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A Mathematical Modeling of Voltage gated Calcium ion channel based Calcium Transient Response in UrinaryBladder Smooth Muscle Cell

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Abstract
Urinary incontinence is leakage of urine without voluntary control that constitutes a social and embarrassing problem in every day's sociallife. Detrusor smooth muscle (DSM) instability is an important pathological reason behind Urinary Incontinence. An increase in cytoplasmic calcium $[Ca^{2+}]_i$ concentration is prerequisite for the activation of contraction of urinary bladder DSM cell. The increase in $[Ca^{2+}]_i$ is accomplished by Ca^{2+} entry mainly via voltage dependent T-type, L-type Ca^{2+} channel and release from intracellular stores. Here, we have developed a biophysically detailed computational model to quantitatively simulate the calcium transient based on ionic currents and calcium dynamics in DSM cell. The model was based on documented electrophysiological data in published experimental studies. Our simulated calcium transient result is validated by matching it with published result from experiments and it shows satisfactory agreements in several parameters. At the present time, our computational model provides an elementary tool to analyze the physiological calcium dynamics and ionic channel kinetics underlying the contractions in DSM cells that successively will explore various hypotheses in genesis of bladder overactivity.
Keywords- Urinary Incontinence; Ca^{2+} channel; PMCA; SERCA; Calcium transient; Computational model;

1 Introduction

Urinary Incontinence (UI) is leakage of urine without voluntary control that makes up a social and embarrassing situation in every day's social life. The International Continence Society (ICS) has classified UI to several types according to their nature. Although the UI is caused by several pathological maneuvers, detrusor smooth muscle instability is considered as a predominant cause behind UI. According to various experimental documented results, DSM cells from several families of species invoke spontaneous contractile activity at different frequency. A good number of empirical and clinical investigations point towards association between calcium dynamics and spontaneous contraction in DSM cell [9]. Transient rise in cytoplasmic calcium $[Ca^{2+}]_i$ controls a wide variety of cellular events. The intracellular $[Ca^{2+}]_i$ elevation is the most essential stage for initiating contraction in DSM cell. In DSM, the intracellular $[Ca^{2+}]_i$ elevation is due to inward flow of Ca^{2+} ions via cell membrane and release from intracellular stores. Influx of extracellular Ca^{2+} ions occurs mainly via voltage gated long lasting (L-type) and transient (T-type) calcium channels. Thesarco/endo-plasmic reticulum (SR) is the principal Ca^{2+} store participating in the initial rapid increase in $[Ca^{2+}]_i$ by supplying Ca^{2+} via Ca^{2+} release mechanism by SR. Ca^{2+} influx via L-type Calcium channel is essential for the rising phase of DSM AP, whereas both voltage gated and calcium dependent potassium channels mediate the repolarization and after hyper-polarization phase of the AP, respectively [5]. Of the large family of mammalian K^+ channel, Ca^{2+} activated potassium channel (I_{BK}), voltage gated potassium channel ($I_{KV1.2}$) and delayed rectified potassium channel (I_{KDR}) are incorporated along with multiple calcium channels (I_{CaL} and I_{CaT}) in AP generation [3]. In novel findings, currents produced by plasma membrane Ca^{2+}-ATPase (PMCA)

pump and sarco/endo-plasmic reticulum Ca^{2+}ATPase (SERCA) pump along with their amplitude and waveform pattern under voltage clamp condition have been identified for DSM cells [9]. Other processes also regulate the calcium dynamics in DSM cell. These include the following: a Ca^{2+} pump in the sarcolemma (I_{PMCA}), buffering of Ca^{2+} ions in the myoplasm and uptake of Ca^{2+} by the SR (I_{SERCA}). A schematic diagram of calcium regulation mechanism is presented in Figure1. The shaded area indicates the presence of Ca^{2+} buffers.

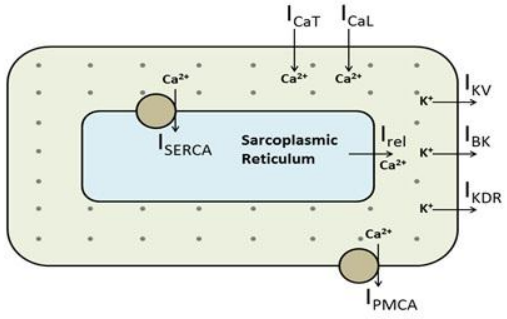

Figure1. Schematic diagram of calcium dynamic of urinary bladder smooth muscle cells

Computational models can compactly quantify the mechanisms of calcium dynamics and permit the user toexplore the contribution of each mechanism in generating cellular mechanical activities. Calcium dynamics models for the smooth muscle cells are at a comparatively emergent stage. This study delivers an elementary realistic calcium dynamic model based on voltage gated calcium channel underlying the kinetic processes in DSM cell. In this study, we constructed a detailed conductance-based model of the DSM cell to investigate how interactions between different Ca^{2+} influx mechanisms in DSM cell produce calcium transient.The model simulates membrane current, action potential and calcium transient response in a fashion similar to experimental recordings. Our simulated results are validated by comparing with experimental results. This single DSM cell model may be integrated to more ion channels and a cell network based syncytium model to look at various pharmacological hypotheses concerning UI.

