
Journal of Physical & Theoretical Chemistry
Islamic Azad University of Iran 2 (3)
(2005)

Science and Research Campus

ISSN: 1735-2126

A simulation study of calcium release channel

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ABSTRACT

The IP3R calcium release channel has been simulated using a stochastic simulation algorithm (SSA; Gillespie algorithm) and De young-Keiser model. A set of different concentration for Ca^{2+} and IP3 have been used. Considering the Number of molecules in each state, a non linear behavior of the system can be seen clearly. The inhibiting role of the Ca^{2+} on the open state (X_{110}) has been studied. The degree of inhibition may depend on the IP3 concentration. Different levels of inhibition have seen in different inhibited states. Using the Gillespie algorithm, the frequency graphs for each set of Ca^{2+} and IP3 concentrations and in different time domains can be achieved. The probability of open channel increases with going from small time domain to large time domain. A good consistency can be inferred comparing with different results from the past studies (Both in theoretical and experimental studies). A reaction pattern in fast time domain has seen, too.

Keywords: Ion channel; DeYoung-Keizer model; Stochasticity; Gillespie algorithm; Monte Carlo; Complex reactions

INTRODUCTION

In a cellular point of view, calcium ion is an important second messenger in cells. There are many cellular processes that can be activated by Ca^{2+} . They have evolved to be Ca^{2+} - dependent and/or Ca^{2+} -regulated. Calcium ions also play an important role in controlling the functioning of all cells of the body by acting as carriers of intracellular messages and are completely remarkable in being able to control numerous processes within the same cell simultaneously [1]. This last ability of calcium to provide a number of signals encoded

by the same ion is a result of the way in which calcium levels can be altered in the cell. For instance, Changes in the cytosolic free Ca^{2+} concentration are used by many cells for signaling. It transmits information as Ca^{2+} waves and oscillations. Ca^{2+} signalling frequently occur as repetitive, but transient, increases in Ca^{2+} concentration [2, 3, and 4]. Changes in cytosolic $[\text{Ca}^{2+}]$ are controlled, in part, by Ca^{2+} channels in the surface membrane and the release of Ca^{2+} from internal stores.

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Opening and closing of the Ca^{2+} channels control release. The organelles such as ER (Endoplasmic Reticulum) and SR (Sarcoplasmic Reticulum) [5-8] employ ion channels in this task.

The channel type in the endoplasmic reticulum membrane is the inositol-1, 4, 5-trisphosphate (IP3) receptor (IP3R). It is the prevailing receptor on the membrane of the ER that controls Ca^{2+} liberation from the ER to the cytosol. The open probability of the IP3R depends on the calcium concentration on the cytosolic side of the channel and the IP3-concentration [2, 9-11]. It increases nonlinearly with the IP3-concentration and the Ca^{2+} concentration. Hence, Ca^{2+} released by one channel increases the open probability of neighboring channels. That provides a self amplifying release mechanism.

The IP3R channel consists of four subunits [12, 13]. Since now three different types of IP3Rs have been found [9, 10, 14-16]. They differ in the characteristics of the regulation by IP3 and Ca^{2+} [14, 15]. All of them share basically the same response with respect to the Ca^{2+} concentration. Inositol 1, 4, 5-trisphosphate receptor channels open and close randomly. The open probability depends on the state of the receptor. The state of the receptor is mainly determined by binding of Ca^{2+} and IP3. The open probability is the highest when a minimum number of activating Ca^{2+} ions and IP3 molecules are bound and very small otherwise. In the other words a subunit in IP3R channel needs to bind IP3 and Ca^{2+} [9, 17, 18-21]. So the open probability of the channel is dependent to IP3 and Ca^{2+} .

The channel is closed in the inhibited state irrespective of binding of IP3 and activating Ca^{2+} . Inhibition is the main process causing a decrease of open probability at high Ca^{2+} concentration.

There are different types of IP3R channel models, such as general models, models of Ca^{2+} pool content and mathematical IP3R channel models. In the last category, many models of IP3Rs have been published to date [17,23-27] most of which designed to reproduce various aspects of the single channel kinetics of the type 1 IP3R, but see[28,29] for models of the IP3R-2 and -3.

