A Quantitative Description of Stimulation-induced Changes in Transmitter Release at the Frog Neuromuscular Junction

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ABSTRACT Endplate potentials were recorded from frog sartorius neuromuscular junctions under conditions of greatly reduced quantal contents to develop a quantitative description of stimulation-induced changes in transmitter release. Four general models relating potentiation, augmentation, and the first and second components of facilitation to transmitter release were developed. These models were then tested by incorporating equations for the kinetic properties of the four components of increased transmitter release and examining the ability of the resulting sets of equations to predict stimulation-induced changes in transmitter release. Three of the models were essentially consistent with the observation that augmentation had a multiplicative type relationship to facilitation. These models could also predict the effect of frequency and duration of stimulation on endplate potential (EPP) amplitude during and after prolonged (40 s) trains including the response to step changes in stimulation rate. These models extend by about two orders of magnitude the duration of stimulation-induced changes in transmitter release that can be accounted for, and show that the combined kinetic properties of potentiation, augmentation, and the two components of facilitation are generally sufficient to account for these changes.

INTRODUCTION

This paper concludes a series of studies directed at developing a quantitative description of stimulation-induced changes in evoked transmitter release. The previous studies described the kinetic properties of the four components that act to increase transmitter release: potentiation (Magleby and Zengel, 1975*a*, *b*), augmentation (Magleby and Zengel, 1976*a*, *b*; Zengel and Magleby, 1982), and the first and second components of facilitation (Zengel and Magleby, 1982). In this paper we (*a*) formulate mathematical models relating these components to transmitter release; (*b*) summarize the equations describing the

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J. GEN. PHYSIOL. © The Rockefeller University Press · 0022-1295/82/10/0613/26 $1.00 613
Volume 80 October 1982 613–638

kinetic properties of the components; (*c*) investigate the relationship between augmentation and facilitation; and (*d*) show that the equations can account for stimulation-induced changes in transmitter release. A preliminary report of some of these results has appeared (Zengel and Magleby, 1977*b*).

MODELS FOR STIMULATION-INDUCED CHANGES IN TRANSMITTER RELEASE

Because of the lack of detailed information concerning the events in the nerve terminal that give rise to stimulation-induced changes in transmitter release, the models developed in this section will not be rigidly tied to specific molecular mechanisms. Instead, more general models will be considered that should serve to characterize and investigate the kinetic properties of transmitter release.

Four general models for stimulation-induced changes in transmitter release will be developed in this section.

Joint Probability Hypothesis

If the four components of increased transmitter release reflect changes in different factors in the nerve terminal whose joint action determines the probability of transmitter release, then stimulation-induced changes in transmitter release would be described by:

$$\mathrm{EPP}/\mathrm{EPP}_0 = (F_1 + 1)\,(F_2 + 1)\,(A + 1)\,(P + 1) \qquad \text{(I)}$$

where EPP_0 is the control EPP amplitude in the absence of repetitive stimulation, EPP is the EPP amplitude during or after repetitive stimulation, and F_1, F_2, A, and P are the magnitudes of the first and second components of facilitation, augmentation, and potentiation. EPP, F_1, F_2, A, and P are all functions of time and the pattern of stimulation.

Scheme I is consistent with the observations that potentiation (Landau et al., 1973; Magleby, 1973*a*, *b*) and augmentation (this paper) have a multiplicative effect on facilitation. This scheme is also consistent with the apparent selective effects of Ba on augmentation and Sr on the second component of facilitation (Zengel and Magleby, 1977*a*, 1980), since the components in this scheme can act independently of one another.

A model with at least two multiplicative factors seems particularly attractive because it is consistent with some of the statistical data on transmitter release. Del Castillo and Katz (1954) found that quantal fluctuations in evoked transmitter release could be accounted for by a model in which release, m, was assumed to arise from the product of two factors, n and p. If increases in n and p give rise to different components of increased transmitter release, then a multiplicative relationship between these two components would be appropriate. (See Wernig, 1972, Bennett et al., 1975, McLachlan, 1975, Bennett and Fisher, 1977 for experiments relating the statistical parameters n and p to stimulation-induced changes in transmitter release.)

A model with multiplicative factors would also be consistent with some of the structural aspects of transmitter release. It has been suggested that a

quantum of transmitter is released each time a synaptic vesicle successfully interacts with specific release sites on the inner side of the presynaptic nerve terminal membrane (del Castillo and Katz, 1956; Heuser et al., 1979; Whittaker and Luqmani, 1980). If this hypothesis is correct, then release would be determined by the product of several factors such that:

$$m = k \begin{bmatrix} \text{effective} \\ \text{number of} \\ \text{synaptic} \\ \text{vesicles} \end{bmatrix} \begin{bmatrix} \text{number of} \\ \text{active} \\ \text{release} \\ \text{sites} \end{bmatrix} \begin{bmatrix} \text{probability} \\ \text{of successful} \\ \text{interaction} \end{bmatrix} \quad (1)$$

where m is the number of quanta discharged with each nerve impulse and k is a constant. If increases in the factors in Eq. 1 give rise to two or more of the components of increased transmitter release, then a multiplicative relationship between these components would be appropriate.

Although the relationships between the quantal factors n and p, the factors in Eq. 1, and the factors in scheme I have not been established, the multiplicative factors in the quantal hypothesis and Eq. 1 do provide a possible basis for multiplicative factors in models for transmitter release.

Stimulation-induced changes in transmitter release are associated with the entry and/or accumulation of Ca in the nerve terminal (Katz and Miledi, 1968; Weinreich, 1971; Younkin, 1974; Erulkar and Rahamimoff, 1978; Charlton et al., 1982). Ca could trigger changes in the quantal factors and/or in the factors in Eq. 1 to give rise to the observed stimulation-induced changes in release. Changes in other agents in the nerve terminal, such as Na, might also be expected to affect these factors in some manner (Birks and Cohen, 1968; Atwood et al., 1975; Erulkar and Rahamimoff, 1978; Lev-Tov and Rahamimoff, 1980).

Unitary Hypothesis (Includes Fourth-Power Residual Calcium Hypothesis)

If stimulation-induced changes in transmitter release result from changes in a single factor in the nerve terminal, then the first and second components of facilitation, augmentation, and potentiation would all have to arise from changes in this unitary factor. Changes in transmitter release would then be described by scheme IV, $EPP/EPP_0 = (F_1^* + F_2^* + A^* + P^* + 1)^4$, if there is a fourth-power relationship between the underlying factor and transmitter release and if F_1^*, F_2^*, A^*, and P^* represent the fractional changes in the factor that gives rise to the components of increased transmitter release.

If the underlying unitary factor is Ca or a Ca complex, then scheme IV becomes a statement of the fourth-power residual Ca hypothesis for stimulation-induced changes in transmitter release (Miledi and Thies, 1971; Rahamimoff and Yaari, 1973). According to this hypothesis, Ca enters and accumulates in the nerve terminal during repetitive stimulation (or is released from internal stores). This residual Ca then combines with the Ca that enters with each nerve impulse to increase transmitter release (Katz and Miledi, 1968). The fourth power indicates that four Ca ions or Ca complexes are needed for transmitter release in this model (Dodge and Rahamimoff, 1967). To account

for the four components of transmitter release with this model, Ca would have to be removed with an apparent four-exponential time course. This might occur if Ca were sequestered through four compartments (see Blaustein et al., 1978).

