Conditional probability analysis for a domain model of Ca^{2+}-inactivation of Ca^{2+} channels

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ABSTRACT The domain model of Ca^{2+} inactivation of Ca^{2+} channels, which has been used to explain rapid inactivation of whole cell Ca^{2+} currents in pancreatic β cells, is applied to single-time and conditional open probability measurements on guinea pig ventricular myocyte Ca^{2+} channels. These two measurements greatly constrain the choice of kinetic constants in the model. Calculations with the model provide a simple quantitative explanation of recent experimental results, including a slow increase in the inactivation rate.

INTRODUCTION

Calcium channels from a variety of tissues (Eckert and Chad, 1984; Rosenberg et al., 1988) exhibit the phenomenon of Ca^{2+}-dependent inactivation of Ca^{2+} currents (Lux and Brown, 1984; Plant, 1988; Hopkins et al., 1991). This type of inactivation is characteristically diminished or absent when Ba^{2+} is the charge carrier and shows up in both whole cell and single channel recordings. For whole cells when calcium channel densities are low and for single-channel recordings, a modified version of the shell model (Chad and Eckhart, 1984), which has been termed the domain model (Sherman et al., 1990), provides a simple explanation of how this might occur.

The domain model relies on results from numerical simulations which show that at the inside mouth of an open channel the average concentration of Ca^{2+} is elevated to the order of a few hundred μM within a few microseconds after opening (Simon and Llinás, 1985). As a consequence, in intact cells the inactivation of an open channel by the binding of a Ca^{2+} ion is vastly more likely than for a closed channel. The concentration of Ca^{2+} in the domain, $Ca_d(V)$, is localized within a few tenths of a micron of the mouth of an open channel (Simon and Llinás, 1985). Its value is determined by the external Ca^{2+} concentration, Ca_0, the membrane potential, V, and the internal Ca^{2+} concentration (Sherman et al., 1990). Sufficiently far from the reversal potential, $Ca_d(V)$ is proportional to the single-channel current, $i(V)$.

The shell model is based on a very different idea about the spatial distribution of Ca^{2+}. In that model, one postulates that Ca^{2+} currents lead to a "shell" of elevated Ca^{2+} concentration underlying the entire plasma membrane. Furthermore, it is the rate of filling of that shell that governs the rate of Ca^{2+} inactivation of the Ca^{2+} current. Finally, because both open and closed channels are subject to Ca^{2+} within the shell, open channels are not inactivated preferentially, as they are in the domain model.

Previously we have shown (Sherman et al., 1990) that the domain model can be used to explain whole cell records of Ca^{2+} inactivation for pancreatic β cells (Plant, 1988; Hopkins et al., 1991; Smolen and Keizer, 1992). Here we extend that work to show that the domain model can explain the surprising "memory" effect found in conditional open probability measurements of single Ca^{2+} channels from guinea pig ventricular myocytes (Yue et al., 1990).

Yue and co-workers (Yue et al., 1990) examined ensembles of single L-type Ca^{2+} channel records from myocytes. After stepping the voltage from a holding potential of -50 mV to $+20$ mV, the time course of the open probability was obtained by averaging repeated records. Ca^{2+} dependent inactivation was observed in the open probability on a time scale of tens of milliseconds. In addition, subensembles of records that contained an open channel in specific time windows subsequent to the step were obtained. By averaging the currents in each subensemble, they were able to construct the time course of the conditional open probability, that is, the probability of finding an open channel at time t' given that it was open at time t. The conditional open probability also showed Ca^{2+} dependent inactivation, but on a faster time scale (less than a millisecond). Furthermore, the conditional open probability had the property that the rate of relaxation towards its equilibrium value increased with the length of time that had elapsed from the depolarizing step. This observation of memory was reinforced by the fact that the existence of openings before the observation window also increased the average relaxation rate over that seen when no prior openings had occurred. Such observations cannot be explained with the earlier shell models of Ca^{2+} inactivation without modification.

STOCHASTIC DOMAIN MODEL

We show here that all of these observations can be explained using the domain model (Sherman et al., 1990), the simplest form of which is

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$$C \rightleftharpoons O \overset{Ca_d}{\rightleftharpoons} B, \quad (1)$$

where C is a closed state, O is an open state, and B is a reversibly blocked state of the channel[1]. Blocking is a bimolecular step attributed to the binding of domain Ca^{2+} as indicated. This is the minimal domain model, which assumes that blocking and binding occur simultaneously.

