Domain model for Ca^{2+}-inactivation of Ca^{2+} channels at low channel density

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ABSTRACT The "shell" model for Ca^{2+}-inactivation of Ca^{2+} channels is based on the accumulation of Ca^{2+} in a macroscopic shell beneath the plasma membrane. The shell is filled by Ca^{2+} entering through open channels, with the elevated Ca^{2+} concentration inactivating both open and closed channels at a rate determined by how fast the shell is filled. In cells with low channel density, the high concentration Ca^{2+} "shell" degenerates into a collection of nonoverlapping "domains" localized near open channels. These domains form rapidly when channels open and disappear rapidly when channels close. We use this idea to develop a "domain" model for Ca^{2+}-inactivation of Ca^{2+} channels. In this model the kinetics of formation of an inactivated state resulting from Ca^{2+} binding to open channels determines the inactivation rate, a mechanism identical with that which explains single-channel recordings on rabbit-mesenteric artery Ca^{2+} channels (Huang Y., J. M. Quayle, J. F. Worley, N. B. Standen, and M. T. Nelson. 1989. *Biophys. J.* 56:1023–1028). We show that the model correctly predicts five important features of the whole-cell Ca^{2+}-inactivation for mouse pancreatic β-cells (Plants, T. D. 1988. *J. Physiol.* 404:731–747) and that Ca^{2+}-inactivation has only minor effects on the bursting electrical activity of these cells.

1. INTRODUCTION

The inactivation of calcium channels by calcium ions has been documented in a variety of cell types (Eckert and Chad, 1984; Lux and Brown, 1984; Kalman et al., 1988). Inactivation is generally observed after sudden depolarizations from membrane potentials at or near the resting potential to potentials near 0 mV and is manifested as a slow decline in the magnitude of the calcium current following a rapid increase. When barium is substituted for calcium as the current-carrying ion, inactivation is greatly reduced, from which it is concluded that Ca^{2+} ions are specifically involved in the inactivation process. Because Ca^{2+} ions bind tightly to proteins, it is commonly assumed that Ca^{2+} binding to the channels is responsible for inactivation, with recent evidence suggesting that binding occurs at the inner face of the membrane (Huang et al., 1989). A number of other inactivation mechanisms for Ca^{2+} channels have been proposed, including phosphorylation and participation of second messengers (Armstrong and Eckert, 1987; Armstrong and Kalman, 1988), and direct inactivation by voltage (Satin and Cook, 1989). Whereas these processes may well be important in many cells on longer time scales, we focus here exclusively on inactivation associated with the direct binding of Ca^{2+} at the inner face of the membrane in cells with low channel density.

Several mechanisms that involve internal calcium binding to channels have been proposed to explain the time course of the inactivation (Standen and Stanfield, 1982; Chad and Eckert, 1984). These mechanisms do not generally distinguish between the effects of Ca^{2+} ions binding to open or closed channels, and they assume that calcium binding to, and consequent blocking of, the channel are rapid with respect to accumulation of calcium near the inner face of the membrane (Chad et al., 1984). The region in which calcium accumulates near the membrane is thought of as a thin "shell" or compartment, whose volume is a small, but not negligible, fraction of the cell volume (Fig. 1). In the shell model slow Ca^{2+}-inactivation of the calcium current is achieved as a consequence of the long time required to increase the calcium concentration within the shell. With suitable adjustment of parameters, such as the rate constant for loss of Ca^{2+} and the volume of the shell, it has been possible to fit this model to experimental data taken from *Aplysia* neurons (Chad and Eckert, 1984; Chad et al., 1984) and insect skeletal muscle (Ashcroft and Stanfield, 1982).

The idea of a shell of elevated Ca^{2+} ion concentration near the membrane is a natural consequence of the localization of calcium channels within the plasma membrane. Although present experimental techniques only allow resolution of space and time averaged Ca^{2+} concentrations directly under the membrane (Hernandez-Cruz et al., 1990), numerical simulation of the concentration profile of Ca^{2+} ions emanating from an open calcium channel (Simon and Llinás, 1985) shows a clear domain structure localized within a few tenths of a micron of the

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inner mouth of the channel. Within such a domain there is an enormous gradient of Ca^{2+} ion concentration, which for physiological conditions ranges from the order of 0.5 mM near the inner mouth of 10 μM at a radius of a few hundredths of a micron (Simon and Llinás, 1985). These calculations also show that high Ca^{2+} ion concentrations at the inner mouth of the channel develop within a few microseconds after the channel opens and disappear on a similar time scale when the channel closes. For membranes containing densities of open channels in excess of 50–100 μm^{-2}, a region of thickness the order of a micron would be formed from the overlap of these localized calcium domains. In this way both open and closed channels at the inner face of the membrane would be subjected to elevated Ca^{2+} ion concentrations, providing justification for the notion of a calcium "shell."

For cells with low channel density the idea of a "shell" is not appropriate, and an alternative mechanism for Ca^{2+}-inactivation is needed. A compelling example is the pancreatic β-cell, reported by Plant (1988) to exhibit Ca^{2+}-inactivation, in which we estimate, using whole-cell currents, a uniform calcium-channel density of ~0.5 μm^{-2}. From the open-channel probability reported by Rorsman and Trube (1986), we conclude that the density of open channels is <0.05 μm^{-2} under physiological conditions. Even if the Ca^{2+} channels are clustered in "hot spots," for which there is some evidence (Rorsman et al., 1988), the local density of open channels is no greater than 2–3 μm^{-2}. Such densities are much too small for the local domains under the open channels to overlap and form a shell of elevated calcium concentration underneath the plasma membrane.

Indeed, the appropriate physical picture for the β-cell seems to be a collection of independent calcium channels. Such a picture is reinforced by the work of Lux and Brown (1984) who discovered that whole-cell inactivation of Ca^{2+} currents could be obtained by superimposing an ensemble of single-channel recordings. In this case regions of high calcium will be localized in "domains" around open channels only (Fig. 1). These domains attain extremely high, quasisteady concentrations of calcium ($\simeq$1 mM) at the inner mouth of the channel within a few microseconds after opening and disappear within a few microseconds after the channel closes. Thus, for the pancreatic β-cell, and other cells with a low surface density of calcium channels, such as rat clonal pituitary cells (Hagiwara and Ohmori, 1983) and bovine chromaffin cells (Fenwick et al., 1982), one expects the effect of elevated calcium near the inner face of the plasma membrane to be felt only by the open channels.