Our interested model in this study is the DeYoung – Keiser model. Using of this model requires a closer look on the IP3 receptor. As said before, measurements on the flux properties have revealed a tetrameric structure. It is known that a subunit expresses binding sites for Ca^{2+} and IP3. However, the exact number of binding sites is still under investigation. Based on the results of Bezprozvanny [17] DeYoung and Keizer proposed a model for a single subunit [24,30]. It consists of three binding sites: an activating and an inhibitory Ca^{2+} binding site (the second Ca^{2+} which produced an inhibited state of the subunit) [31] and an activating IP3 binding site. Therefore the state of a subunit can be specified by a binary triplet ijk . The first index represents the IP3 binding site, the second the Ca^{2+} activating and the last the Ca^{2+} inhibiting binding site.

An index equals 1 when a site is occupied and 0 otherwise. Hence the state 110 refers to IP3 and Ca^{2+} bound to the activating sites, respectively, and no Ca^{2+} attached to the inhibiting binding site. The resulting eight states of a subunit are shown in Figure 1. The binding rate constants for IP3 activation are given by a_1 and a_3 , where as a_2 and a_4 refer to Ca^{2+} inhibition. Ca^{2+} activation is controlled by a_5 .

The dissociation rates for the above processes are denoted by b_1 through b_5 , with respecting to $b_i = a_i d_i$. The reactions that occur at a subunit are binding and unbinding of Ca^{2+} and IP3. They determine the state of one subunit.

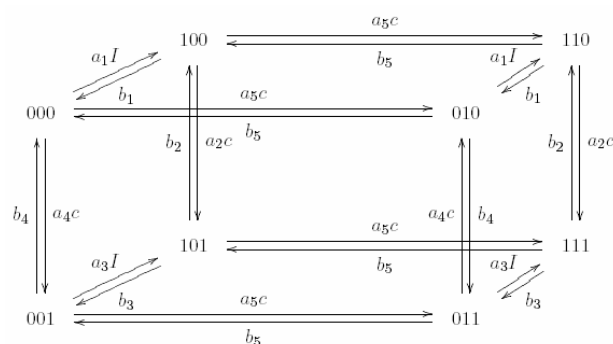


Figure1. Transition scheme of the De Young Keizer model [23, 32].

These subunits are treated as independent and identical; only when three of the subunits have both IP3 and an activating Ca^{2+} bound is the IP3 receptor regarded as in the open state. Almost in all the case of studies, differential equations based on mass-action kinetics have been used. Here in this study we have used a stochastic simulation algorithm, called Gillespie algorithm.

METHODOLOGY

The time behavior of a spatially homogeneous chemical system can be mathematically described in two formalism: The deterministic approach regards the time evolution as a continuous, wholly predictable process which is governed by a set of coupled, ordinary differential equations (the “reaction-rate equations”); the stochastic approach regards the time evolution as a kind of random-walk process which is governed by a single differential-difference equation (Chemical master equation or simply master equation). Chemical master equation describes the transition of the system from one state to another state by using probability methods. In this approach the transition of the state of the system is described through changing the probability of the system being in a certain state. Simple kinetic theory arguments show that the stochastic formulation of chemical kinetics has a firmer physical basis than the deterministic formulation, but unfortunately the stochastic master equation is often mathematically intractable,

because it tries to write a set of (differential) equations and solve them simultaneously for all possible states. So this approach can be used just for very simple systems.

The problem is solving by use of an exact numerical calculations within the framework of the stochastic formulation without having to deal with the master equation directly. It is a relatively simple digital computer algorithm which uses a strictly derived Monte Carlo procedure to numerically simulate the time evolution of the given chemical system. This Stochastic Simulation Algorithm (SSA) correctly accounts for the inherent fluctuations and correlations that are necessarily ignored in the deterministic formulation, as well as the master equation

Gillespie developed two exact stochastic simulation algorithms, "Direct method" and "First reaction method". Both of the two Gillespie algorithms, make two basic assumptions: the system is homogenous and well-mixed. Unlike the master equation, at each time step system is exactly in one state. The transition consists of execute of a reaction, so there can be r probable transition from a specified state. Using a uniform random number (URN) generator, we can use random numbers to select the transitions.

The Direct method, exactly calculates which reaction occur next (that is shown by random number of μ) and when it occurs (which is shown by random number of τ).

In other words, the aim is to generate a random couple of (τ , μ) in correspondence with the suitable probability density.

The latter method generates for each reaction μ , a putative time τ_μ at which reaction occurs. Then select the first reaction with the smallest τ_μ^* and execute it in time τ_μ^* .