Schemes I and IV can be viewed as two extreme models for stimulation-induced changes in transmitter release. In scheme I, the four components of increased transmitter release arise from four different factors in the nerve terminal, each with fairly simple kinetics. In scheme IV, the components arise from a single factor that has very complex kinetics. Two additional models will be considered that combine features from both these schemes. These models, together with schemes I and IV, are listed below.

$$\mathrm{EPP/EPP_0} = (F_1 + 1)\,(F_2 + 1)\,(A + 1)\,(P + 1) \quad \text{(I)}$$

$$\mathrm{EPP/EPP_0} = (F_1^* + F_2^* + 1)^3\,(A + 1)\,(P + 1) \quad \text{(II)}$$

$$\mathrm{EPP/EPP_0} = (F_1^* + F_2^* + A^* + 1)^3\,(P + 1) \quad \text{(III)}$$

$$\mathrm{EPP/EPP_0} = (F_1^* + F_2^* + A^* + P^* + 1)^4 \quad \text{(IV)}$$

When the underlying factors F_1^*, F_2^*, A^*, and P^*, which give rise to the components of increased transmitter release, are not expressed as a power, then the changes in the underlying factors are the same as the changes in the components, so the components are used directly in the equations.

Scheme II is listed with a third power to be consistent with the experimental examples in the previous paper (Zengel and Magleby, 1982). We have also tested scheme II with fourth and second powers. The fourth power describes the data as well as the third power, whereas the second power describes the data slightly less well. If all four components in schemes I–IV are triggered by Ca, then a second power in scheme II, a third power in scheme III, and a fourth power in scheme IV would be consistent with the observed fourth-power relationship between Ca and transmitter release described by Dodge and Rahamimoff (1967). A fourth-power relationship could also rise by other means so that possible values for the power are not necessarily restricted by this reasoning.

To test schemes I–IV for stimulation-induced changes in transmitter release, it is necessary to incorporate the kinetic properties of each of the four components. The kinetic properties are summarized in the next section.

KINETIC PROPERTIES OF FACILITATION, AUGMENTATION, AND POTENTIATION

Facilitation

The first and second components of facilitation decay with time constants of ~ 60 and 400 ms. The change in the underlying factors, F_1^* and F_2^*, that give rise to these components can be described by

$$dF_1^*/dt = J(t)f_1^* - k_{F_1^*}F_1^*, \qquad J(t) = \begin{cases} 1 \text{ at the time of the} \\ \quad \text{nerve impulse} \\ 0 \text{ at all other times} \end{cases} \quad (2)$$

$$dF_2^*/dt = J(t)f_2^* - k_{F_2^*}F_2^*, \qquad J(t) = \begin{cases} 1 \text{ at the time of the} \\ \quad \text{nerve impulse} \\ 0 \text{ at all other times} \end{cases} \tag{3}$$

where $k_{F_1^*}$ and $k_{F_2^*}$ are the rate constants for removal of F_1^* and F_2^* from their sites of action, $J(t)$ represents a train of unit impulses (delta functions) occurring at an interval of 1/(stimulation rate), and f_1^* and f_2^* are the incremental increases in F_1^* and F_2^* with each impulse (Zengel and Magleby, 1982). Since $J(t)$ is 1 at the time of each nerve impulse and is 0 at all other times, the increments f_1^* and f_2^* are added instantaneously to F_1^* and F_2^*, respectively, at the instant of each nerve impulse. This leads to step increases in F_1^* and F_2^* of magnitude f_1^* and f_2^*. Notice from Eqs. 2 and 3 that F_1^* and F_2^* are functions of time and stimulation rate.

Augmentation

Augmentation decays approximately exponentially with a time constant of ~7 s. The change in the underlying factor, A^*, that gives rise to augmentation can be described by the simultaneous equations

$$dA^*/dt = J(t)a^* - k_{A^*}A^*, \qquad J(t) = \begin{cases} 1 \text{ at the time of the} \\ \quad \text{nerve impulse} \\ 0 \text{ at all other times} \end{cases} \tag{4}$$

$$a^* = a_0^* Z^{JT} \tag{5}$$

where k_A^* is the rate constant for removal of A^*, $J(t)$ represents a train of unit impulses (delta functions) occurring at an interval of 1/(stimulation rate), and a^*, the incremental increase in A^* with each impulse, can increase during repetitive stimulation as described by Eq. 5; a_0^* in this equation is the increment in A^* in the absence of repetitive stimulation (i.e., the increment added by the first impulse in the train), Z is a constant that determines the rate of increase a^* with each impulse, J is the stimulation rate, and T is the duration of stimulation (Zengel and Magleby 1982).

Potentiation

Repetitive stimulation has a dual effect on potentiation, increasing both its magnitude and time course (Rosenthal, 1969; Magleby and Zengel, 1975*a*; Woodson et al., 1978). Potentiation decays approximately exponentially with a time constant that ranges from tens of seconds after a few impulses to minutes after hundreds of conditioning impulses. The decay of potentiation can be described by

$$P = P(T)e^{-t/\tau_P} \tag{6}$$

where P is potentiation t seconds after a conditioning train, $P(T)$ is the initial magnitude of potentiation immediately after a conditioning train of T seconds duration, and τ_P is the time constant of decay of potentiation. The time constant increases with the magnitude of potentiation during repetitive stimulation as

$$\tau_P = \tau_{P_0}e^{P(T)/B} \tag{7}$$

where τ_{P_0} (same as A in Magleby and Zengel, 1975*a*, *b*) and B are constants determined from a semilogarithmic plot of τ_P against $P(T)$.

For the purpose of this paper, the time constant for the decay of potentiation can be viewed as being set to a larger value by each nerve impulse in a train; during the decay of potentiation after the train, the time constant remains essentially fixed at its value at the end of the train. In the period between trains, the factor affecting the time constant of decay of potentiation then returns to its normal state with a time constant of ~130 s. A detailed kinetic analysis of this factor, the time constant factor, is presented in Magleby and Zengel (1976*c*); a similar factor has been described by Woodson et al. (1978). These studies suggest that the underlying mechanisms controlling the magnitude of potentiation are different from those controlling its time constant; the relationship expressed by Eq. 7 (which can be misleading in terms of mechanism) most likely arises because both the magnitude and time constant are a function of the stimulation rate and number of conditioning impulses.

The change in the underlying factor P^* that gives rise to potentiation has been described by

$$dP^*/dt = J(t)p^* - k_{P^*}P^*, \qquad J(t) = \begin{cases} 1 \text{ at the time of the} \\ \quad \text{nerve impulse} \\ 0 \text{ at all other times} \end{cases} \tag{8}$$

where k_{P^*} is the rate constant for removal of P^*, $J(t)$ represents a train of unit impulses (delta functions) occurring at an interval of 1/(stimulation rate), and p^* is the incremental increase in P^* with each impulse (Magleby and Zengel, 1975*a*, *b*).

Our initial study on potentiation (Magleby and Zengel, 1975*a*, *b*) where the model described in Eqs. 6–8 was developed, was done using hand-measured data from experiments in which the response was large enough to measure adequately from film. We have subsequently examined over 40 additional preparations using computer-sampled and -analyzed data. In these additional experiments, we found that potentiation usually increased less rapidly during repetitive stimulation than in our previous study. The later experiments were typically done at lower quantal contents and data were collected for longer periods of time than in the earlier study; this may account for some of the difference in results. It is also possible that we underestimated augmentation and overestimated potentiation in the previous study because of the difficulty in obtaining enough data on film to define adequately the decay of potentiation.

In the later experiments, the observed rate of increase in potentiation during repetitive stimulation was typically less than would be expected on the basis of a simple linear addition model and appeared similar to that observed by McNaughton (1982) in the hippocampus. A reduced response might occur if a partial depression with a time constant of recovery similar to that of potentiation developed during the conditioning train (see Magleby and Zengel, 1976*b*), or if some form of saturation phenomenon developed during the train. It is not known whether one of these or other possible mechanisms

gives rise to a reduced response, but incorporating an equation for possible saturation into our previous model led to an adequate prediction of potentiation in the later experimental data. The equation used was

$$P = \{(P^* + 1)/[(P^*/G) + 1]\} - 1 \quad (9)$$

where G is a constant. Since P appears to decay exponentially (Magleby and Zengel, 1975*a*), P^* would not necessarily decay exponentially if the relationship between P and P^* is described by Eq. 9. In this case, then, k_{P^*} in Eq. 8 would be a function of P^*. In solving Eqs. 6–9 for potentiation, the decay of P^* between impulses and after conditioning trains was not calculated using k_{P^*}, but was calculated from the decay of P as follows: the value of P immediately after each impulse was calculated from P^* with Eq. 9. This value of P was then allowed to decay as described by Eqs. 6 and 7 until just before the next impulse. The value of P^* just before the next impulse was then calculated from P with a rearranged version of Eq. 9. An increment of p^* was then added to the calculated value of P^* at the time of the nerve impulse. The value of P immediately after the nerve impulse was then calculated from the new value of P^* with Eq. 9. P was then allowed to decay again. This process was repeated for each impulse in the train (Magleby and Zengel, 1975*b*).

