

CALCIUM COOPERATIVITY IN COUPLING NEUROTRANSMITTER RELEASE TO NERVE TERMINAL DEPOLARIZATION: FACILITATION

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Abstract

The observed dependence of neurotransmitter release on extracellular calcium concentration and on nerve terminal depolarization is consistent with a model in which the influx of calcium into the nerve terminal via voltage-gated channels is a saturating function of extracellular Ca^{2+} with cooperativity exponent r , and release is a saturating function of internal Ca^{2+} , with cooperativity exponent n . However, the experimental data from vertebrate endplates can be fitted to this model only if r is at least 2 and n is probably 2 and no more than 3. On the basis of this model, facilitation can be ascribed to residual Ca^{2+} , that is, Ca^{2+} caused to enter by nerve impulses summates with Ca^{2+} persisting within the nerve terminal after antecedent nerve impulses. However, to fit experimental data regarding the rate of transmitter release between and after nerve impulses it becomes necessary to have an n of at least 4. This inconsistency implies that the residual Ca^{2+} model for facilitation is untenable.

1. Introduction

At the skeletal neuromuscular junction the quantal release of neurotransmitter by the nerve terminal is very steeply dependent upon the concentration of Ca^{2+} in the external bathing medium, with a maximum slope of the log of end-plate potential amplitude (e.p.p.) vs. log external $[\text{Ca}^{2+}]$ close to 4 (Jenkinson, 1957; Dodge and Rahamimoff, 1967; Hubbard, Jones and Landau, 1968; Cooke, Okamoto and Quastel, 1973). This has led to the proposition that 4 atoms of Ca^{2+} must cooperate to produce release of a quantum of neurotransmitter (Dodge and Rahamimoff, 1967). The frequency of miniature end-plate potentials ($F_{\text{m.e.p.p.}}$) is increased by nerve terminal depolarization, either by application of electric current, or by increase of $[\text{K}^+]$ in the bathing medium (Liley, 1956; Landau, 1969). Parnas and Segel (1981) have recently presented a rather detailed analysis of the data of Cooke *et al.* (1973), which provided experimental observations of $F_{\text{m.e.p.p.}}$ over a wide range of C_e and depolarization, as well as curves of e.p.p. height vs. C_e . They have shown that a model in which release of each quantum of transmitter is supposed to require a fixed number of Ca^{2+} ions (Dodge and Rahamimoff, 1967) can indeed account for the observation that the maximum slope of log release vs. $\log C_e$ varies continuously with the degree of nerve terminal depolarization. It is my purpose here to point out that this model, as

formalised by Parnas and Segel (1981), with the parameters necessary to account for the data of Cooke *et al.* (1973), cannot at the same time account for "facilitation" on the basis of residual Ca^{2+} in the nerve terminal, following one or more conditioning stimuli.

2. The Relation Between Release and External Calcium

As emphasized by Cooke *et al.* (1973) and Parnas and Segel (1981) the data of Cooke *et al.* (1973) show: (i) A sigmoidal relation between $\ln L$ (either $F_{\text{m.e.p.p.}}$ or average number of quanta per e.p.p.) and $\ln C_e$, with a maximum slope (m_{max}), and minimal and maximal values of L , that are increasing functions of nerve terminal depolarization (D), and (ii) the value of C_e , C_e^{max} , at which the slope of $\ln L$ vs. $\ln C_e$ is maximal (i.e., at m_{max}) is a decreasing function of D .

In addition the data show: (iii) Either at any D , or with e.p.p.s, the data could be closely fitted by the function $\ln L = \alpha + \beta/[1 + (\gamma/C_e)^\theta]$, where α , β , γ , and θ are fitting parameters, and least square fitting always gave θ very close to 2.0, under conditions where $L_{\text{max}} (= \exp(\beta + \alpha))$ and $L_0 (= \exp(\alpha))$, and $\gamma (= C_e^{\text{max}})$, varied over a wide range. For this function m_{max} , the maximum slope of $\ln L$ vs. $\ln C_e$ is $\theta \ln(L_{\text{max}}/L_0)/4$. Thus the ratio of m_{max} to $\ln(L_{\text{max}}/L_0)$ was experimentally observed to be 0.5.