At first look, it may seem that these two algorithms have a great difference with each other, but it can be proved that they are the same [33, 34]. Although this algorithm solves the master equation exactly, but it needs a large amount of computational effort for simulating of a complex system.

Gibson made an improvement of Gillespie algorithm by his "Next Reaction Method". This method improves time performance of Gillespie algorithm while keeping the exactness of it [35]. Also Gillespie have presented the "Tau-leaping method" which is produce significant gains in the computational speed, without losing unacceptable accuracy [36].

Gillespie algorithm has been applied in too many in silico simulations, recently [37-39] but it has not been used for an IP3R calcium release channel since now.

For a review of Monte Carlo simulation methods applicable to stochastically gating ion channel models see [40,41]. Briefly, time is discretized and at each time-step the channel is given an opportunity to change state. In a short time interval of length Δt , the probability that the channel makes a transition between state i and j

states is calculated and show by elements of W_{ij} in a W matrix.

The row of W corresponding to the current state needs to be calculated at every time step. The non-zero entries of this row will always sum to one and can be used to partition the unit interval. Next, a pseudo-random number generator produces a random variable uniformly distributed on [0,1] and the transition from state i to j occurs if the random number falls on the partition associated with column j of W . We chose Δt small enough so that the diagonal entries of W are greater than 0.9 when the $[Ca^{2+}]$ is it's maximum value ($c = c_{ss}$); thus, most time-steps do not result in a state change for the channel.

RESULTS AND DISCUSSION

The IP3R channel has been simulated by using the Gillespie algorithm and the DeYoung-Keiser reaction model. This model has been considered in its complete form, not in any simplified forms. An initial value of 10000 molecules in the first state (X_{000}) have been used in all runs.

The rpd (reaction per dot) notation has been used for all resulted graphs. It shows the number of reaction that occurs between each dot that appears on a simulation output graph [33]. All the plots are 100 rpd plots.

A set of different concentration for IP3 and calcium have been considered. Since, the concentration of Ca^{2+} free in the cytosol is generally less than 0.2 micro molar [42], three different level of concentration have been used in this study up to maximum value of 0.2 micro molar: 0.1, 0.15 and 0.2 micro molar.

IP3 may have different concentrations in different cells. A range of 0.15 to 0.6 micro molar has been considered [4, 28 and 42].

Because of having no truly cutoff range for the complex reactions, like biological reaction systems, we have to study the effect of time evolution in such systems and then select a cutoff range.

Time evolution in range of several minutes has not much effect on the system. This behavior have been checked for different selections of calcium and IP3 concentrations. As an instance, the results for concentrations of 0.2 and 0.3 micro molar for calcium and IP3, respectively have been given (Table1). So, the simulations have been performed for the first five minutes.

We have performed twelve simulations, each for a group selection of calcium and IP3 concentrations. The results have been reported in three time domains: fast domain (small t : 10^{-4} seconds), intermediate domain (t : 10^{-3} seconds) and slow domain (large t : 10^{-2} and in some cases 10^{-1} seconds) (Table2).

Table2 shows that the whole reactions have stochastic nature and the reactants in different states are dependent to each other. They show a non-linear behavior. (For instance, see Figure2).

