CALCIUM DOMAINS ASSOCIATED WITH INDIVIDUAL CHANNELS CAN ACCOUNT FOR ANOMALOUS VOLTAGE RELATIONS OF CA-DEPENDENT RESPONSES

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ABSTRACT Computer-assisted modeling of calcium influx through voltage-activated membrane channels predicted that buffer-limited elevation of cytoplasmic free calcium ion concentration occurs within microscopic hemispherical "domains" centered upon the active Ca channels. With increasing depolarization, the number of activated channels, and hence the number of Ca domains, should increase; the single-channel current should, however, decrease, thereby decreasing Ca^{2+} accumulation in each domain relative to the macroscopic current. Such voltage dependence of the microscopic distribution of Ca^{2+} may influence relations between total Ca^{2+} entry and Ca-dependent processes. Ca-mediated inactivation of Ca channels in *Aplysia* neurons exhibits behavior consistent with the calcium domain hypothesis.

INTRODUCTION

The entry of calcium ions through voltage-activated channels produces increases in intracellular free calcium ion concentration, Ca_i, starting from a low resting level (<0.1 μM; DiPolo et al., 1976; Alvarez-Leefmans et al., 1981). Elevations in Ca_i have profound effects on membrane function, including activation of Ca-dependent currents (Meech, 1978; Colquhoun et al., 1981; Bader et al., 1982), inactivation of calcium current (Eckert et al., 1981; Ashcroft and Stanfield, 1981), and release of synaptic transmitter (Katz and Miledi, 1967; Llinás et al., 1981). The spatial distribution of Ca_i relative to the inner surface of the cell membrane should influence the relationship between the macroscopic Ca current, I_{Ca}, and Ca-dependent responses. Ion-sensitive electrodes suggest rapid changes in Ca_i close to the membrane during Ca current flow, with smaller, slower transients further from the membrane toward the cell interior (Levy et al., 1982; Deitmer et al., 1983; Chad et al., 1984*a*). The restricted region of transiently increased Ca_i and the steep spatial profile, also indicated with Ca-sensitive dyes (Smith and Zucker, 1980; Connor et al., 1981), is believed to be produced by the retardation of diffusion of Ca^{2+} by cytoplasmic buffering mechanisms.

Evidence for the acute localization of Ca near its site of entry was obtained in the squid giant synapse where it was observed that calcium entering the terminal through Na channels did not contribute to transmitter release (Llinás et al., 1982), presumably because most of the distributed sodium channels are too distant from localized release sites. In an analysis of synaptic transmission, Llinás et al. (1981) modeled the elevation of Ca_i within the terminal in response to the passage of a 200 nA Ca current through a single active zone containing many Ca channels. These calculations predicted steep spatial gradients of Ca_i within the terminal, even in the absence of a Ca buffering system.

Models of Ca^{2+} binding and diffusion (Smith and Zucker, 1980; Stockbridge and Moore, 1982; Zucker and Stockbridge, 1983) based upon uniformly distributed Ca^{2+} influx predict that significant elevations in Ca_i are initially restricted to a layer extending ~1 μm from the inner membrane surface. However, influx is punctate, Ca entering through individual channels; we have therefore constructed a model of radial dispersion that predicts that a single Ca channel primarily influences Ca_i within a hemispherical domain centered on that channel. The distribution of Ca channels plus the size and overlap of calcium domains should influence the microscopic distribution of calcium ions following their entry into the cell through a population of activated channels. This concept is illustrated in Fig. 1, which shows a section of membrane containing Ca channels at two dissimilar potentials. A brief, small depolarization will open few channels (Fig. 1 *A*), producing localized regions of high Ca_i. A brief, large depolarization (Fig. 1 *B*) selected to produce the same macroscopic I_{Ca} will activate more channels, but the single-channel currents will be weaker because of the reduced driving force and constant field considerations. Thus, the stronger

