Differential Effects of Ba\(^{2+}\), Sr\(^{2+}\), and Ca\(^{2+}\) on Stimulation-induced Changes in Transmitter Release at the Frog Neuromuscular Junction

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ABSTRACT Endplate potentials (EPP) were recorded from the frog sartorius neuromuscular junction under conditions of low quantal content to study the effect of Ba\(^{2+}\), Sr\(^{2+}\), and Ca\(^{2+}\) on the changes in evoked transmitter release that occur during and after repetitive stimulation. The addition of 0.1–1 mM Ba\(^{2+}\) or Sr\(^{2+}\) to the Ca\(^{2+}\)-containing bathing solution, or the replacement of Ca\(^{2+}\) with 0.8–1.4 mM Sr\(^{2+}\), led to a greater increase in EPP amplitudes during and immediately after repetitive stimulation. These changes in release were analyzed in terms of the four apparent components of increased transmitter release that have previously been distinguished on the basis of their kinetic properties. The Ba\(^{2+}\)-induced increase in EPP amplitudes was associated with an increase in the magnitude but not the time constant of decay of augmentation. Ba\(^{2+}\) had little effect on potentiation or the first and second components of facilitation. The Sr\(^{2+}\)-induced increase in EPP amplitudes was associated with an increase in the magnitude and the time constant of decay of the second component of facilitation. Sr\(^{2+}\) had little effect on potentiation, augmentation, or the first component of facilitation. The selective effects of Ba\(^{2+}\) on augmentation and of Sr\(^{2+}\) on the second component of facilitation were reversible and could be obtained in the presence of the other ion. The addition of 0.1–0.3 mM Ca\(^{2+}\) to the bathing solution had little effect on potentiation, augmentation, or the two components of facilitation. These results provide pharmacological support for the proposal that there are four different components of increased transmitter release associated with repetitive stimulation and suggest that the underlying factors in the nerve terminal that give rise to these components can act somewhat independently of one another.

INTRODUCTION

The amount of transmitter released from a synapse by each nerve impulse varies as a function of previous synaptic activity (del Castillo and Katz, 1954 b; Liley, 1956; Hubbard, 1963). When quantal content is decreased by increasing Mg\(^{2+}\) or decreasing Ca\(^{2+}\) levels in the bathing solution, repetitive stimulation leads to a progressive increase in evoked transmitter release.
Testing impulses applied after a train of conditioning impulses show that the increase in evoked transmitter release decays back to the control level with a multieponential time-course. These changes in evoked transmitter release have been separated into components on the basis of differences in kinetic properties and time constants of decay of the components. The components are: first and second components of facilitation, which decay with time constants of ~50 and 300 ms, respectively (Mallart and Martin, 1967; Magleby, 1973a; Younkin, 1974); augmentation, which decays with a time constant of ~7 s (Magleby and Zengel, 1976a and b; Erulkar and Rahamimoff, 1978); and potentiation, which decays with a time constant that ranges from tens of seconds to minutes (Rosenthal, 1969; Magleby, 1973b; Magleby and Zengel, 1975a and b; Barondes et al., 1977). The factors in the nerve terminal that give rise to these four components are not known, but marked differences in some of their kinetic properties suggest that there are some differences in underlying mechanisms (Landau et al., 1973; Magleby, 1973b; Magleby and Zengel, 1976b and c; Erulkar and Rahamimoff, 1978). If it could be established that these components, which were initially distinguished on the basis of their kinetic properties, are also affected differentially by pharmacological agents, further support would be lent to the suggestion that the components are separable on the basis of differences in their underlying mechanisms. In this paper we show that Ba$^{2+}$ and Sr$^{2+}$, two ions that enter the nerve terminal with depolarization, as Ca$^{2+}$ does (Katz and Miledi, 1969), have different effects on the components of transmitter release. Sr$^{2+}$ increases the magnitude and time-course of the second component of facilitation, whereas Ba$^{2+}$ increases the magnitude of augmentation. These effects provide a new way to distinguish among the components and suggest that the underlying factors in the nerve terminal that give rise to these components are separable and that they can act relatively independently of one another. A preliminary report of some of this work has appeared (Zengel and Magleby, 1977a).

In the following paper (Zengel et al., 1980) we use the differential effects of Ba$^{2+}$ and Sr$^{2+}$ to identify the components of increased transmitter release in the rabbit superior cervical ganglion.