If this is not the case, the domain model must be extended to (Smolen and Keizer, 1992)

$$C \rightleftharpoons O \overset{Ca_d}{\rightleftharpoons} O' \rightleftharpoons B, \quad (2)$$

where the state O' represents the open state with a bound Ca^{2+} ion. This form of the domain model has been used to analyze Ca^{2+}-inactivation for mouse β cells (Hopkins et al., 1991; Smolen and Keizer, 1992). Clearly, the mechanism in Eq. 1 can not explain the memory effect observed by Yue et al., (1990) because no matter at what time an open state is observed, it will certainly be state O and, because the model is Markovian, the conditional open probability will relax at a rate that is independent of the time elapsed from depolarization. As we show here, the extended model in Eq. 2 can explain the conditional observations because it contains two open states, O and O'. If they have the same conductance, then for a sufficient lapse of time after the voltage step, an "open" state is more likely to be the state O', which obviously inactivates or "blocks" more rapidly than the state O.

It is implicit in Eq. 2 that both open states have the same conductance and, therefore, cannot be distinguished in a single channel record. This is compatible with the observation of unitary currents of a fixed size (0.35 pA) for ventricular myocytes. We should also note that when Ba^{2+} is the charge carrier, the states O' and B are inaccessible as long as Ba^{2+} does not bind to the channel. This is consistent with the observation (Yue et al., 1990) that Ba^{2+} currents do not inactivate nor, in that case, does the rate of decay of the conditional open probability depend upon the time lapsed from the depolarization step.

The statistical properties of the model in Eq. 2 are governed by a master equation (Hill 1977; Keizer, 1987) consisting of four linear ordinary differential equations that describe the time rate of change of the probability of the four states. Assuming that the only state that occurs at -50 mV is the closed state, C, we have solved these equations for the single-time probability, $W(i, t)$ ($i = C$, O, O', B), using the initial condition $W(C, 0) = 1$, where the depolarization step occurs at $t = 0$.

As is well known (Lux and Brown, 1984) the temporal behavior of $W(\text{open}, t)$ is proportional to the whole cell current. Thus, by averaging the current in a large number single-channel records in which the depolarization occurred at time zero, Yue et al. (1990) were able to obtain the single-time open probability. The phrase single-time is used here to differentiate this probability from the conditional open probability, which involves two time points in its determination. Theoretically the single-time open probability is given by the expression $W(\text{open}, t) = W(O, t) + W(O', t)$ because both O and O' are open. The relative fraction of the two states in the single time ensemble are, therefore, given by $w_0(t) = W(O, t)/W(\text{open}, t)$ and $w_{0'}(t) = W(O', t)/W(\text{open}, t)$. We will refer to these fractions as the weight factor for each state.

To calculate the single-time open probability we have selected a set of rate constants for the model in Eq. 2 that provide a reasonable fit to the all of the measurements of Yue et al. (1990), which were performed at $V = +20$ mV. The single-channel current was determined experimentally to have a value of 0.35 pA (Yue et al., 1990). For the pancreatic β cell, it was observed that the magnitude of the domain calcium concentration, in units of mM, roughly equaled the magnitude of the current, in units of pA (Sherman et al., 1990). Using this provides an estimate of 0.35 mM for Ca_d. Combining this with the value of the pseudo first-order rate constant, $k_2^+ = K \cdot Ca_d = 2.3\ \text{m s}^{-1}$, then allows us to predict that the bimolecular binding rate constant, K, of Ca^{2+} to the channel is the order of $10^7\ \text{M}^{-1}\ \text{s}^{-1}$. This is smaller than the diffusion-controlled value, but comparable to the binding rate constant for charybdotoxin to Ca^{2+}-activated potassium channels (Miller, 1991). Using this value for k_2^+ and the other rate constants in the legend to Fig. 1, we have calculated the time dependence of the single-time open probability shown in Fig. 1 *A*. The maximum and asymptotic values ($\approx$0.047 and 0.00) and the time course of the inactivation are in reasonable agreement with that observed by Yue et al. (1990).