To account for the experimental observations (i) and (ii), Parnas and Segel (1981) proposed that there exists in the nerve terminal a constant basal level of Ca^{2+} , C_0 , independent of external Ca^{2+} (C_e). When the nerve terminal is depolarized, internal Ca^{2+} is augmented by Ca^{2+} that enters the nerve terminal. To summarize their model,

$$(1) \quad L = \lambda / (1 + K_\lambda / C^n)$$

$$(2) \quad C = C_0 + \varepsilon / (1 + K_\varepsilon / C_e^r)$$

where λ , K_λ , and K_ε are constants, C is internal Ca^{2+} concentration, and n and r are positive integers that are "Hill coefficients" for Ca^{2+} action internally, and Ca^{2+} binding to an external receptor, respectively. The parameter ε (equivalent to $f(V)$ in Cooke *et al.*, 1973) is considered to rise with depolarization. These equations give rise (Parnas and Segel, 1981) to the close approximation for m_{max} , the maximum slope of $\ln L$ vs. $\ln C_e$,

$$(3) \quad m_{\text{max}} = nr \frac{S-1}{S+1},$$

$$(4a) \quad \text{where } S = [1 + \varepsilon/C_0]^{0.5} = K_\varepsilon / (C_e^{\text{max}})^r$$

$$(4b) \quad \text{and } L_0 = \lambda / [1 + K_\lambda / C_0]^n \text{ and } L_{\text{max}} = \lambda / [1 + K_\lambda / (C_0 + \varepsilon)]^n$$

Since $(S-1)/(S+1)$ can be small, it is possible to produce a series of curves where m_{max} and L_{max}/L_0 increase with ε and therefore with D , and C_e^{max} declines with D , as observed experimentally.

Parnas and Segel (1981) correctly observe that the product nr must be about 4 and the values $n=4$ and $r=1$ were chosen by them in order to be able to account

quantitatively for observed data *re* facilitation (see below). However, their theoretical curves for the relation between $\log L$ and $\log C_e$, at various values of D (Figs. 6 and 7 in Parnas and Segel, 1981) do not fit observation (iii) above. With the parameters chosen by them, i.e., $n=4$, $r=1$, $\varepsilon=D/10$, $C_0=.001$, $\lambda=10$ (Fig. 6) (or $\lambda=D$, in Fig. 7), $K_\lambda=0.015$, the ratio of m_{max} to $\ln(L_{\text{max}}/L_0)$ is always less than 0.25, being 0.248 at $D=0.01$, 0.23 at $D=0.1$, 0.18 at $D=1.0$ and 0.16 at $D=10.0$. By comparing Fig. 6 or Fig. 7 of Parnas and Segel (1981) (where the axes are in \log_{10}) with Fig. 1a or Fig. 1b (where the axes are in \log_e), it can be seen that, for curves with similar m_{max} , $\ln(L_{\text{max}}/L_0)$ is at least twice as large for the theoretical curves as for the experimental curves.