**METHODS**

**Experimental Procedure**

For most experiments, extracellular endplate potentials (EPP) were recorded with a surface electrode from endplate regions of frog (*Rana pipiens*) sartorius nerve-muscle preparation. In a few experiments intracellular EPPs and miniature EPPs were recorded using conventional microelectrode techniques. The standard bathing solution had the composition (mM): NaCl, 115; KCl, 2; CaCl$_2$, 1.8; Na$_2$HPO$_4$, 2.16; NaH$_2$PO$_4$, 0.85; glucose, 5; choline, 0.03. This solution was modified by reducing the Ca$^{2+}$ concentration to 0.3–0.8 mM and adding 5 mM Mg$^{2+}$ to greatly decrease transmitter release. Osmolarity was maintained by changing the NaCl concentration. The term "Ca$^{2+}$ Ringer" will be used to refer to the low-Ca$^{2+}$-high-Mg$^{2+}$ Ringer's bathing solution. "Ca$^{2+}$ + Ba$^{2+}$ Ringer" refers to Ca$^{2+}$ Ringer plus the specified
amount of Ba$^{2+}$. "Ca$^{2+} + Sr^{2+}$ Ringer" refers to Ca$^{2+}$ Ringer plus the specified amount of Sr$^{2+}$. "Ba$^{2+}$ Ringer" refers to Ca$^{2+}$ Ringer with the Ca$^{2+}$ replaced by the specified amount of Ba$^{2+}$. "Sr$^{2+}$ Ringer" refers to Ca$^{2+}$ Ringer with the Ca$^{2+}$ replaced by the specified amount of Sr$^{2+}$. The pH of all solutions was adjusted to 7.2-7.4. Under the low quantal content conditions created by these bathing solutions, extracellular recording gives a good measure of average intracellular activity and greatly reduces the number of conditioning-testing trials required to determine the underlying response (Mallart and Martin, 1967; Magleby, 1973 a; Magleby and Zengel, 1976 a). All experiments were performed at 20°C.

A PDP-11 computer (Digital Equipment Corp., Maynard, Mass.) was used to generate the stimulation patterns, measure and store EPP amplitudes during the experiment, and analyze data. The intervals between successive EPPs during the conditioning trains and in the conditioning-testing periods were sufficiently large that EPPs did not fall on the tails of previous EPPs. Thus, EPP amplitudes could be estimated by measuring from the projected baseline, which was determined just before each nerve stimulus, to the peak of the resulting EPP. Details of the stimulating, recording, and data analysis techniques can be found in Magleby (1973 a) and Magleby and Zengel (1976 a) and will be only briefly presented here as is necessary in the discussion of the new types of experiments.

In the basic experiment described in this paper we determined the effects of various ions on transmitter release (measured as changes in EPP amplitudes) during and after repetitive stimulation. Data were collected and analyzed in terms of trials consisting of control, conditioning, and testing impulses. Details of the various conditioning-testing trials are presented in the text and figure legends. Because of the low levels of transmitter release, the quantal fluctuations (del Castillo and Katz, 1954 a), even with surface recording, were often considerable. Therefore, it was usually necessary, depending on the type of experiment, to average data from five to several hundred identical conditioning-testing trials to smooth the fluctuations in the response. In general, 100-300-impulse conditioning trains were presented once every 5-8 min, whereas 10-impulse conditioning trains were presented once every 30-60 s. Under these conditions the testing EPP amplitudes after the trains returned to the control level before the next train was delivered.

Because the transmitter release properties of a preparation can change with successive trials and time (Magleby and Zengel, 1976 b), the experiments were designed to control for possible drift in the properties of the preparation. For example, in a typical experiment in which the effects of the addition of a small amount of Ba$^{2+}$ to the bathing solution (Figs. 1-4, and 9 B) were examined, a series of conditioning-testing trials was first collected in Ca$^{2+}$ Ringer. This solution was then replaced with Ca$^{2+} + Ba^{2+}$ Ringer and an identical series of conditioning-testing trials was collected. The Ca$^{2+} + Ba^{2+}$ Ringer was then replaced with Ca$^{2+}$ Ringer and additional conditioning-testing trials were collected. (When changing solutions we usually emptied the preparation chamber before adding the new solution, repeating this procedure 2-5 times over a 5-15-min period.) The trials collected during the two periods in Ca$^{2+}$ Ringer were then usually averaged (Fig. 1 A) for comparison with the data collected in the Ca$^{2+} + Ba^{2+}$ Ringer trials (Fig. 1 B). Data from trials collected during the first 5-50 min after the solution change were excluded from the averages to give sufficient time for the preparation to approach a consistent response in the new solution. Similar experimental procedures were used to investigate the effects of Sr$^{2+}$ (Figs. 6-9 A) and various concentrations of Ca$^{2+}$ (Fig. 11). In additional experiments for each ion, the sequence of presentation of the solutions was reversed (B-A-B instead of A-B-A) or extended (A-B-A-B-A), with similar results.
Analysis of Data

