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An Application of Reverse Quasi Steady State Approximation over SERCA

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Abstract : *Many cell types encode signals in the frequency component of calcium oscillations. In non-excitable cells calcium oscillations are induced by calcium influx from inositol trisphosphate receptors (IP3R) or/and ryanodine receptors (RyR). The frequency of Calcium oscillations induced in such cases is primarily by the way intracellular stores like endoplasmic reticulum, sarco-endoplasmic reticulum are refilled. Sarco-endoplasmic reticulum calcium ATPase (SERCA) is the major pathway by which this refilling process is effected. Most of the mathematical models proposed thus far used Michaelis-Menten kinetics to formulate SERCA pump. In this paper more emphasis has been given over the mathematical formulation of SERCA pump. In particular reverse quasi steady-state approximation (rQSSA) is used to formulate SERCA pump. Comprehensive comparative analysis is performed with the previous SERCA pump formulation. An apparent increase in frequency and amplitude of calcium oscillation is observed.*

Keywords - *SERCA, Michelis-Menten, rQSSA, Enzyme Kinetics.*

I. Introduction

Calcium (Ca^{2+}) ions play a pivotal role in a number of cell processes like synaptic plasticity, muscle contraction, secretion, etc. Cells are known to encode signals in the frequency of Ca^{2+} oscillations while avoiding its toxic higher concentrations. In non-excitable cells Ca^{2+} oscillations are induced by an agonist binding to its receptor which can cause via G-protein link to phospholipaseC (PLC) the cleavage of phosphotidylinositol (4,5)-bisphosphate (PIP_2) into inositol triphosphate (IP_3) and water soluble diacylglycerol (DAG). This IP_3 is free to diffuse inside the cytosol and binds with IP_3R causing an efflux of Ca^{2+} from IP_3R located at the ER surface. Since we know that higher concentration of Ca^{2+} is known to be toxic for the cell thus their exist Ca^{2+} pump which transports Ca^{2+} ions back inside ER and outside the cell.

Ca^{2+} pump or Ca^{2+}-ATPases constitute a large family of proteins that fall into two distinct groups, the sarco(endo)plasmic reticulum Ca^{2+}-ATPase (SERCA), and the plasma membrane Ca^{2+}-ATPase (PMCA). As we know that, Ca^{2+} ions are transported from cytosol to ER through the SERCA pump, to accomplish this transportation these Ca^{2+} ions bound to pump proteins on the cytosolic side of the membrane and the protein undergoes a change in conformation. This change in conformation is done by the energy released from the conversion of ATP to ADP.

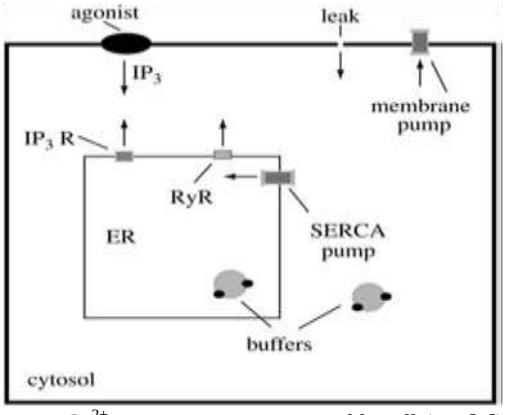

Fig. 1 Ca^{2+} transport in a non-excitable cell (see [1])

Moreover mathematically SERCA pump is modeled using Hill equation with a Hill coefficient of '*n*' and is of the form

$$J_{SERCA} = \frac{V_m[Ca^{2+}]^n}{K_m^n + [Ca^{2+}]^n} \tag{1}$$

where V_m is the maximum pumping rate, K_m is Michaelis-Menten constant and [Ca^{2+}] is the calcium concentration. The Hill equation governing SERCA pump formulation is based on standard quasi steady state

approximation (sQSSA). The hypothesis of sQSSA is based on the assumption that the substrate concentration [*S*] has to greatly exceed that of the enzyme [*E*], (first discussed by Laidler [2]) i.e.