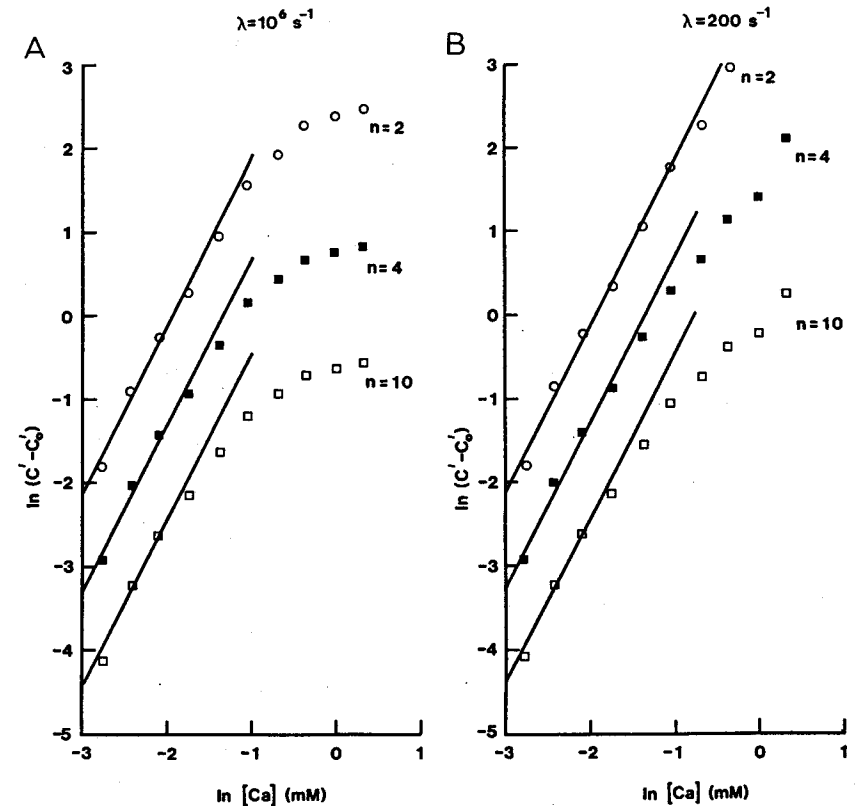


Fig. 1. Plots of $\ln(C - C_0)$ vs. $\ln(C_e)$ for $n=2, 4$, and 10 , for $\lambda \gg L_{\text{max}}$ (A) and for λ close to L_{max} (B). The lines are drawn with a slope of 2.0. The data are from a junction where $F_{\text{m.e.p.p.}}$ was followed as C_e was raised from 0 to 14 mM, in the presence of 20 mM K^+ (Fig. 4. in Cooke *et al.*, 1973). Similar graphs are obtained with any positive n , and with any $\lambda > L_{\text{max}}$, for any of the sets of data relating transmitter release to C_e , in Cooke *et al.*, (1973).

Evidently, Parnas and Segel (1981) did not recognize that the experimental data do not permit assignment of values to n and r on the basis of their model. This can be done as follows.

Rearranging equation (1) gives $C^n = [K_\lambda L / (\lambda - L)]$, and it therefore follows that for any arbitrarily chosen n and λ , each observed value for transmitter release rate gives a value for $[L / (\lambda - L)]^{1/n} = C / K_\lambda^{1/n}$. Meanwhile, from equation (2), above,

$$C - C_0 = \varepsilon / (1 + K_e / C_e)$$

$$d \ln(C - C_0) / d(\ln C_e) = r / (1 + C_e / K_e) = r [1 - (C - C_0) / \varepsilon]$$

Hence, if we define $C' = [L / (\lambda - L)]^{1/n}$ and $C'_0 = C / K_\lambda^{1/n}$, a plot of $\ln(C' - C'_0)$ vs. $\ln C_e$ will give r , as the limiting slope where C_e is small. When this is done, for any of the sets of data in Cooke *et al.* (1973), it is observed that r is close to 2.0, whatever the value chosen for n , or λ , the only constraint being that $\lambda > L_{\max}$ (Fig. 1). That this result must generally be true will now be shown analytically.

Defining $y = \ln(L / L_0)$, we have, from the empirical result (iii), above,

$$y = \beta / [1 + (\gamma / C_e)^\theta]$$

$$dy / d(\ln C_e) = \theta y (1 - y / \beta)$$

Defining $B = (K_\lambda L / \lambda)^{1/n}$, and putting $B = B_0$ when $C_e = 0$, we have,

$$C^n = B^n \cdot \lambda / (\lambda - L)$$

$$B = B_0 (L / L_0)^{1/n} = B_0 e^{y/n}, \quad B / (B - B_0) = 1 / (1 - e^{-y/n})$$

$$n d(\ln B) / dy = 1; \quad n d(\ln C) / dy = \lambda / (\lambda - L); \quad dC / dB = [\lambda / (\lambda - L)]^{(1+1/n)}$$

$$\frac{d(\ln(B - B_0))}{d(\ln C_e)} = \frac{d(\ln(B - B_0))}{d(\ln B)} \frac{d(\ln B)}{dy} \frac{dy}{d(\ln C_e)}$$

$$= \frac{B}{(B - B_0)} \frac{1}{n} \theta y (1 - y / \beta)$$

$$= \frac{y/n}{1 - e^{-y/n}} \theta (1 - y / \beta)$$

We now observe that for all positive values of y/n the expression $(y/n) / (1 - e^{-y/n})$ has a minimum value of 1; when y/n and y/β are small the slope of $\ln(B - B_0)$ vs. $\ln C_e$ has the value, θ .