The first component of facilitation, the second component of facilitation, augmentation, and potentiation are all defined in a similar manner: each is given by the fractional increase in a test EPP amplitude over a control when the other processes equal 0, such that

\[
\frac{F_1(t)}{P(t)} = 0 \quad A(t) = 0 \quad P(t) = 0
\]

\[
\frac{F_2(t)}{P(t)} = 0 \quad A(t) = 0 \quad P(t) = 0
\]

\[
\frac{A(t)}{P(t)} = 0 \quad F_1(t) = 0 \quad P(t) = 0
\]

\[
\frac{P(t)}{P(t)} = 0 \quad F_1(t) = 0 \quad F_2(t) = 0 \quad A(t) = 0
\]

where \(F_1(t)\) and \(F_2(t)\) are the first and second components of facilitation, \(A(t)\) is augmentation, \(P(t)\) is potentiation, \(v(t)\) is the EPP amplitude at time \(t\), and \(v_0\) is a control EPP amplitude when \(F_1(t)\), \(F_2(t)\), \(A(t)\), and \(P(t)\) all equal 0. Experimentally, however, it is not always possible to measure one process in the absence of the others. Consequently, the magnitude and time-course of the individual processes are derived from the fractional change in EPP amplitude, which is given by

\[
V(t) = \frac{v(t)}{v_0} - 1,
\]

where \(V(t)\) is the fractional change in a testing EPP amplitude at time \(t\) compared with a control EPP amplitude.

The experimentally determined estimation of the contribution that facilitation, augmentation, and potentiation make to increasing transmitter release during repetitive stimulation relies on the fact that these processes have distinct and nonoverlapping time constants which characterize their decays. Facilitation decays with time constants of \(\sim 50\) and \(300\) ms (Mallart and Martin, 1967), augmentation decays with a time constant of \(\sim 7\) s (Magleby and Zengel, 1976 a and b), and potentiation (posttetanic potentiation) decays with a time constant that ranges from \(\sim 30\) s after a few impulses to minutes after thousands of impulses (Rosenthal, 1969; Magleby and Zengel, 1975 a and b). After a conditioning train, the first component of facilitation decays to insignificant levels in \(\sim 200\) ms, the second component decays in \(\sim 2\) sec, and augmentation decays in \(\sim 20\) s. Potentiation can then be estimated in the absence of the faster decaying components from the data points collected after facilitation and augmentation have decayed. Because augmentation and the two components of facilitation fall on one or more slower decaying components, these faster decaying components can only be estimated in terms of a model for transmitter release that defines the relationship between the appropriate components and transmitter release. Since the specific mechanism of transmitter release is not known, the data have been analyzed in terms of three general models to determine to what extent the findings of this study are generally applicable and to what extent they are model dependent. The
rationale for the selected models will be presented in Results. The three models are:

**additive model**

\[
V(t) = F_1^*(t) + F_2^*(t) + A^*(t) + P^*(t),
\]

**multiplicative model**

\[
V(t) = \left[ F_1^*(t) + F_2^*(t) + 1 \right] \left[ A^*(t) + 1 \right] \left[ P^*(t) + 1 \right],
\]