MODELS OF TRANSMITTER RELEASE

The simultaneous Eqs. 2–9, which describe the kinetic properties of facilitation, augmentation, and potentiation, together with schemes I–IV, which describe the manner in which the components interact, represent formal descriptions of the models of transmitter release to be examined in this paper.

METHODS

The preparation, recording technique, solutions, and data analysis were the same as those in the previous paper (Zengel and Magleby, 1982); recordings of endplate potentials (EPPs) were obtained with a surface electrode (and in a few experiments with intracellular recording) from frog (*Rana pipiens*) sartorius nerve–muscle preparations under conditions of greatly reduced quantal contents obtained by raising the $[Mg^{2+}]$ in the bathing solution to 5 mM and decreasing the $[Ca^{2+}]$ to 0.4–0.6 mM.

Solutions of Equations

The simultaneous Eqs. 2–9, together with the model of transmitter release under consideration (scheme I, II, III, or IV), were solved by numerical methods on a digital computer. A discussion of how this is done for potentiation is presented in Magleby and Zengel (1975*b*). In testing each scheme, the parameters used in the equations were obtained by assuming that the components of transmitter release interact as described by the scheme under examination. Detailed examples of the method of obtaining the parameters used in the calculation of faciliation, augmentation, and potentiation for scheme II are presented in Magleby and Zengel (1975*a*, *b*) and the proceding paper (Zengel and Magleby, 1982). Some details for obtaining the parameters for scheme IV (power model) are given in Zengel and Magleby (1980).

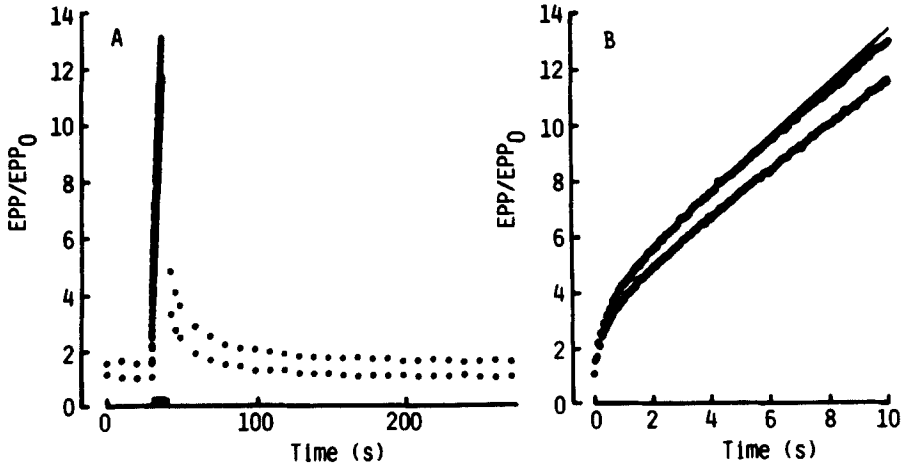

FIGURE 1. Multiplicative relationship between facilitation and augmentation of transmitter release. (A) Plot of EPP amplitudes against time for conditioning-testing stimulation in which the nerve was stimulated with pairs of impulses separated by a 33-ms interval. See text for stimulation details. Each vertical pair of points represents the response to a pair of impulses; the higher point in each case is the second EPP amplitude of the pair. The plotted points for the successive pairs of EPP amplitudes overlap during the train and appear as two thick lines. The plots in this and each of the following figures represent the average response from six or more trials using surface recorded EPPs. (B) EPP amplitudes obtained during the train plotted on an expanded time scale (large dots). Predicted EPP amplitudes calculated with scheme II and Eqs. 2–9 are plotted as small dots. Experimental and calculated EPP amplitudes are superimposed except during the last 3 s of the train, where the predicted amplitudes to the second impulse of each pair are slightly higher than the observed. The parameters used in the calculations were obtained as described for Fig. 3 and were: $p^* = 0.02$, $\tau_{P_0} = 40$ s, $B = 1.08$, $G = 2.04$, $a_0^* = 0.007$, $\tau_{A^*} = 9.8$ s, $Z = 1.0037$, $f_1^* = 0.17$, $\tau_{F_1^*} = 64$ ms, $f_2^* = 0.038$, $\tau_{F_2^*} = 440$ ms. (C) Plot of the difference beween the first and second EPP amplitudes of each pair for the data shown in A divided by the control difference before the conditioning train. The difference increases during the train (indicated by bar on abscissa) and returns to the control level thereafter. Continuous line: calculated difference if there is a multiplicative relationship between augmentation and potentiation as described by schemes I and II. Dotted and dashed line: calculated difference for scheme III. Double-dotted and dashed line: calculated difference for scheme IV (fourth-power residual Ca model). Dashed line: calculated difference if augmentation and facilitation add and have a multiplicative relationship to potentiation as described by Eq. 10. Dotted line: calculated difference if the four components of transmitter release add as described by Eq. 11. All calculations were performed assuming the increments of f_1^* and f_2^* added by each impulse remain constant during and after the conditioning stimulation.

RESULTS

Relationship between Augmentation and Facilitation

Landau et al. (1973) and Magleby (1973b) observed a multiplicative relationship between facilitation and potentiation. We performed the same type of experiment to determine the relationship between facilitation and augmentation. This was done by testing for facilitation with pairs of impulses when augmentation was present. If there is a multiplicative relationship between these two components (scheme I and II), then it can be shown that the difference between the first and second EPP amplitude of each pair, which is mainly caused by facilitation, should be proportional to augmentation. If the factors leading to facilitation and augmentation add and their sum raised to

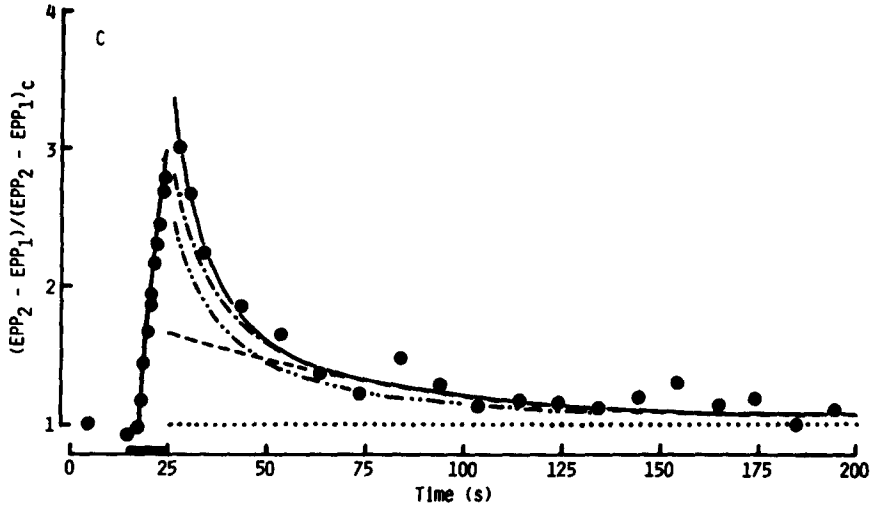

FIG. 1C

a power governs transmitter release (schemes III and IV), it can be shown that the difference between two EPPs of a pair is still highly dependent on the level of augmentation. Finally, if facilitation and augmentation simply add in their net effects, the difference in EPP amplitudes in a pair should be independent of augmentation. This interpretation is based on the assumption that the incremental changes, f_1^* and f_2^*, in the factor(s) that gives rise to facilitation are the same for each impulse in the train.