$$\text{Since,} \quad \frac{d(\ln(C - C_0))}{d(\ln C_e)} = \frac{d(\ln(B - B_0))}{d(\ln C_e)} \frac{d(\ln(C - C_0))}{d(\ln(B - B_0))}$$

to obtain the slope of $\ln(C - C_0)$ vs. $\ln C_e$, we must first evaluate the slope of $\ln(C - C_0)$ vs. $\ln(B - B_0)$,

$$\begin{aligned} \frac{d(\ln(C - C_0))}{d(\ln(B - B_0))} &= \frac{B - B_0}{C - C_0} \frac{dC}{dB} \\ &= \frac{L^{1/n} - L_0^{1/n}}{[L\lambda / (\lambda - L)]^{1/n} - [L_0\lambda / (\lambda - L_0)]^{1/n}} \left(\frac{\lambda}{\lambda - L} \right)^{(1+1/n)} \\ &= \frac{\lambda}{\lambda - L} \frac{L^{1/n} - L_0^{1/n}}{(L^{1/n} - L_0^{1/n}) W} \end{aligned}$$

where

$$W = [(\lambda - L) / (\lambda - L_0)]^{1/n}$$

For any values of λ , L and L_0 where $0 < L_0 < \lambda$ which is necessarily the case, and for any $n \geq 1$, this expression has a minimum value of 1 and increases as L approaches λ . Hence

$$\frac{d(\ln(C - C_0))}{d(\ln C_e)} \geq \frac{d(\ln(B - B_0))}{d(\ln C_e)} = \theta \frac{y/n}{1 - e^{y/n}} (1 - y/\beta)$$

i.e., $\frac{d(\ln(C - C_0))}{d(\ln C_e)} > \theta$, when $y (= \ln L)$ is small compared to $\beta(\ln L_{\max})$.

However, this same limiting slope, $d(\ln(C - C_0)) / d(\ln C_e)$, is also r in equation (2). Therefore, the observation of Cooke *et al.* (1973) that the modulation of L with C_e fits very closely to the empirical equation $\ln L = \alpha + \beta / [\gamma / C_e]^\theta$, with $\theta = 2$, implies, in terms of the Parnas-Segel model, that $r \geq \theta = 2$, whatever the value of n and however closely L may approach λ .

An alternative derivation of the same result, differing from the previous in that it makes use of more experimental data points, may be obtained using the close approximations in equations (3) and (4). From the rearrangement of equation (1) to give $C^n = K_\lambda L / (\lambda - L)$, and since L_{\max} corresponds to $C = C_0 + \varepsilon$ and L_0 to $C = C_0$,

$$[(C_0 + \varepsilon) / C_0]^n = (1 + \varepsilon / C_0)^n = S^{2n} = \frac{L_{\max}}{L_0} \frac{\lambda - L_0}{\lambda - L_{\max}}$$

Taking logarithms of both sides of the last equality one obtains

$$\frac{1}{n} = \frac{2 \ln S / \ln(L_{\max} / L_0)}{1 + q}$$

$$\text{where } q = \ln \frac{(\lambda - L_0)}{(\lambda - L_{\max})} \ln \frac{L_{\max}}{L_0}$$

Using the empirical result (iii) $2m_{\max} = \ln(L_{\max} / L_0)$ and the close approximation (equation 3), $m_{\max} = n r (S - 1) / (S + 1)$

$$r = \frac{1}{n} m_{\max} \frac{(S + 1)}{(S - 1)} = \frac{2 \ln S}{2 m_{\max}} \frac{1}{1 + q} m_{\max} \frac{(S + 1)}{(S - 1)}$$

or

$$r = \frac{\ln S(S+1)/(S-1)}{1+q}$$

In this equation the numerator has a value close to 2 for any value of S close to 1, rising to 2.5 at $S=6$ and to 3.0 at $S=13$. The term q has a low value whenever L_{\max} is substantially less than λ , which must have been the case for much of the data of Cooke *et al.* (1973). For example, using steady depolarization of nerve terminals by 15 mM K^+ , $\ln(L_{\max}/L_0)$ was about 5, while with 20 mM K^+ , $\ln(L_{\max}/L_0)$ was about 7. This gives λ at least e^7 , and a value of q of <0.03 for 15 mM K^+ . A more realistic estimate of λ is obtained from the observation that the spontaneous transmitter release rate (L_0) is normally about 1/sec, while with normal (~ 2 mM) C_e an e.p.p. corresponds to release of about 10^5 m.e.p.s/sec, maintained for about 1 msec (Miledi and Thies, 1971). This gives a value of $(1 + K_\lambda/C_0^n)$ which is λ/L_0 , at least e^{11} making $q \ll 1$. Therefore, unless one ascribes the increase of transmitter release by depolarization primarily to factors other than entry of Ca^{2+} (which would be a model very different from that considered by Parnas and Segel, 1981), it must be concluded again, that the data of Cooke *et al.*, (1973) can be fitted to the Parnas-Segel model only if $r \geq 2$.

