Detailed Model of Ryanodine Receptor-Mediated Calcium Release in Purkinje Cells

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Abstract
Ryanodine receptor-mediated calcium release was modeled in a compartmental model of the Purkinje cell with a detailed representation of buffered calcium diffusion. For several important parameters no constraining experimental data were available. The importance of the threshold of activation of release and of the unbinding rate of the main calcium buffer in the stores (calsequestrin) is described. Using reasonable assumptions for these parameters, calcium influx during voltage-gated calcium spikes can activate ryanodine receptors in the submembrane region without noticeably changing the firing pattern of the model.

Introduction
It has been known for a long time that dendrites of cerebellar Purkinje cells contain high densities of intracellular Ca^{2+} stores,[1] making these cells a popular preparation for the study of Ca^{2+} release mechanisms. Calcium release from stores can be activated by IP_3 receptors, subsequent to synaptic stimulation of metabotropic glutamate receptors,[2] or by Ca^{2+} itself activating ryanodine receptors.[3,4] But the physiological role of these processes remains unclear and positive evidence for Ca^{2+} release under normal *in vivo* conditions is still lacking. As a first step towards a better understanding, we have modeled the effect of ryanodine receptor-mediated Ca^{2+} release, also called Ca^{2+}-induced Ca^{2+} release (CICR), in Purkinje cells.

Methods
These modeling studies used a new version of a highly detailed compartmental model of the Purkinje cell with voltage-gated channels in the soma and dendrites.[5] In particular, the dendrite contained a P-type and a T-type Ca^{2+} channel and two types of Ca^{2+}-activated K^+ channels and a persistent K^+ channel, while in addition the soma also contained a fast and a

persistent Na^+ channel, a delayed rectifier and A-current K^+ channel, and an anomalous rectifierchannel.[5] The new version of the model incorporated a detailed representation of calcium dynamics based on buffered, radial diffusion of calcium.[6] It also computed the electrogenic effect of the plasma membrane Ca^{2+}-ATPase pump and of the Na^+-Ca^{2+} exchanger.[6]

Radial diffusion of Ca^{2+} was modeled using concentric shells with a *Dr* of 0.2 mm, resulting in a total of 7471 shells for the 1604 compartments in the model. We used a D_{Ca} of 2.10^{-6} cm^2sec^{-1}, based on measurements of Ca^{2+} diffusion rates in cytoplasm.[7] A very high buffer capacity has been estimated for Purkinje cells.[3,8] The non-mobile buffer capacity was put at 2080 by including 4 mM of a slow buffer with a K_d of 1.9 mM. Additionally fura-2 was simulated, represented as 75 mM of a mobile buffer (D_{fura} 2.10^{-6} cm^2sec^{-1} with a K_d of 0.2 mM). The fura-2 estimated [Ca^{2+}] was computed using standard methods.[6,9]

Calcium stores were modeled as pools occupying 20 % of the volume of each diffusion shell, based on an electron microscopic reconstruction of the ER in Purkinje cells.[1] This store volume was converted into store surface using the assumption that the ER could be represented as a long tube with a diameter of 0.050 mm.[10] Calcium uptake by the Ca^{2+}-ATPase pump and Ca^{2+} release by ryanodine receptor activation were modeled using equations based on those of Goldbeter *et al*.[11] These Hill-function based equations were modified to include a time constant of activation[12] and release was proportional to the concentration gradient between store and cytoplasm.[6] Stores contained a moderately high Ca^{2+} concentration at rest (200 mM) and 100 mM of the low affinity buffer calsequestrin (K_d 1 mM).

Results

Tuning of Free Parameters

CICR is in first analysis a regenerative system as the released Ca^{2+} can activate more release. Most modeling studies of CICR have assumed that CICR continues on timescales of seconds to minutes till the stores are empty.[11,13] It is unlikely that the complete ER in a region of the Purkinje cell dendrite empties, as in the model the stores contain 99.985% of the Ca^{2+} present in the cell. In fact, instantaneous release would cause a cytoplasmic [Ca^{2+}] of more than 100 mM! Slower complete emptying of stores might of course be possible, but the experimental evidence argues against this in Purkinje cells[3,4] and in other preparations.[14] We have assumed that CICR operates only in a limited range of cytoplasmic [Ca^{2+}]; below an activation threshold there was no CICR[4,12] and at higher concentrations the Ca^{2+}-ATPase up-take (SERCA3[15]) dominated. The parameters for uptake into the stores were: V_{max} of 4.10^{-9} $mM^{-2}msec^{-1}$ and K_d of 1 mM; and for CICR:

V_{max} of 10^{-8} $mM^{-2}msec^{-1}$ or 3.10^{-8} $mM^{-2}msec^{-1}$, time constant of 1.2 msec and K_d of 0.3 mM.[6] Another potential mechanisms limiting the ryanodine-receptor mediated release would be inactivation of the receptor,[12] but this inactivation is not complete and was not included in the model.