Because at low channel densities there is no "shell" to be filled, there must be other factors that determine the rate of Ca^{2+}-inactivation. For inactivation with time scales in the range 10–100 ms, one can exclude mechanisms based solely on bulk cytosolic Ca^{2+}, which is at a concentration three to four orders of magnitude lower than in the "domains," and which changes relatively slowly. For example, in β-cells there is theoretical and experimental evidence that cytosolic Ca^{2+} oscillates on a time scale of seconds (Perez-Armendariz and Atwater, 1986) and minutes (Grapengiesser et al., 1988). Again we are led to the idea that inactivation is due to domain calcium. However, because the formation of localized calcium domains occurs on the microsecond time scale, it is not possible to relate the time scale of inactivation to the time required for domains to be formed. We, therefore, consider which processes are responsible for the time course of inactivation in cells with low channel densities. In their pioneering work on Ca^{2+}-inactivation, Chad and Eckert (1984) suggested that a slow step in "the calcium–dependent process" might be the culprit, although they did not pursue this idea. If we accept that Ca^{2+} binding to the channel is involved in inactivation, then the logical calcium–dependent process to consider is the inactivation step, itself, that occurs when a Ca^{2+} ion binds to a site on the channel. The rate-limiting step could be either binding itself or a conformational change that blocks the channel following binding (Huang et al., 1989).

In the remainder of this paper we develop a simple domain mechanism for explaining the Ca^{2+}-inactivation of calcium channels in cells with low channel densities. The mechanism involves the binding of a single Ca^{2+} ion to an open channel, where the concentration of Ca^{2+} under the channel mouth may be in the millimolar range. The time course of Ca^{2+} current is determined by the kinetics of the opening, closing, and Ca^{2+}-blockage of the channels. In section II we derive the equations and show that the domain mechanism provides a simple, quantitative explanation of the U-shaped Ca^{2+}-inactivation curves, h, as a function of membrane potential, V, that are obtained in the standard two-pulse experimental measurements of Ca^{2+}-inactivation. In addition, we find that the characteristic time for inactivation to occur, $\tau_B(V)$, is roughly proportional to the Ca^{2+}-inactivation function, $h(V)$. This implies that $\tau_B(V)$ decreases and then increases as the membrane potential is increased from the resting potential, as has been noted experimentally (Plant, 1988). We also show that in such a model, inactivation gives the appearance of being a function of total calcium entry into the cell, in spite of the fact that average cytosolic calcium is not the agent of inactivation. We, thus, show that the experimental observation of the foregoing features does not necessarily imply a "shell" model, although this has often been tacitly assumed in the literature.

We compare our results with recent whole-cell

voltage-clamp experiments by Plant (1988) on the Ca^{2+}-inactivation of calcium channels in cultured mouse pancreatic β-cells (Rorsman et al., 1988; Keahey et al., 1989) and find good agreement with experiment. These cells exhibit bursting electrical activity with a period of tens of seconds, which is thought to be regulated by a slow process. Using numerical simulation of a model (Chay and Keizer, 1983; Sherman et al., 1988), we demonstrate that Ca^{2+}-inactivation of the Ca^{2+} channels over hundreds of milliseconds is compatible with a bursting mechanism based on the build-up and removal of macroscopic cytosolic Ca^{2+}. We find that whereas inactivation modifies the spikes, it is not capable by itself of producing bursts. In our concluding section, we note that our inactivation mechanism is identical to that which Huang et al. (1989) used to explain recent single-channel recordings on Ca^{2+}-inactivation in smooth muscle, and that our conclusions are compatible with patch clamp measurements by Lux and Brown (1984) on *Helix* cells.

II. DERIVATION OF THE MODEL AND QUALITATIVE PROPERTIES

Experimentally one can quantify steady-state inactivation using a two-pulse protocol in which the ratio of the peak calcium current attained after a voltage clamp prepulse to that attained in the absence of a prepulse is calculated. We follow customary usage and define the inactivation, h, independently of experimental procedure as the fraction of channels which at any moment are not inactivated. We will derive several properties of this theoretical h and show that the experimental definitions reduce to it in limiting cases.

One of several experimental results that our model will account for is the U-shaped dependence of h on the pulse potential, V_p. That is, h first decreases then increases with depolarization, generally with a minimum near the minimum of the whole-cell calcium current (Plant, 1988). In the case of the pancreatic β-cell Plant further observes that the time constant of inactivation, here denoted τ_B, is also U-shaped, with a minimum ~ -20 mV. Chad and Eckert (1984) report and model similar behavior in *Aplysia*.

Our model is based on the following assumptions: (*a*) There are small localized domains of high Ca^{2+} concentration ($\simeq 1$ mM) immediately under the inner mouths of open channels. Domains are established instantaneously (in microseconds) relative to the time scale of channel activation and inactivation kinetics and vanish instantaneously when the channel closes (Simon and Llinás, 1985). (*b*) Inactivation results from the binding of domain Ca^{2+} (Ca_d) to open channels, and the time scale of inactivation is determined by the kinetics of Ca^{2+} binding to and blocking the channel. This differs from previous models for Ca^{2+}-inactivation (Standen and Stanfield, 1982; Chad and Eckert, 1984), which assume that Ca^{2+} binding to the channels is instantaneous and that the time scale of inactivation is due to the rate of filling of a submembrane compartment or "shell" (Fig. 1). Note that we implicitly assume that the dissociation constant of the chennels for Ca^{2+} is sufficiently high that binding to closed channels, which see the bulk cytosolic Ca^{2+} concentration, is negligible. (*c*) The time constant of inactivation (tens of milliseconds) is much slower than that of activation (~ 1 ms). (*d*) Channel density is sufficiently low that domains of different channels do not overlap.

