STOCHASTIC MODELING OF INOSITOL-1,4,5-TRISPHOSPHATE RECEPTORS IN PURKINJE CELL SPINE

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ABSTRACT

Transient rises in cytosolic calcium concentration play a crucial role in initiating long-term depression (LTD) of synaptic activity. Calcium release from endoplasmic reticulum is particularly important in LTD. In Purkinje cells, the release is mediated by inositol-1,4,5-trisphosphate (IP_3) receptors (IP_3Rs) that are highly expressed in dendritic spines. The small volume of spine and the small number of molecules involved increase stochasticity in biochemical processes. We studied the effects of stochasticity by comparing stochastic and deterministic simulations for two different IP_3R models. We found a significant difference between the responses when using small initial concentration of calcium or IP_3. Deterministic simulations of IP_3R activation do not produce realistic results under all conditions.

1. INTRODUCTION

Transient rises in the cytosolic Ca^{2+} concentration have an important functional role in neurons. In cerebellar Purkinje cell (PC) dendritic spines, they are essential for generation of LTD of synaptic strength [1, 2]. These temporary rises are due to Ca^{2+} entry from the extracellular space and Ca^{2+} release from intracellular stores such as endoplasmic reticulum (ER). In PC spines, IP_3Rs are responsible for the Ca^{2+} release from the ER and are relatively highly expressed.

Mathematical modeling is one of the important tools when trying to understand the complex behavior of proteins within networks and pathways. Several models have been proposed to describe the behavior of IP_3R (for a comprehensive review, see, for example, [3]). All the IP_3R models and simulations were deterministic until recent years. Deterministic models show the average behavior of the system, i.e. do not include any kind of randomness. However, when biochemical reactions occur in very small volumes, such as in dendritic spines, the number of molecules is low even with fairly large concentrations. The small number of molecules increases the possibility for stochastic effects in reactions. Both the randomness of molecular encounters and the fluctuations in the transitions between the conformational states of proteins become relevant. Given the small volume of the PC spine, it is of interest to test the stochastic nature of the system and to take the stochasticity into account to obtain biologically realistic simulations. Even though the deterministic approach is adequate in some cases, it fails to reflect the detailed nature of the biological system.

The aim of this work was to study the concentration levels at which the effects of stochasticity on the function of IP_3R can not be ignored. Among many mathematical models of IP_3R two recent ones were chosen as test cases. The models were implemented into two different software, GENESIS/Kinetikit [4, 5] for deterministic simulations and STEPS [6] for stochastic simulations, to perform two types of simulations, open probability simulations and dynamic simulations.

2. MATERIALS AND METHODS

2.1. IP_3R models

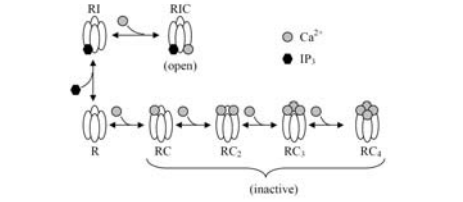
2.1.1. Model of Doi et al.

The IP_3R model of Doi et al.[7] was originally published as a part of a larger model for Ca^{2+} dynamics in the cerebellar PC spine and parameter values of this model were determined based on experimental data from Purkinje cells [7]. The model was originally implemented as deterministic. A schematic representation of the model is shown in Figure 1a.

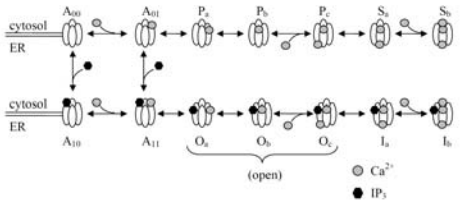
All the reactions and their rate constants can be found in Supplemental material of the original article [7]. Briefly, in this model IP_3R needs to bind both IP_3 and Ca^{2+} to open and thus provide Ca^{2+} flux from ER lumen to cytosol. IP_3R has only one open state, RIC, in this model.