$$\frac{[E_0]}{[S_0]} << 1 \tag{2}$$

where $[E_0]$ and $[S_0]$ are the initial concentrations of enzyme and substrate respectively. By means of simulation modeling on a digital computer, and by considering the time-dependent process, Stayton and Fromm found that sQSSA is generally hold for substrate-enzyme ratios greater than 100 [3]. Later, a more general condition was given for the validity of sQSSA [4], [5], which states that

$$\frac{[E_0]}{K_m + [S_0]} << 1 \tag{3}$$

Condition (3) is stronger than (2) and guaranties the validity of sQSSA. For the basic enzyme reaction, condition (3) is usually satisfied in most *in vitro* assays. Assumption (3) is intended to guarantee that significant fraction of the substrate do not bound to the enzyme during the assay and thus the formation of the enzyme-substrate complex does not diminish significantly the concentration of the substrate [4], [6], [7], [8]. However, the sQSSA condition breaks down in biological (i.e. *in vivo*) conditions as intra-cellular concentrations of enzyme are usually higher or at least of the same magnitude as their substrates [9]. Consequently, a significant fraction of *S* can be bound as enzyme-substrate complexes. Furthermore, high affinity of an enzyme for a substrate may lead to binding of a significant proportion of substrate to the enzyme. To utilize the full potential of the enzyme, substrate concentration within the cells are in the neighborhood of their K_m values (these values range from about 10^{-6} to 10^{-2} M) [8], [10], [11], [12].

Therefore when enzyme concentration is high in comparison to substrate the methods based on sQSSA are not expected to be valid. Segel and Slemrod proposed a reverse quasi steady state approximation (rQSSA) in which the substrate *S* is in a QSS with respect to the enzyme–substrate complex [5]. Recently Schnell and Maini discussed the validity of rQSSA and derived the solution for it [13]. In the present work we have modeled SERCA pump using rQSSA and studied its impact over cytosolic Ca^{2+} oscillations.

II. Mathematical Model

Present mathematical model is for a non-excitable cell exhibiting cytosolic Ca^{2+} oscillations. The mathematical model governing Ca^{2+} oscillations is based on early remarkable work of De Young and Keizer [14], Keizer and De Young [15] which was simplified by Jafri and Keizer [16]. Although the work is based on the work presented by Jafri and Keizer [16], it contains a number of modifications and advancements to their theory as per the arguments given in the previous section (see fig. 1). It should be pointed out that in fig. 1 RyR is also present but it has not been incorporated in the mathematical model to keep the model simple. The whole cell model comprises of a number of components which are elaborated below.

2.1 SERCA PUMP

SERCA pump is a p-type ATPase expressed in most cell types. The mechanism of CA^{2+} ion regulation by SERCA pump can be framed with the help of following bimolecular reaction.

$$2[Ca^{2+}] + [E] \underset{k_{-1}}{\overset{k_1}{\rightleftarrows}} [E(Ca^{2+})_2] \xrightarrow{k_2} [E] + 2[Ca^{2+}]_{ER} \tag{4}$$

In this reaction first reaction is representing the formation of enzyme calcium ion complex $E(Ca^{2+})_2$ from two ions of Ca^{2+} and enzyme in the cytosolic side. This reaction is reversible with forward and reverse rate constants k_1 and k_{-1} respectively. The second reaction irreversibly yields two ions of Ca^{2+} in the ER side and enzyme becomes free with a rate constant k_2. Applying law of mass action governing (4) we get the following system of nonlinear differential equations,

$$\frac{d[Ca^{2+}]}{dt} = -k_1([E_0] - [E(Ca^{2+})_2])[Ca^{2+}]^2 + k_{-1}[E(Ca^{2+})_2] \tag{5}$$

$$\frac{d[E(Ca^{2+})_2]}{dt} = k_1([E_0] - [E(Ca^{2+})_2])[Ca^{2+}]^2 - (k_{-1} + k_2)[E(Ca^{2+})_2] \tag{6}$$

$$\frac{d[Ca^{2+}]_{ER}}{dt} = k_2[E(Ca^{2+})_2] \tag{7}$$

and the enzyme substrate conservation law

$$[E_0] = [E] + [E(Ca^{2+})_2] \tag{8}$$

where, $[E_0]$ is the initial enzyme concentration.