Thus, our kinetic mechanism is

$$C \underset{\beta}{\overset{\alpha}{\rightleftharpoons}} O$$

$$O + Ca \underset{k_-}{\overset{k_+}{\rightleftharpoons}} B,$$

where the states represent closed (C), open (O), and inactivated or blocked (B), and Ca is domain calcium. Although one can easily imagine more elaborate kinetic schemes that include more channel states, this scheme has recently been shown to suffice in analyzing the kinetics of single Ca^{2+} channel recordings from rabbit artery muscle (Huang et al., 1989). The second step in this kinetic scheme represents blocking of the calcium channel by binding of a calcium ion at the inner face of the channel. It could correspond to (*a*) a true bimolecular binding kinetics resulting in a bound state that blocks the channel or (*b*) the net effect of rapidly equilibrating, nonsaturating binding of calcium to the channel, followed by a slow conformational change to the blocked state. In the latter case $k_+ = k_f/K_{dis}$, where k_f is the rate constant for the conformation change, and K_{dis} is the dissociation con-

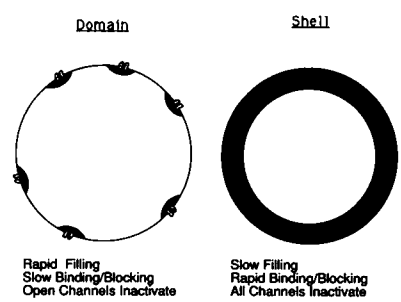

FIGURE 1 In cells with low channel density high Ca^{2+} will be found only in the domains underneath the inner mouths of open channels. In cells with high density domains may overlap to form a continuous submembrane compartment "shell" of elevated Ca^{2+}.

stant. In either case, the small net transition rate from O to B gives rise to the slow inactivation of the channel.

The equations for this process are:

$$\frac{dC}{dt} = -\alpha(V)C + \beta(V)O \tag{1a}$$

$$\frac{dB}{dt} = -k_{-}B + k_{+}Ca_d(V)O \tag{1b}$$

$$O = 1 - B - C. \tag{1c}$$

The differential equations, Eq. 1 *a* and *b*, are coupled through the algebraic conservation law, Eq. 1 *c*. The variables *C*, *O*, and *B* represent the fraction of channels in the closed, open, and inactivated (blocked) states, respectively. The quantity Ca_d represents the average Ca^{2+} concentration in a domain localized around the inner mouth of an open channel. According to simulations of three-dimensional diffusion with constant calcium flux through a channel (Simon and Llinás, 1985), the domain Ca^{2+} concentration close to the channel reaches a steady-state in microseconds. We, therefore, take Ca_d to be instantaneously proportional to the steady-state flux at the mouth of a single channel. Indeed, using the diffusion equation with a source at the channel and modeling buffering as a reduction in the effective diffusion coefficent (Fogelson and Zucker, 1985) in the cytosol it is easy to show that the steady-state concentration is proportional to the current. Thus, we make the further assumption that

$$Ca_d(V) = Ai(V), \tag{1d}$$

where *A* converts the units from current to concentration and is negative because an inward flux of positive ions is a negative current. The magnitude of *A* can be changed experimentally. It can be decreased by adding mobile buffers like EGTA or BAPTA. Similarly, it is possible to swamp the contribution of the open-channel current to Ca_d as given in Eq. 1 *d* by use of concentrated Ca^{2+} solutions in perfused whole-cell or excised patches. In these ways the rate of inactivation by domain Ca^{2+} can be manipulated experimentally. As a final caveat on this assumption, we note that Eq. 1 *d* breaks down at the reversal potential where $i(V)$ changes sign and the small ($\simeq$0.1 μm) background Ca^{2+} concentration in the cytosol becomes significant. A more detailed model would be required in that situation.

The fraction of channels not inactivated is $h = 1 - B$. Its steady-state value, h_∞, can be found by setting the left hand sides of Eqs. 1 equal to zero, which gives

$$h_\infty(V) = 1 - B_\infty(V) = \frac{1}{1 + \frac{k_+}{k_-}Ai(V)m_\infty(V)}. \tag{2}$$

Here m_∞ is the fraction of noninactivated channels which are open at steady-state,

$$m_\infty(V) = \frac{\alpha(V)}{\alpha(V) + \beta(V)} = \frac{O_\infty(V)}{O_\infty(V) + C_\infty(V)}. \tag{3}$$

Eq. 2 can be verified using Eq. 3 and noting that at equilibrium

$$\frac{k_+}{k_-}Ai(V) = \frac{B_\infty(V)}{O_\infty(V)}.$$

Inspecting Eq. 2 we find h_∞ must approach one at very negative voltages because m_∞ goes to zero, and that it must approach one near the Ca^{2+} reversal potential because $i(V)$ is zero there. Furthermore, because m_∞ increases with *V* and the magnitude of *i* decreases with *V*, h_∞ is U-shaped with a single minimum. We have plotted h_∞ in Fig. 2 *a* using parameters appropriate for the β-cell. From Eq. 2 we can also see that h_∞ decreases (i.e., there is more inactivation) as the external Ca^{2+} concentration increases because the flux through the channel increases. This is consistent with the fact that Rorsman and Trube (1986) observed less inactivation in pancreatic β-cells using 2.5 mM Ca_o than did Plant (1988) using 10 mM Ca_o.