For the same parameter values, the time course of the weight factor of the open state, O', is shown in Fig. 1 *B*. It exhibits a complicated behavior, rapidly increasing from zero in the first few milliseconds, leveling off for $\sim$20 ms, and then slowly increasing to nearly unity. This reflects the initial rapid shift in the relative probability of the two open states from O to O' following the depolarization step, followed by the slower equilibration of channels predominantly into state O' as equilibrium is achieved. The weight factor can be written as a quotient of sums of the equilibrium value and three exponentials, whose relaxation times are $\tau = .099$, 0.20, and 10.7 ms for the indicated rate constants.

To obtain the conditional open probability for the model in Eq. 2, we calculated the single-state conditional probabilities weighted by their time-dependent probability of occurrence, i.e.,

[1] The idea of reversible blocking, rather than inactivation to an absorbing state, is supported by experiment (Yue et al., 1990).

$$P(\text{open}, t \mid \text{open}, t')$$
$$= w_0(t) \cdot [P(O \mid O, t' - t) + P(O \mid O', t' - t)]$$
$$+ w_{0'}(t) \cdot [P(O' \mid O, t' - t) + P(O' \mid O', t' - t)]. \quad (3)$$

The left-hand side of Eq. 3 represents the open probability at time t', conditioned on an observation of an open state at time t, in an ensemble in which all states are C at $t = 0$ ($0 < t < t'$). This is equal to the right hand side of Eq. 3, where the P's are single-state conditional open probabilities and depend only on the time interval, $t' - t$, following the observation of the first state. They are calculated from the master equation by making the appropriate choice of initial condition, e.g., $P(O \mid O', t' - t) = W(O', t' - t)$ if $W(O, 0) = 1$. The two terms in square brackets in Eq. 3 are the "mixed" open probabilities conditioned on the initial state O or O', i.e., $P(O \mid \text{open}, t' - t)$ and $P(O' \mid \text{open}, t' - t)$, respectively.

The time course of the "mixed" conditional probabilities are shown in Fig. 2 *A*. The upper curve is for O as the initial state, and the lower curve is for O'. Thus, the open probability conditioned initially on state O' relaxes more rapidly than when O is the initial state. As is obvious from the connectivity of the states in Eq. 2, this is a general property of the model although the difference in relaxation rates is determined by the precise values of the rate constants.

While the "mixed" conditional open probabilities cannot be measured directly, using the expressions in Eq. 3 they can be combined with the weight factors in Fig. 1 *B* to obtain the conditional open probability, which has been measured (Yue et al., 1990). The results are shown in Fig. 2 *B*, where we have selected our observation "windows" at 10, 50, and 170 ms after the depolarization step.[2] Although we have not attempted to adjust the kinetic constants systematically to optimize agreement with experiment, the results for the three open probabilities in Fig. 2 *A* are similar in shape and duration to those found by Yue et al. (1990). All three curves fall essentially to zero within a millisecond, and the rate of relaxation is increased significantly if one waits for 50 or 170 ms after the depolarization step. Note that it is the rapid (less than a millisecond time scale) relaxation in Fig. 2 *B* that corresponds to the effect of domain calcium blocking the channel, just as seen by Yue et al., (1990).

The time course of the single-time open probability taken together with the conditional probabilities provide significant constraints on the choice of rate constants for our model. Three distinct pieces of kinetic information are provided by the single-time probability: its rise time just after depolarization, its relaxation time toward equi-

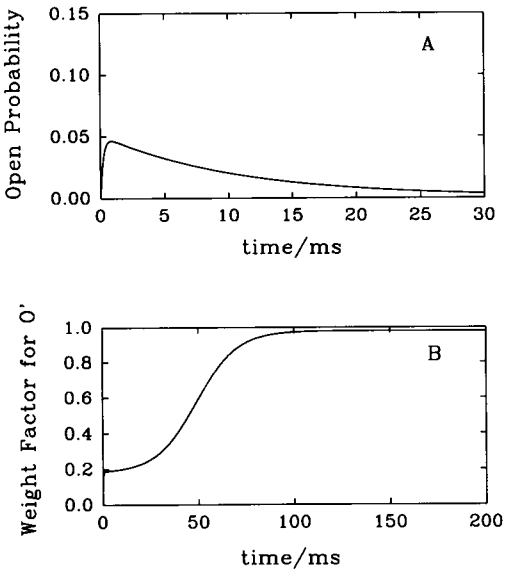

FIGURE 1 *A* shows the single-time open probability for the four-state domain model in Eq. 2. The calculation was made using the following values of rate constants, where the steps in Eq. 2 are labeled left to right, the superscript $^+$ is the forward step and the $^-$ is the backward step: $k_1^+ = 0.2$, $k_1^- = 2.5$, $k_2^+ = K \cdot Ca_d = 2.3$, $k_2^- = 0.05$, $k_3^+ = 10$, $k_3^- = 0.005$. The units are all ms^{-1}. *B* shows the weight factor for state O' as a function of time calculated with the same rate constants.