For the experiment in Fig. 1, the interval between the nerve impulses in each pair used to test for facilitation was 33 ms. The nerve was first stimulated with a pair of impulses every 10 s to establish a control response. The nerve was then conditioned with 100 pairs delivered at a rate of 10 pairs/s, and the effect of the conditioning stimulation was followed by testing with a pair of impulses once every 3 s for 9 s and then once every 10 s for 300 s. The points in Fig. 1A plot EPP amplitude against time for the conditioning-testing trial; Fig. 1B presents a plot on an expanded time scale of the EPP amplitudes

during the train. Each vertical pair of points in these figures represents the response to a pair of impulses; the higher point in each case is the second EPP amplitude of the pair. Notice that both the first and second EPP amplitudes of each pair and the difference between the EPP amplitudes of each pair increase during the train and recover thereafter.

Fig. 1C plots this difference divided by the control difference before the train. The continuous line, which describes the data, is the calculated difference if augmentation and potentiation have a multiplicative effect on facilitation as described by schemes I and II. The value of facilitation used in calculating the difference after the train was obtained directly from the average facilitation of the second EPP amplitude over the first for the pairs before the train. Values of augmentation and potentiation were obtained from the decay of the first EPP amplitudes of each pair after the train. Since ≥3 s separated each pair of impulses after the train, no facilitation was present for the first impulse of each pair and consequently the decay of the first EPP amplitude of each pair indicated the decay of augmentation and potentiation. The continuous line during the train was calculated with scheme II incorporating Eqs. 2–9 as described in the following sections.

The dashed line in Fig. 1C is the calculated difference if facilitation and augmentation have an additive relationship to each other and a multiplicative relationship to potentiation such that

$$\mathrm{EPP}/\mathrm{EPP}_0 = (F_1 + F_2 + A + 1)(P + 1). \tag{10}$$

The dotted line is the calculated difference if facilitation, augmentation, and potentiation all have an additive relationship such that

$$\mathrm{EPP}/\mathrm{EPP}_0 = (F_1 + F_2 + A + P + 1). \tag{11}$$

Clearly, neither of these two models describes the data. Thus, if each impulse adds a constant increment of f_1^* and f_2^*, the assumption used in these calculations, then the models described by Eqs. 10 and 11 can be excluded.

Scheme III in which F_1^* F_2^*, and A^* add and are then expressed as a power underpredicted the difference slightly (dotted and dashed line). Scheme IV, in which all the factors add and are expressed as a power (fourth-power residual Ca hypothesis), underpredicted the difference considerably (double-dotted and dashed line).

In the seven experiments of the type shown in Fig. 1 (the intervals between the impulses of each pair ranged from 33 to 150 ms in these experiments), schemes I and II described the data or overpredicted the difference slightly, scheme III fit or slightly over- or underpredicted the difference, and scheme IV consistently underpredicted the difference, giving worse fits than the other three schemes. It is interesting that scheme III, in which facilitation and augmentation add and are expressed as a power, gives an apparent multiplicative relationship between facilitation and augmentation. The findings in these experiments are consistent then with the data of Landau et al. (1973); although augmentation had not been defined at the time their experiments

were performed, it appears to be present in their data and appears to have a multiplicative relationship to facilitation.

The results of this section suggest that there is a multiplicative relationship between augmentation and facilitation that can be reasonably well described by schemes I–III. Linear relationships as described by Eqs. 10 and 11 did not describe the data in any of our experiments.

Predicting Transmitter Release during Repetitive Stimulation Using Parameters Obtained from the Decay of Testing EPP Amplitudes after the Train

In this and the following sections we examine further whether schemes I–IV can account for stimulation-induced changes in transmitter release. A variety of experiments are presented that, when taken together, provide a rigorous test of the models.

Fig. 2A presents observed and predicted EPP amplitudes during a 200-impulse train delivered at 20 impulses/s. Predicted EPP amplitudes were calculated with scheme II using Eqs. 2–9 for the kinetic properties of potentiation, augmentation, and the two components of facilitation. The parameters used in the equations were obtained from the decay of EPP amplitudes after conditioning trains of 100 and 200 impulses (see below). The predicted rise is an excellent description of the experimental data; the predicted and observed EPP amplitudes are superimposed, except at the end of the train, where the predicted response is slightly higher.

Figs. 2B–F present the calculated increases in potentiation, augmentation, and the two components of facilitation during the train. The plotted values are those at the time of the nerve impulses; the fluctuating response that occurs between nerve impulses (see Zengel and Magleby, 1981, 1982) is not presented. The increase in EPP amplitudes during the first 2 s of the train is mainly caused by facilitation; the increase thereafter is caused by augmentation and potentiation.

It is important to note that the parameters used in the calculation of EPP amplitudes during the train in Fig. 2 were not free but were essentially fixed by the decays of EPP amplitudes after the train. The parameters determining the rate constants of decay of facilitation (τ_{F_1}* and τ_{F_2}*) and augmentation (τ_{A}*) were determined by least-squares fits to the decays of EPP amplitudes after a 200-impulse train as shown in Fig. 1 in Zengel and Magleby (1982). f_1* and f_2* were determined from the zero time intercept of the decays using Eqs. 14 and 15 in Zengel and Magleby (1982). The parameters determining the decay of potentiation (τ_{P_0} and B) were determined from least-squares fits to decays of EPP amplitudes after 100- and 200-impulse trains (Magleby and Zengel, 1975*a*). The parameters a_0*, Z, P*, and G were not fixed precisely by their method of determination from the decays of EPP amplitudes after 100- and 200-impulse trains (see Magleby and Zengel, 1975*a*, *b*; Zengel and Magleby, 1982), but variation of these parameters within the range of uncertainty had small effects (less than about ±10% in total) on predicted EPP amplitude during the first second or two of repetitive stimulation and had little effect on predicted EPP amplitude after ~3 s of stimulation.

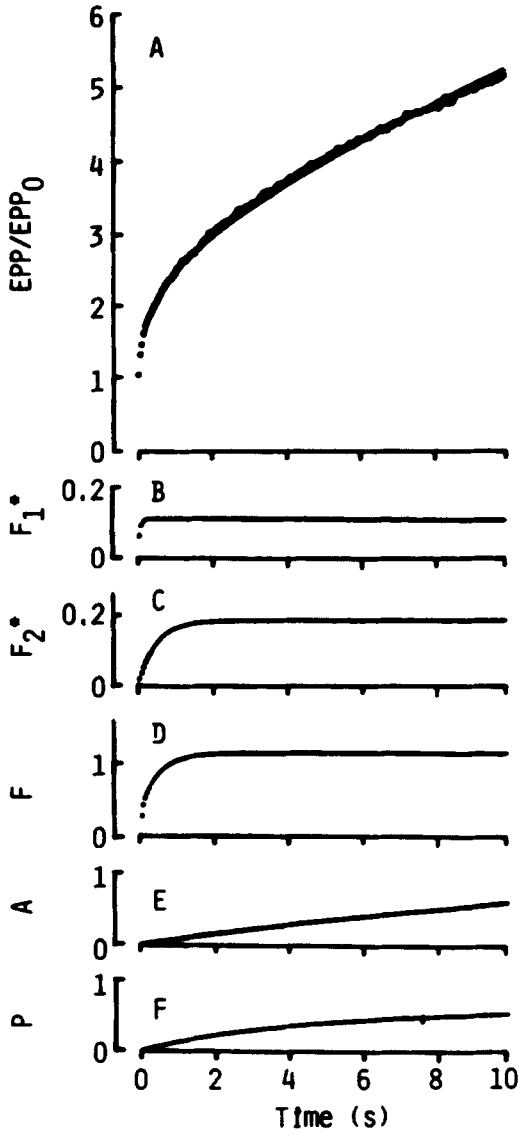

FIGURE 2. Predicting transmitter release during repetitive stimulation using parameters obtained after the train. (A) Plot of observed and predicted EPP amplitudes during a 200-impulse conditioning train delivered at 20 impulses/s. The observed EPP amplitudes are plotted with filled circles that overlap after the first few impulses and appear as a thick line. The predicted amplitudes are plotted with smaller dots that overlap and appear as a fine line. Observed and predicted responses are superimposed except near the end of the train where the predicted response is slightly higher than the observed. The predicted response was calculated with scheme II and Eqs. 2–9. (B–F) Plots of the calculated increase in the factors underlying the predicted response; F_1* and F_2* are the underlying factors that give rise to the first and second components of facilitation, F is the total facilitation resulting from these factors; A is augmentation,