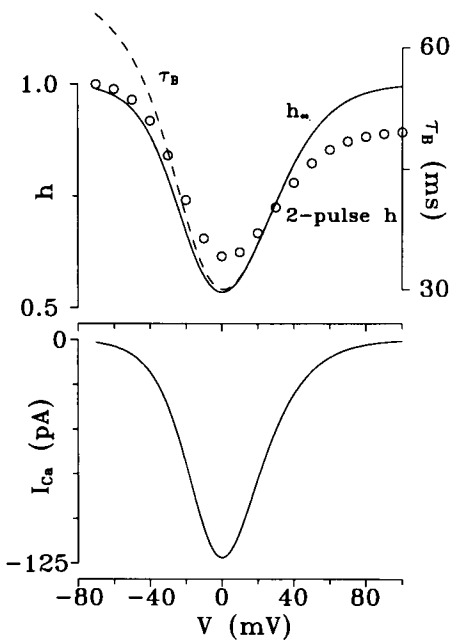

FIGURE 2 (*a*) (*Solid, left scale*) Steady-state inactivation, h_∞, (Eq. 2). (*Dashed, right scale*) The time constant of inactivation, τ_B, plotted against prepulse potential, V_p. The circles represent values of *h* calculated from the two-pulse protocol in Fig. 3. (*b*) Theoretical peak Ca^{2+} current (Eq. 10) plotted against prepulse potential. Note that *h* and I_{Ca} have minima at the same voltage. τ_B is approximately proportional to *h* as predicted by the fast activation theory (Eq. 8) except at negative voltages.

Eq. 2 is an exact steady-state solution of Eqs. 1 *a–c* for any set of initial conditions. Eqs. 1 are linear with constant coefficients for fixed V and all time-dependent solutions can be obtained explicitly. To simplify this we consider first the special case of a single voltage step from a holding potential, V_h, where the Ca^{2+} current is essentially zero to a pulse potential, V_p, and exploit our assumption that the "activation" step in our kinetic scheme is much faster than "inactivation." According to Eqs. 1 *a* and *b*, this implies that

$$\frac{k_- + k_+ Ai(V)}{\alpha + \beta} \ll 1. \tag{4}$$

Under this assumption, the processes of activation and inactivation can be approximately separated in time and there will be two well-separated time constants, a fast one for activation (τ_m) and a slow one (τ_B) for inactivation.

In typical whole-cell, one-pulse experiments the holding potential is very negative and all the channels are in the closed state. Therefore, initially $C = 1$ and $O = B = 0$. When the voltage is stepped to a more positive potential (V_p), O and C rapidly go to a quasisteady state, with O rising exponentially from 0 to $m_\infty(V_p)$ with time constant

$$\tau_m(V_p) = \frac{1}{\alpha(V_p) + \beta(V_p)}. \tag{5}$$

When Eq. 4 holds, we can treat this rise as instantaneous, with B remaining near zero. Inactivation then slowly increases while the ratio of O to C remains fixed:

$$\frac{O}{C} = \frac{\alpha(V_p)}{\beta(V_p)}. \tag{6}$$

Substituting this into Eqs. 1 *b* and *c* we get a simplified equation for B during the inactivation phase:

$$\frac{dB}{dt} = k_+ Ai(V_p)m_\infty(V_p) - [k_- + k_+ Ai(V_p)m_\infty(V_p)]B.$$

Thus, in the limit of fast activation/slow inactivation, B rises exponentially from 0 to a steady-state value, denoted $B_\infty(V_p)$, with time constant

$$\tau_B(V_p) = \frac{1}{k_- + k_+ Ai(V_p)m_\infty(V_p)}. \tag{7}$$

The fraction of open channels, O, falls from $m_\infty(V_p)$ to its steady-state value, $O_\infty(V_p)$, with the same time constant, τ_B.

Comparing Eq. 2 to Eq. 7 we have the remarkable result that

$$h_\infty(V_p) = k_- \tau_B(V_p). \tag{8}$$

This means that τ_B is U-shaped, and in fact has the same shape as h_∞, provided k_- is voltage independent and Eq. 4 holds. In Fig. 2 *a* we have plotted h_∞ and τ_B against V_p using parameter values appropriate for mouse beta cells as described in the Appendix. The curve for τ_B is not strictly a multiple of h_∞ because for the beta cell Eq. 4 is only approximately true at sufficiently negative potentials.

Note that the expression in Eq. 7 is not simply the time constant that Eq. 1 *b* would have if it were uncoupled to Eq. 1 *a*, but has an additional factor $m_\infty(V_p)$. This can also be obtained from the analytic solution of Eqs. 1 by expanding the eigenvalues in powers of the left-hand side of Eq. 4.

The whole-cell calcium current is computed by multiplying the single-channel current by the total number of Ca^{2+} channels, N_{Ca}, and the fraction which are open, O, i.e.,

$$I_{Ca}(t) = N_{Ca}O(t)i(V). \tag{9}$$

In the limit of fast activation the peak Ca^{2+} current during a single pulse from rest to V is

$$\bar{I}_{Ca}(V) = N_{Ca}m_\infty(V)i(V), \tag{10}$$

and we can use Eqs. 2 and 10 to express h_∞ in terms of the peak current,

$$h_\infty(V) = \frac{1}{1 + \tilde{A}\bar{I}_{Ca}(V)}, \tag{11}$$

where $\tilde{A}$ is independent of V. Differentiating Eq. 11 we find that, not only does h_∞ inherit the U-shaped functional dependence of $\bar{I}_{Ca}$ on V, but that the two curves have a minimum at the same voltage (Fig 2, *a* and *b*).

This relationship between h_∞ and $\bar{I}_{Ca}$ is striking because total Ca^{2+} current plays no explicit role in the model, but it can be understood as follows. Eq. 2 implies that $(1 - h_\infty)/h_\infty$ is proportional to $m_\infty(V)i(V)$. Multiplying through by h_∞ shows that the equilibrium fraction of channels inactivated, B_∞, is proportional to $O_\infty(V)i(V)$; it is proportional to i because we assume that domain calcium is proportional to i, and it is proportional to O_∞ because we assume that calcium acts on open channels.