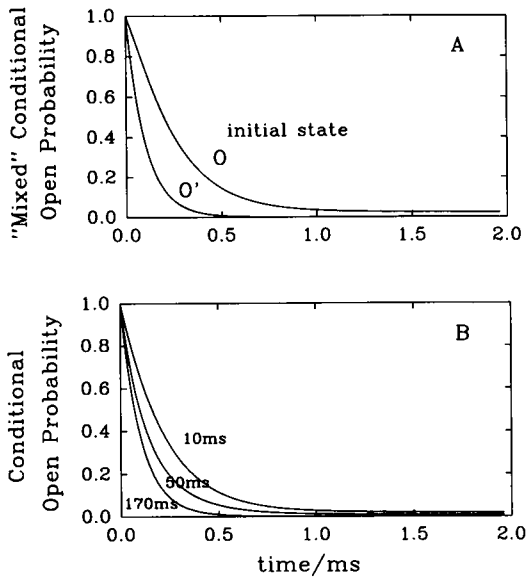

FIGURE 2 *A* shows the "mixed" conditional probabilities for the four-state domain model in Eq. 2 calculated with the same parameters as in Fig. 1. The upper curve is for the initial condition O and the lower curve is for O'. *B* gives the conditional open probabilities for the four state domain model, where the initial open condition is observed at 10, 50, and 170 ms after the depolarizing step to +20 mV.

[2] To obtain a sufficient number of data, Yue et al. (1990) used small time "windows" of increasing length centered at the point 10, 50, and 170 ms. For simplicity, our analysis uses precise initial points as described by Eq. 3. Otherwise, we would need to average our results over the time windows.

librium, and its asymptotic equilibrium value (cf. Fig. 1 *A*). The time course of the conditional probability measured at 10 and 170 ms helps define the inactivation rates from state O and O' (cf. Fig. 2), whereas the measurement at 50 ms provides information about the time scale on which the weight factor, $w_{0'}(t)$, changes (cf. Fig. 1 *B*). Indeed, the magnitude of the rate constants dictated by these constraints (see Fig. 1, legend) are in keeping with those that can be inferred for L-type Ca^{2+} channels in whole cells (Lux and Brown, 1984; Plant, 1988) and bilayers (Rosenberg et al., 1988).

Additional kinetic information, which would serve as a check on the assignment of kinetic constants, could come from measurements of the distribution of open times (Lux and Brown, 1984). Because the ensemble of channels following depolarization is nonstationary, the distribution of open times will depend of the time interval, t, after depolarization. For openings that commence at time t, the probability density for open times of length τ can be shown to be:

$$P_{\text{open},t}(\tau) = w_0(t)\cdot[k_1^-\bar{P}(O|O,\tau) + k_3^+\bar{P}(O|O',\tau)] + w_{0'}(t)\cdot[k_1^-\bar{P}(O'|O,\tau) + k_3^+\bar{P}(O'|O',\tau)], \quad (4)$$

where, for example, $\bar{P}(O|O,\tau)$ represents the probability of a channel being in state O at τ given that its state was O at $\tau = 0$ and that it stayed open (O or O') throughout $[0, \tau]$. $\bar{P}$ is easy to calculate because it is identical to the single-state conditional probability calculated with $k_1^+ = k_3^- = 0$ (see Eq. 2). Using Eq. 4 we have calculated $P_{\text{open},t}(\tau)$ and find, as expected, that for small or large values of t, it is similar in shape to the 10 and 170 ms curves in Fig. 2 *B*. Using Eq. (4) and the fact that $w_0(t) = 1 - w_{0'}(t)$, it further follows that

$$P_{\text{open},t}(0) = k_1^- + (k_3^+ - k_1^-)\cdot w_{0'}(t). \quad (5)$$

According to Eq. 5, the distribution of open times could provide both a check on the values of k_1^- and k_3^+ and independent information about the weight factor, $w_{0'}(t)$.