Even when constrained by the above assumptions, the model was very sensitive to the values of its parameters, most of which were not better than educated guesses. In particular, CICR produced reasonable results only if calsequestrin was assumed to have extremely slow backward binding rates (1 sec^{-1} or 10 sec^{-1}). Unfortunately these binding rate are not known as only the steady state kinetics of calsequestrin have been measured, though indirect evidence exists for slow unbinding.[16] With faster unbinding rates Ca^{2+} release dumped the complete contents of the stores fast enough to raise the cytoplasmic [Ca^{2+}] beyond 20 μM.

We used also higher threshold values for induction of Ca^{2+} release (120 nM or 200 nM) than reported for Purkinje cells,[4] because the experimental values are distorted by the spatial averaging of the fura-2 signal.[6] In fact, the apparent [Ca^{2+}] threshold for CICR (80 nM) that could estimated from the computed fura-2 signal in a spiny dendrite of both the 120 nM and 200 nM threshold models was very close to the experimentally observed values.[4]

Finally, we tuned the parameters of the CICR model so that CICR was activated during somatic current injection without causing noticeable changes to the firing pattern of the model. This assumption was based on the experimental observation that Purkinje cell firing patterns look normal in cells where subsequent addition of low concentrations of caffeine evokes large Ca^{2+} signals due to CICR[4,17] and that Ca^{2+} influx through voltage-gated channels can activate CICR.[3] The model could reproduce the effects of caffeine application on spontaneous firing patterns,[4,17] simulated by increasing the V_{max} of CICR (results not shown).

In the rest of this paper we will compare two versions of the release model: the low-threshold model (120 nM of cytoplasmic [Ca^{2+}] needed to cause CICR in the same shell) had a V_{max} of 10^{-8} $mM^{-2}msec^{-1}$ and a 1 sec^{-1} backward binding rate for calsequestrin, while the high-threshold model (200 nM) had a V_{max} of 3.10^{-8} $mM^{-2}msec^{-1}$ and a 10 sec^{-1} unbinding rate.

Calcium Release During Spontaneous Dendritic Spiking

Under these conditions the model showed several interesting results. Figure 1 demonstrates that the firing pattern of the two release models with CICR (1**B** and **C**) looked very similar to that of a Purkinje cell model without Ca^{2+} stores (1**A**). In fact, the only effect was a decreased delay to the first dendritic spike after onset of the somatic current injection and a slightly sharper dendritic spike as recorded in the soma. While these differences may seem significant when these models are compared to each other, it should be noted that they fall within the natural variability as observed during

recordings of Purkinje cell activity in slice[18]. In other words, the traces produced by the two models with CICR look like any normal Purkinje cell, demonstrating that significant Ca^{2+} release is possible (Figure 2) without causing a specific change in the cell's firing pattern.
The ryanodine receptor-mediated calcium release caused complex changes to the cytoplasmic Ca^{2+} levels during the dendritic spikes. Each dendritic spikes evoked CICR in the smooth dendritic compartment shown, causing prolonged increases of the submembrane [Ca^{2+}] (Figure 2, compare to Figure 6.6 of De Schutter and Smolen[6] for the [Ca^{2+}] changes in a model without CICR). A constant observation in our simulations was that this CICR, which was triggered by the voltage-gated Ca^{2+} entry, always remained localised to the submembrane