2.1.2. Model of Fraiman and Dawson

The IP_3R model of Fraiman and Dawson [8] (see Figure 1b) is the only model that has a Ca^{2+} binding site inside the ER in addition to the cytosolic binding sites found in other models. The parameter values used in this work can be found in Errata for the original article [8]. This model was originally simulated stochastically, as a Markov process.



(a)



(b)

Figure 1. Schematic representation of the states and transitions of the IP_3R models. (a) Doi et al. (b) Fraiman and Dawson.

Originally, the six states, O_a, O_b, O_c, P_a, P_b, and P_c, are considered as open. However, IP_3R needs IP_3 to reach a stable open conformation [9, 10]. For this reason, three of the original open states were neglected in the present work and only states O_a, O_b, and O_c were considered as open. Also in the original article [8], the rate constant of the transition from A_{10} to A_{00} is defined as 'detailed balance'. We fixed the parameter by testing three values with deterministic open probability simulations (data not shown). Simulations were done as described in Section 2.3.1. The parameter values of 0 s^{-1} and 200 s^{-1} produced identical results while the value of 2000 s^{-1} slightly upraised the left side of the open probability curve. Based on these test simulations the value of 200 s^{-1} was chosen.

2.2. Simulation software

2.2.1. Genesis/Kinetikit

The GENESIS (GEneral NEural SImulation System) [4] simulation environment can be extended with Kinetikit [5] that is an extension for simulating reaction kinetics in well-mixed conditions. GENESIS/Kinetikit can be used to model and simulate the behavior of molecular networks and pathways. In this work, GENESIS version 2.2.1 for Cygwin and Kinetikit version 10 were used to obtain deterministic simulation results. Deterministic versions of the IP_3R models used are based on the law of mass action. The differential equation system was numerically solved (simulated) with the Exponential Euler method [4].

2.2.2. STEPS

STEPS (STochastic Engine for Pathway Simulation) [6] performs full stochastic simulation of reactions and diffusion of molecules in three dimensions. It extends the stochastic simulation algorithm (SSA) described by Gillespie [11]. In this work, STEPS developmental version 0.1.3 was used. Simulations were run both on a computer cluster and in a Cygwin environment on a standalone machine.

In the SSA, all reactions must be unidirectional. For this reason forward and backward parts of reversible reactions are defined as two separate reactions in the STEPS input file. In this early version of the software, the compartments of the modeled system are geometrically modeled as cubic shapes that are then discretized into small voxels. It is possible to define walls or surfaces between voxels that belong to different compartments. This enables modeling of surface bound molecules, such as ion channels, in their natural location.

2.3. Simulations

2.3.1. Open probability simulations

It has been experimentally shown that the open probability of IP_3R is dependent on the cytosolic Ca^{2+} concentration ($[Ca^{2+}]$) [12]. This dependence is bell-shaped with logarithmic x-axis. Originally, both models were built to reproduce this dependency.

In the deterministic open probability simulations, the behavior of a single IP_3R is simulated in an environment with constant $[Ca^{2+}]$ (several points, see Figure 2) and $[IP_3]$ (10 μM) until steady-state is achieved. The cytosol and also the ER had a volume of 0.1 μm^3 which is an experimentally defined average volume for PC spine cytosol [13]).

Deterministic simulations, using GENESIS/Kinetikit, were run for 5 or 15 s with a time step of 1 μs. The open probability of IP_3R was obtained at the end of simulation. In stochastic simulations with STEPS the models were simulated for 20 s using a sampling frequency of 0.1 s. In stochastic simulations the steady-state was achieved before 10 s time. For each initial Ca^{2+} concentration, 100 simulations were run with different seed values for the random number generator. The open probability was calculated as an average of the open IP_3Rs for the time interval 10-20 s over the 100 iterations.

