\frac{1}{C_m}(I_{Ca} + I_{KCa} + I_{Kv} + I_l) \tag{2}$$

All ionic currents were modeled according to the Hodgkin-Huxley formalism, which is expressed by the following equation.

$$I = \overline{g} m^x h^y (V_m - E_{rev}) \tag{3}$$

where $\overline{g}$ is the maximum conductance, E_{rev} is the ion's Nernst/reversal potential, m and h are the dimensionless activation and inactivation gating variables.

Both m and h are dependent upon membrane potential and time. First order differential equations are used to express the time dependent properties of both m and h. The following differential equation represents the dynamics of 'm' variable.

$$\frac{dm}{dt} = \frac{(m_\infty - m)}{\tau_m} \tag{4}$$

where m_∞ is the steady-state value of the m and τ_m, is the time constant for reaching the steady-state value. These are also functions of voltage and/or ionic concentrations.

In addition, the steady-state inactivation and activation values for all ion channels are described by the following Boltzman equation.

$$m_{\infty} = {}^{1}/_{1} + \exp\left((V_m + V_{\frac{1}{2}})/s\right) \tag{5}$$

Where $V_{1/2}$ is the half activation potential and S is the slope factor. For our model, both $V_{1/2}$ and S are taken from the published experimental data.

The sAPs were induced in the whole-cell model by applying an external stimulus current as brief rectangular pulses or synaptic input.

3 Results

There is an array of ion channels discovered in USM cell electrophysiology to regulate the cell's excitability. It includes T and L-type voltage-gated Ca^{2+} channels (I_{CaL} and I_{CaT}), ATP-dependent K^+ channel (I_{KATP}), two outward rectifying voltage-gated K^+ channel (I_{KA} and I_{Kv}), Ca^{2+}, and voltage-dependent large-conductance K^+ channel (I_{KCa}), Ca^{2+} dependent Cl^- channel (I_{ClCa}) and the leakage channel (I_{Leak}). The biophysical details of one inward current (I_{CaL}) is presented in the following section.

L-type Calcium Current (I_{CaL})

Several researcher groups have elucidated the presence of two types of Ca^{2+} channel (Transient and long-lasting type) in USM cell electrophysiology. However, the L-type (Long-lasting) Ca^{2+} channel (I_{CaL}) is responsible for the major inward current in USM cells [5, 15]. It is demonstrated that I_{CaL} is activated first between $V_m \approx -35$ and -20 mV; the peak magnitude of the current-voltage (I–V) relationship curve appears at $V_m \approx 10$mV. The half-activation potentials for both steady-state activation and inactivation curve are –3.4 mV and –24.8 mV respectively. The Nerst potential E_{CaL} is fixed at 45mV. The equations of I_{CaL} incorporate both activation (m) and inactivation (h) gating variables. The biophysical parameters for the I_{CaL} are extracted from the published experimental data in human USM electrophysiology [21]. Figure 2(A) shows the steady-state activation and inactivation curve with respect to membrane potential.

The red and black solid lines represent simulated steady-state curves for inactivation and activation parameters respectively. The superimposed filled squares and triangles represent the experimental data [21]. The whole-cell current I_{CaL} is simulated according to the voltage clamp protocol for a duration of 200 ms. The holding potential is –70 mV. Simulated tracings of I_{CaL} are shown in Fig. 2(B).

AP Simulation

The AP can be evoked either by the external current injection via the inserted electrode or by the induced synaptic input from the neighbor nerve. Seven numbers of ionic conductances are incorporated into this single USM cell model. The USM cell model successively responded to both current and synaptic input stimuli by showing all-or-none AP firing properties.

A current input is a step input pulse with different amplitudes and durations. A synaptic input is also mimicked by the alpha function to evoke AP in our model. The

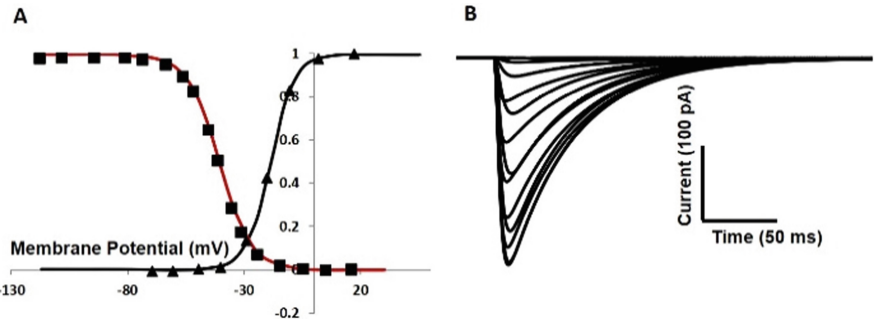

Fig. 2. USM I_{CaL} model. A steady state activation and inactivation parameter curve and B shows the current traces from the voltage clamp protocol.

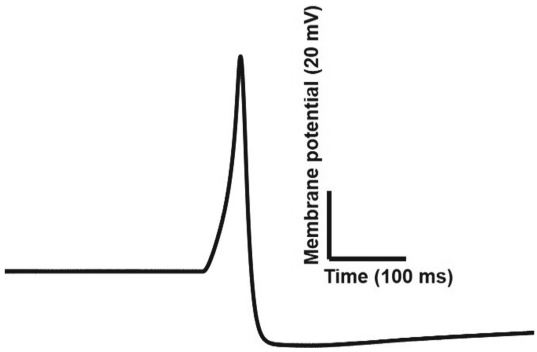

Fig. 3. The simulated AP in the USM model.

voltage threshold is ≈ -35 mV. Figure 3 presents the simulated AP after inducing a synaptic input to mimic the experimental AP in [22].

Table 1 compares simulated AP with experimental one [22] in terms of RMP, peak amplitude, AP duration and AHP (after hyperpolarization potential).

Table 1. Comparison between simulated AP and the experimental AP [22]

	RMP (mV)	Peak (mV)	AHP (mV)	Duration (ms)
Experiment	–40	47	–53	38
Simulation	–40	55	–52	35

4 Discussion

The primary objective of this study was to develop and validate a computational model of a USM cellular electrophysiology. The model description integrates those ion channels

that were significantly contributing to generate the USM cell AP. The ion channel kinetics are characterized by the Hodgkin and Huxley formalism after extracting all parameter values from the literature on USM electrophysiology. The model has demonstrated its' ability by simulating the experimental AP successfully.

The assumptions and simplification approaches are concerned about developing a perfect mathematical model.

A better physiologically realistic model is always based on enough electrophysiological data obtained from a single species. However, due to experimental setup complexity, these data are not always available from the same species. We, therefore, made assumptions driven from values obtained from USM in different species (rat, human, mouse, pig, guinea pig, and rabbit) and under various experimental conditions. Some debate also exists with regard to the ionic conductances that are involved in the repolarizing phase. It has been suggested that more than one K^+ conductance (for example fast A-type K^+ current [15] may carry a portion of the outward current. However, due to a lack of experimental evidence, this model doesn't include this channel. Another question can also be raised towards simulating the experimental AP when the single USM cell is coupled to the other cell.

In the present state, this model is at an elementary stage. Integration of other active channels, Na^+- Ca^{2+} exchanger, Ca^{2+} ATPase pump and sarcoplasmic reticulum Ca^{2+} releasing mechanism will improve this model towards a more comprehensive stage. In addition, the expansion of this single-cell model to syncytium or network level will help to establish a better physiologically realistic computational model for investigating the SUI.

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