Scheme I predicted the data in Fig. 2A almost as well (not shown) as scheme II. Schemes III and IV described the data less well, overpredicting release 8 and 15 percent after 2 s of stimulation, respectively; the difference between observed and predicted results then decreased until it was negligible by the end of the train (not shown). In testing the different schemes, the parameters used in the equations were obtained by assuming the components of transmitter release interact as described by the scheme under examination.

Similar results were found in four additional experiments of the type shown in Fig. 2, including experiments with 400-impulse trains; schemes I and II predicted EPP amplitudes during the train using parameters obtained from the decay of EPP amplitudes after the train, scheme III gave reasonably good descriptions of the data, and scheme IV gave the worst fits. The agreement between observed and predicted results for schemes I and II was typically similar to that shown in Fig. 2, but in some cases the difference became significant (10–20 percent) for all four transmitter release models. Since scheme II typically gave the best description of the data in these experiments and those to be presented below, further consideration of schemes I, III, and IV will be reserved for a later section.

Predicting Stimulation-induced Changes in Transmitter Release for Low- and High-Response Preparations

The rate of increase and final magnitude of stimulation-induced changes in transmitter release during a conditioning train can vary widely, depending on the state of the preparation (Magleby and Zengel, 1976*b*). Figs. 3A and C plot EPP amplitude during 400-impulse conditioning trains (20 impulses/s) for two preparations that encompass the range of response observed in our experiments. EPP amplitudes increased over 27 times in the high-response preparation (C), whereas they increased only about 10 times in the low-response one (A). (Conditioning–testing trials for the high response preparation are presented in Fig. 2 of Zengel and Magleby, 1982.) The lines, which are reasonably good descriptions of the data, are the predicted EPP amplitudes calculated for each preparation with scheme II and Eqs. 2–9.

Figs. 3B and D present the calculated increases in facilitation, augmentation, and potentiation for both the low- and high-response preparations. The difference in response is mainly due to a much greater increase in augmentation in the high-response preparation.

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and P is potentiation. In this and the following figures the magnitude of the underlying factors is plotted only at the time of the nerve impulses. The fluctuating response in these factors that occurs between nerve impulses is shown in Zengel and Magleby (1982). The parameters used in the calculations were obtained from the decay of EPP amplitudes after repetitive stimulation as described in the text and were: $f_1^* = 0.156$, $\tau_{F_1^*} = 56$ ms, $f_2^* = 0.02$, $\tau_{F_2^*} = 470$ ms, $a_0^* = 0.0038$, $\tau_{A^*} = 7.2$ s, $Z = 1.003$, $p^* = 0.014$, $\tau_{P_0} = 48$ s, $B = 2.3$, $G = 2$.

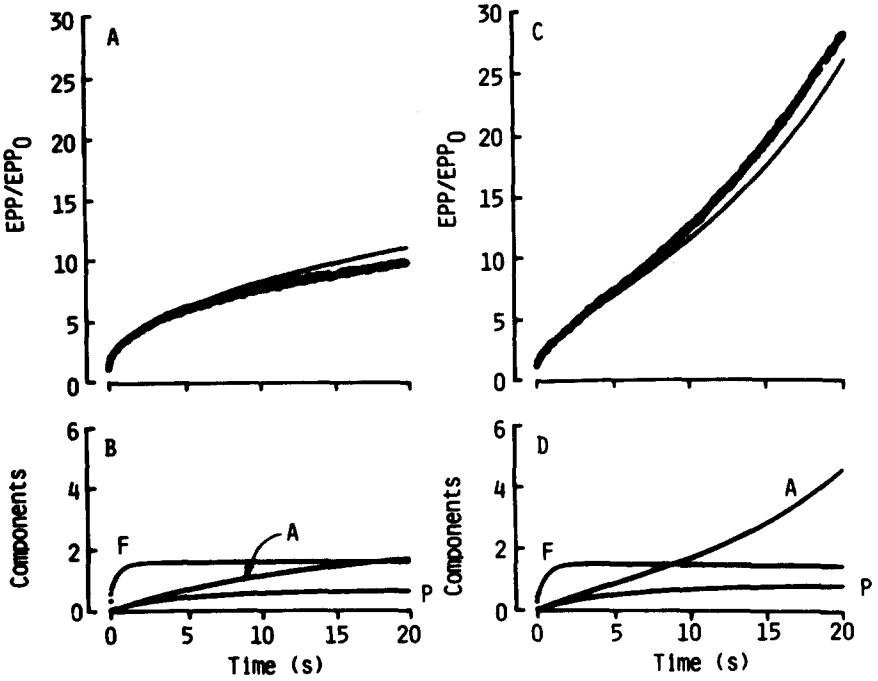

FIGURE 3. Predicting stimulation-induced changes in transmitter release for low- and high-response preparations. (A and C) Plots of observed and predicted EPP amplitudes for two preparations during 400-impulse conditioning trains delivered at 20 impulses/s. The observed EPP amplitudes are plotted with filled circles that overlap and appear as a thick line. The predicted amplitudes are plotted with smaller dots that overlap and appear as a fine line. The predicted response was calculated with scheme II and Eqs. 2–9. (B and D) Calculated increases in facilitation, augmentation, and potentiation underlying the predicted response. The parameters for augmentation and potentiation were determined from the decay of EPP amplitudes after conditioning trains as described in Magleby and Zengel (1976*a*, *b*; Zengel and Magleby, 1982). The facilitation parameters were obtained by numerical solution of scheme II and Eqs. 2–9 to describe the rise of EPP amplitudes during the first second of repetitive stimulation, as described in Zengel and Magleby (1982). The parameters used in the calculations were determined separately for each preparation. For A they were: $f_1^* = 0.17$, $\tau_{F_1^*} = 65$ ms, $f_2^* = 0.023$, $\tau_{F_2^*} = 500$ ms, $a_0^* = 0.008$, $\tau_{A^*} = 8.4$ s, Z = 1.0011, $p^* = 0.018$, $\tau_{P_0} = 30$ s, $B = 0.8$, $G = 1.85$; and for B were: $f_1^* = 0.15$, $\tau_{F_1^*} = 55$ ms, $f_2^* = 0.023$, $\tau_{F_2^*} = 570$ ms, $a_0^* = 0.0095$, $\tau_{A^*} = 5.5$ s, $Z = 1.0048$, $p^* = 0.014$, $\tau_{P_0} = 42$ s, $B = 1.26$, $G = 2.3$.