CONCLUSIONS

The origin of the two time scales in the domain model (rapid blocking of open channels and slow increase in blocking rate) can be traced to two features of Eq. 2. First, the existence of two open states with the same conductance, one free of Ca^{2+} (O) and the other with Ca^{2+} bound (O'). The state O' is, thus, primed to be reversibly blocked. This insures that the "mixed" conditional probability relaxes more rapidly when O' is the initial state. The second feature is the existence of more than two relaxation times. This allows the single-time open probability to relax within tens of milliseconds, while the fraction of open states that are O' increases on a longer time scale (cf. Fig. 1). In fact we can improve the agreement with the published data of Yue et al. (1990) by adding an additional closed state to the model. This allows us to reduce the maximum single time open probability in Fig. 1 *A* from 0.047 to 0.03, which is more in line with experiment. While there is evidence for additional closed states of the ventricular myocyte Ca^{2+} channel (Imredy and Yue, 1991), they do not seem crucial for mimicking the open probability measurements.

Our purpose in this note has been two-fold. First, to show how the domain model (Sherman et al., 1990) of Ca^{2+} inactivation of Ca^{2+} channels can be used to analyze conditional open probability measurements. And second, to show that this analysis can be used to explain both the single time and conditional open probability measurements of Yue et al. (1990) for guinea pig ventricular myocytes. This latter analysis requires selection of particular rate constants for the model in Eq. 2, but otherwise requires no changes in its basic features. The additional kinetic information contained in the conditional open time probabilities provides important constraints on the selection of kinetic constants, which could be augmented, as we have shown, by a knowledge of the nonstationary distribution of open times. In light of this, it would be interesting to measure the voltage dependence of the conditional open probabilities and to see how well the domain model agrees with those experiments when the appropriate voltage dependence in the rate constants is included.

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REFERENCES

Chad, J. E., and R. Eckert. 1984. Calcium domains associated with individual channels can account for anamolous voltage relations of Ca-dependent responses. *Biophys. J.* 45:993–999.

Eckert, R., and J. E. Chad. 1984. Inactivation of calcium channels. *Prog. Biophys. Mol. Biol.* 44:215–267.

Hill, T. L. 1977. Free Energy Transduction in Biology. John Wiley and Sons, Inc., New York.

Hopkins, W., L. Satin, and D. Cook. 1991. Inactivation kinetics and pharmacology distinguish two calcium currents in mouse pancreatic β cells. *J. Membr. Biol.* 119:229–239.

Imredy, J. P., and D. T. Yue. 1991. Poster, Biophysical Society Meeting, February, 1991.

Keizer, J. 1987. Statistical Thermodynamics of Nonequilibrium Processes. Springer-Verlag, New York.

Lux, H. D., and A. M. Brown. 1984. Single channel studies on inactivation of calcium currents. *Science (Wash. DC).* 225:432–434.

Miller, C. 1991. Diffusion-controlled binding of a peptide neurotoxin to its K^+ channel receptor. *Biochemistry.* 29:5320–5325.

Plant, T. D. 1988. Properties and calcium-dependent inactivation of

calcium currents in cultured mouse pancreatic β cells. *J. Physiol. (Lond.)*. 404:731–747.

Rosenberg, R. L., P. Hess, and R. Tsien. 1988. Cardiac calcium channels in planar lipid membranes. L-type channels and calcium permeable channels open at negative membrane potential. *J. Gen. Physiol.* 92:27–54.

Sherman, A., J. Keizer, and J. Rinzel. 1990. Domain model for Ca^{2+}-inactivation of Ca^{2+} channels at low channel density. *Biophys. J.* 58:985–995.

Simon, S. M., and R. R. Llinás. 1985. Compartmentalization of the submembrane calcium activity during calcium influx and its significance in transmitter release. *Biophys. J.* 48:485–498.

Smolen, P., and J. Keizer. 1992. Slow voltage-inactivation of Ca^{2+} currents and bursting mechanisms for the mouse pancreatic β-cell. *J. Membr. Biol.* In press.

Yue, D. T., P. H. Backx, and J. P. Imredy. 1990. Calcium-sensitive inactivation in the gating of single calcium channels. *Science (Wash. DC)*. 250:1735–1738.