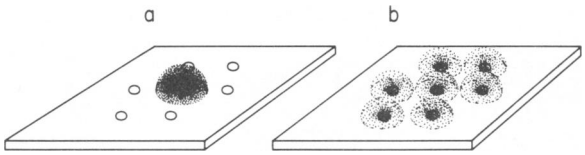

FIGURE 1 Schematic representation of the Ca domain concept. The inner face of the membrane is shown as a horizontal surface punctured by distributed Ca channels. Calcium ions passing through active channels increase Ca_i within hemispheric "domains" indicated by density of stippling. (*A*) A small depolarization causes few channels to open, but the single channel currents are large, resulting in a few domains of high Ca_i. (*B*) A large depolarization activates many channels, but the single channel currents are small, resulting in a more diffuse distribution of Ca^{2+}.

depolarization will produce a more diffuse distribution of Ca^{2+}. Differences in distribution of entering Ca^{2+} may have important implications for a Ca-mediated response taking place close to the membrane, particularly if the response is saturable, and does not have a simple first-order (linear) relationship to Ca_i.

In this paper we consider the domain hypothesis in the interpretation of experiments on *Aplysia* neurons where Ca-current inactivation appears to be solely Ca dependent (Chad et al., 1984). The data support the existence of Ca domains associated with individual Ca channels. We also present a spatio-temporal model of Ca^{2+} buffering and diffusion that considers the limitations of a single domain. Preliminary results were reported earlier (Chad et al., 1983*b*).

METHODS

Pharmacologically isolated calcium currents were recorded from voltage-clamped neurons (L2-L6) of *Aplysia californica* utilizing the methods of Chad et al., 1984*b*). Currents recorded during long pulses were digitized at 1 kHz and were analyzed with the aid of a DEC 11-23-based computer (Digital Equipment Corp., Marlboro, MA).

Current traces were fitted by computer simulation, using the relationship (Chad et al., 1983*a*, 1984*b*; Eckert et al., 1982)

$$I_{Ca} = I_{max}[m_\infty - (m_\infty - m_0)e^{-t/\tau_m}]^2 \cdot (1 + KS)^{-1} \qquad (1)$$

in which m_0 and m_∞ represent probabilities at the holding potential and after infinite time at the test potential, respectively, and τ_m is inversely proportional to the rate of change between these probabilities. The parameter S represents free calcium ion concentration at the inactivation site and K the apparent potency of calcium available to the inactivation site. The constant I_{max} represents the maximal current that would flow at infinite time if m_∞ were unity and there were no inactivation. The equation was solved numerically using a time increment of 0.5 ms, and fits were achieved while maintaining constant all parameters determining the relationship of Ca_i to I_{Ca}. The values of the activation parameters m_∞ and τ_m and the potency parameter K were varied to optimize the fit as judged by eye. The value of I_{max} was set at 420 nA throughout.

The cytoplasmic Ca^{2+} buffer is assumed to have a concentration of 4×10^{-4} M and an affinity of 2×10^{-6} M, which is comparable with published values for "calcium binding protein" found in *Aplysia* neurons (Christakos et al., 1983; Breddermann and Wasserman, 1974).

RESULTS

Spatio-temporal Model of a Calcium Domain

To determine the feasibility of Ca^{2+} being restricted to a minute domain centering on the inner opening of a calcium channel, we calculated the cytoplasmic distribution in both space and time of Ca^{2+} entering through a single channel. Calcium was assumed to be buffered by evenly distributed binding sites with the same kinetic parameters utilized earlier (K_D, 2×10^{-6} M; concentration 4×10^{-4} M), and was assumed to diffuse radially from the channel at the rate predicted for aqueous solution between bindings (Hodgkin and Keynes, 1957). The effect of saturation of buffer on the probability of buffering, and hence on the effective rate of diffusion (Fischmeister and Horackova, 1983) was included in our formulation. This differs from the formulation of Zucker and Stockbridge (1983), who used a single value of buffering probability, and did not consider Ca_i gradients other than those perpendicular to the membrane inner surface.