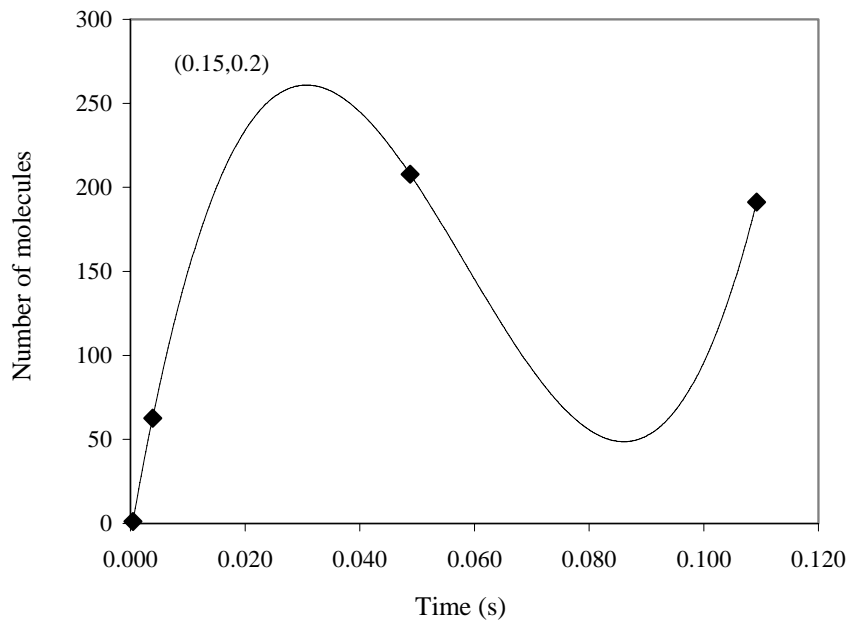


Figure2. The Number of molecules in the open state (X110) vs. time
Initial concentrations are 0.15 and 0.2 micro molar for IP3 and calcium, respectively.

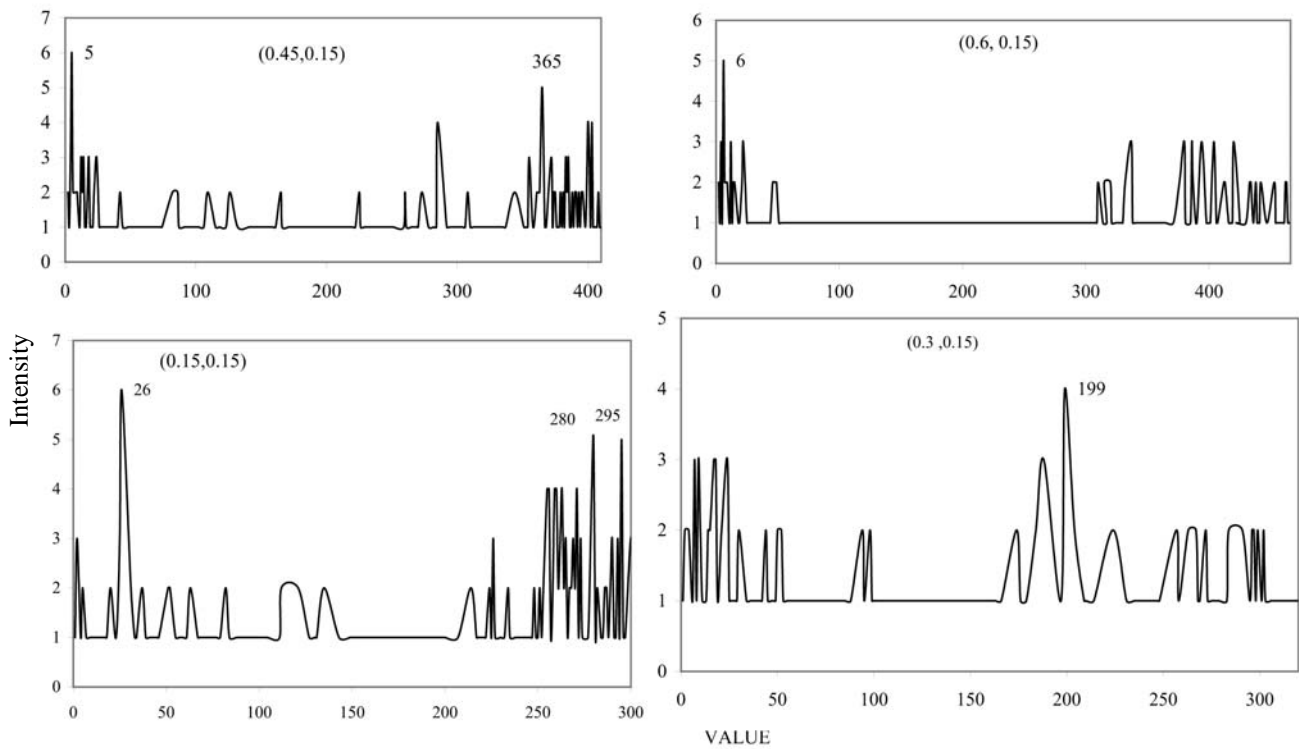


Figure3. Frequency graphs:Monte Carlo simulations of a Ca²⁺-activated channel using different concentrations of IP3 with a concentration of 0.15micromolar for Ca²⁺.