2 Methods

Mathematical interpretation of a physical system is the first step in computational modelling. Here the mathematical interpretation of DSM cell membrane is established on traditional Hodgkin-Huxley formalism. The excitable cell membrane is representedas a parallel resistor capacitor (RC) circuit consisting of a membrane capacitance C_m and a number of variable ion channel conductances. The parallel conductance model is presented in Figure 2, where I_{cap} is membrane capacitance current, I_{ion} is summation of ionic currents, and I_{tot} is total current. Our DSM cell model is cylindrical in shape having length of 200 µm and diameter of 6 µm. The value of membrane capacitance (C_m) and membrane resistance (R_m) are taken as 1µF/cm^2 and 138MΩ–cm^2 respectively. The axial resistance is taken as 181Ω-cm [6]. We have adapted "NEURON" [2] software as the simulation platform for this model because it has been meant for realistic modelling of biological excitable cells. Equation one represents the time dependence characteristics of V_m.

$$\frac{dV_{m(t)}}{dt} = -\left[\frac{I_{ion\,(t)}+I_{stim\,(t)}}{C_m}\right] \qquad (1)$$

Where V_m (mV) represents the membrane potential, I_{ion} represents ionic current, and I_{stim} represents injected stimulation current in cell membrane.
Voltage dependent calcium channels (I_{Ca}), voltage gated potassium channel (I_{Kv}), Calcium activated potassium channel (I_{KCa}) and back ground leakage currents (I_l) are incorporated to generate APs and calcium transient. After applying Kirchhoff's current law in

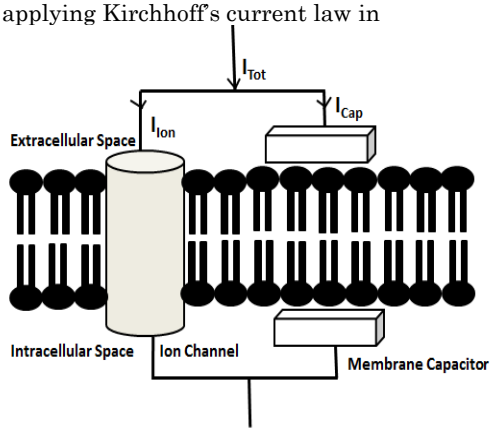

Figure 2. Schematic diagram of parallel conductance model of urinary bladder smooth muscle cell

Parallel conductance model, we got the following differential equations with respect to change in membrane potential V_m:

$$\frac{dV_{m(t)}}{dt} = -\frac{1}{C_m}\left(I_{Ca} + I_{KCa} + I_{Kv} + I_l\right) \qquad (2)$$

In ourmodel, all ion channels are presentedby conventional Hodgkin-Huxley approach in equation (3):

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

where $\bar{g}$ is maximum conductance, E_{rev} is Nernst potential for individual ion, m is activation gating variable and h is inactivation gating variable.
First order differential equation is used to describe time and voltage dependent nature of gating variables m and h.

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

In equation 4, m_∞ and τ_m are defined as steady-state value and time constant of 'm' respectively.
The Boltzman equation is used to describe steady state inactivation and activation gating variables for all ion channels.

$$m_\infty = {}^{1}\!/\!_{1 + ex\,p\,1\,((V_m + V_{\frac{1}{2}})/s)} \qquad (5)$$

In the Boltzman equation (5), $V_{1/2}$ is the half activation potential and S is the slope factor for activating gating variable 'm'. The values of $V_{1/2}$ and S are borrowed from experimental published data.