Experiments using Ca^{2+}-sensitive dyes suggest that Ca^{2+} buffering becomes less effective toward the center of the cell (Tillotson and Gorman, 1980). As we are primarily concerned with a region close to the membrane surface, we have assumed that Ca^{2+} buffering by the cytosol is uniform and predominates at short times over other mechanisms of Ca^{2+} regulation. Experiments comparing TTX-treated, and Tris-substituted blockade of the Na current suggest that Na/Ca exchange is at most a minor factor in determining the time course of removal of Ca-dependent conductance changes following a pulse of Ca^{2+} entry in *Aplysia* neurons (Deitmer and Eckert, in preparation). A similar low sensitivity to extracellular Na^+ was reported by Barish and Thompson (1983) for neurons of dorid nudibranchs. We considered a series of concentric hemispherical shells (1 to n) of thickness Δr centered on the inner opening of a Ca channel. The outer radius of the nth shell is thus $n \cdot \Delta r$ and the inner radius $(n - 1) \cdot \Delta r$. Diffusion takes place between adjacent shells at a rate dependent upon the diffusion constant, D, for Ca^{2+} in aqueous solution (6×10^{-6} cm^{2}s^{-1}; Hodgkin and Keynes, 1957), the cross-sectional area, and the concentration gradient. Therefore if $Ca_{(n)}$ is the free Ca concentration in shell n, then the rate of diffusion to the adjacent, $n + 1$, shell is given by

$$\frac{\text{d moles of Ca}}{dt} = \frac{D \cdot [Ca_{(n)} - Ca_{(n+1)}] \cdot 2\pi(n \cdot \Delta r)^2}{\Delta r}. \qquad (2)$$

The change of concentration within shell n is thus given by

$$\frac{dCa_{(n)}}{dt} = \frac{D \cdot [Ca_{(n)} - Ca_{(n+1)}] \cdot 3 \cdot n^2}{\Delta r^2 \cdot [n^3 - (n-1)^3]}. \qquad (3)$$

However, Ca ions entering a layer will be buffered with a probability $P_{B(n)}$ which depends upon the free buffer concentration and dissociation constant, K_D. If the binding reaction is rapid, such that Ca is effectively in equilibrium with buffer, it can be shown (Chad et al., 1984, Eqs. 3 and 4) that

$$P_{B(n)} = \frac{B_{tot}}{B_{tot} + Ca_{(n)} + K_D} \quad (4)$$

where B_{tot} is the total concentration of calcium buffer. Thus, the change in Ca concentration is given by

$$\frac{dCa_{(n)}}{dt} = \frac{D \cdot (1 - P_{B(n)} \cdot 3 \cdot \{(n-1)^2[Ca_{(n-1)} - Ca_{(n)}] - n^2 \cdot [Ca_{(n)} - Ca_{(n+1)}]\}}{\Delta r^2[n^3 - (n-1)^3]}. \quad (5)$$

In the case of the first shell or hemisphere ($n = 1$) we consider only Ca influx via the channel (i_{Ca}) and diffusion to the adjacent shell ($n = 2$). In this particular case

$$\frac{dCa_{(1)}}{dt} = \frac{i_{Ca} \cdot 3 \cdot (1 - P_{B(n)})}{4 \cdot F \cdot \pi \cdot \Delta r^3} - \frac{D \cdot (1 - P_{B(n)}) \cdot 3 \cdot [Ca_{(1)} - Ca_{(2)}]}{\Delta r^2}. \quad (6)$$