**power model**

\[
V(t) = \left[ F_1^*(t) + F_2^*(t) + A^*(t) + P^*(t) + 1 \right]^4 - 1,
\]

where \( V(t) \) is the fractional increase in transmitter release as defined by Eq. 5, and \( F_1^*(t), F_2^*(t), A^*(t), \) and \( P^*(t) \) represent the fractional changes in the underlying factor(s) in the nerve terminal that give rise to \( F_1(t), F_2(t), A(t), \) and \( P(t) \). Since it is the properties of the underlying factors that must be defined to describe the kinetics and understand the mechanism of transmitter release, we estimate and plot \( F_1^*(t), F_2^*(t), A^*(t), \) and \( P^*(t) \) in this paper. It should be noted that previous studies have also made distinctions between the observed response and the proposed underlying factor(s) referred to as \( Ca^* \) (Miledi and Thies, 1971), \( Y \) (Barrett and Stevens, 1972), \( CaX \) (Rahamimoff and Yaari, 1973; Zucker, 1974; Younkin, 1974), or \( R^*(t) \) (Magleby and Zengel, 1975). For the additive model, the numerical values of the observed components and underlying factors are the same, so it is not necessary to distinguish between them (Mallart and Martin, 1967; Magleby, 1973; Magleby and Zengel, 1976).

**Estimating \( F_1^*(t), F_2^*(t), A^*(t), \) and \( P^*(t) \)**

**ADDITIVE MODEL** Testing EPP amplitudes expressed as \( V(t) \) are plotted semilogarithmically against time, and \( P^*(t) \) is estimated by a least squares fit to the data points beyond about 30 or 40 s after the ends of the conditioning trains (lines in Fig. 1 C and D). The intercept of the lines with the ordinate gives \( P^*(T) \), the initial magnitude of potentiation at the end of the conditioning trains of \( T \) s duration. The time constant of decay of \( P^*(t) \), \( \tau_{P^*} \), is given by the time required for \( P^*(t) \) to fall to \( 1/e \) of its initial value. The data are then corrected for the contribution from potentiation by subtracting \( P^*(t) \) (see Magleby and Zengel, 1976). \( A^*(t) \) is then estimated from semilogarithmic least squares fits to the corrected data points falling between about 1.5 and 20 s (Fig. 1 F). The data are then corrected for the contribution from augmentation by subtracting \( A^*(t) \), and \( F_2^*(t) \) is estimated from semilogarithmic fits to the data points falling between about 200 and 1,000 ms. Finally, the data are corrected for the contribution from the second component of facilitation by subtracting \( F_2^*(t) \), and \( F_1^*(t) \) is estimated from semilogarithmic fits to the data points falling between about 30 and 150 ms (Fig. 3 D).

**MULTIPLICATIVE MODEL** Estimates of \( P^*(t) \) are obtained as described for the additive model. Once estimates of \( P^*(t) \) are made, values of \( A^*(t) \) can be obtained by rearranging Eq. 7 and deleting the facilitation terms to obtain \( A^*(t) = \frac{V(t) + 1}{[P^*(t) + 1]} - 1 \). \( A^*(T) \) and \( \tau_{A^*} \) are then estimated from semilogarithmic least squares fits to the corrected data points falling between about 1.5 and 20 s. Once estimates of \( A^*(t) \) and \( P^*(t) \) are made, the data points describing \( F_1^*(t) \) and \( F_2^*(t) \) can be obtained by rearranging Eq. 7 to \( F_1^*(t) + F_2^*(t) = \frac{V(t) + 1}{[A^*(t) + 1][P^*(t) + 1]} - 1 \). These points are plotted and \( F_1^*(t) \) and \( F_2^*(t) \) are estimated from semilogarithmic fits to the data points as shown in Fig. 3 E.
POWER MODEL. The method of estimating $P^*(t)$, $A^*(t)$, $F_2^*(t)$ and $F_1^*(t)$ is similar to that for the additive model, except that the successive subtraction of the exponentials describing each of the components was carried out on the fourth root of the data points obtained by rearranging Eq. 8 to

$$F_1^*(t) + F_2^*(t) + A^*(t) + P^*(t) = [V(t) + 1]^{1/4} - 1.$$

Notice from Eqs. 6 and 7 that estimates of potentiation obtained with either the additive or multiplicative models are identical. Unless otherwise indicated, estimates of potentiation presented in this paper are those that would be obtained with these models and estimates of augmentation are those that would be obtained with the multiplicative model.

Data are presented as mean ± SD.