Predicting the Effect of Stimulation Rate on Transmitter Release

A further test of scheme II would be to determine whether parameters obtained under one set of experimental conditions can predict transmitter release under a different set of conditions. Fig. 4 presents an experiment of this type. EPP amplitudes during 200-impulse conditioning trains delivered at 10 and 20 impulses/s are presented in Fig. 4A. The lines, which are excellent

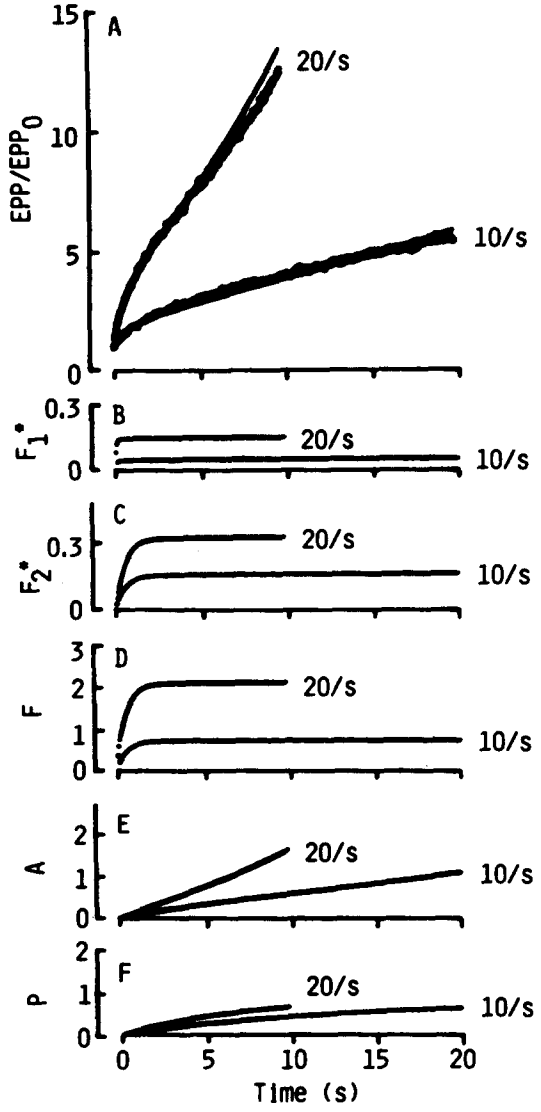

FIGURE 4. Predicting the effect of stimulation rate on transmitter release. (A) Plot of observed and predicted EPP amplitudes during 200-impulse conditioning trains delivered at 10 and 20 impulses/s. The observed (large dots) and predicted (smaller dots) responses are superimposed except at the end of the 20/s train where the predicted response is slightly higher. The predicted response for both trains was calculated with scheme II and Eqs. 2–9 using parameters obtained from conditioning–testing trials with 20/s trains. The parameters were obtained from these trials as described in Fig. 3. (B–F) Calculated increases in the factors underlying the predicted responses for the two stimulation rates. The parameters used in the calculations were: $f_1^* = 0.18$, $\tau_{F_1^*} = 60$ ms, $f_2^* = 0.03$, $\tau_{F_2^*} = 550$ ms, $a_0^* = 0.0075$, $\tau_{A^*} = 6.9$ s, Z = 1.0055, $p^* = 0.011$, $\tau_{P_0} = 86$ s, $B = 7.8$, $G = 2.5$.

descriptions of the experimental data, were calculated with scheme II and Eqs. 2–9 using parameters obtained from the 20/s data. The 10/s data were thus predicted without free parameters.

Figs. 4B–F present the calculated increases in potentiation, augmentation, and the two components of facilitation underlying the increase in EPP amplitudes during the 10 and 20/s conditioning trains. EPP amplitudes increased at a greater rate and reached a higher level during the 20/s stimulation than during the 10/s stimulation because the increments of

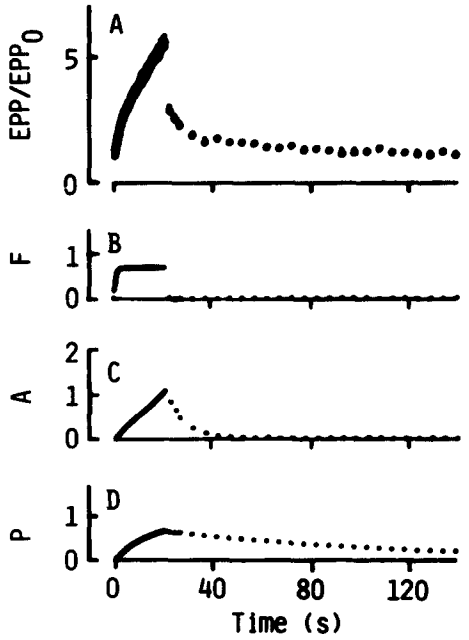

FIGURE 5. Predicting the decay of EPP amplitudes. (A) Plot of observed and predicted EPP amplitudes during and after a 200-impulse conditioning train delivered at 10 impulses/s. The observed response (large dots) and predicted response (smaller dots) are essentially superimposed. The predicted response was calculated with scheme II and Eqs. 2–9 using parameters obtained from 20/s data. (B–D) Calculated changes in the factors underlying the predicted response. Same experiment and parameters as Fig. 4.

potentiation, augmentation, and the two components of facilitation added by each impulse were added at twice the rate for the 20/s stimulation and because there was less time beween impulses at the faster stimulation rate for the components to decay. The two components of facilitation reached higher steady state levels at the faster stimulation rate, and then these higher values remained constant throughout the trains. The effect of the higher stimulation rate on augmentation and potentiation accumulated throughout the trains.

Predicting the Decay of EPP Amplitudes

The larger points in Fig. 5A plot the experimentally observed rise and decay of EPP amplitudes during and after the 10/s train shown in Fig. 4. The

smaller points, which are essentially superimposed on the experimental data, plot the rise and decay calculated with scheme II and Eqs. 2–9 using parameters obtained from the 20/s data. The calculated rise and decay of the underlying components is shown in Figs. 5B–D. Facilitation decays to insignificant levels in the first few seconds after the train; augmentation decays more slowly, and potentiation is slower still.

Similar results were found in 11 additional experiments of the type shown in Figs. 4 and 5, including experiments in which the duration of stimulation was extended to 40 s for the 10/s data. Although the fit between observed and predicted results was not always as close as in these figures (predicted results often started to deviate from the observed after the first half of the conditioning trains, and in a few experiments this deviation reached 20–30 percent of the observed amplitudes by the end of the conditioning trains), the data were typically reasonably well described.

Predicting Transmitter Release during Step Changes in Stimulation Rate

An additional test of the transmitter release models would be to examine their ability to describe step changes in stimulation rate. Since step changes lead to rapid changes in the faster-decaying components, this type of experiment examines whether the models can account for the interactions between the faster- and slower-decaying components. An experiment of this type is shown in Fig. 6. The preparation was presented with three types of stimulation during 200-impulse conditioning trains: constant 20/s stimulation (not shown), alternating step changes in the stimulation rate between 20 and 10 impulses/s (Fig. 6A), and repeated 2-s bursts of stimulation with 2-s latent periods (Fig. 6G). Observed EPP amplitudes are plotted as large dots. Predicted EPP amplitudes calculated with scheme II and Eqs. 2–9 are plotted as small dots. The parameters used in the calculations were obtained from the conditioning–testing trial with the constant 20/s stimulation during the train. For most EPPs the predicted response fell amost exactly on the observed and is not visible. Notice especially that the change in EPP amplitude after each step change in stimulation rate was predicted, without free parameters, using values obtained from the constant stimulation data.

Figs. 6B–F and H–L present the calculated increases in potentiation, augmentation, and the two components of facilitation at the time of each EPP for the two stimulation patterns. The dashed lines in H–L are the calculated decay of the components during the 2-s latent periods. It can be seen that the rapid changes in EPP amplitude with step changes in stimulation rate are caused mainly by rapid changes in facilitation. Augmentation and potentiation changed only slowly during these stimulation patterns. These results support the explanation offered for the differential effects of Ba and Sr on bursts of EPP amplitudes. A comparison of Fig. 6 with Fig. 9 in Zengel and Magleby (1980) shows that the effect of Sr is consistent with a selective increase in the magnitude of the second component of facilitation, whereas the effect of Ba is consistent with a selective increase in the magnitude of augmentation.