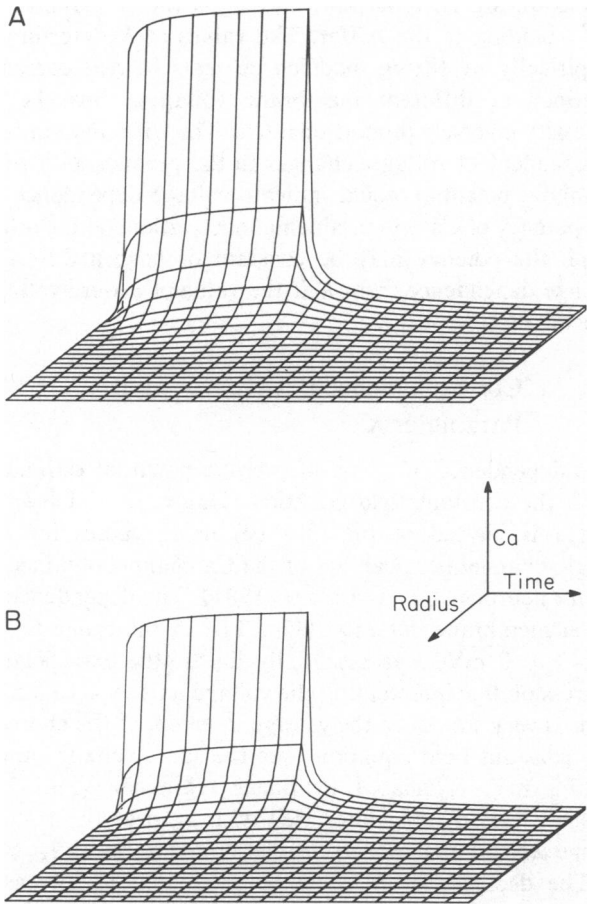

FIGURE 2 Three-dimensional plots of Ca_i within one domain, centered upon a single Ca channel, through space and time. Calibrations are 4×10^{-6} M, 0.5 μm, and 10 ms for the y, z, and x axes, respectively. Activated channel has a probability of $(1 + K \cdot Ca_i)^{-1}$ of not being inactivated where K is 0.2×10^{-6} M. (*A*) Depolarization to −20 mV for 20 ms activates a channel with i_{Ca} equal to 0.8 pA. (*B*) Depolarization to 0 mV for 20 ms activates a channel with i_{Ca} equal to 0.4 pA.

These equations were solved numerically using values of Δr and Δt sufficiently small to give consistent results (Δr = 0.05 μm, Δt = 0.01 ms). Values used for Ca^{2+} influx through a single channel were based on values reported for the unitary current, i_{Ca}, reported for Ca channels in *Helix* neurons (Lux and Nagy, 1981; Brown et al., 1982), with a voltage dependence predicted by the constant field equation. The channel was taken to be activated for a proportion of time, P_A, dependent on potential (cf. m_∞^2 value in Methods) and was taken to be "not inactivated" (open) for a variable proportion of time based on the probability of being inactivated by Ca_i. Thus $P_{open} = P_A/(1 + K \cdot Ca_1)$ in which P_{open} is the probability of being open, and Ca_1 is the value of Ca_i at the inner opening of the channel. For simplicity P_A was set to 1.0 in each case. Fig. 2 *A* illustrates the predictions of Ca_i through space and time for a depolarization to −20 mV where i_{Ca} is taken to be 0.8 pA. Ca_i in the immediate vicinity of the channel rapidly reaches a plateau; at greater radial distances plateau values are reached later. For a depolarization to 0 mV i_{Ca} is taken to be 0.4 pA (Fig. 2 *B*). The pattern of Ca distribution is similar to the above but the peak values of Ca_i are reduced. The calculated steady-state values of Ca_i close to the channel mouth are directly related to the single-channel flux, whereas Ca_i at greater distances from the channel is related more closely to the integral of Ca entry.

Domain Concept Applied to Ca Channel Inactivation

The voltage dependence of the apparent potency of Ca^{2+} in producing inactivation of the Ca channel (contained in the parameter K, Eq. 1) was examined by eliciting a family of currents in response to step potentials ranging from −24 to 0 mV (Fig. 3 *A*). Each current (noisy trace) was fitted by eye with a computer-simulated current (smooth trace). The term m_∞ primarily describes the probability of activation, whereas the term K determines inactivation. The fits show that the value of m_∞ increases with depolarization as expected (Byerly and Hagiwara, 1982), whereas the value of K determined by computer fits of modeled currents to the recorded currents was found to decrease (Chad et al., 1984*b*). The values of K (■) are plotted against membrane potential in Figure 3 *B*. The solid line was given by a linear regression, and has an extrapolated voltage intercept of +16.5 mV. If the Ca-binding site were to lie within the membrane field, the effect of increased depolarization should be to increase the probability of Ca^{2+} binding and

