A BUFFERING MODEL FOR CALCIUM-DEPENDENT NEUROTRANSMITTER RELEASE

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ABSTRACT A simple model is proposed, whereby a single buffering system for intracellular calcium accounts for the steep external Ca dependence of neurotransmitter release during depolarization of the presynaptic nerve terminal. Ca entry and buffering in the nerve terminal are assumed to be saturable; release is assumed to be proportional to intracellular Ca. The novel feature of this model is that it explains the apparent cooperative relationship between transmitter release and extracellular calcium, without invoking cooperative Ca binding.

INTRODUCTION

When the presynaptic nerve terminal is depolarized by an invading action potential, there is an increased conductance for calcium ions (Ca). Ca enters the nerve ending, moving down a steep electrochemical gradient; the subsequent rise in the intraterminal ionized Ca concentration (Ca_i) triggers neurotransmitter release by an unknown process (for reviews, see Katz, 1969; Llinas, 1977). The Ca dependence of neurotransmitter release has been studied and carefully quantified in a variety of preparations (see Ginsborg and Jenkinson, 1976; Martin, 1977). As first described by Jenkinson (1957) for the frog neuromuscular junction, nerve-evoked release increases steeply as Ca is added to the bathing solution. In recent years it has become evident that Ca buffering plays a crucial role in the metabolism of Ca in presynaptic nerve terminals (for a review, see McGraw et al., 1982). In this manuscript we propose a simple model in which buffering of Ca underlies the nonlinear relationship between transmitter release and external Ca (Ca_o).

Dodge and Rahamimoff (1967) examined the Ca_o dependence of transmitter release in detail and developed a cooperative model to describe their results. They followed the hypothesis of Del Castillo and Katz (1954), assuming that Ca combined with a membrane site, X, to form a CaX complex necessary for release. The cooperative assumption of Dodge and Rahamimoff was that n Ca ions had to bind to X (or that n CaX complexes had to be formed) before release could occur. If formation of the Ca_nX complex was rate limiting, release would be a saturating power function of the external Ca concentration. The data of Dodge and Rahamimoff were consistent with a value of 4 for n at the frog neuromuscular junction. Lower values of n, however, have been determined for the Ca dependence of release in other preparations (for a review, see Martin, 1977). At the crayfish neuromuscular junction, for instance, n has a value of ~1 (Bracho and Orkand 1970), and at the squid giant synapse, n has a value of 3 or less (Katz and Miledi, 1970). If n actually represents a physical parameter related to Ca binding, it is difficult to understand why it should vary among different types of preparations.

Hubbard et al. (1968) suggested a more complicated model in which several complexes of Ca (e.g., CaX, Ca_2X, and Ca_3X) triggered release with differing degrees of efficacy. This model accounts for the Ca dependence of release under a variety of experimental conditions (e.g., with electrical depolarizatoin and high potassium), and allows n to assume different values.

A serious drawback of all cooperative models is that it has been difficult to identify the cooperative Ca binding site, X. Ca transport across cell membranes (for a review, see Hagiwara and Byerly, 1981), including the presynaptic nerve terminal membrane (Nachshen and Blaustein, 1980; Llinas et al. 1981), is noncooperative. Also, in studies that may be relevant, Baker and Knight (1978) examined noradrenaline release from bovine adrenal medullary cells made leaky to external ions. They found that release was dependent on Ca_i, but in a noncooperative manner.

A noncooperative model described by Cooke et al. (1973) is based on the notion that quantal release is continuously graded with the amount of a simple calcium complex (CaX) inside the nerve terminal. Other assumptions are (*a*) that the activity of X, the internal Ca receptor, is increased by depolarization, (*b*) that Ca entry increases exponentially with depolarization, and (*c*) that Ca is transported across the membrane by a Ca_2Y complex. This complicated model could empirically describe the Ca dependence of release over a wide range of experi-

mental conditions. The underlying assumptions, however, have yet to be verified experimentally.