Scheme II also predicted EPP amplitudes during step changes in stimulation

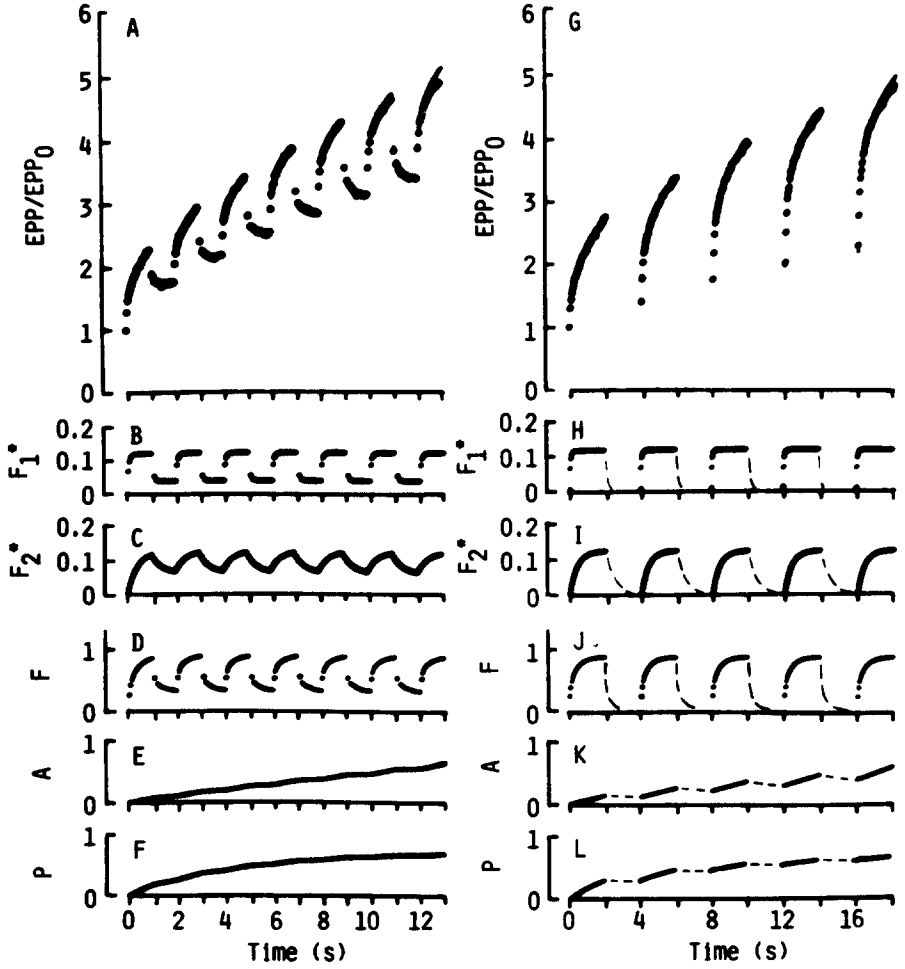

FIGURE 6. Predicting transmitter release during step changes in stimulation rate. (A) Observed and predicted EPP amplitudes during alternating changes in stimulation rate between 20 and 10 impulses/s; an impulse was delivered every 50 ms for 1 s, then every 100 ms for 1 s, then every 50 ms for 1 s, etc., until 200 impulses were delivered. (B–F and H–L) Calculated increases in the factors underlying the predicted response for the data in A and G, respectively. The dashed lines in H–L indicate the decay of the factors between the bursts of stimulation. (G) Observed and predicted EPP amplitudes during repeated 2-s bursts of stimulation at 20/s with 2-s latent periods. In both A and G the predicted (small dots) and observed responses (large dots) are superimposed except at the end of the trains where the predicted response is slightly higher. The predicted response for each train was calculated with scheme II and Eqs. 2–9 using parameters obtained from separate conditioning–testing trials in which the conditioning trains consisted of 20/s stimulation without changes in stimulation rate. The parameters were obtained from these trials as described in Fig. 3. The parameters used in the calculations for both stimulation patterns were: $f_1^* = 0.15$, $\tau_{F_1^*} = 60$ ms, $f_2^* = 0.016$, $\tau_{F_2^*} = 400$ ms, $a_0^* = 0.0033$, $\tau_{A^*} = 9.4$ s, $Z = 1.0045$, $p^* = 0.02$, $\tau_{P_0} = 45$ s, $B = 1.65$, $G = 2.1$.

rate in additional experiments in which a variety of stimulation patterns were used. An example of one such experiment is shown in Fig. 1B where the response to pairs of impulses (33-ms interval) delivered at a rate of 10 pairs/s is predicted almost exactly. The response in these additional experiments was not always predicted as closely as in Figs. 1B and 6, but the predictions did give reasonably good descriptions of the data.

Schemes I and III Describe the Data About as Well as Scheme II

For the experiments presented in the previous sections, scheme I typically predicted EPP amplitude about as well as scheme II, and scheme III predicted the response only slightly less well. For example, for the experiment in Fig. 6, scheme III underpredicted the step changes in EPP amplitude ~10 percent during the conditioning trains compared with the smaller overprediction with scheme II. The predicted responses with schemes I and III have not been presented because the difference in response among schemes I–III was sufficiently small that it could only be observed if the results were superimposed. Although the expected differences in response between these schemes are small, they can be emphasized with some experiments (Fig. 1) and may be useful in separating the schemes when more is known about the underlying assumptions.

Alternative Assumptions for Transmitter Release Models

If we assumed that the saturation aspect of potentiation described by Eq. 9 applied only to P^* and not to the other components, then scheme IV described the data in a qualitative manner that underpredicted the step changes in EPP amplitude ~20 percent by the end of the trains in Fig. 6. When the saturation term was applied to all four underlying factors in scheme IV before the power was calculated (to be consistent with the fourth-power residual Ca model, where saturation might be expected to apply to all four terms), then the data were markedly underpredicted.

The selective saturation version of scheme IV could be made to describe the data if we made the additional assumption that the increments of one or both components of facilitation added by each impulse, f_1^* and f_2^*, increased with repetitive stimulation. However, the rate of increase required to describe the data was typically less than that described by Z for augmentation (Eq. 5), and therefore, there seemed little basis for the assumption at the time; a residual Ca model might suggest about the same accelerating increases in the increments of all four components.

The results in this section suggest that fourth-power residual Ca models can be constructed that will describe stimulation-induced changes in EPP amplitude if the appropriate assumptions are made. Some of these assumptions appear to have little apparent basis at this time, but if support arises, then this type of model can be explored in greater detail.

DISCUSSION

The results presented here show that the transmitter release models described by schemes I–III, when taken together with the kinetic properties of potentia-

tion, augmentation, and the two components of facilitation (Eqs. 2–9), describe stimulation-induced changes in transmitter release at the frog neuromuscular junction under the low quantal content conditions of our experiments. These models predict with reasonable accuracy the effects of frequency and duration of stimulation as well as the effects of step changes in the stimulation rate on transmitter release, and they do so for extended periods of stimulation. The equations thus extend by about two orders of magnitude (when compared with the pioneering studies of Mallart and Martin [1967]), the duration of stimulation-induced changes in transmitter release that can be described. In most cases, the parameters used to solve the equations were obtained under one set of experimental conditions and then used to predict transmitter release under a different set; the equations could thus predict changes in transmitter release without free parameters.

Although the equations are consistent with plausible general mechanisms (see the Introduction), the agreement between observed and predicted results does not, of course, establish that any of the considered mechanisms are correct. What we feel is established is that the combined kinetic properties of potentiation, augmentation, and the two components of facilitation are sufficient to account for stimulation-induced changes in transmitter release. Each nerve impulse adds an incremental increase to each component, the components decay with approximate first-order kinetics with characteristic time constants, and there is a multiplicative relationship among the components.