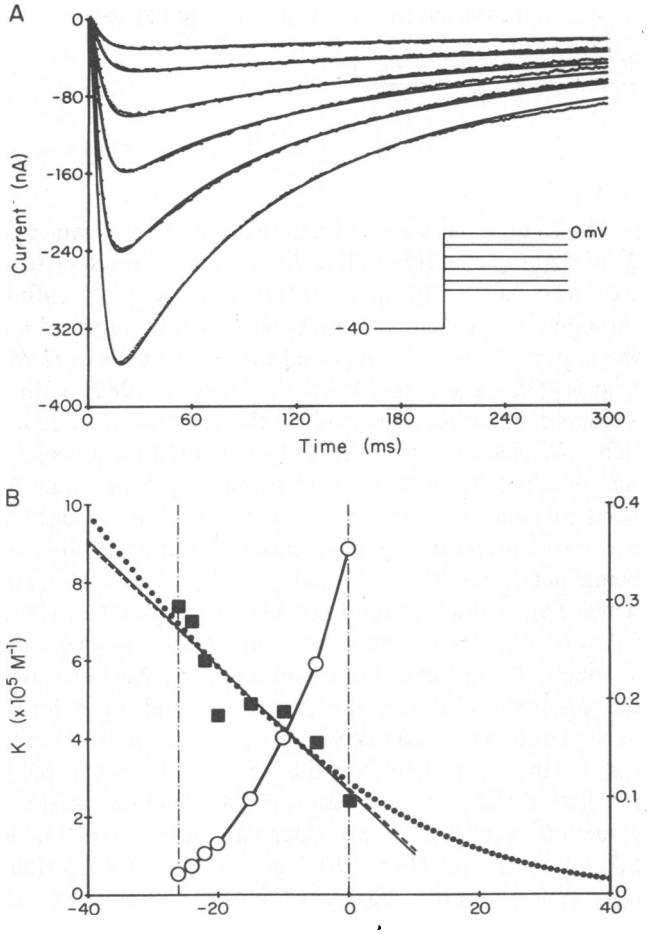

FIGURE 3 (*A*) Calcium currents (noisy traces) recorded in response to a set of voltage clamp steps from a holding potential of −40 mV (inset). Computer model fits (smooth traces) are plotted for each step potential, the fitted value of potency parameter K decreasing with depolarization. Parameters used were fixed as stated in the text except for the values (given in the order m_∞, τ_m and K) for each potential: −24 mV, 0.05, 6.1 ms, 7.0×10^5 M^{-1}; −20 mV, 0.08, 5.6 ms, 4.6×10^5 M^{-1}; −15 mV, 0.17, 5.2 ms, 4.9×10^5 M^{-1}; −10 mV, 0.27, 5.4 ms, 4.7×10^5 M^{-1}; −5 mV, 0.42, 4.8 ms, 3.9×10^5 M^{-1}; 0 mV, 0.51, 4.8 ms, 2.4×10^5 M^{-1}. (*B*) Parameter K, obtained from the fits, plotted against membrane potential (■). The least squares linear regression (—) intercepts the voltage axis at +16.5 mV. The constant field prediction for dependence of i_{Ca} on voltage (●) was calculated (permeability 3×10^{-14} cm^3 s^{-1}; Ca_i, 0.1 μM; Ca_o, 20 mM) and scaled to the data with a least squares routine, maximum current −0.4 pA. The measured peak macroscopic current, I_{Ca} (○), was also plotted against voltage, maximum current −0.4 μA. The chord to the constant field equation over the same voltage range as the data (---) has a voltage intercept of 17.5 mV.

hence increase the potency of Ca^{2+}. If all else remained constant, this increased potency should be reflected as an increase in the parameter K, which relates the degree of inactivation to the macroscopic Ca_i (Chad et al., 1984*b*).