In this regard it may be noted that experiments do indeed show some increase of "spontaneous" transmitter release with prolonged or repeated presynaptic depolarization (Cooke *et al.*, (1973), which could be attributable to increase in C_0 , increase in λ , or decrease of K_λ . However, this takes considerable time to develop (Cooke and Quastel, 1973) and can play little role in release of transmitter evoked by brief depolarizing pulses or single presynaptic action potentials, where the relation between L and external Ca^{2+} also fits well to the same empirical formula, $\ln L = a + \beta/[1 + \gamma/(Ca)^2]$, as when nerve terminals are continuously depolarized by raised K^+ .

With the conclusion that $r \geq 2$, it must also be concluded that n has a value of about 2, and must certainly be no more than 3. This arises because with e.p.p.s, m_{\max} is close to 4 and no more than 5 (Jenkinson, 1957; Dodge and Rahamimoff, 1967; Hubbard *et al.*, 1968; Cooke *et al.*, 1973). Here ϵ/C_0 must be fairly large, whatever the value of n , to account for an L_{\max}/L_0 of about e^{11} , and $(S-1)/(S+1)$ must therefore approach unity. Moreover, the shifts with depolarization of C_e^{\max} (equivalent to γ) observed by Cooke *et al.*, 1973, are incompatible with nr more than about 4, given (equations 3 and 4) that

$$C_e^{\max} = (K_\epsilon/S)^{1/r} \cong K_\epsilon^{1/r} (L_0/L_{\max})^{1/(2nr)}$$

Indeed, the observed shifts in C_e^{\max} are in excess of those predicted by this equation even with $nr=4$, a result which calls into question whether the Parnas-Segel model can in fact provide a good fit to the data as a whole.

It should be noted that the data of Cooke *et al.* (1973) can be fitted just as well (or better) to a model in which the probability of release of a quantum of transmitter is continuously graded with the local amount of a bound intracellular Ca^{2+} (CaX). As a

first approximation this leads to an exponential relation between overall release rate and concentration of CaX (Cooke *et al.*, 1973; Quastel, 1974). Such a model predicts a constant ratio of m_{\max} to $\ln(L_{\max}/L_0)$, as observed experimentally (i.e., " θ " constant at 2.0); the Parnas-Segel model predicts that this ratio should progressively diminish with increasing L_{\max}/L_0 . However, the 'exponential' model fails to account adequately for the large increase in L_{\max}/L_0 that takes place, in conjunction with reduction of C_e^{\max} , when the nerve terminal is depolarized (Cooke *et al.*, 1973).

3. Facilitation

It is well known that following one or a train of nerve impulses transmitter release by nerve impulses is enhanced; e.p.p.s are enlarged (del Castillo and Katz, 1954). The time course of this process is complex and various components, termed "facilitation", "augmentation" and "potentiation", can be distinguished (Zengel and Magleby, 1980, 1981, 1982). For the present purposes these will all be considered under the one term "facilitation". Short term facilitation was examined experimentally by Katz and Miledi (1968), who found that Ca^{2+} appeared to be necessary to the process and suggested that "a residue of the 'active calcium' which enters the terminal axon during the nerve impulse is responsible". The fact that single pulse facilitation could be as much as 9-fold (with low C_e) was explained in terms of cooperativity in Ca^{2+} activation of release. In particular, if n were 4, facilitated release could be as high as 2^4 or 16 times the control. Since that time it has become usual to attribute all components of facilitation to residual Ca^{2+} cooperating with Ca^{2+} brought in by the nerve impulse, acting together at the same Ca^{2+} receptor sites (e.g., Parnas and Segel, 1981). In this section it will be shown that existing data require that with this model n must be at least 4, which is incompatible with experimental data regarding the dependence of transmitter release on external Ca^{2+} (see section 2).