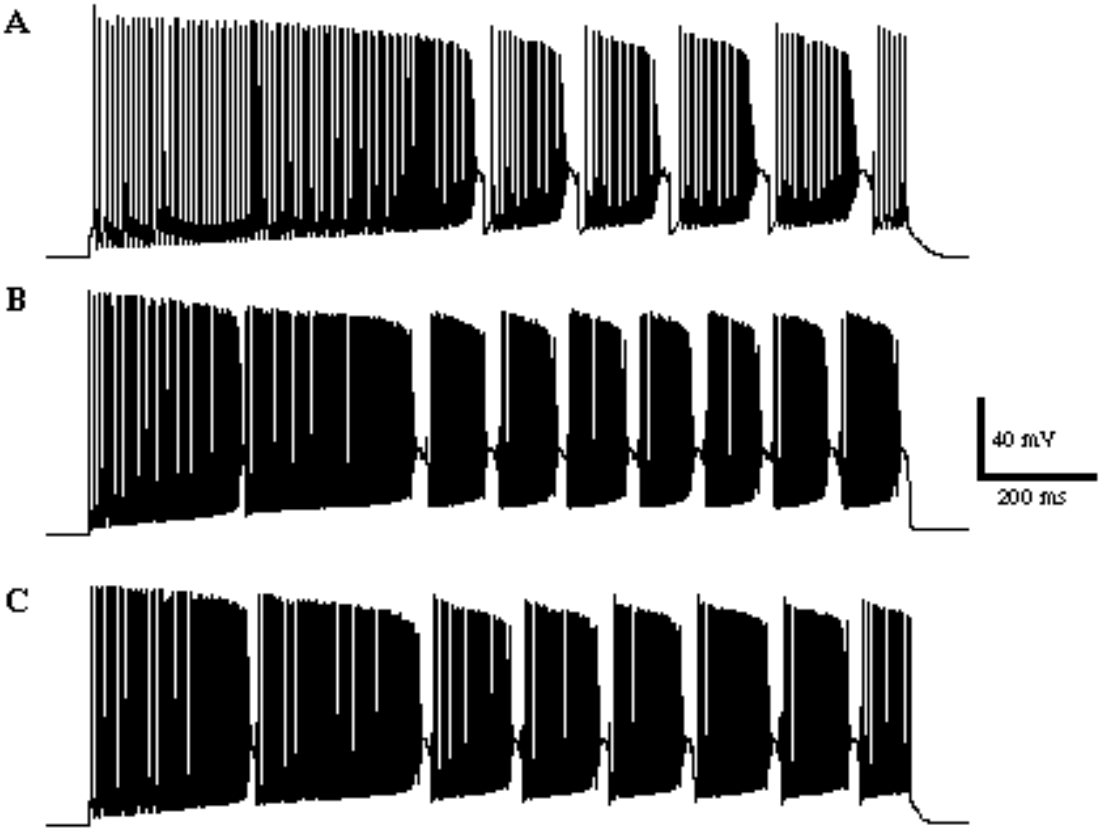

Figure 1
The activation of ryanodine receptor mediated calcium release may have little effect on membrane potential recordings. Somatic recordings from models without (**A**), with high-threshold (**B**) or low-threshold (**C**) calcium stores during a 1.5 nA current injection in the soma.