RESULTS

Ba$^{2+}$ Increases EPP Amplitudes during and after Repetitive Stimulation

The addition of small amounts of Ba$^{2+}$ to the solution bathing the neuromuscular junction leads to a greater increase in EPP amplitudes during and after repetitive stimulation but has little effect on EPP amplitudes in the absence of repetitive stimulation. This is shown in Fig. 1 A and B, which presents plots of EPP amplitudes against time for conditioning-testing trials from the same preparation in the absence and presence of 0.15 mM Ba$^{2+}$. For each conditioning-testing trial, the nerve was first stimulated once every 5 s to establish a control response. The nerve terminal was then conditioned by stimulation at 20 impulses per second for 300 impulses, and the effect of this conditioning stimulation was followed by testing once every 2 s for three impulses and then once every 5 s for 75 impulses. The first nine points in Fig. 1 A and B represent the control EPP amplitudes before the conditioning train. During the conditioning stimulation the EPP amplitudes increase rapidly and then decay back to the control level after the train. Notice that in Ca$^{2+}$ + Ba$^{2+}$ Ringer the EPPs during the conditioning train reach almost twice the amplitude of those in Ca$^{2+}$ Ringer, and that the EPPs evoked by the first few testing impulses after the conditioning train are also greater in amplitude in Ca$^{2+}$ + Ba$^{2+}$ Ringer.

The Ba$^{2+}$-induced Increase in EPP Amplitudes during Repetitive Stimulation

Results from Increased Transmitter Release

It has been established that increases in EPP amplitudes during repetitive stimulation like those shown in Fig. 1 A result from changes in the number of quanta released from the nerve terminal (del Castillo and Katz, 1954 b; Magleby and Zengel, 1976 a). To determine whether the Ba$^{2+}$-induced increase in EPP amplitudes shown in Fig. 1 B is also a result of increases in the amount of transmitter released, we recorded miniature endplate potentials (MEPP) during a series of conditioning-testing trials like that shown in Fig. 1 B. Mean MEPP amplitude (estimated from 20–40 MEPPs in each case) was $0.27 ± 0.09$ mV (mean ± SD) in the control periods before the trains, $0.28 ± 0.05$ mV during the first 5 s of the trains, $0.27 ± 0.06$ mV during the last 3 s of the trains, $0.26 ± 0.06$ mV during the first 2 s after the trains, and $0.25 ± 0.05$ mV 20 s after the trains. The observation that mean MEPP amplitude
did not change in this or in other similar experiments indicates that postsynaptic sensitivity and quantal size remained constant during and after the conditioning trains. While doing these experiments with intracellular recording, we also observed, as expected since surface recording gives a good measure of average intracellular activity (Mallart and Martin, 1967; Magleby, 1973 a), changes in augmentation comparable to those seen with surface recording. Thus, the Ba\(^{2+}\)-induced increase in EPP amplitudes during and after repetitive stimulation results from an increase in the number of quanta released from the nerve terminal by each impulse.

A Working Hypothesis of Transmitter Release for the Analysis of Data

In the working hypothesis that we have used in designing and interpreting the experiments reported in this paper we assume that changes in transmitter release during and after repetitive stimulation can be described in terms of four kinetically distinguishable components (or processes) in the nerve terminal that act to increase transmitter release. Each nerve impulse is assumed to produce an increment in each of these components, and during repetitive stimulation each component builds up at a rate and to a level that is a function of the stimulation rate, the magnitude of the increment of the component added by each impulse, and the decay rate of each component (Mallart and Martin, 1967; Magleby and Zengel, 1975 a and b, and 1976 a and b). After repetitive stimulation, each of the four components decay with their characteristic time constants. In terms of this hypothesis, it is the changes in these four components that give rise to the observed components of transmitter release described as the first component of facilitation, the second component of facilitation, augmentation, and potentiation (see Introduction).

The justification for using this working hypothesis to interpret the data is: (a) models consistent with this working hypothesis can account for the effect of repetitive stimulation on facilitation (Mallart and Martin, 1967; Magleby, 1973 a and b; Younkin, 1974), augmentation (Magleby and Zengel, 1976 a and b, and 1977) and potentiation of transmitter release (Magleby and Zengel, 1975 a and b); (b) this working hypothesis is consistent with the interpretations used by Erulkar and Rahamimoff (1978) and Zengel and Magleby (1978) to describe the effects of repetitive stimulation on changes in the frequency of miniature endplate potentials; (c) this working hypothesis can describe quantitatively the effects of repetitive stimulation on transmitter release (Zengel and Magleby, 1977 b); and (d) this working hypothesis is consistent with the data to be presented in this paper and provides a unifying explanation for the complicated changes in transmitter release that result as a function of changes in stimulation rate and exposure to Ba\(^{2+}\) and Sr\(^{2+}\).