We consider now why the parameter K might undergo an apparent anomalous decrease with increasing positive membrane potential. In the simplified formulation of the binding site model (Chad et al., 1984*b*) used to generate the fits shown in Fig. 3 *A*, calcium ions were presumed to cross the membrane at an effectively infinite number of sites and uniformly enter a buffer-limited submembrane compartment of arbitrary depth (1 μm). Within this compartment diffusion was assumed to be rapid enough that buffering was in equilibrium with the entering Ca^{2+}, allowing the calculation of buffering probabilities. Best fits between measured trajectories of macroscopic I_{Ca} and model calculations were obtained when it was presumed that no loss occurred from this initial layer, which, in the simple version of the model, has a fixed volume for all potentials. Because of the discrete nature of Ca channels, however, the assumption of a fixed volume may be erroneous, thus producing an apparent decrease in K as increased depolarization leads to a more distributed entry of calcium ions. Central to this problem is the presence in Eq. 1 of the $K \cdot S$ product, in which

$$\Delta S = \frac{\int I dt \cdot (1 - P_B)}{2Fv} \tag{8}$$

where v is the volume of the compartment into which Ca^{2+} enters during current flow, and P_B is the probability of Ca^{2+} binding to the buffer. The values of K determined empirically by fitting modeled currents to real currents obtained at different membrane voltages, must be of necessity inversely proportional to v. Thus, if v were in fact independent of voltage, changes in the product $K \cdot S$ with changing potential would indicate voltage dependence of the potency of Ca^{2+} in producing inactivation; on the other hand, the potency may be constant or have a different voltage dependence from K if the value of v were voltage dependent.

Correspondence Between i_{Ca} and Parameter K

The dependence of i_{Ca} on membrane potential estimated from the constant field equation (Hagiwara and Byerly, 1981) is plotted in Fig. 3 *B* (●) using values for the single-channel permeability of the Ca channel obtained in *Helix* neurons (Lux and Nagy, 1981). The dependence of K on membrane potential (Fig. 3 *B*) in the range tested (−40 to 0 mV) was essentially linear, the extrapolated regression line intercepting the voltage axis at +16.5 mV. This is very similar to the voltage intercept of the chord to the constant field equation over the same voltage range, +17.5 mV, calculated by linear regression (Fig. 3 *B*, dashed line). K correlates well with i_{Ca} over the voltage range tested but shows no correlation to total peak I_{Ca} (○).

The decrease in K could be attributed to increased current density causing an increased rate of diffusion due to local saturation of Ca buffers (see Fischmeister and Horackova, 1983). This seems unlikely, however, because the same behavior is seen for two currents of the same magnitude recorded at different membrane potentials (Fig. 4 *A*). Cadmium block of Ca channels was used to reduce the current recorded in response to a step to 0 mV from its control value, trace *a*. Currents were recorded at