We find that a single Ca buffering system can give rise to apparent cooperativity in the absence of cooperative Ca binding without complicating assumptions about the internal Ca receptor or the release mechanism.

BUFFERING MODEL

We first evaluate Ca entry into the nerve terminal through Ca channels that are opened by a depolarizing nerve impulse. The rate of net Ca entry through the open Ca channels, J_{Ca}, is given by

$$J_{Ca} = P[Ca_o - Ca_i \exp(ZFV/RT)]/1 - \exp(ZFV/RT). \quad (1)$$

P is the permeability of the open Ca channel to Ca, Ca_o is the external Ca concentration, Ca_i^* is the resting ionized Ca concentration before the depolarization, and Z, F, V, R, and T have their usual thermodynamic meanings. We assume that the depolarization caused by the action potential can be approximated by a square voltage pulse with an excursion from the resting membrane potential (about −60 mV) to 0 mV. We also assume that $Ca_i^* \ll Ca_o$. Therefore, Eq. 1 can be rewritten as

$$J_{Ca} = P\,Ca_o. \quad (2)$$

In many types of cells (see review by Hagiwara and Byerly, 1981), including neurons (Akaike et al., 1978; Nachshen and Blaustein, 1980; Llinas et al., 1981), the Ca channel has a site to which Ca binds during translocation. P therefore is a saturating function of Ca_o, and the following relationship holds (see Hille, 1975; Hagiwara, 1975) when the membrane potential is zero:

$$P = \frac{J_{Ca(max)}}{Ca_o + K_C}. \quad (3)$$

$J_{Ca(max)}$ is the maximal rate of Ca entry into the terminal through the open Ca channels, and K_C is the half-saturation constant of the channel.

By combining Eqs. 2 and 3, we obtain

$$J_{Ca} = \frac{J_{Ca(max)}\,Ca_o}{Ca_o + K_C}. \quad (4a)$$

The time course of Ca entry is undoubtedly complicated by the time dependence of Ca channel opening and closing. For the sake of simplicity, we assume that Ca is injected instantaneously into the nerve terminal. The initial increase in Ca concentration, the Ca load (Ca_L), is therefore

$$Ca_L = \frac{Ca_{L(max)}\,Ca_o}{Ca_o + K_C}. \quad (4b)$$

$Ca_{L(max)}$ is the maximal concentration to which Ca_L can initially rise.

We now evaluate buffering of the Ca load prior to the release of neurotransmitter. We assume that the Ca load is buffered instantaneously, according to the reaction scheme: $Ca_i + B \rightleftharpoons [CaB]$, where B is the free buffer concentration, $[CaB]$ is the concentration of the Ca-buffer complex, and their sum is the total concentration of buffer (B_T). The equilibrium constant for Ca buffering, K_B, is given by

$$K_B = \frac{Ca_i \cdot B}{[CaB]}. \quad (5)$$

The net Ca_i will therefore be

$$Ca_i = Ca_L - [CaB]. \quad (6)$$

Combining Eqs. 5 and 6, and solving for Ca_i, we obtain

$$Ca_i = \{Ca_L - B_T - K_B + [(B_T + K_B - Ca_L)^2 + 4\,Ca_L \cdot K_B]^{1/2}\}/2 \quad (7)$$

where Ca_L is defined by Eq. 4b.

Ca_i triggers transmitter release by an unknown mechanism. Subsequently, release is terminated within 1–2 ms, apparently due to a rapid return of Ca_i to its resting level (Katz and Miledi, 1968). Because both the actions of Ca_i on transmitter release and the time course of Ca_i removal are unknown, we assume simply that release is proportional to Ca_i.