Quantitatively the residual Ca^{2+} hypothesis can be phrased as follows (see Miledi and Thies, 1971; Zengel and Magleby, 1981; Parnas and Segel, 1981). A control nerve impulse injects a set amount of Ca^{2+} into the nerve terminal and transiently increases internal $[Ca^{2+}]$ (C) from the resting level C_0 to $C_0 + E$. Then C gradually declines towards C_0 , but at any time there is a residuum over C_0 , say G . A test nerve impulse now increases C to $C_0 + G + E$. Now, we may rewrite equation (1) as:

$$(5) \quad R = \frac{\lambda' \Delta t}{1 + K'_\lambda / (C_0 + E + G)^n}$$

where R is the amount of transmitter release by a nerve impulse, λ' and K'_λ are the values of L and K_λ appropriate to a nerve impulse (i.e., we admit the possibility that λ may be increased and K_λ may be decreased by the nerve impulse) and Δt is the duration of the relatively high release rate produced by the nerve impulse. At the same time, we have:

$$(6) \quad L = \frac{\lambda}{1 + K_\lambda / (C_0 + G)^n}$$

where L is the frequency of m.e.p.p.s. To evaluate the relative effect of G on R and on L , we consider the limit as G becomes small, i.e., differentiate the above expressions and put G negligible relative to C_0 and E , to obtain:

$$(7) \quad \frac{d \ln R}{dC} = \frac{n}{C_0 + E} \frac{1}{1 + (C_0 + E)^n / K'_\lambda} < \frac{n}{C_0} \frac{1}{1 + C_0^n / K'_\lambda} = \frac{d \ln L}{dC}$$

Thus, a small residue of Ca^{2+} after one impulse or a series of impulses causes a multiplication of R , i.e., facilitation of the e.p.p., which (1) is always less than the multiplication of L (m.e.p.p. frequency) and (2) most closely approaches the same value as the multiplication of L when $(C_0 + E)^n \ll K'_\lambda$, i.e., when $R/\Delta t \ll \lambda'$. In view of the latter result, it is permissible to pursue the argument considering only the case where the internal Ca^{2+} (C) is small relative to K_λ and K'_λ , i.e., $R/\Delta t$ is considerable less than λ' , and (see above) L much less than λ . This corresponds to facilitation observed when e.p.p.s are reduced in amplitude by using low C_e and/or raised external Mg^{2+} , which is the usual experimental situation at the vertebrate end-plate. Then,

$$\begin{aligned} (8a) \quad \text{spontaneous (resting) } F_{m.e.p.p.} &= L_0 = k_L C_0^n \\ (8b) \quad \text{release (e.p.p.) by first impulse} &= R_1 = k_R (C_0 + E)^n \Delta t \\ (8c) \quad F_{m.e.p.p.} \text{ before test impulse} &= L_1 = k_L (C_0 + G)^n \\ (8d) \quad \text{release (e.p.p.) by test impulse} &= R_1 = k_R (C_0 + G + E)^n \Delta t \end{aligned}$$

where k_L is λ/K_λ and k_R is λ'/K'_λ . Again, it is evident that R_1 must be larger than R_1 (i.e., there is facilitation):

$$R_1/R_1 = \frac{(C_0 + E + G)^n}{(C_0 + E)^n} = [1 + G/(C_0 + E)]^n < (1 + G/E)^n$$

Since for single pulse facilitation, G/E is necessarily less than one, the above relation gives 2^n as the maximal possible single pulse facilitation. Moreover, if $E \gg C_0$, R_1/R_1 is dependent only on the ratio of G to E , and not on the absolute magnitude of either. This agrees with the experimental observation that facilitation is indeed essentially independent of external Ca^{2+} , provided R is small and the same external Ca^{2+} is present for conditioning and test nerve impulses (Hubbard, 1963; Rahamimoff, 1968; Katz and Miledi, 1968). However, it should be noted that if n is large, E will not necessarily be much greater than C_0 , and the above equation predicts facilitation that rises with C_e .

In addition, from the above equations,

$$L_1/L_0 = (1 + G/C_0)^n > [1 + G/(C_0 + E)]^n = R_1/R_1$$

In agreement with this (and with equation 7), it has long been recognized that at the neuromuscular junction facilitation of frequency of miniature end-plate potentials is consistently greater than the facilitation of e.p.p.s (Hubbard, 1963; Zengel and Magleby, 1981), i.e., $L_1/L_0 > R_1/R_1$. However, the ratio L_1/L_0 is observed never to be very much larger than R_1/R_1 , and, as will be shown below, this puts an important constraint on the residual Ca^{2+} model for facilitation.