The quantity h_∞ is usually measured indirectly by two-pulse experiments. In these experiments (Plant, 1988) voltage is stepped from the holding potential, V_h, to a series of prepulse potentials, V_p, for a time t_p. The voltage is then stepped back down to V_h for a gap-time t_g, and finally stepped up to a test potential, V_t as shown in Fig. 3. The inactivation function, h, is defined as the ratio of the peak calcium current attained during the second pulse to V_t after a prepulse of duration t_p to that attained in the absence of a prepulse. Equivalently,

$$h(V_p) = \frac{\text{peak } I_{Ca}(V_p)}{\text{peak } I_{Ca}(V_h)}. \tag{12}$$

The actual values obtained for h depend somewhat on V_t,

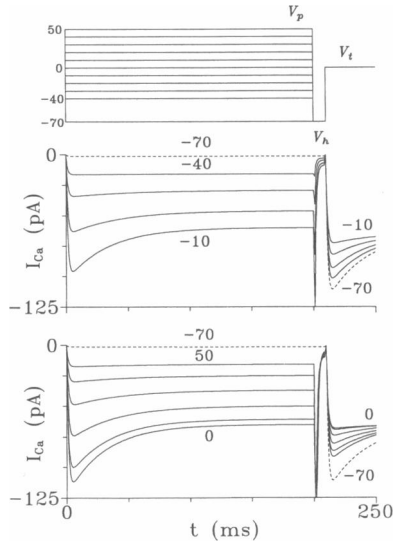

FIGURE 3 Simulated voltage-clamp Ca^{2+} currents for two-pulse protocol. Membrane potential is stepped from the holding potential, $V_h = -70$ mV, to the prepulse potential, V_p, for 200 ms. V_p is varied from -40 to $+50$ mV in steps of 10 mV (*voltage protocol at top*). Voltage is then stepped back to the holding potential, $V_h = -70$ mV, for a gap of 10 ms, and finally stepped to the test potential, $V_t = 0$ mV. Current records for prepulses to (*a*) -40, -30, -20, and -10 mV, (*b*) 0.10, . . . , 50 mV. Tail currents are clipped at -125, pA for display purposes. Compare with Plant (1988) Fig. 1 *A*.

t_p, and t_g. In the limit of fast activation, long prepulse duration, and no gap, peak $I_{Ca}(V_p)$ is proportional to $m_\infty(V_t)h_\infty(V_p)$, because the activation instantaneously adjusts to the test potential while inactivation remains at the value it had at the end of the prepulse. On the other hand, peak $I_{Ca}(V_h)$ is proportional to $m_\infty(V_t)h_\infty(V_h)$. Because $h_\infty(V_h) \approx 1$, the ratio would indeed yield $h_\infty(V_p)$.

III. APPLICATION TO THE PANCREATIC β-CELL

We now specialize our model to account for the experiments of Plant (1988) on mouse pancreatic β-cells, which show inactivation of Ca^{2+} channels. Although there is some evidence for two Ca^{2+} channels in rat β-cells (Hiriart and Matteson, 1988; Satin and Cook, 1985), rat insulinoma RINm5f β-cells (Velasco, 1988), and HIT cells (Satin and Cook, 1988; but see also Keahey et al., 1988), Plant (1988) and Rorsman et al. (1988) conclude that in mouse β-cells there is only one type of Ca^{2+}-inactivating Ca^{2+} channel. To compare with Plant's experiments on the β-cell, values of the activation–rate constants α and β were taken from the experiments of Rorsman and Trube (1986). Single-channel currents were calculated from the Goldman-Hodgkin-Katz formula (Goldman, 1943; Hodgkin and Katz, 1949). The values of $k_+A = 0.028$ pA^{-1} ms^{-1} and $k_- = 0.018$ ms^{-1} (see Appendix) were determined to match Plant's whole-cell currents. Using the computations of Simon and Llinás (1985) to estimate a value of $Ca_d \simeq 0.35$ mM for the domain Ca^{2+} concentration implies that $k_+ = 0.036$ $mM^{-1}ms^{-1}$ for the β-cell, about an order of magnitude smaller than the values obtained from single-channels measurements in rabbit mesenteric arteries by Huang et al. (1989). The effective dissociation constant in our mechanism, $K_d = k_-/k_+$, is on the order of 0.5 mM, i.e., comparable to the estimated domain Ca^{2+} concentration. This is about two orders of magnitude larger than has been invoked in the usual "shell" models (Chad and Eckert, 1984). If the K_d is this large, and channel density is sufficiently low that there is no "shell," then closed channels will make no significant contribution to inactivation, whether or not their Ca^{2+} binding properties inherently differ from those of open channels.

Whole-cell peak calcium currents (I_{Ca}), the inactivation (h) and inactivation time constant (τ_B) using the fast-activation/slow-inactivation approximation in Eqs. 2, 7, and 10 are shown in Fig. 2. The results are in good quantitative agreement with Plant's experimental measurements. Whereas Plant does not provide numerical data for the dependence of τ_B on the potential, he does note that the rate of inactivation increases as the voltage is stepped up to -20 mV only to decrease at larger depolarizations. The fact that the minimum of h_∞ as measured by Plant occurs at a slightly higher voltage than the minimum of I_{Ca} could be incorporated into our model by having k_+ decrease with an exponential Boltzmann factor, although we neglect this refinement in the interest of simplicity.

Plant observed that when the external Ca^{2+} is increased from 1 to 10 mM at a fixed potential of -10 mV, the peak whole-cell currents, $\bar{I}_{Ca}$, are unchanged, but the rate and extent of inactivation increase (Plant, 1988, Fig. 2). As Plant notes, the "shell" model incorrectly predicts that the inactivation rate and extent should be the same. As it stands our domain model makes the same incorrect prediction (see Eqs. 8 and 11). If we postulate, however, that more than one calcium is needed to bind to a channel to block it, additional factors of the single-channel current, $i(V)$, would appear in the denominators of Eqs. 7 and 11. Because increasing external Ca^{2+} increases $i(V)$, both h_∞ and the inactivation time constant would decrease, as found experimentally, even though $\bar{I}_{Ca}$ remains constant.

The result of our simulated two-pulse experiments, obtained by integrating Eqs. 1 *a–c* numerically, are displayed in Figs. 3 *a* and *b* and agree well with two-pulse current measurements (see Plant, 1988; Figs. 1 *A* and 3 *A*). The decline of the Ca^{2+} current becomes steeper

and faster as the prepulse voltage, V_p, is increased from -40 to -10 mV (Fig. 3 *a*) and becomes shallower and slower as V_p is increased further from 0 to 50 mV (Fig. 3 *b*). The dependence of the inactivation, h, based on these simulations and Eq. 12 is shown as circles in Fig. 2 *a*. Note that these agree rather well with the approximate values predicted for the case of fast-activation/slow-inactivation. Most of the discrepancy is the result of redistribution of channel states during the gap. In particular, gap effects are responsible for the failure of h to return to one as V_p approaches the Ca^{2+} reversal potential, a phenomenon which was reported by Ashcroft and Stanfield (1982) in stick insect muscle.