Solution of Eq. 7 (combined with Eq. 4b) involves four parameters: $Ca_{L(max)}$, K_C, B_T, and K_B. Reasonable limits can be set for some of these parameters. (*a*) $Ca_{L(max)}$ is $\simeq 10^{-6}$ M (Llinas, 1977; McGraw et al., 1982). This value is based on the assumption that Ca_L is distributed homogeneously throughout the nerve terminal. During the 0.2 ms that it takes for Ca ions to trigger exocytosis subsequent to entry (Llinas, 1977), they will probably diffuse <0.2 μm away from the presynaptic membrane (Parsegian, 1977). Furthermore, because Ca ions may, perhaps, enter the terminal only at restricted regions (e.g., at the active zones where vesicles are localized; see Llinas and Heuser, 1977), a more realistic assumption is that Ca_L near the zone of transmitter release is far greater, possibly several hundred micromolar (Llinas et al., 1981). (*b*) K_C has been estimated by several different methods as 0.2–1 mM (Dodge and Rahamimoff, 1967; Silinsky, 1977; Nachshen and Blaustein, 1980). (*c*) The value of B_T is unknown. (*d*) Although K_B is also unknown, a range of values can be estimated from the half-saturation constants of identified Ca buffering systems (see McGraw et al., 1982). These include Ca binding proteins, smooth endoplasmic reticulum ($K_{0.5} = 10^{-7} - 10^{-6}$ M), and, possibly, synaptic vesicles and mitochondria ($K_{0.5} = 10^{-5} - 10^{-4}$ M).

We next consider how Ca_i varies as a function of Ca_o when the values of each parameter are changed. The functions have been plotted in Fig. 1 on log-log coordinates to show variations of Ca_i over a wide range of Ca_o values. (*a*) As $Ca_{L(max)}$, the maximal Ca load entering the terminal, is increased, the value of Ca_i becomes larger for all values of Ca_o (Fig. 1 *A*). Furthermore, the relationship between log Ca_i and log Ca_o becomes sigmoidal. Thus, small variations in Ca_o cause greater changes in Ca_i as $Ca_{L(max)}$ is increased. (*b*) Changing K_C, the half-saturation constant of the Ca channel, has little effect on the shape of the log Ca_i vs. log Ca_o function (Fig. 1 *B*). With increasing values of K_C, the curves are shifted along the Ca_o axis, i.e., higher concentrations of Ca_o are required to obtain equivalent values of Ca_i. A similar shift would be caused by competitive blockers of the Ca channel. (*c*) Decreasing B_T, the maximal buffering capacity of the terminal, is qualitatively similar to increasing $Ca_{L(max)}$: the relationship between log Ca_i and log Ca_o becomes more sigmoidal, and Ca_i becomes larger at all values of Ca_o (Fig. 1 *C*). At very low B_T concentrations, however, the relationship becomes less steep. (*d*) Increasing the value of K_B, the half-saturation constant of the buffering system, has little effect on the log Ca_i vs. log Ca_o relationship at high values of Ca_o (Fig. 1 *D*). At lower values of Ca_o, however, there is a marked decrease in Ca_i for any given value of Ca_o with increasing values of K_B.

DISCUSSION

Fig. 1 shows that the relationship between log Ca_i and log Ca_o has a slope >1 over a limited range of Ca_o if appropriate values are chosen for the four parameters required to solve Eq. 7. For example, with $Ca_{L(max)} = 200$ μM, $K_C = 500$ μM, $B_T = 50$ μM, and $K_B = 0.2$ μM (Fig. 1, curves with asterisks), the log-log function has an average slope of ~4 in the Ca_o range of 100–300 μM (compare with straight line in Fig. 1 *A*). This is the Ca_o range over which Dodge and Rahamimoff (1967, Fig. 3 *B*) found a slope of 4 for the relationship between log release and log Ca_o ($Mg_o = 0.5$ mM) at the frog neuromuscular junction.