Schnell and Maini challenged the basic assumption $d[E(Ca^{2+})_2]/dt \approx 0$ with the aid of the rQSSA when the enzyme reaction (4) occurs at high enzyme concentration [13]. Segel and Slemrod examined sQSSA and

showed that it holds if initial substrate (Ca^{2+}) concentration is much larger than enzyme [5]. When the concentration of the substrate (Ca^{2+}) is high enough, it seems reasonable to say that the enzyme–substrate complex ($E(Ca^{2+})_2$) is in a quasi steady state, because in this condition free enzyme E will immediately combine with another molecule of Ca^{2+}. However, this condition does not hold if there is an excess of enzyme *E*, compared to substrate [5], [17]. In this case, all the molecules of substrate Ca^{2+} will immediately combine with the molecules of *E* implying that the substrate will be depleted, and thus the approximation $d[Ca^{2+}]/dt \approx 0$ will be valid for a considerable period of time. Therefore, at high enzyme concentration it seems to be more reasonable to propose that Ca^{2+} is in quasi steady state with respect to $E(Ca^{2+})_2$, rather than saying $(E(Ca^{2+})_2)$ is in quasi steady state with respect to Ca^{2+}.

On the basis of rQSSA, Schnell and Maini obtained a uniform approximation for the total time evolution ($0 < t < \infty$) of the reactant concentration [13], given as follows,

$$[Ca^{2+}](t) = [Ca^{2+}]_{\infty} \exp(-k_1[E_0]t) + \frac{K_s[Ca^{2+}]}{[E_0]}\left(\exp(-k_2 t) - \exp(-k_1[E_0]t\right) \tag{9}$$

$$[E(Ca^{2+})_2](t) = [Ca^{2+}]_{\infty}\left(\exp(-k_2 t) - \exp(-k_1[E_0]t\right) \tag{10}$$

where $K_s = k_{-1}/k_1$ is the equilibrium dissociation constant of $[Ca^{2+}]$ from $[E(Ca^{2+})_2]$. Using rQSSA the SERCA pump can be modeled as follows,

$$J_{SERCA} = \frac{k_2 E_0 [Ca^{2+}]^2}{K_s^2 + [Ca^{2+}]^2} \tag{11}$$

where the dynamics of $[Ca^{2+}]$ is governed by (9).

2.2 Ip_3r Receptor

The IP_3R receptor model is based on the kinetics proposed by Keizer and Jafri [16] (see fig. 2). In the model there are four states X_{100}, X_{101}, X_{110} and X_{111}. Among these four states two states X_{101} and X_{111} are inactivated, state X_{100} is the closed state and X_{110} is open state i.e. the conducting state.

Applying law of mass action in the kinetics described in fig. 2, we get the following system of differential equations governing open and closed state of IP_3R channel,

$$\frac{dX_{100}}{dt} = -a_2[Ca^{2+}]X_{100} - a_5[Ca^{2+}]X_{100} + b_5 X_{110} \tag{12}$$

$$\frac{dX_{110}}{dt} = -a_2[Ca^{2+}]X_{110} + b_2 c_2[IP_3] + a_5[Ca^{2+}]X_{100} - b_5 X_{110} \tag{13}$$

where $b_2 = a_2 \cdot d_2$ and $b_5 = a_5 \cdot d_5$, while a_2, a_5, d_2, d_5 and c_2 are as described in the Table I. In (13) $[IP_3]$ denotes the cytosolic IP_3 concentration suitable for inducing cytosolic Ca^{2+} oscillation.

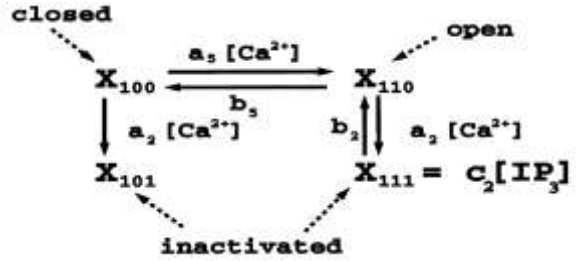

Fig. 2 Schematic of IP_3R kinetics [16]

2.3 Membrane Fluxes

We have included two fluxes across the plasma membrane. Flux through the plasma membrane calcium pump is denoted by J_{pm} and is modeled by a Hill equation with a Hill coefficient of 2 and the influx from outside the cell is denoted by J_{in} and is of the form given by Sneyd et al. [18]. These fluxes are as follows,

$$J_{pm} = \frac{V_{pm}[Ca^{2+}]^2}{K_{pm}^2 + [Ca^{2+}]^2}, \qquad J_{in} = 0.2 + 12[IP_3] \tag{14}$$

where V_{pm} is the maximum pumping rate and K_{pm} is the Ca^{2+} concentration when pumping rate is half of its maximum and $[IP_3]$ is as described earlier.