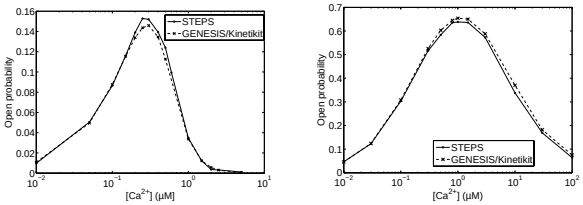
2.3.2. Dynamic simulations

A cell is a constantly evolving dynamic system. It is therefore important to study the dynamic behavior of intracellular functions in addition to steady-state properties. In this work, we studied the cytosolic Ca^{2+} concentration as a function of time. In the dynamic simulations, the Ca^{2+} flux through the open IP_3R was modeled in addition to IP_3R state transitions. In GENESIS/Kinetikit, the flux is modeled using the *kchan* entity which describes a ligand-gated channel. The equation for flux behind *kchan* is not published, but it is known to depend on the concentration gradient over the membrane and the rate of the flux is controlled with a parameter defined by the user. In STEPS, the flux is also dependent on the concentration gradient. Based on test simulations (data not shown) the equations for the flux are almost identical in GENESIS/Kinetikit and in STEPS.

Rate parameters of the flux were estimated for both simulators separately. It is estimated that 5400 Ca^{2+} ions go through open IP_3R during one opening and that the

Table 1. Initial conditions for dynamic simulations.

Species	Value
Number of IP_3Rs (naive state)	16
$[IP_3]$	0.1 μM, 0.2 μM, 0.5 μM, 1.0 μM, 5.0 μM
$[Ca^{2+}]_{cyt}$	0.01 μM, 0.05 μM, 0.1 μM, 0.2 μM, 0.5 μM, 1 μM
$[Ca^{2+}]_{ER}$	150 μM



(a) (b)

Figure 2. Results of open probability simulations. (a) Doi et al. (b) Fraiman and Dawson.

mean open time of IP_3R is 3.7 ms in physiological conditions [14]. The estimated parameter values for flux functions were 595 (unit not known) for GENESIS/Kinetikit and $5.8{\cdot}10^8$ $M^{-1}s^{-1}$ for STEPS.

The compartments in these dynamic simulations had the same volume as in open probability simulations and the volumes were considered as well-mixed (i.e diffusion was not taken into account). The initial conditions used in dynamic simulations are given in Table 1. The average number of IP_3Rs in a PC spine has been estimated to be 16 (see Supplemental material of [7]). There are five different initial concentrations for IP_3 and six for cytosolic Ca^{2+}. All combinations of the initial concentrations were used in simulations. A deterministic simulation response and 100 stochastic simulation responses were obtained for each situation. Data analysis was done with MATLAB®.

3. RESULTS

3.1. Open probability simulations

The results from open probability simulations are presented in Figure 2. The open probability curves obtained from deterministic (GENESIS/Kinetikit) and stochastic simulations (STEPS) are consistent. This expected result shows that both models were correctly implemented in both simulation environments.

3.2. Dynamic simulations

To study the dynamic behavior of the two IP_3R models, cytosolic $[Ca^{2+}]$ was followed as a function of time. Examples of simulation results with both IP_3R models are shown in Figure 3. The 100 individual stochastic iterations are shown as thin gray curves, their mean as thick solid curve, and the deterministic curve as dashed line for comparison. The variation in stochastic simulations increases, i.e. the gray curves are more spread out, when initial $[IP_3]$ and $[Ca^{2+}]$ are decreased.

The data was examined in two ways. First, the maximum Ca^{2+} concentration reached during simulations was measured as a function of the initial $[IP_3]$ and initial cytosolic $[Ca^{2+}]$ (data not shown) for the deterministic and for the mean of the stochastic cases. Second, the time at which half of the maximum cytosolic Ca^{2+} concentration was reached was measured as a function of the initial $[IP_3]$ and initial cytosolic $[Ca^{2+}]$. This is a convenient way to compare the curve slopes at the steepest region.