Defining $z = (R_1/\Delta t)/L_0$, one obtains from equations (8a–8d)

$$\begin{aligned} (C_0 + E)/C_0 &= (z \cdot k_L/k_R)^{1/n} = X_1 \\ G/(C_0 + E) &= (R_1/R_1)^{1/n} - 1 = X_2 \end{aligned}$$

and therefore

$$(9) \quad L_1/L_0 = (1 + G/C_0)^n = (1 + X_1 X_2)^n$$

Thus, given k_L/k_R , z , and R_1/R_1 , any specified n will yield a predicted L_1/L_0 , which can be compared with the value observed experimentally. To evaluate k_L/k_R , we note that, in the absence or near absence of external Ca^{2+} (i.e., $C_e = 0$), E is zero and $k_R/k_L = (R/\Delta t)/L$. Experimentally, the latter ratio appears to be near unity when C_e is very low, and is certainly less than 10 (e.g., Cooke *et al.*, 1973). A typical value of z is obtained with $R_1 = 1$, $\Delta t = 1$ ms (Miledi and Thies, 1971) and $L_0 = 1/s$, giving $z \cong 1,000$. Equation 9 now allows a minimum estimate of L_1/L_0 for any given degree of facilitation of R . Invariably, experimental data (e.g., Hubbard, 1963; Miledi and Thies, 1971; Zengel and Magleby, 1981) show values of L_1/L_0 much less than predicted by equation (9), for any observed value of R_1/R_1 , unless n is chosen to be at least 4. For example, data in Hubbard (1963) show, immediately after a train of 10 impulses, $R_1/R_1 = 2.5$ and $L_1/L_0 = 8.5$. With $n = 2$ and $k_L/k_R = 10$, equation 9 gives $L_1/L_0 = 46$, while with $n = 4$ the equation gives $L_1/L_0 = 11$. If $k_R = k_L$, which is most consistent with experimental observations, equation 9 gives, with $n = 2$, $L_1/L_0 = 375$ and with $n = 4$, $L_1/L_0 = 52$. Of course, by arbitrarily increasing n (or k_R/k_L), it is always possible to obtain a predicted value for L_1/L_0 that comes close to that experimentally observed. For the above data of Hubbard (1963), the observed value of L_1/L_0 is obtained with $n = 4.7$, if $k_R/k_L = 10$, and with $n = 7.4$, if $k_R = k_L$. The data are compatible with $n = 4$ if $k_R/k_L = 17.5$, and with $n = 2$ if $k_R/k_L = 92$. Such high values of k_R/k_L would be experimentally obvious as e.p.p.s in the absence of external Ca^{2+} , which evidently do not exist (e.g. Cooke *et al.*, 1973).

The conclusion that n must be at least 4, and probably more, if one is to account for observed R_1/R_1 and L_1/L_0 , on the basis of the Parnas-Segel model, can be drawn more directly from equation (7). That is, given that $R/\Delta t \ll \lambda'$ and $L \ll \lambda$,

$$\frac{d(\log L)/dC}{d(\log R)/dC} = \frac{C_0 + E}{C_0} = \left(\frac{R_1/\Delta t}{L_0} \cdot \frac{k_L}{k_R} \right)^{1/n}$$

Evidently, as one increases the chosen value of n , the e.p.p. is attributed to lower values of E/C_0 , G/C_0 approaches $G/(C_0 + E)$ and the predicted L_1 (i.e., frequency of m.e.p.p.s at the time when facilitated e.p.p.s are recorded) becomes minimized. Thus, the failure of m.e.p.p. frequency to be facilitated much more than the e.p.p. forces choice of high n when one attempts to fit experimental data to the model.

It may also be noted that the exact expression, corresponding to (9), applicable when λ or λ' is not much larger than L_1 , $R_1/\Delta t$, or $R_1/\Delta t$ is obtained by replacing L_1/L_0 by A_1/A_0 and R_1/R_1 by B_1/B_1 , where $A_0 = L_0/(1 - L_0/\lambda)$, $A_1 = L_1/(1 - L_1/\lambda)$,

$B_1 = (R_1/\Delta t)/[1 - (R_1/\Delta t)/\lambda']$, and $B_1 = (R_1/\Delta t)/[1 - (R_1/\Delta t)\lambda]$. Unless L_1 is closer to λ than $R_1/\Delta t$ is to λ' , the predicted value of L_1/L_0 is increased, for any given R_1/R_1 . That is, using the approximation (equation 9) tends to *reduce* predicted L_1/L_0 .