Another way to test our model is to plot the two-pulse inactivation, h, against total Ca^{2+} entry during the prepulse (Plant, 1988, Fig. 4). For the β-cell, Plant found that inactivation appears to be a function only of Ca^{2+} entry, which could be fit by an exponential plus a constant. The dependence of the Ca^{2+} inactivation on the total calcium entry, Ca_{entry}, at a fixed membrane potential has been used as an indication that accumulation of calcium in a shell is responsible for inactivation of calcium channels. What is interesting is that, in the limit of very fast activation, a similar result holds for our domain model.

This can be seen in the following way. To obtain the total Ca^{2+} entry we integrate the instantaneous Ca^{2+} current (Eq. 9)

$$Ca_{entry} = [N_{Ca} i(V)/2F] \int_0^{t_p} O(t)\, dt \qquad (13)$$

with F Faraday's constant. In the limit of fast activation O decreases exponentially with time constant τ_B during the prepulse from a maximum of $m_\infty(V)$ to a steady-state value $m_\infty(V)h_\infty(V)$ and Eq. 13 implies that

$$Ca_{entry} = \frac{N_{Ca}[1 - h_\infty(V)]}{2Fk_+A} \cdot \{[1 - h_\infty(V)][1 - e^{-k_- t_p/h_\infty(V)}] + k_- t_p\}. \qquad (14)$$

This equation shows that Ca_{entry} is a function of h_∞ for any fixed value of t_p because all the voltage dependence is in h_∞. Whereas this would not be strictly true if k_+ or k_- were voltage-dependent, it would be approximately true if the voltage-dependence were weak. Inverting Eq. 14 gives h_∞ as a function of Ca_{entry}. If t_p is made large, the second term dominates and the dependence on h_∞ becomes approximately linear, not exponential as found by Plant (1988). Nonetheless, we think it noteworthy that an exact, monotonically decreasing relationship exists between inactivation and the integral of total Ca^{2+} current in our model even though it is single-channel current which determines inactivation. Clearly a correspondence between h and Ca^{2+} cannot be taken as diagnostic of inactivation due to filling of a submembrane "shell" as some authors have assumed.

These results satisfy us that a simple domain model can account for the voltage-clamp measurements of Plant (1988) on pancreatic β-cells, namely: (*a*) the U-shaped inactivation curve; (*b*) the U-shaped dependence of the inactivation time constant on voltage; (*c*) the anomalous increase in the inactivation rate at -10 mV when external Ca^{2+} is raised from 1 to 10 mM; (*d*) the dependence of inactivation on total Ca^{2+} influx during the prepulse; and (*e*) the time course of whole-cell Ca^{2+} current measurements. We have also confirmed that the fast-activation/slow-inactivation theory of section II is a good approximation to the behavior of the complete model.

Beta cells in pancreatic islets undergo electrical bursting when exposed to stimulatory amounts of glucose (Atwater et al., 1989) and Plant (1988) has speculated that Ca^{2+}-inactivation might have a role in bursting. Indeed, before Plant's work Chay and colleagues (Chay, 1987; Chay and Kang, 1987, 1988) proposed models in which Ca^{2+}-inactivation is an essential ingredient in the bursing mechanism. We have investigated this possibility by incorporating our domain model, fit as described above to Plant's and Rorsman and Trube's data, into a recent version of the Chay-Keizer bursting model (Sherman et al., 1988). That model employs three ionic currents, the voltage-gated, delayed-rectifying, K^+ current (I_K), the voltage-gated Ca^{2+} current (I_{Ca}), and the calcium-activated K^+ current (I_{K-Ca}) in the total current balance equation,

$$C_m \frac{dV}{dt} = -I_K - I_{Ca} - I_{K\text{-}Ca}. \qquad (15)$$

The only significant changes from Sherman et al. (1988) are in the form of the Ca^{2+} current. Here we include the effect of inactivation, which has a similar time scale to K^+ activation, by solving Eqs. 1 *a–c* to determine the open fraction, O, as a function of time and obtain the current from Eq. 9. In addition, the activation curves have been shifted to the right to account for the screening caused by high external Ca^{2+} (Frankenhaeuser and Hodgkin, 1957). Further discussion of the derivation and properties of the family of models to which this one belongs can be found in Chay and Keizer (1983) and Rinzel (1985).

Fig. 4 shows bursing solutions obtained with the model. The unusually large spike amplitude (Fig. 4 *a*) is due to the high levels of Ca_0 (10 mM), and is qualitatively what one expects based on experiment (Ribalet and Beigelman, 1980; Atwater et al., 1980). More typical bursts are obtained with $Ca_0 = 2.5$ mM and activation parameters shifted back to account for the reduced Ca^{2+} screening effect (not shown). The onset and termination of bursts is regulated by oscillations in average cytosolic calcium, shown in Fig. 4 *b*. When the decrease of calcium during