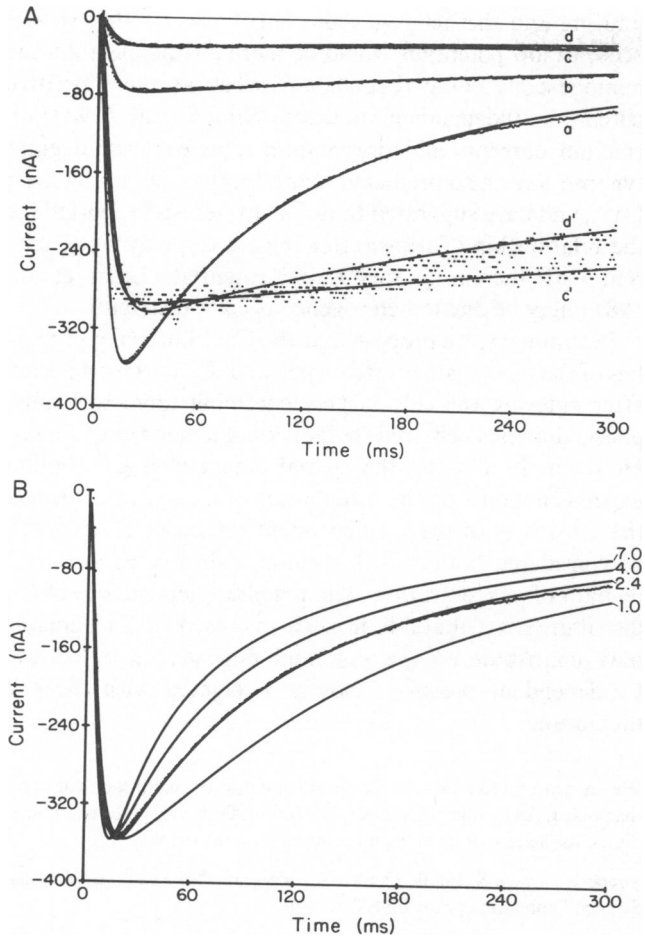

FIGURE 4 Calcium currents elicited by potential steps to 0 mV. (*A*) Before (*a*) and after (*b*, *c*) bath addition of 0.5 mM Cd. Current *d* was obtained by a step to −24 mV before addition of Cd. Currents *c* and *d* are shown with X10 gain (*c'*, *d'*) to illustrate slower inactivation at the more depolarized potential (*c'*). (*B*) Calcium current (uneven trace) obtained in response to a step to 0 mV compared with model curves generated with various values of K (1.0, 2.4, 4.7 × 10^5 M^{-1}).

different intervals after external addition of Cd^{2+}, which produced a progressive decrease (trace *b* → trace *c*).

We assume that the blocking reaction is slow compared with inactivation, and that Cd^{2+} reduces the number of open channels without affecting the single channel current. This change was modeled according to the competitive blocking equations of Hagiwara and Takahashi (1967), using a K_{Cd} of 10^{-5} M. The values of m_∞ and K in the model were set to the fit to trace *a*, and a single parameter corresponding to cadmium concentration was adjusted to yield the fits for *b* and *c*. The peak current elicited by a step to 0 mV in the presence of Cd (trace *c*) is close to that obtained for a step to −24 mV in the absence of Cd (trace *d*). For closer comparison, these currents and fits are displayed with X10 gain (*c'* and *d'*), showing marked differences in the rate of inactivation, which was slower at the more depolarized potential.

To illustrate the resolution in determination of K, the peak current elicited by a step to 0 mV is shown with a family of model currents that differ in the values of parameter K (Fig. 4 *B*); the values of m_∞ were adjusted to make the peaks coincident. Variations of K on the order of 5% produce discernible differences in the time course of inactivation in the modeled currents, and so the measured variation in K with potential (Fig. 3) appears to be an accurate reflection of a kinetic change. It is noteworthy that the values of K determined by fitting modeled currents to real currents appear to be closely correlated with single-channel current and negatively correlated with the macroscopic Ca current.

DISCUSSION

In *Aplysia* neurons the Ca-dependent inactivation of the Ca channels resulting from a given macroscopic Ca^{2+} influx becomes weaker with increasing depolarization (Eckert and Ewald, 1982; Eckert et al., 1982). This behavior, which at first seemed anomalous or paradoxical, was evaluated with the aid of a binding site model of Ca-mediated inactivation (Chad et al., 1984*b*). Central to this model is the potency parameter K, which includes the efficiency with which intracellular Ca^{2+} mediates the inactivation of the Ca channel. Isolation of parameter K presumes a knowledge of the volume, v, of Ca^{2+} dispersal in these formulations. When v is considered to be fixed, the value of K in computer fits of recorded currents was found to decrease for increasing depolarizations (Fig. 3 *A*). It appears to be significant that K determinations based on this premise varied in a manner consistent with the voltage dependence of the unitary Ca channel current, as shown in Fig. 3 *B*. In contrast, the value of K varied inversely with the voltage dependence of the macroscopic current in the range −30 to 0 mV.

We postulate that this close correlation between K and i_{Ca} results from a limited dispersal of Ca^{2+} from the site of entry. Calculations based on physiological values of buffering and diffusion indicate that a channel would be predicted to predominately control Ca_i within a small hemispherical "domain." Based on Eqs. 5 and 6, Ca_i should undergo a steep drop in value with distance from the inner opening of the channel. Thus, Ca^{2+} entry through a given channel should determine primarily the inactivation of that channel, and Ca-mediated inactivation should be determined primarily by i_{Ca} rather than I_{Ca}.