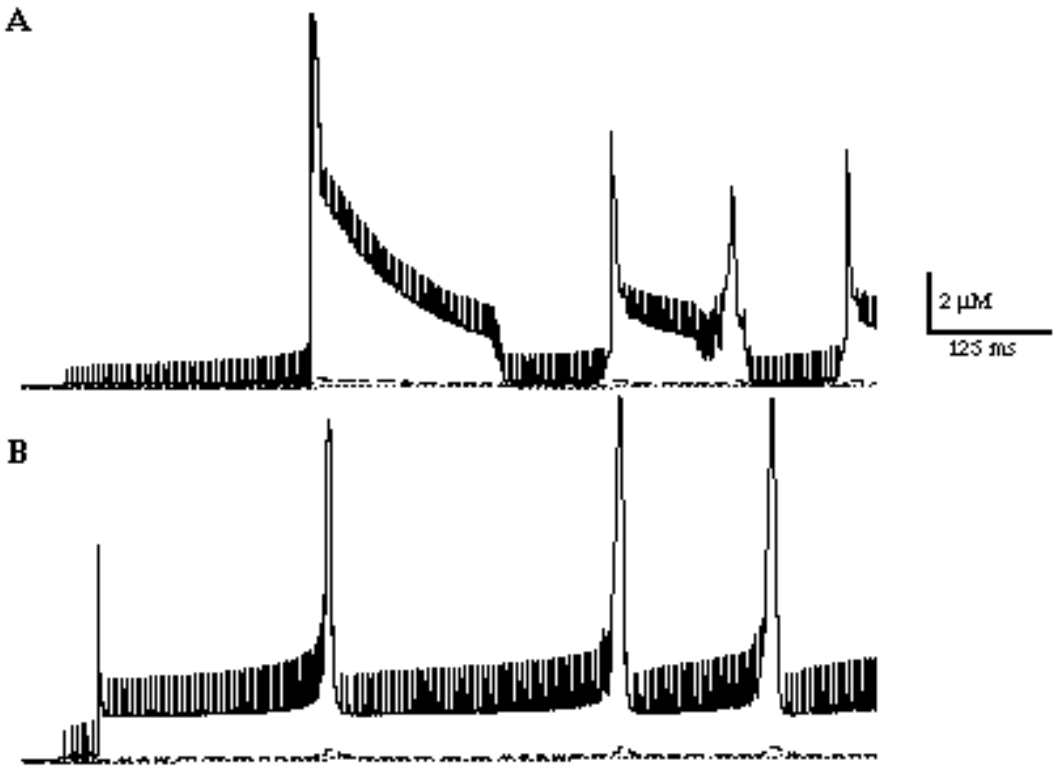

Figure 2

Changes in cytoplasmic calcium concentration in the smooth dendrite (diameter 8.6 mm) during activation of ryanodine receptor mediated calcium release. Recordings from models with high-threshold (**A**) or low-threshold (**B**) calcium stores during a 1.5 nA current injection in the soma. The concentrations in the submembrane shell (full line), in a shell 2 mm lower (broken line) and in the core (stippled line) are shown.

Region of Large Compartments

This can be observed in Figure 2, where the trace from the shell 2 mm below the membrane (broken line) is almost flat. This lack of propagation towards deeper regions can be explained by the relative high threshold for CICR initiation and suggests that the conditions for quantal release[14] may be present. We could not evoke Ca^{2+} waves[13] traveling towards the center in the model, as has been observed in the somata of some neurons[19] but not in Purkinje cells.[4]

Next we consider the differences between the low- and high-threshold models and more in particular the effect of the unbinding rate of calsequestrin on cytoplasmic calcium profiles. Figures 3 and 4 show the changes in the stores and cytoplasm in a spiny dendrite for the high-threshold and low-threshold model respectively. An important difference with the much larger smooth dendrite shown in Figure 2 is that here almost no cytoplasmic [Ca^{2+}] gradient existed both before and during the CICR. Looking first at the high-threshold model in Figure 3 it is obvious that, after the initial peak in [Ca^{2+}] caused by each dendritic spike, the [Ca^{2+}] transients followed the inverse of the free calsequestrin curve. In other words, the evolution of Ca^{2+} release to a steady state was rate-limited by the unbinding

of Ca^{2+} from the store buffer, emphasising the importance of this parameter. The low-threshold model (Figure 4) had even slower buffer kinetics. Because of the time scales used, it is more difficult to notice the buffer unbinding effect. It is, however, obvious that it limits the amount of Ca^{2+} that can be released from the stores in the low-threshold model (which also had a lower V_{max} for release). The low threshold also caused low levels of release before the first dendritic spike (Figure 2**B**).

Two observations are common to both models. First, the Ca^{2+} uptake was not homogenous over the shells. The submembrane stores removed a large part of the voltage-gated Ca^{2+} in-flux into the compartment, causing a constant rise in their concentration before the phase of release. Because the rate of release was sensitive to the store concentration,[6] this rise contributed to the inititation of the first release phase. Second, the steady state cytoplasmic [Ca^{2+}] rose to rather high levels in spiny dendrites that are probably unrealistic, though data from such tiny dendrites are lacking.[4] This high [Ca^{2+}] can be explained by either too high a V_{max} for release in the model or, more likely, by the absence of a mechanism which can end CICR.