The calcium transient obtained in figure 1 is given by

$$\frac{d[Ca^{2+}]_i}{dt} = J_{CaL} + J_{CaT} + J_{SR} - J_{PMCA} - J_{SERCA} + \frac{([Ca^{2+}]_\infty - [Ca^{2+}]_i)}{\tau} \qquad (6)$$

In above equation, J_{CaL} is the flux caused by the L-type Ca^{2+} channel, J_{SR} is the flux caused by Ca^{2+} release

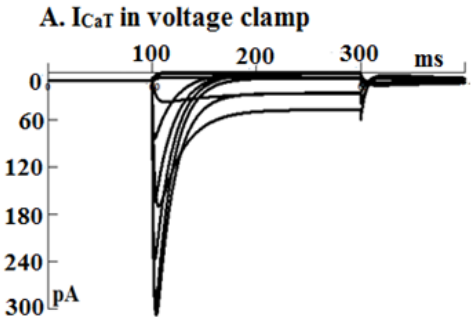

Figure 3. Simulated T-type calcium current from voltage clamp in DSM cells

compartment from intracellular stores, J_{PMCA} is the pumping activity to the outside of the cell, J_{SERCA} is the Ca^{2+} uptake to the SR, $[Ca^{2+}]_\infty$ is the equilibrium concentration of Ca^{2+} and τ is the time constant of Ca^{2+} extrusion (200 ms in this model). Action potentials and Calcium transients were simulated by injecting an external brief square stimulus current (I_{stim}).

3 Results

A. T-type Calcium current – (I_{CaT})

Transient type Calcium current (I_{CaT}) in our model appears at V≈ –60 mV [6, 7] and the peak of current-voltage curve is valued between –20 mV and –30 mV. The inactivation time constant varies between 7-35 ms in experimental current tracings. The model generated peak current for I_{CaT} is ≈ –300 pA at V = –12 mV. I_{CaT} is shown in Figure 3 with respect to applied voltage clamp in our model. The current unit is translated to absolute pA unit from $pApF^{-1}$ unit for simplification.

B. L-type Calcium current – (I_{CaL})

I_{CaL} is considered as the most dominating inward current in DSM cells [6, 7]. L type calcium channels opens between V ≈ –28 to –19 mV; the maximum amplitude of the current-voltage (I–V) curve appears at V ≈ 12 mV. To fit Boltzman equation in activation and inactivation curve, the half-activation potentials are documented at– 3.4 mV and –24.8 mV. It is also mentioned in several experimental studies that L-type calcium channels permits other cations after channel activation. However there aren't any published data for detrusor smooth muscle cells. Thus, the Goldman-Hodgkin-Katz formulation with multiple permeabilities is not used here; rather, the Nernst potential for L-type calcium channel in the model is kept constant of 45 mV to avoid computational complexities. The Hodgkin-Huxley formalism for L - type Ca^{2+} channel corporate gating variable "b" and "g" as activating and inactivating variable respectively.

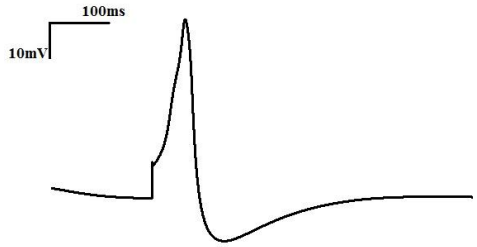

Figure 4. Simulated AP in DSM model.

This model has generated I_{CaL} after applying voltage clamp approach for 200 ms time period. The holding potential is –80 mV as suggested in published documents. Simulated tracings of I_{CaL} are compared with experimental findings [6, 7]. This model also incorporates Voltage-gated K^+ channels and Ca^{2+} activated K^+ channels, which are modeled according to Hodgkin-Huxley formalism with parameters adapted from literatures [3, 5, 7].

C. AP and Calcium Transient Simulation

An external stimulus current of 1-3 nA is injected for 0.5-1 ms to generate APs and Calcium transients in our computational model. Five numbers of active ion channels are integrated intothis single DSM cell model. Injecting both current and synaptic input, this model can generate DSM cell action potential. It is observed that 3 nA current for a time period of 0.6 ms generates the first spike with voltage threshold at –30 mV. The simulated action potential (Figure 4) and calcium transient (Figure 5) are compared with experimental spike and calcium transient obtained from DSM literature [9]. Calcium transient is obtained at 2 µm depth of cytoplasm from the surface membrane. The resting $[Ca^{2+}]_i$ is set to 100 nM. The L-type channel is the major contributor for the rise in $[Ca^{2+}]_i$. The peak $[Ca^{2+}]_i$ transient obtained is 1900 nM in this model. The similarity of the shape of $[Ca^{2+}]_i$ transient and peak value with the experimental results reported in some experiments[9] demonstrates that the kinetics of channels, pumps and calcium transient are represented to a good degree of accuracy in our model.

4 Discussion

We have developed a model of calcium transient in detrusor smooth muscle. To the best of our knowledge, this is the first biophysically based basic model of calcium dynamics in DSM cell.