A second way of describing the relation between Ca current and resulting inactivation of Ca channels is that the volume of dispersal is not fixed but is related to the number of active channels. Thus, at greater depolarizations more channels are active, and I_{Ca} enters a greater effective volume, thus appearing to be less effective per mole-equivalent unit. This would be most pronounced if the density of active channels were such that domains did not have significant overlap (diagrammed in Fig. 1). Direct determination of active single Ca channel density in molluscan neurons can best be obtained by patch clamp techniques (*Helix pomatia*, Lux and Nagy, 1981; Brown

et al., 1982). Pipettes with openings of 0.5 μm radius seal patches of membrane containing one to a few Ca channels. Assuming a minimum disk of membrane with the same diameter, this leads to an approximate density of $5/\mu m^2$. (This estimate of channel density is undoubtedly high since the area of membrane sucked into a patch pipette is generally believed to be greater than the pipette diameter due to deformation of the membrane patch.) Similar Ca channel densities can be deduced for rat clonal pituitary cells (Hagiwara and Ohmori, 1983) and for bovine chromaffin cells (Fenwick et al., 1982). The inter-channel distances determined from those data ($\approx$0.4 μm for even distribution) are such that Ca domains would not overlap, and therefore effective independence of Ca_i in each domain is feasible.

An important qualification is that the rate of channel "cycling" not be so high as to even out Ca^{2+} distribution among all available Ca channels. If essentially all channels were equally active, exhibiting open times that varied uniformly with potential throughout the channel population, Ca distribution would be effectively homogeneous, and our arguments based on heterogeneity of domains would not apply. Evidence from patch clamp experiments on calcium channels in rat clonal pituitary cells (Hagiwara and Ohmori, 1983) indicate at least two components in the distributions of mean closed times. The slower component implies that the channels do not cycle rapidly during sustained depolarization. Thus, at any given potential the active channels appear statistically to be a subset of the total population, and this should contribute to heterogeneity in the distribution of Ca^{2+}.

Kinetic models of Ca-mediated responses typically require a term related to the integral of Ca entry. However, it is evident from the present calculations and those of Simon (1984) that Ca_i immediately at the mouth of the Ca channel rapidly approaches a steady value directly related to i_{Ca}. Thus, the physiological correlate(s) of the integrater term may be one or both of the following: (*a*) the relatively slow rise in Ca_i at a receptor site located some distance from the center of the domain, or (*b*) a relatively slow rate-limiting step in the development of the Ca-dependent process.

Heterogeneous, potential-dependent Ca^{2+} distribution together with nonlinear dependence of responses on Ca_i may produce potential dependence of the relationship between the kinetics of a Ca-dependent process and the macroscopic Ca current. Thus, at low potentials few channels are activated but i_{Ca} is large, producing a heterogeneous Ca^{2+} distribution with saturation of the Ca^{2+}-mediated response in some regions. However, at more positive potentials Ca influx is more homogeneously distributed because of the greater number of activated channels, thus, less Ca^{2+} should be "wasted" in regions of saturated response, producing a larger overall response. The converse is predicted for the condition of lowered external Ca. In that circumstance Ca^{2+} entry would be limiting and the heterogeneous entry of Ca^{2+} that should occur at low potentials would be more efficacious than the homogeneous entry that should occur at more positive potentials. Independent studies by Simon et al. (1984) on calcium currents and presynaptic release at squid giant synapse have also predicted acute localization of entering Ca^{2+}, and have suggested that the "hysteresis" reported for the relationship of transmitter release to presynaptic current with increasing membrane potential (Linás et al., 1981) may be due to heterogeneous Ca^{2+} distribution.

In summary, we propose that the Ca^{2+} buffering properties of the cytoplasm retard dispersal of Ca ions for a period after entering the cell, largely confining them to hemispheric domains centered on individual active Ca channels. However, in a sense, the spatial extent of a Ca domain depends not only on the distribution of Ca ions, but also on the sensitivity of the Ca-dependent response. Thus, it can be considered both as a domain of influence as well as a domain of concentration. The potential dependence of Ca distribution attributable to such microscopic Ca domains may contribute to the potential dependence of certain Ca-dependent processes closely associated with the cell membrane.

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