2.4 Calcium Buffer

We have assumed the following bimolecular enzyme kinetic reaction between calcium and buffer inside the cytosol,

$$[Ca^{2+}]+[B] \underset{k_1^-}{\overset{k_1^+}{\rightleftarrows}} [CaB] \tag{15}$$

In this reaction k_1^+ and k_1^- are the buffer association and dissociation rate constants respectively. On applying the law of mass action we get the following system of nonlinear differential equations,

$$\frac{d[Ca^{2+}]}{dt} = -k_1^+[Ca^{2+}][B] + k_1^-[CaB] \tag{16}$$

$$\frac{d[B]}{dt} = -k_1^+[Ca^{2+}][B] + k_1^-[CaB] \tag{17}$$

$$\frac{d[CaB]}{dt} = k_1^+[Ca^{2+}][B] - k_1^-[CaB] \tag{18}$$

If we assume that there is no source or sink present for buffer then applying the conservation law for buffer,

$$[B_T] = [B] + [CaB] \Rightarrow [B] = [B_T] - [CaB] \tag{19}$$

we get reduced system of nonlinear differential equations governing the reaction kinetics given by (15),

$$\frac{d[Ca^{2+}]}{dt} = -k_1^+[Ca^{2+}]\left([B_T] - [CaB]\right) + k_1^-[CaB] \tag{20}$$

$$\frac{d[CaB]}{dt} = k_1^+[Ca^{2+}]\left([B_T] - [CaB]\right) - k_1^-[CaB] \tag{21}$$

Similar bimolecular reactions can be assumed inside the ER as given in (15) and on applying the same procedure described above gives the system of differential equations governing the $[Ca^{2+}]_{ER}$ and $[CaB]_{ER}$ kinetics as follows,

$$\frac{d[Ca^{2+}]_{ER}}{dt} = -k_2^+[Ca^{2+}]_{ER}\left([B_T]_{ER} - [CaB]_{ER}\right) + k_2^-[CaB]_{ER} \tag{22}$$

$$\frac{d[CaB]_{ER}}{dt} = k_2^+[Ca^{2+}]_{ER}\left([B_T]_{ER} - [CaB]_{ER}\right) - k_2^-[CaB]_{ER} \tag{23}$$

2.5 The Whole Cell Model

The complete cell model using equations (4-23) (see fig. 1) giving the effect of an rQSSA SERCA pump over intracellular Ca^{2+} dynamics can be written with the help of following,

$$\frac{d[Ca^{2+}]}{dt} = c_1\left(v_2 + v_1 X_{110}^3\right)\left([Ca^{2+}]_{ER} - [Ca^{2+}]\right) - J_{SERCA} - \frac{V_{pm}[Ca^{2+}]^2}{\left(K_{pm}^2 + [Ca^{2+}]^2\right)} + \left(0.2 + 12[IP_3]\right) - k_1^+[Ca^{2+}]\left([B_T] - [CaB]\right) + k_1^-[CaB] \tag{24}$$

$$\frac{d[Ca^{2+}]_{ER}}{dt} = \frac{1}{c_1}\left(c_1\left(v_2 + v_1 X_{110}^3\right)\left([Ca^{2+}]_{ER} - [Ca^{2+}]\right) + J_{SERCA}\right) - k_2^+[Ca^{2+}]_{ER}\left([B_T]_{ER} - [CaB]_{ER}\right) + k_2^-[CaB]_{ER} \tag{25}$$

$$\frac{dX_{100}}{dt} = -a_2[Ca^{2+}]X_{100} - a_5[Ca^{2+}]X_{100} + b_5 X_{110} \tag{26}$$

$$\frac{dX_{110}}{dt} = -a_2[Ca^{2+}]X_{110} + b_2 c_2 IP_3 + a_5[Ca^{2+}]X_{100} - b_5 X_{110} \tag{27}$$

$$\frac{d[CaB]}{dt} = k_1^+[Ca^{2+}]\left([B_T] - [CaB]\right) - k_1^-[CaB] \tag{28}$$

$$\frac{d[CaB]_{ER}}{dt} = k_2^+[Ca^{2+}]_{ER}\left([B_T]_{ER} - [CaB]_{ER}\right) - k_2^-[CaB]_{ER} \tag{29}$$