Within the constraints of this working hypothesis, several different models can be formulated to define the relationship of facilitation, augmentation, and potentiation of evoked transmitter release. In this paper the data are analyzed in terms of three models to investigate to what extent the conclusions of the paper depend on the model assumed. The models used for the analysis, an additive model, a multiplicative model, and a power model, represent three general classes of models that are commonly considered when the mechanism
of transmitter release is being investigated (see Mallart and Martin, 1967; Dodge and Rahamimoff, 1967; Miledi and Thies, 1971; Magleby, 1973; Younkin, 1974; Magleby and Zengel, 1975; Erulkar and Rahamimoff, 1978). The equations describing the models are presented in Methods, together with a description of how the different components are estimated for each model. A detailed discussion of the mechanisms consistent with these models will be reserved for future papers concerned with a quantitative description of transmitter release.

Figure 1. Effect of Ba\(^{2+}\) on the increase in EPP amplitudes during and after repetitive stimulation. (A and B) Plot of EPP amplitudes against time for conditioning-testing trials recorded in 0.4 mM Ca\(^{2+}\) Ringer (A) and 0.4 mM Ca\(^{2+}\) plus 0.15 mM Ba\(^{2+}\) Ringer (B). Bars indicate time of 300-impulse conditioning trains. For stimulation details, see text. The data in A represent the average response from 50 trials, 20 recorded before exposure to Ba\(^{2+}\) and 30 recorded after Ba\(^{2+}\) was washed from the preparation; B is the average response of 20 trials in the presence of Ba\(^{2+}\). (C and D) Decay of potentiation after the conditioning trains in Ca\(^{2+}\) Ringer (C, continuous line, \(P^*(T) = 0.84, \tau_p = 53\) s) and Ca\(^{2+}\) + Ba\(^{2+}\) Ringer (D, broken line, \(P^*(T) = 0.81, \tau_p = 52\) s). (E–G) Decay of \(A^*(T)\) in Ca\(^{2+}\) Ringer (continuous lines) and Ca\(^{2+}\) + Ba\(^{2+}\) Ringer (broken lines) for three models of transmitter release. (E) Multiplicative model: Ca\(^{2+}\), \(A^*(T) = 1.84, \tau_{A^*} = 8.0\) s; Ba\(^{2+}\), \(A^*(T) = 3.7, \tau_{A^*} = 8.6\) s. (F) Additive model: Ca\(^{2+}\), \(A^*(T) = 3.4, \tau_{A^*} = 7.7\) s; Ca\(^{2+}\) + Ba\(^{2+}\), \(A^*(T) = 6.9, \tau_{A^*} = 8.0\) s. (G) Power model: Ca\(^{2+}\), \(A^*(T) = 0.32, \tau_{A^*} = 11.2\) s; Ca\(^{2+}\) + Ba\(^{2+}\), \(A^*(T) = 0.54, \tau_{A^*} = 11.8\) s. See text for details.

Ba\(^{2+}\) Increases the Magnitude of Augmentation While Having Little Effect on Potentiation

In terms of our working hypothesis, the Ba\(^{2+}\)-induced increase in transmitter release shown in Fig. 1 could arise from increases in one or more of the
underlying components in the nerve terminal that act to increase transmitter release. To determine whether this is the case, we looked at the effect of Ba^{2+} on the decay of facilitation, augmentation, and potentiation after repetitive stimulation.

The decay of EPP amplitudes after the conditioning trains recorded in Ca^{2+} Ringer and in Ca^{2+} + Ba^{2+} Ringer shown in Fig. 1 A and B are plotted semilogarithmically against time (circles) in Fig. 1 C and D. The lines, obtained by least squares fits to the data points beyond ~40 s, represent the decay of $P^*(t)$, the factor responsible for potentiation, which had a time constant of decay of 53 s in Ca^{2+} Ringer and 52 s in Ca^{2+} + Ba^{2+} Ringer. $P^*(T)$, the magnitude of $P^*(t)$ at the end of the conditioning trains, is given by the intercept of the lines with the ordinate at 0 time and was 0.84 in Ca^{2+} Ringer and 0.81 in Ca^{2+} + Ba^{2+} Ringer. Thus, even though Ba^{2+} almost doubled the magnitude of EPPs during the conditioning train, it had little effect on the magnitude or time constant of decay of potentiation. The estimates of $P^*(T)$ presented in Fig. 1 C and D are those that would be obtained with either the additive (Eq. 6) or the multiplicative (Eq. 7) model (see Methods). Ba^{2+} also had no effect on potentiation in this experiment when estimates were obtained with the power model (Eq. 8).