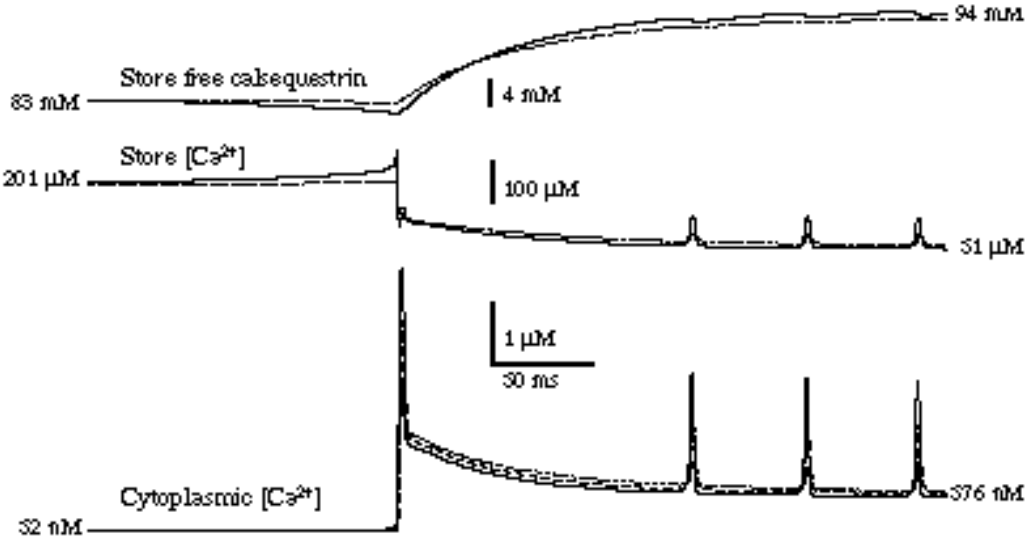

Figure 3

Dynamics in a spiny dendrite (diameter 1.3 mm) of the high-threshold release model with slow store buffer kinetics. Changes in (from top to bottom) the free buffer and calcium concentrations in the stores and in the cytoplasmic calcium concentration during the current injection shown in Figure 1**B**. For each loca-tion the concentrations in the submembrane shell (full line) and in the core (stippled line) are shown. The fura-2 estimated cytoplasmic calcium concentration is shown by the broken line. For each set of traces the initial concentration in the submembrane shell is shown at the left and the final concentration at the right.

Discussion

Our earlier model of the Purkinje cell,[5,20] with simple Ca^{2+} dynamics, was much more constrained by experimental data than the current model. As such, the earlier model has been quite successful in predicting new

experimental results, like the importance of inhibition[21,22] and the dendritic Ca^{2+} inflow caused by focal parallel fiber activation.[23,24] The current model should be considered to be much more explorative; it inspires us to think about how CICR could function under normal conditions.
Nevertheless, the current model reproduced many experimental results, like the effect of high CICR rates on firing patterns[17] and the threshold for activation and the activation by complex spikes.[4,6] Moreover, it showed that CICR may occur during bursting without visibly affecting the membrane potential record (Figure 1). This is a very important result, because intuitively this was not expected. Similarly, the presence of a threshold in cytoplasmic $[Ca^{2+}]$ for CICR activation hinders propagation to low $[Ca^{2+}]$ regions (Figure 2).

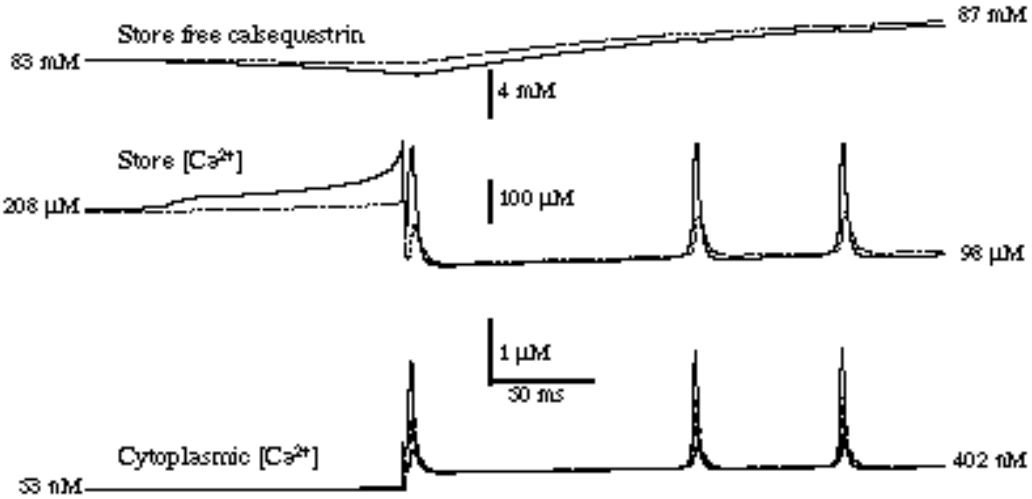

Figure 4
Dynamics in a spiny dendrite of the low-threshold release model with very slow store buffer kinetics during the current injection shown in Figure 1**C**. Same conventions as in Figure 3.

Another important conclusion is that if CICR persists beyond the first few milliseconds of release, it will be dominated by the unbinding kinetics of the store buffers (Figures 3, 4). Unfortunately this important parameter has never been measured directly! Indirect evidence for very slow unbinding can be found, however, in Ikemoto et al.[16] who show that caffeine-induced release from sarcoplasmic reticulum vesicles begins with a fast rate followed by a slow release rate of about 2 sec^{-1}. The authors show that this second phase is related to binding to the calsequestrin buffer and we propose that the slow rate probably reflects the buffer unbinding rate, while the faster initial rate follows free Ca^{2+} being released at V_{max}

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