Calcium controls the IP3R in a biphasic manner. In other words, high calcium concentrations inhibit release through the IP3R channel. In such a case the channel is closed, whether the calcium and IP3 are bound or not. The findings by Mak et al. suggest that inhibition is a prolongation of closed time [7]. Calcium inhibition together with activation by calcium and modulation of IP3 binding by calcium generates a biphasic dependence on calcium concentration [17,19,43,44]. The degree of inhibition may depend on the IP3 concentration and type of receptor, too. Several studies report the Ca^{+2} - inhibition is weak or not present at high IP3 concentrations [10, 45,46]. The results of stochastic simulations are in consistency with these concepts (Table3 and 4).

Theoretical and experimental results, indicate the domain $[Ca^{+2}]$ may change by tens of micro molar in a millisecond [42, 47, 48]. By plotting of the time repetition of the repeated values of molecules in the

calcium activated state (X_{110}) vs. their values, intensity graphs resulted. They show, for each group selection of concentrations, the open probability in each concentration. Using the frequency definition and comparing these graphs, we see that the frequencies for small t domain (10^{-4} s) increase and decrease quickly, for large t domains (10^{-3} or 10^{-2} s) frequencies fluctuate more slowly (for instance see Figure 3).

The open probability increases with increasing calcium concentration and with going through small domain to large domain .This can be confirmed by the values are given in Table5.

Considering the different time domains and using the results given in Table2, we can see a stable reaction pattern in the small time domain, but not in large time domains. In small time domain there is a stable four state pattern. In this domain and independently from the different concentrations of IP3 and Ca^{+2} , we can see such a pattern, (Figure 4).

Table1. Standard deviations for IP3 =0.3, c =0.2, after 5,10, 15 minutes in different time domains

	5minutes	10 minutes	15 minutes	σ_n	σ_{n-1}
Time domain(s)					
1.00E-04					
X000	8807.444	8809.37	8807.444	0.905	1.109
X100	1130.962	1129.111	1130.962	0.872	1.068
X110	4.259	4.259	4.259	0	0
X010	55	54.925	55	0.0353	0.043
X001	2.259	2.259	2.259	0	0
X101	0.074	0.074	0.074	0	0
X111	0	0	0	0	0
X011	0	0	0	0	0
1.00E-03					
X000	3106.674	3107.662	3106.974	0.413	0.506
X100	3422.73	3421.64	3422.258	0.445	0.545
X110	116.707	116.831	116.764	0.0506	0.062
X010	177.078	177.022	177.089	0.029	0.035
X001	92.719	92.876	92.539	0.137	1.168
X101	24.044	24.011	24.022	0.013	0.016
X111	0.191	0.191	0.191	0	0
X011	0.629	0.629	0.629	0	0
1.00E-02					
X000	49.157	43.169	42.96	2.873	3.519
X100	570.636	338.697	339.232	109.211	133.755
X110	388.458	386.874	387.003	0.718	0.879
X010	188.397	181.492	181.567	3.237	3.965
X001	287.821	262.071	262.292	12.086	14.803
X101	88.164	80.392	80.482	3.642	4.461
X111	1.321	8.953	8.907	3.586	4.393
X011	14.78	25.176	25.117	4.886	5.985
1.00E-01					
X000	0	51.543	51.395	0.74	0.104
X100	0	124.751	124.201	0.247	0.388
X110	0	362.865	363.064	0.099	0.14
X010	0	156.865	157.755	0.445	0.629
X001	0	189.838	191.179	0.67	0.948
X101	0	60.993	61.345	0.176	0.248
X111	0	16.53	16.345	0.0925	0.13
X011	0	51.241	51.05	0.0955	0.135

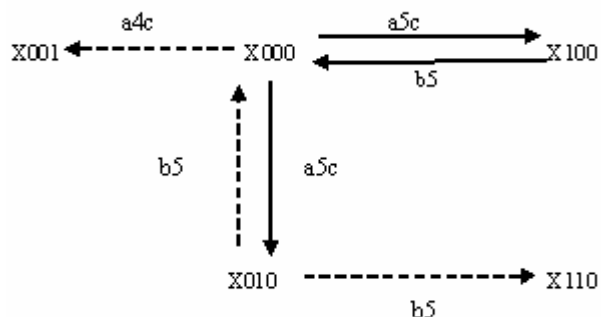


Figure 4. The reaction pattern in small time domain.