Since L_1/L_1 always remains higher than R_1/R_1 (as far as one can tell from the available data), data can presumably always be accommodated to a residual Ca^{2+} model provided a sufficiently high n is chosen. A high n , of course, also permits high values for single pulse facilitation. It is of course possible to circumvent the requirement that n must be large, to avoid the false prediction of very high m.e.p.p. frequencies (L_1) during facilitation, by postulating that there is a component of L_0 (and L_1) that is independent of intracellular Ca^{2+} . However, any such model predicts a time course of facilitation of spontaneous release, i.e., of L_1 , much briefer than the time course of facilitation of e.p.p.s (i.e., of R_1) — this is contrary to the experimental observations (e.g. Zengel and Magleby, 1981).

Two aspects of the residual calcium model require emphasis: (i) The number n in the model is the minimum number of intracellular Ca^{2+} atoms that *must* cooperate for release of a single quantum (rather than a number that may sometimes cooperate). This arises because the high n is required to *minimize* the expected release by the hypothetical residual Ca^{2+} during facilitation. (ii) The model is independent of how internal Ca^{2+} (C) is governed, and of the time course of G/E . Indeed, it is implicit that for any observed time course of facilitation (and augmentation and potentiation), of either evoked or spontaneous release, any arbitrarily chosen n will dictate an inferred time course for C .

Thus, explanation of observed post-activation changes in evoked and spontaneous release in terms of residual Ca^{2+} and the Parnas-Segel model is possible only if the relation between increment of release rate and increment of internal Ca^{2+} corresponds to a power function with exponent, n , of 4 at the very least and probably 5 or more. This high n is required to account for the observation that facilitation of spontaneous release is not very much greater than facilitation of e.p.p.s. However, such a high value for n is not consistent with the observed dependence of transmitter release on extracellular Ca^{2+} (see section 2). With the model in which the probability of release for each quantum of neurotransmitter is continuously graded with the number of CaX at critical sites and overall transmitter release is therefore exponentially related to local internal CaX (Cooke *et al.*, 1973), a residual Ca^{2+} would produce a multiplication of the e.p.p. (and of m.e.p.p. frequency) that is conspicuously graded with C_e ; at low C_e , facilitation (as R_1/R_1) would be exponentially related to C_e . Since it is clear that this does not generally occur, the "graded probability" model does not allow residual Ca^{2+} as the basis for facilitation, except, perhaps, for very fast components. Experimentally, facilitation appears as a slowly developing and slowly decaying component in the response of $F_{\text{m.e.p.p.}}$ to nerve terminal depolarizations, that becomes relatively prominent in the presence of low Ca^{2+} / high Mg^{2+} , and is very nearly equal as a multiplier of spontaneous release and as a multiplier of the fast, Ca^{2+} — dependent, component of the response (Cooke and Quastel, 1973; Cooke *et al.*, 1973). In terms of the Parnas-Segel model, this could be 'explained' by a cumulative and persistent increase in λ , and/or decrease in K_λ , and/or increase in ϵ .

4. Summary and Discussion

In summary, existing data relating release of neurotransmitter to external Ca^{2+} concentration (C_e) can be fitted to a model in which entry of Ca^{2+} into the nerve terminal is a saturating function of C_e , and a fixed number, n , of Ca^{2+} ions must cooperate (at saturable sites) to induce release of a single quantum of transmitter (Dodge and Rahamimoff, 1967; Cooke *et al.*, 1973; Parnas and Segel, 1981). However, contrary to the conclusion of Parnas and Segel (1981, 1982), the experimental data for the vertebrate end-plate require that with this model $n \approx 2$, and must be less than 3 at this junction. However, when one seeks to account for facilitation on the basis of this model, with residual Ca^{2+} , values of n less than 4 are inconsistent with existing experimental data (Hubbard, 1963; Katz and Miledi, 1968; Miledi and Thies, 1971; Zengel and Magleby, 1981). Thus, one must reject the hypothesis that facilitation reflects simply the summation of the Ca^{2+} that each nerve impulse causes to enter the nerve terminal, with the residue of internal Ca^{2+} left over from one or more conditioning nerve impulses, all this Ca^{2+} "cooperating" at one species of internal "receptor" that mediates transmitter release. These considerations do not exclude the possibility that facilitation may indeed reflect internal Ca^{2+} , which is now known to persist in a nerve terminal for considerable time (Charlton, Smith and Zucker, 1982), acting via a "receptor" distinct from that involved in the rapid release of transmitter that occurs in response to a nerve impulse. Alternatively, or in addition, it may be postulated that persistent effects of nerve terminal depolarization on transmitter release are not all due to opening of voltage-sensitive Ca^{2+} channels and influx of Ca^{2+} (Cooke *et al.*, 1973; Quastel, 1974).

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