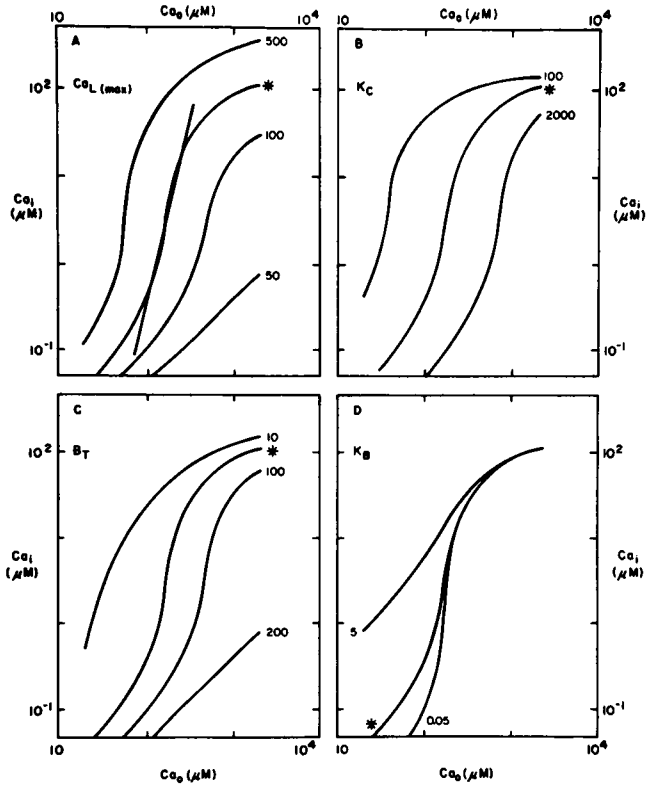

FIGURE 1 Ca_i as a function of Ca_o. The logarithm of the ionized internal calcium concentration near the membrane release sites (ordinate) is plotted as a function of the logarithm of the external calcium ion concentration (abscissa). The curves with asterisks were generated from Eq. 7, using the following values (in μM): $Ca_{L(max)} = 200$; $K_C = 500$; $B_T = 50$; $K_B = 0.2$. The other curves were generated by allowing one parameter to vary, as indicated by the values next to the plots in each set: (*A*) $Ca_{L(max)}$, (*B*) K_C, (*C*) B_T, (*D*) K_B. The straight line in *A* has a slope of 4.

The model we present conveniently explains the variability of the "cooperative" number (n) observed in different preparations (see Introduction). Changes in the values of $Ca_{L(max)}$, B_T, and K_B can alter the maximal slope of the release-log Ca_o function. For example, a terminal with a very small or a very large buffering capacity (B_T) would be expected to have a less steep relationship between release and Ca_o (Fig. 1 *C*) than a terminal with a buffering capacity in the intermediate range.

At high concentrations of external calcium, release is less steeply dependent on Ca_o (see Dodge and Rahamimoff, 1967, Fig. 6), as predicted by both the cooperative and buffering models. The models can, however, be distinguished at low concentrations of Ca_o: the cooperative model of Dodge and Rahamimoff predicts that the Ca_o dependence of release remains steep, whereas the buffering model predicts that the Ca_o dependence decreases. Measurements of release at low Ca_o yield conflicting data. Cooke et al. (1973) and Crawford (1974) observed a decrease in the Ca dependence of release at low Ca_o. Andreu and Barrett (1980) also observed a decrease, in the presence of Mg, and at high frequencies of nerve stimulation; however, they observed a fourth-order relationship between release and low Ca_o in the presence of Mn or Co, and at low frequencies of nerve stimulation. Clearly, it is necessary to clarify further the mode of action of Mg, Mn, and Co before either model can be excluded, as some of these ions have complicated effects on transmitter release (e.g., see Kita et al., 1981).

It should be remembered that the buffering model is a greatly simplified representation of the events occurring during synaptic transmission. It is possible, for example, to extend the range over which Ca_i and Ca_o show a steep interrelationship if a second, higher affinity buffering system is included in the calculation (not shown). However, more complex analysis is unwarranted in view of the limited information about Ca metabolism at the nerve terminal. Nonetheless, even with the simplest buffering model, an apparent cooperative relationship between transmitter release and Ca_o can be obtained without cooperative binding of Ca. Thus, sequestration of Ca, by intraterminal organelles or Ca binding proteins, may control the relationship between transmitter release and Ca_o at a variety of synapses.

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