Along with initial conditions

$$[Ca^{2+}] = 0.1\ \mu\text{M},\ [Ca^{2+}]_{ER} = 10.9\ \mu\text{M},\ X_{100} = 0.291,\ X_{110} = 0.281,\ [CaB] = 2.3\ \mu\text{M}\ and\ [CaB]_{ER} = 2.3\ \mu\text{M} \tag{30}$$

The above set of equations (24-30) has been solved numerically with the help of Gears algorithm implemented in MATLAB. The whole cell model has been simulated for one second using variable time step. The time taken to simulate whole cell model for one second is about 920 millisecond, when simulated on a

system having 3 GB RAM and Intel Centrino Core2 Duo Processor with processing speed 1.67 GHz. Parameter values, taken from the literature are given in Table 1.

TABLE I
Ca^{2+} Regulatory Mechanism Parameters (see [16])

Parameter	Definition	Value
c_1	Ratio of ER volume to cytosolic volume	0.185
c_2	Proportionality constant X_{111} to $[IP_3]$	0.2 μM
v_1	Maximum $[IP_3]$ receptor flux	300 s^{-1}
v_2	Ca^{2+} leak rate constant	2 s^{-1}
a_2	Inhibitory receptor binding constant	0.2 μM^{-1} s^{-1}
a_5	Activation receptor binding constant	20 μM^{-1} s^{-1}
d_2	Inhibitory receptor dissociation constant	1.0 μM
d_5	Activation receptor dissociation constant	82.3 nM
$[IP_3]$	Basal concentration of $[IP_3]$	0.5 μM

TABLE II
Parameter Values Of The Model

Parameter	Value
k_1	15.58 μM^{-1} s^{-1}
k_2	0.6 s^{-1}
K_s	0.7 μM
k_1^+	1.5 μM^{-1} s^{-1}
k_1^-	0.3 s^{-1}
k_2^+	100 μM^{-1} s^{-1}
k_2^-	37 s^{-1}
$[B_T]$	5 μM
$[B_T]_{ER}$	5 μM
V_{pm}	12 μM s^{-1}
K_{pm}	0.1 μM

III. Results And Discussion

In this section results relevant to cytosolic Ca^{2+} oscillations are shown. The biophysical parameters used during the course of simulation are listed in Table I and Table II.

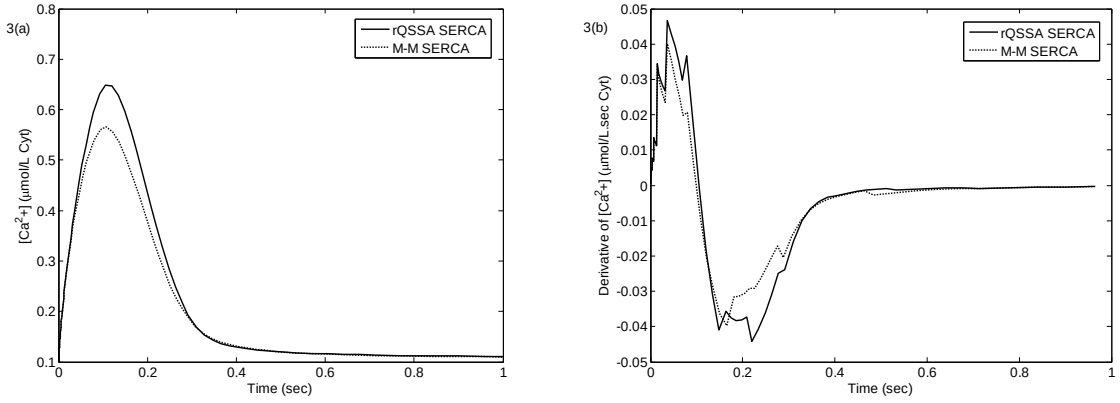

Fig. 3 Ca^{2+} oscillations and rate of change of Ca^{2+} oscillations for rQSSA and M-M SERCA pumps

Fig 3(a) – 3(b) depicts the change in cytosolic Ca^{2+} concentration and $d[Ca^{2+}]/dt$ with respect to time for different SERCA pump formulations viz. reverse Quasi-Steady State Approximation (rQSSA) and Michaelis–Menten (M–M) formulations. All the parameters, reaction equations and constants are same for both the curves. But, the only difference is the way net influx into ER via SERCA pump is modeled i.e. using rQSSA and M-M formulations. It will seem from fig. 3(a) that there is no change in frequency of Ca^{2+} oscillation for both the formulations. But, there is a decrease in frequency of Ca^{2+} oscillation when we consider more realistic rQSSA formulation in place of M-M formulation. This becomes more apparent if one looks at fig. 3(b) where the rate of change of Ca^{2+} with respect to time is plotted. It is clear from fig. 3(b) that M-M curve is quick to attain its crest and trough values while the rQSSA curve always lags behind the M-M curve signifying the change in frequency of Ca^{2+} oscillation in both the cases.