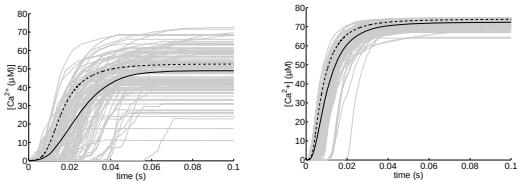
The maximum cytosolic Ca^{2+} concentration attained in the deterministic simulations with both models is dependent only on initial $[IP_3]$, not on initial $[Ca^{2+}]$. The latter might be due to the quick response to the rising $[Ca^{2+}]$. $[Ca^{2+}]$ rises when the channel opens and so the initial concentration does not have much influence on the maximum concentration. In the stochastic simulations, the results are similar to the deterministic ones above initial cytosolic $[Ca^{2+}]$ of 0.1 μM. Below this concentration value, the maximum $[Ca^{2+}]$ might be also dependent on the initial $[Ca^{2+}]$. This concentration threshold is identical for both models.

The time at which half of the maximum cytosolic Ca^{2+} concentration was reached is dependent on the initial $[IP_3]$ in deterministic and stochastic simulations. However, in the deterministic simulations, only a minor dependence on the initial $[Ca^{2+}]$ can be seen, whereas, in stochastic simulations, dependence on the initial $[Ca^{2+}]$ is more emphasized. In stochastic simulations, the dependence on both $[IP_3]$ and $[Ca^{2+}]$ is evidently seen. These results are consistent in both models.

To study the difference between deterministic and stochastic simulation results in times at which half of the maximum cytosolic Ca^{2+} concentration was reached the deterministic plots were subtracted from the stochastic plots for both models. The difference between stochastic and deterministic simulation results is shown in Figure 4. Furthermore, a threshold, below which the effect of stochasticity seems to be significant, can be determined from these plots. In the case of IP_3R model of the Doi et al. the thresholds for the initial $[IP_3]$ is around 1.0 μM and for the initial cytosolic $[Ca^{2+}]$ between 0.1 μM and 0.2 μM. In the case of the IP_3R model of Fraiman and Dawson the thresholds are slightly lower, namely 0.5 μM for $[IP_3]$ and 0.1 μM for $[Ca^{2+}]$. Our work implies that there is a difference when having 100 or less molecules. An important thing to notice is that the thresholds for $[Ca^{2+}]$ are close to the resting level of Ca^{2+} concentration, 70 ± 29 nM, if we apply results from hippocampal pyramidal neuron [15] to PC spines.

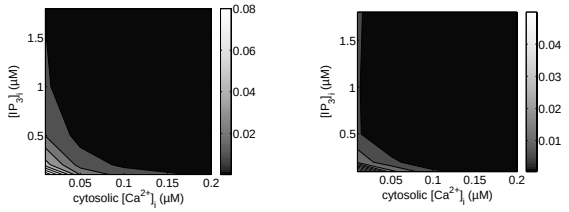
4. CONCLUSIONS

In this work, the importance of stochasticity in simulation of IP_3 receptor function was determined. The stochastic simulation algorithm gives more realistic results than the deterministic one because it takes random fluctuations



(a) (b)

Figure 3. Examples of dynamic simulations. Results from deterministic simulations (dashed) and the mean value (solid) of 100 stochastic simulations (thin gray) are shown. Initial concentrations: $[IP_3]$ = 0.2 μM, $[Ca^{2+}]$ = 0.1 μM. (a) Doi et al. (b) Fraiman and Dawson.



(a) (b)

Figure 4. Difference (gray scale) between deterministic and stochastic simulation results as a function of initial $[IP_3]$ and $[Ca^{2+}]$. (a) Doi et al. (b) Fraiman and Dawson.

into account. Based on dynamic simulation results of both models, we evaluated that there exists a threshold for initial IP_3 and cytosolic Ca^{2+} concentrations below which the effect can not be neglected. The threshold for Ca^{2+} concentration is close to the resting level of Ca^{2+} concentration in spines and thus it corresponds to the resting state of a spine before Ca^{2+} signals are induced. The present study strongly advocates for stochastic modeling and simulation of protein function.

5. ACKNOWLEDGMENTS

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