The black lines show the stable reactions, and the dashed lines show the next path which may be selected by the reactants. It seems that the X_{010} is a determinative barrier. From this state reaction may select the path to X_{110} or the only inhibited state in this domain, X_{001} . It seems that the selecting of these two paths is dependent to the concentration of calcium. Increasing the calcium concentration, increase the probability of selecting X_{110} path and so a four state pattern can be seen. In the other hand, with increasing the IP3 concentration this probability decrease and reaction goes to selecting X_{001} path.

In going through small to large time domains, there is no stable pattern for determining the behavior of reactants. What can be seen clearly, is the increasing of inhibited states and increasing the number of molecules in them. For instance the number of molecules in the X_{101} state is more than X_{111} and X_{011} states.

Table2. Number of IP3R states for different concentration of IP3 and Calcium, in different time domains

* Concentrations are given in μM ** the results are averages in 100 rpd (run per dot)

concentrations*	Number								
	Time	X000	X100	X110	X010	X001	X101	X111	X011
IP3 =0.15 c= 0.1	4.729E-04	93554	609.33	0.733	34.4	0.133	0	0	0
	4.343E-03	5073.506	2829.93	47.05	139.92	38.88	5.012	0	0.1012
	3.213E-02	124.06	681.817	178.451	180.02	163.344	24.795	0.376	2.559
IP3 =0.3 c= 0.1	4.778E-04	8824.962	1150.666	1.851	21.962	0.555	0	0	0
	4.402E-03	3252.912	3610.659	69.637	94.78	51.021	13.747	0.021	0.34
	2.359E-02	184.242	1165.766	239.233	134.9065	172.093	55.242	1.233	2.719
IP3 =0.45 c= 0.1	4.667E-04	8354.108	1625.135	2.054	16.54	2.162	0	0	0
	4.572E-03	2849.42	3658.369	108.019	97.656	64.624	24.738	0.305	0.439
	1.220E-02	392.731	3896.439	301.634	137.682	190.756	82.731	1.121	1.78
IP3 =0.6 c= 0.1	4.633E-04	7921.234	2056.361	2.574	17.231	0.191	2.404	0	0
	4.565E-03	1902.745	4996.068	101.259	37.647	32.573	68.401	0	0
	1.340E-02	252.732	2984.966	272.355	82.627	107.271	177.813	0.254	0.237
IP3 =0.15 c= 0.15	4.662E-04	9344.866	607.933	0.466	46.133	0.6	0	0	0
	4.419E-03	4839.629	2787.814	74.851	212.296	56.925	7.395	0.0246	0.098
	4.700E-02	44.671	412.993	267.503	253.412	195.551	31.054	1.244	5.7
IP3=0.3 c= 0.15	4.730E-04	8818.962	1139.37	4.407	35.888	1.333	0.037	0	0
	4.155E-03	3196.741	3512.393	89.932	123.37	64.842	17.595	0	0.337
	1.525E-02	118.396	1355.264	271.981	152.792	204.943	61.358	0.735	1.698
IP3=0.45 c= 0.15	4.626E-04	8356.54	1608.648	4.567	27.702	2.243	0.297	0	0
	4.271E-03	2229.697	4044.541	113.239	101.531	69.135	26.614	0.489	0.479
	2.290E-02	41.568	937.836	361.043	128.681	209.396	99.06	3.456	4.275
IP3=0.6 c= 0.15	4.562E-04	7928.652	2023.304	7.456	35.086	5.13	0.369	0	0
	4.537E-03	1478.312	4058.031	171.166	99.739	107.666	51.552	1.291	0.927
	1.680E-02	55.443	1328.829	401.84	116.579	243.477	149.909	7.204	8.681