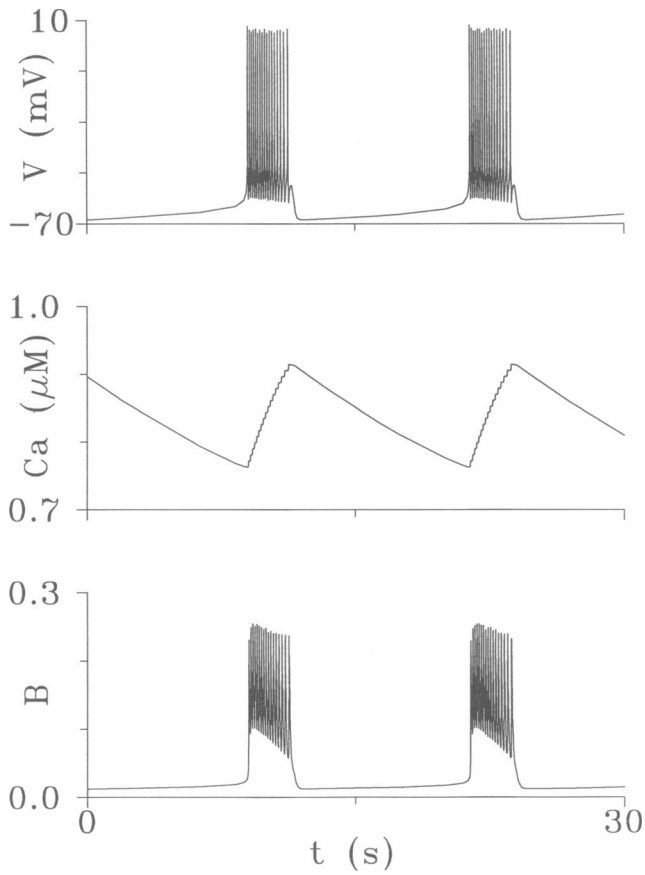

FIGURE 4. Time courses of bursting electrical activity in the presence of glucose are obtained by numerical solution of the equations of Sherman et al. (1988) as modified in the text, and with 10 mM external Ca^{2+} (see Appendix). (*a*) Membrane potential, *V*. (*b*) Free intracellular Ca^{2+}, Ca_i. The slow oscillations of Ca_i trigger the onset and termination of bursting by activating K-Ca channels. (*c*) The instantaneous fraction of Ca^{2+} channels inactivated, *B*.

the silent phase reduces the K-Ca conductance to a critical minimum value, the membrane depolarizes, triggering the voltage-gated Ca^{2+} and delayed rectifying K^+ channels and initiating rapid oscillations. The spikes bring Ca^{2+} into the cell which raises the K-Ca conductance to a critical maximum value and terminates the burst. Fig. 4 *c* shows the instantaneous fraction of Ca^{2+} channels which are inactivated (*B*). This quantity oscillates rapidly, following the membrane potential changes through Eqs. 1 rather than the slow changes in average cytosolic Ca^{2+} concentration.

Thus, our model for inactivation of Ca^{2+} channels by domain Ca^{2+} can be incorporated into the paradigm of bursting based on slow Ca^{2+} feedback into K-Ca channels. The differences caused by inactivation are small quantitatively, and reside primarily in the form of the spikes. We emphasize that the results do not rest on the use of the K-Ca channel; Ca^{2+} activation of K-ATP channels in addition to or instead of the K-Ca channel (Keizer and Magnus, 1989) or even slow voltage-dependent inactivation of the Ca^{2+} channels would work as well. We conclude that Ca^{2+}-induced inactivation of Ca^{2+} channels plays only a secondary role in the bursting activity of the β-cell.

IV. CONCLUDING REMARKS

Our model of Ca^{2+} inactivation of calcium channels is based on the calcium domain that has been predicted theoretically to form near the inner mouth of an open calcium channel a few microseconds after the channel opens. The calcium concentration at the inner mouth of an open channel is almost instantaneously fixed by the single-channel current, $i(V)$, and, therefore, depends on the membrane potential. We assume that domain Ca^{2+}, Ca_d, is proportional to $i(V)$, and assume that inactivation is a result of the binding of calcium to open channels. If the rate-limiting step in inactivation is binding, the kinetic rate of inactivation by calcium is directly proportional to Ca_d. If the rate-limiting step is a subsequent conformational change, the kinetic rate is proportional to the number of bound channels, and if the dissociation constant for the binding step is large, the rate is again proportional to Ca_d. In either case, the transition rate to the inactivated state is proportional to $i(V)$. The activation rate of calcium channels from their closed state, on the other hand, is determined by the intrinsic voltage-dependent rate at which closed and open channels interconvert, which is generally more rapid than the inactivation. This mechanism is a departure from previous "shell" models for Ca^{2+} inactivation (Standen and Stanfield, 1982; Chad and Eckert, 1984) which are based on accumulation of Ca^{2+} in a macroscopic submembrane compartment (Fig. 1). Shell models assume that Ca^{2+} binds rapidly to both open and closed channels and that the rate-limiting step in inactivation is the time for the shell Ca^{2+} concentration to increase. Our domain mechanism is appropriate for membranes with low densities of open calcium channels (less than a few per square micron) in which local calcium domains around open channels do not overlap to form a true shell underneath the membrane. For such membranes, closed channels are not subjected to significantly elevated calcium concentrations and are not involved in the Ca^{2+} inactivation process.

The domain mechanism of inactivation has several interesting kinetic features, derived in section II, in the limit that the activation rate is much faster than the

inactivation due to calcium. First, the steady-state Ca^{2+} inactivation, $h_\infty(V)$, is a U-shaped function of the membrane potential. Second, Ca^{2+} inactivation occurs as a single exponential with a characteristic time, $\tau_B(V)$, which as a function of membrane potential is also U-shaped and is proportional to the inactivation curve. These two features hold approximately even when activation is simply faster, but not infintely faster, than the Ca^{2+} inactivation, as judged by our numerical simulations of the standard two-pulse experimental measurements of the inactivation. If one assumes that only a single Ca^{2+} ion is required to bind to a channel to inactivate, then one gets the stronger result that h_∞ and $\bar{I}_{Ca}$ are functionally related by Eq. 11. In particular, the minimum of h_∞ is located where the magnitude of peak calcium current, $\bar{I}_{Ca}(V)$, is maximum.

For the pancreatic β-cell the domain model predicts the major experimental features of whole-cell Ca^{2+}-inactivation (Plant, 1988). We believe it to be a more plausible description of calcium inactivation than the shell model because of the low density of calcium channels in these cells. Although a number of investigators (Plant, 1988; Chay, 1987; Chay and Kang, 1987, 1988; Satin and Cook, 1989) have speculated that Ca^{2+}-inactivation may play an important role in the bursting electrical activity of the pancreatic β-cell, we find that if inactivation is modeled as due to domain Ca^{2+} in a manner consistent with Plant's voltage-clamp data, then its effect on bursting is slight. Indeed, examining the calculations of bursting in Fig. 4 *c*, one sees that the fraction of inactivated channels follows the rapid changes in membrane potential rather than the slow changes in internal calcium. This is a consequence of the fact that in the domain model calcium acts only on open channels with a rate that is dependent on the open-channel current at a given potential.