Our detrusor smooth muscle cell model is built upon nine ion channels described by Hodgkin-Huxley formalism, where the parameters are borrowed from literature on

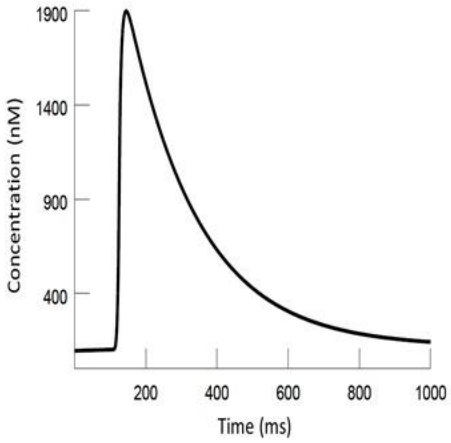

Figure 5. Simulated Calcium transient in DSM cell

DSM electrophysiology. The calcium transient and AP generated from simulation resembles experimental result up to a great context. We also agree that this model is not a complete realistic model for following reasons. The primary concern is that the structure of the model itself. For an ideal biophysical realistic model, all modeling parameters should have been extracted from a same species. Unfortunately, due to experimental set up complexity in electrophysiological recordings, these data are not consistently available. We tend to borrow all equation parameters from the detrusor smooth muscle cells in varied species under different pharmacological conditions. Additional discussion also exists with connection to incorporation of active ion channels that are concerned within the repolarizing period of AP. In several research papers it is documented that multiple types of potassium ion channel might participate in repolarization phase of action potential, however current-voltage relationship of all potassium conductances have not been well studied through patch-clamp experiments. Calcium diffusion is applied for a single pool section in our model; however multiple calcium pool can also modulate the intracellular calcium release profile.

In our future perspectives of this work, we will add additional active ion channels, Sodium Calcium exchanger, calcium dynamics based on activation of IP3 and Ryanodine receptors, plasma membrane Ca^{2+} ATPase pump (PMCA) and sarcoplasmic reticulum Ca^{2+} ATPase pump (SERCA) for an advance level model. We are looking forward to integrate our calcium transient model in a smooth muscle syncytium to investigate pathological conditions in UI.

APPENDIX

Mathematical Equations for I_{CaT}

$$I_{CaT} = \overline{g_{CaT}}\, bg(V - E_{CaT}) \tag{7}$$

$$b_\infty = \frac{1}{1+\exp\left(\frac{-(V+54.23)}{9.88}\right)} \tag{8}$$

$$g_\infty = 0.02 + \frac{0.98}{1+\exp\left(\frac{(V+72.98)}{4.64}\right)} \tag{9}$$

$$\tau_b = 0.45 + \frac{3.9}{1+\left(\frac{(V+66)}{26}\right)^2} \tag{10}$$

$$\tau_g = \left(150 - \frac{150}{\left(1+exp\left(\frac{(V-417.43)}{203.18}\right)^1\right)\left(1+\exp\left(\frac{-(V+61.11)}{8.07}\right)\right)}\right) \tag{11}$$

References

[1] Brading, A. F., and K. L. Brain. "Ion channel modulators and urinary tract function." *Urinary Tract*. Springer Berlin Heidelberg, 2011. 375-393.

[2] Hines, Michael L., and Nicholas T. Carnevale. "The NEURON simulation environment." *Neural computation* 9.6 (1997): 1179-1209.

[3] Hristov, Kiril L., et al. "Suppression of human detrusor smooth muscle excitability and contractility via pharmacological activation of large conductance Ca^{2+}-activated K^+ channels." *American Journal of Physiology-Cell Physiology*302.11 (2012): C1632-C1641.

[4] Liu, Li, et al. "Role of plasma membrane Ca2+-ATPase in contraction-relaxation processes of the bladder: evidence from PMCA gene-ablated mice." *American Journal of Physiology-Cell Physiology* 290.4 (2006): C1239-C1247.

[5] Petkov, Georgi V. "Role of potassium ion channels in detrusor smooth muscle function and dysfunction." *Nature Reviews Urology* 9.1 (2011): 30-40.

[6] Sui, G. P., C. Wu, and C. H. Fry. "A description of Ca^{2+} channels in human detrusor smooth muscle." *BJU international* 92.4 (2003): 476-482.

[7] Sui, G-P., C. Wu, and C. H. Fry. "Inward calcium currents in cultured and freshly isolated detrusor muscle cells: evidence of a t-type calcium current." *The Journal of urology* 165.2 (2001): 621-626.

[8] Thorneloe, Kevin S., and Mark T. Nelson. "Properties and molecular basis of the mouse urinary bladder voltage-gated K^+ current." *The Journal of physiology*549.1 (2003): 65-74.

[9] Ganitkevich, V., and G. Isenberg. "Depolarization-mediated intracellular calcium transients in isolated smooth muscle cells of guinea-pig urinary bladder." *The Journal of Physiology* 435.1 (1991): 187-205.