Limitations of Equations

Although the equations could account for long trains (40 s) of low-frequency stimulation (5–20 impulses/s) and short trains (100 ms) of high-frequency stimulation (100 impulses/s), we did not apply stimulation rates >20 impulses/s for extended durations because the response was not reproducible under these conditions. The equations do not account for stimulation-induced changes in miniature endplate potential (MEPP) frequency, but can be expanded to approximate MEPP frequency (see below). The equations also do not account for depression of transmitter release (Takeuchi, 1958; Thies, 1965) that can occur at higher quantal contents than used in our experiments. However, since all four components of increased transmitter release are present at normal quantal contents (Pallotta and Magleby, 1979), the equations may be of some use to extend the studies of Mallart and Martin (1978) and Betz (1970) on the relationship between depression and stimulation-induced increases in transmitter release.

Applicability to Other Synapses

Components of increased transmitter release are a consistent feature of synaptic transmission at a variety of synapses (Martin and Pilar, 1964; Kuno, 1964; Porter, 1970; Woodson et al., 1978; Charlton and Bittner, 1978; Zengel et al., 1980; McNaughton, 1980; Ohmori et al., 1981), which suggests that the equations may have some general applicability. However it should be noted that the parameters used in the equations will most likely be different for

different synapses; f_1^* and f_2^* are about twice as large in the toad as in the frog (Zengel and Magleby, 1982), and f_2^* and a_0^* are about 10 times larger in the mammalian sympathetic ganglion than in the frog (Zengel et al., 1980). Hence, the relative contributions of the different components to increasing transmitter release during and after repetitive stimulation will have to be determined for each synapse.

An additional component will have to be added to the equations to account for long-term potentiation, a process in the hippocampus (Bliss and Lomo, 1973; McNaughton, 1982) and sympathetic ganglion (Brown and McAfee, 1982) that leads to increases in transmitter release that last for hours and appear to be different from facilitation, augmentation, and potentiation.

Multiplicative Relationship among the Components

The observed multiplicative relationship among the four components of increased transmitter release could arise because of a true multiplicative relationship as described by scheme I. An important finding of this study is that an approximate multiplicative relationship among components could also arise if the underlying factors that give rise to the components sum linearly and are then taken to a power. Schemes II–IV incorporate to various degrees this feature, which is a characteristic of fourth-power residual Ca models for stimulation-induced changes in transmitter release. Only when the observed and predicted results were superimposed on the same plot did distinctions between the transmitter release models described by schemes I–III become readily apparent. It is rather interesting that such conceptually different models can lead to rather similar kinetics for EPP amplitude. The kinetics of stimulation-induced changes in MEPP frequency can be markedly different for the different schemes, however (see below).

Alternative Interpretation for G in Eq. 9

It is possible that the apparent saturation parameter, G, used in Eq. 9 may be describing depression phenomena rather than saturation. The method used to estimate the various components would make a slight masked depression appear as a decrease in the magnitude of potentiation (see Magleby and Zengel, 1976*b*). If this is the case, then the observed and predicted curves for potentiation should be viewed as reflecting both potentiation and the interaction of depression on transmitter release. The depression phenomena would also act on the other components in schemes I–III because of the multiplicative relationships among the components in these schemes.

Which Model?

Our results were not described by models that assumed linear relationships between the components of increased transmitter release (Eqs. 10 and 11). They were described by models in which there were multiplicative relationships or in which the underlying factors that give rise to the components add and are expressed to a power. The results were best described by scheme II, which incorporates both multiplicative and power features. Schemes I and III

described the data almost as well as scheme II, with scheme I giving slightly better fits than scheme III. Scheme IV (with selective saturation of P) gave qualitative, if not quantitative, descriptions of the data.

These conclusions apply to evoked quantal transmitter release that gives rise to EPPs. If the residual Ca hypothesis *as described by scheme IV* is the basis for all stimulation-induced increases in transmitter release, then it might be expected that scheme IV should also give an approximate description of stimulation-induced changes in MEPP frequency because this scheme is based on general assumptions that would be expected to apply to both types of release. Scheme IV, however, predicts that MEPP frequency should increase about 10 times more than is observed and decay 3–4 times slower than is observed (Zengel and Magleby, 1981). Ca, which has presumably entered the nerve terminal by liposomes, also has less of an effect on MEPP frequency than might be expected on the basis of simple residual Ca model (Rahamimoff et al., 1978; Kharasch et al., 1981).

The differential effects of extended stimulation and time on the components of transmitter release are also not easily explained by scheme IV (Magleby and Zengel, 1976*b*), nor are the differential effects of Ba and Sr (Zengel and Magleby, 1980). A further argument against scheme IV comes from the suggestion of Erulkar and Rahamimoff (1978) and Lev-Tov and Rahamimoff (1980) that augmentation of MEPP frequency is dependent on entry of extracellular Ca into the nerve terminal, whereas potentiation of MEPP frequency is not. These authors suggest that potentiation is Na dependent with Na acting through Ca. Consistent with the idea that several factors affect potentiation, Magleby and Zengel (1976*c*) and Woodson et al. (1978) have presented evidence that different factors control the magnitude and decay rates of potentiation.

Some of the difficulties with scheme IV would also apply to some extent to schemes I–III if it is assumed that one or more of the factors F_1^*, F_2^*, A^*, and P^* represent residual Ca. Thus, there does appear to be somewhat of a paradox between the attractively simple residual Ca hypothesis for stimulation-induced changes in transmitter release and the properties of stimulation-induced changes in EPP amplitude and MEPP frequency. This may result because stimulation-induced changes in transmitter release arise from several factors acting together to increase transmitter release, as is the case for schemes I—III, or because one or more of the assumptions used to formulate and test scheme IV, are incorrect. Perhaps other ions such as Mg accumulate and act at the release sites (Andreu and Barrett, 1980; Karasch et al., 1981).

Stimulation-induced Changes in MEPP Frequency

If the restrictions of the residual Ca hypothesis are ignored, that is, if F_1^*, F_2^*, A^*, and P^* do not represent residual Ca in the classical sense, then it is a simple matter to expand the transmitter release models so that they can approximate both MEPP frequency and EPP amplitude. This can be done by making the probability of transmitter release much higher at the time of

the nerve impulse than between nerve impulses. For scheme I,

$$\text{Release} = K(F_1 + 1)(F_2 + 1)(A + 1)(P + 1) \qquad \text{(IA)}$$

where release is expressed in quanta/s, K = 1,000 quanta/s for a 1-ms period at the time of each action potential, and K = 1 quanta/s in the absence of nerve impulses (see Miledi and Thies, 1971; Zengel and Magleby, 1981). These values of K apply under the conditions of our experiments where in the absence of repetitive stimulation quantal content was ~1 and resting MEPP frequency was ~1/s. During repetitive stimulation, both MEPP frequency and EPP amplitude would increase in a similar manner with scheme IA, but the rate of asynchronous release (MEPP frequency) would always be about 1,000 times less than the rate of synchronous release at the time of the endplate potential. This is similar to what is observed experimentally (Zengel and Magleby, 1981). The transient increase in K at the time of the nerve impulse could arise from the transient increase in Ca at the release sites at the time of the nerve impulse (Miledi and Thies, 1971). Similar equations could be written for schemes II–IV.

Although scheme IA could account for stimulation-induced changes in EPP amplitude and roughly approximates stimulation-induced changes in MEPP frequency, it should be viewed as only a starting point with which to study the relationship between these two types of quantal transmitter release. This equation would not be able to account for stimulation-induced changes in MEPP frequency in the presence of Ba or Sr where increases in MEPP frequency are many times greater than increases in EPP amplitude (Zengel and Magleby 1981).

Conclusion

The equations presented here describe the kinetic properties of stimulation-induced changes in transmitter release. Not until all of these properties are accounted for in terms of specific underlying mechanisms will the dynamic properties of transmitter release be understood. These equations should provide a means with which to explore mechanisms by providing a framework with which to interpret experimental results.

This work was supported by National Institutes of Health grant NS 10277.

Received for publication 8 October 1981 and in revised form 24 May 1982.

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