Recently it has been possible to explore mechanistic features of Ca^{2+}-inactivation using patch clamp measurements on single calcium channels (Lux and Brown, 1984; Rosenberg et al., 1988). The work of Lux and Brown, in particular, provides further evidence for our domain model. Using a whole-cell arrangement, these authors showed that the time course of whole-cell inactivation could be obtained by averaging an ensemble of single-channels records. Because this was also true when Ca^{2+} channels external to the patch were blocked, they deduced that inactivation is a property of isolated Ca^{2+} channels. Furthermore, by averaging separately single-channel records with low and high initial activity, they found that initial bursts of openings did not supress subsequent current levels. Both of these observations are compatible with the domain model, in which Ca^{2+}-inactivation inherits its voltage dependence from single-channel currents, but they rule out the shell model. The fact that Lux and Brown obtain similar results for *Helix* cells, rat and guinea pig ventricular myocytes, and avian dorsal root ganglion suggests that these conclusions are rather general.

Shortly after this paper was submitted for publication, Huang et al. (1989) published single channel recordings of Ca^{2+}-inactivation kinetics for rabbit mesenteric artery. They successfully analyzed their results using the same kinetic scheme that we have used, namely one in which only open channels are inactivated. Furthermore, they observe that inactivation occurs when elevated Ca^{2+} (2 mM) is applied to the cytoplasmic face of the patch from which they conclude that calcium binds to an internal site not in the pore. By increasing the frequency cut-off of their noise filter to 2 kHz they observed flickering of the open state, from which they were able to deduce values for the inactivation rate constants, k_+ and k_-. For mesenteric artery k_+ lies between 0.3 and 0.7 $mM^{-1}ms^{-1}$ and k_- between 1.7 and 4.6 $ms.^{-1}$. Their values of k_- are several orders of magnitude greater than the value of 0.018 ms^{-1} that we need to explain Plant's data whereas their values of k_+ are about an order of magnitude larger than the value that we estimate for the β-cell. In light of their work, frequency resolved single-channel measurements on β-cell Ca^{2+} channels would seem to be in order.

APPENDIX

The rate constants $\alpha(V)$ and $\beta(V)$ were determined using Eqs. 3 and 5. We took a constant value of $\tau_m = 1.25$ ms from Rorsman and Trube (1986), who fit the activation with the Boltzmann function

$$m_\infty(V) = \frac{1}{1 + \exp[(V_m - V)/S_m]}.$$

At low-external Ca^{2+} (2.5 mM) they found $V_m = 4.0$ mV and $S_m = 14.0$ mV. To match Plant's *I–V* curves, which were obtained with high external Ca^{2+} (10 mM), we have set $V_m = -5.0$ mV and $S_m = 10.0$ mV (Frankenhauser and Hodgkin, 1957). We use the Goldman-Hodgkin-Katz formula (Goldman, 1943; Hodgkin and Katz, 1949) to describe the single-channel current:

$$i(V) = \hat{g}_{Ca} P \frac{2FV}{RT}\left[\frac{Ca_o - Ca_{in}\exp(2FV/RT)}{1 - \exp(2FV/RT)}\right].$$

Here $\hat{g}_{Ca}$ is the single-channel conductance, P converts concentration to membrane potential. By matching the *I–V* curves obtained by Plant with $Ca_o = 10$ mM (Fig. 2 *b*) we arrived at a value $P = 14$ mVmM^{-1}. We use 7 pS for the single-channel Ca^{2+} conductance, $\hat{g}_{Ca}$, and 200 for the total number of Ca^{2+} channels, N_{Ca}, to give a maximal Ca^{2+} conductance of 1,400 pS as previously (Sherman et al., 1988). For simplicity we omitted the dependence of $i(V)$ on intracellular Ca^{2+} in our simulations because replacing Ca_{in} by Ca_i or, in the worst case, Ca_d (Eq. 1 *d*) has only small effects. Therefore, in the model, the Ca^{2+} current never reverses, but it is nearly 0 for V larger than 80 mV (Fig. 2 *b*).

We chose k_- and k_+ to approximate the rate and degree of inactivation in Plant's voltage clamp currents. We obtained reasonable

agreement by using $k_- = 0.018$ ms^{-1} and setting $k_+A = 10k_-/\hat{g}_{Ca}$ (ms^{-1}pA^{-1}). Typical values for the pseudo first-order rate constant for Ca^{2+} binding, $k_+Ai(V)$, are then 0.01 – 0.1 ms^{-1}, being larger at negative voltages where the single-channel Ca^{2+} current is larger. In order to obtain a value for k_+ separately, one must know A. If $A = 0.7$ mMpA^{-1} then $Ca_d = 0.35$ mM when the single-channel current is 0.5 pA, consistent with the diffusion calculations of Simon and Llinás (1985). Then k_+ is about 0.036 mM^{-1}ms^{-1}, well below the diffusion controlled limit of 10^3 mM^{-1}ms^{-1} (Hague, 1971). Thus, either the calcium binding site must be partially inaccessible or the bimolecular process in Eq. 1 *b* must correspond to an instantaneous binding equilibrium followed by a conformational change, as described in section II. With the same choice of A the dissociation constant, $K_d = k_-/k_+$, is ~0.5 mM. In general, K_d will be proportional to A and Ca_d.

Some of the calculations for this study were carried out at the NCI Advanced Scientific Computing Laboratory. We thank Dr. J. Maizel for providing computer time, Dr. Eduardo Rojas for reading of the manuscript, and an anonymous reviewer for pointing out the work of Lux and Brown.

Dr. Keizer acknowledges support from National Science Foundation grant CHE 86-18647 and the Agricultural Experiment Station at UC Davis. Dr. Sherman was supported by an NRC-National Institutes of Health Research Associateship.

Received for publication 25 September 1989 and in final form 27 March 1990.

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