Table2. continued

concentrations*	Number	X000	X100	X110	X010	X001	X101	X111	X011
	Time								
IP3=0.15 c= 0.2	4.582E-04	9345.4	595.6	1.26	56.866	0.866	0	0	0
	3.875E-03	4256.92	1963.42	62.74	198.98	48.66	7.22	0.06	0
	4.880E-02	23.803	181.794	207.745	193.558	132.98	20.666	1.803	9.225
	1.092E-01	22	28.8125	191.25	154.1875	85.25	14.3125	3.5	19.3125
IP3=0.3 c= 0.2	4.693E-04	8807.444	1130.962	4.259	55	2.259	0.074	0	0
	4.133E-03	3106.674	342.73	116.707	177.078	92.719	24.044	0.191	0.629
	2.880E-02	49.157	570.636	388.458	188.397	287.821	88.164	1.321	14.78
IP3=0.45 c= 0.2	4.600E-04	8358.081	1592.351	6.783	38.675	3.513	0.594	0	0
	4.237E-03	2168.892	3837.494	145.935	119.344	99.268	35.526	0.535	0.29
	2.040E-02	68.063	956.603	431.63	147.738	262.378	121.378	7.153	11.198
IP3=0.6 c= 0.2	4.560E-04	7929.543	2022.434	4.456	35.062	5.13	0.369	0	0
	4.536E-03	1478.322	4058.114	171.104	99.739	107.583	51.583	1.291	0.927
	1.570E-02	59.626	1459.186	391.866	117.946	240.28	147.52	6.626	7.48

Table3. The inhibiting role of calcium on X110 states

IP3	0.15	0.3	0.450	0.6
t =1.0E-04 (s)				
c = 0.1	0.733	1.851	2.054	2.574
c= 0.15	0.466	4.407	4.567	7.456
c = 0.2	1.26	4.259	6.783	4.456
t =1.0E-03 (s)				
c = 0.1	47.05	69.637	108.019	101.259
c= 0.15	74.851	89.932	113.239	171.166
c = 0.2	207.745	116.707	145.935	171.104
t =1.0E-02 (s)				
c = 0.1	178.451	239.233	301.634	272.355
c= 0.15	267.503	271.981	361.043	401.840
c = 0.2	207.745	388.458	431.63	391.860

Table4. The effect of increasing the concentration of calcium and IP3 on the inhibited states

Ca+2 conc.		IP3 =0.15			
		X001	X101	X111	X011
c =0.1		189	36	3	6
c =0.15		237	43	6	13
c =0.2		167	31	6	22
Ca+2 conc.		IP3 =0.3			
		X001	X101	X111	X011
c =0.1		199	75	3	7
c =0.15		241	79	2	4
c =0.2		322	109	13	28
Ca+2 conc.		IP3 =0.45			
		X001	X101	X111	X011
c =0.1		214	103	1	3
c =0.15		239	125	10	11
c =0.2		294	152	16	23
Ca+2 conc.		IP3 =0.6			
		X001	X101	X111	X011
c =0.1		205	134	2	2
c =0.15		275	176	14	20
c =0.2		274	174	13	17

Table5. Open probability of the channel in different IP3 and Calcium concentrations, in different time domains
* Concentrations are given in μM

concentrations*	Number		concentrations*	Number		concentrations*	Number	
	Time	P open		Time	Popen		Time	Popen
IP3 =0.15 c= 0.1	4.729E-04	7.75E-06	IP3=0.3 c= 0.15	4.730E-04	4.41E-04	IP3=0.45 c= 0.2	4.600E-04	6.78E-04
	4.343E-03	5.78E-03		4.155E-03	1.28E-02		4.237E-03	2.27E-02
	3.213E-02	0.1316		1.525E-02	0.1255		2.040E-02	0.2151
IP3 =0.3 c= 0.1	4.778E-04	1.85E-04	IP3=0.45 c= 0.15	4.626E-04	4.57E-04	IP3=0.6 c= 0.2	4.560E-04	4.46E-04
	4.402E-03	9.82E-03		4.271E-03	1.71E-02		4.536E-03	2.86E-02
	2.359E-02	0.1223		2.290E-02	0.2022		1.570E-02	0.1612
IP3 =0.45 c= 0.1	4.667E-04	2.05E-04	IP3=0.6 c= 0.15	4.562E-04	7.46E-04			
	4.572E-03	0.0158		4.537E-03	0.0286			
	1.220E-02	0.062		1.680E-02	0.178			
IP3 =0.6 c= 0.1	4.633E-04	2.57E-04	IP3=0.15 c= 0.2	4.582E-04	1.26E-04			
	4.565E-03	0.0141		3.875E-03	9.60E-03			
	1.340E-02	0.0702		4.880E-02	0.2692			
			1.092E-01	0.3687				
IP3 =0.15 c= 0.15	4.662E-04	4.66E-05	IP3=0.3 c= 0.2	4.693E-04	4.26E-04			
	4.419E-03	4.58E-03		4.133E-03	0.03022			
	4.700E-02	0.2206		2.880E-02	0.2445			

LITERATURE CITED

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