The change in ER Ca^{2+} concentration and ER bound buffer concentration is shown in fig. 4. As and when the IP_3 channel opens ER Ca^{2+} starts decaying and reaches its resting level, see fig. 4(a). Similar phenomenon is also evident from fig. 4(b), apart from a small phase where bound Ca^{2+} concentration rises

initially. It happens because as the reaction starts ER Ca^{2+} get bound to ER buffer as per the scheme given in (15). Since, we know that ER buffer concentration is lesser than ER Ca^{2+} concentration as a result of which very soon total ER buffer concentration gets saturated by binding of ER Ca^{2+} concentration. Afterwards, as ER Ca^{2+} concentration depreciates bound Ca^{2+} concentration also depreciates to reach its resting concentration.

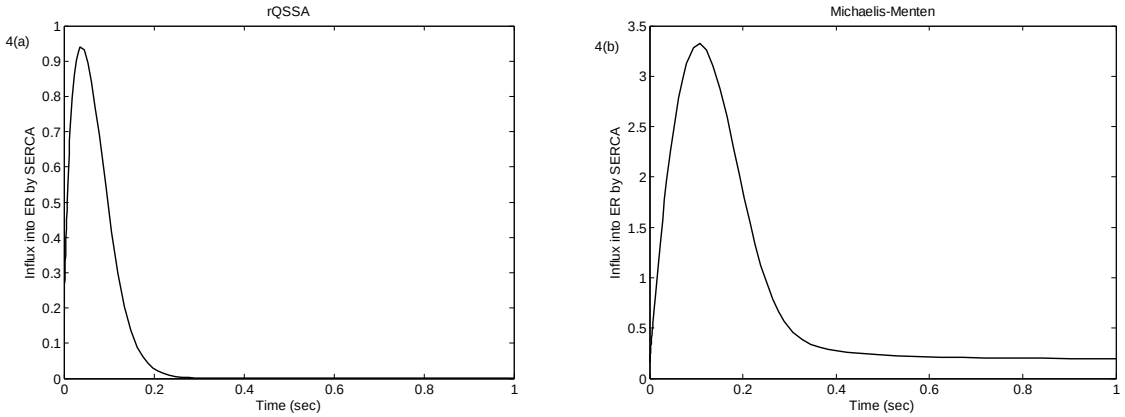

Fig. 4 Change in ER Ca^{2+} and bound Ca^{2+} concentration for rQSSA model against time

Fig. 5 gives an illustration of change in bound cytosolic Ca^{2+} concentration with respect to time. There is not much of a change in cytosolic bound buffer concentration as they rapidly get saturated after opening of IP_3R Ca^{2+} channel. This thing is also evident from fig. 5 by an initial increase in bound Ca^{2+} concentration followed by a gradual decrease in bound Ca^{2+} concentration.

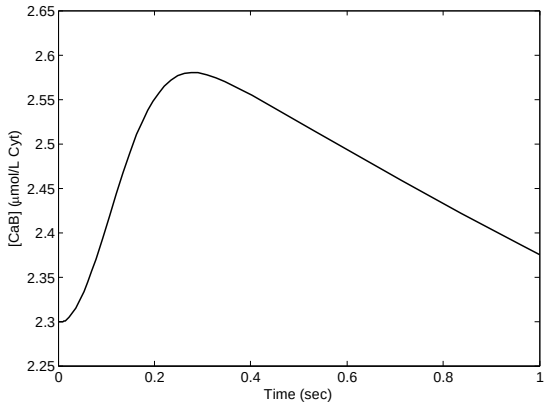

Fig. 5 Cytosolic bound Ca^{2+} concentration for rQSSA model

This article highlights the importance of SERCA pump over cytosolic Ca^{2+} oscillations. This article also aims to suggest a different formulation for SERCA pump which is biologically more sound. This article also shows the effect of different SERCA pump formulations over the frequency of cytosolic Ca^{2+} oscillations.

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