The deviation of the data points from the lines in Fig. 1 C and D at times <30 s results from augmentation (Magleby and Zengel, 1976 a). Because augmentation falls on the more slowly decaying potentiation, estimates of its decay depend on the assumed relationship of augmentation, potentiation, and transmitter release. Fig. 1 E-G presents estimates of the decay of $A^*(t)$ after the conditioning trains shown in Fig. 1 A and B for multiplicative, additive, and power models of transmitter release. $A^*(t)$ represents the fractional changes in the underlying factor in the nerve terminal that gives rise to augmentation. The continuous lines in Fig. 1 E-G represent the decay of $A^*(t)$ in Ca^{2+} Ringer and the broken lines the decay in Ca^{2+} + Ba^{2+} Ringer. The intercept of these lines with the ordinate at 0 time indicates $A^*(T)$, the magnitude of $A^*(t)$ at the end of the conditioning trains. It can be seen that, independent of the model assumed for transmitter release, the addition of Ba^{2+} leads to an increase in the magnitude of augmentation. For example, Ba^{2+} increased $A^*(T)$ from 1.8 to 3.7, assuming a multiplicative model (Fig. 1 E). Ba^{2+} had little effect on the time constant of decay of $A^*(t)$ for any of the models, as indicated by the parallel decays in the presence and absence of Ba^{2+}.

In three other experiments like that shown in Fig. 1, similar results were obtained. The presence of 0.15 mM Ba^{2+} increased $A^*(T)$ about twofold (1.9 ± 0.4) after 300-impulse conditioning trains but had little effect on the time constant of decay of augmentation or on the magnitude and time constant of decay of potentiation. A Ba^{2+}-induced increase in $A^*(T)$, with little effect on the time constant of decay of augmentation or on the magnitude and time constant of decay of potentiation, was also seen in 14 preparations in which Ba^{2+} concentrations ranged from 0.05–1 mM and the conditioning trains ranged from 50–400 impulses. The magnitude of the Ba^{2+} effect appeared to
be relatively independent of the duration of stimulation (perhaps decreasing slightly) in the three experiments in which data were obtained after various durations in the same preparation. For example, in one experiment the presence of 0.05 mM Ba\(^{2+}\) increased \(A^*(T)\) about three times (from 0.57 to 1.68) after a 50-impulse train, about 2.8 times (from 1.5 to 4.2) after a 100-impulse train, and about 2.6 times (from 3.8 to 9.7) after a 200-impulse train.

**Time-course of the Ba\(^{2+}\) Effect**

The Ba\(^{2+}\)-induced increase in the magnitude of augmentation always occurred, developed slowly after the addition of Ba\(^{2+}\) to the bathing solution, and was slowly reversible. The time-course of the effect is shown in Fig. 2, which shows, as a function of time, estimates of \(A^*(T)\) and \(\tau_{A^*}\) made after conditioning trains of 100 impulses delivered at 20 impulses/s. Conditioning-

![Figure 2. Time-course of Ba\(^{2+}\) effect. Estimates of \(A^*(T)\) (●) and \(\tau_{A^*}\) = (○) after 100-impulse conditioning trains are plotted against trial number and time. The bathing solution always contained 0.36 mM Ca\(^{2+}\) to which 0.135 mM Ba\(^{2+}\) was added as indicated. The estimates were obtained with the multiplicative model, and similar conclusions were reached with the additive and power models.](image)

testing trials were continuous, each taking about 7 min. The plotted points represent average values from 3–10 consecutive trials. Notice that although Ba\(^{2+}\) had a large effect on \(A^*(T)\) it had little effect on \(\tau_{A^*}\). In this experiment, \(A^*(T)\) was still increasing after 2 h in Ca\(^{2+}\) + Ba\(^{2+}\) Ringer; in other experiments, \(A^*(T)\) would sometimes approach a constant level after 1–2 h in Ca\(^{2+}\) + Ba\(^{2+}\) Ringer. Even in the absence of Ba\(^{2+}\), the magnitude of augmentation typically increases, and its time constant of decay decreases slightly with successive trials and with time (Magleby and Zengel, 1976b). This effect can also be seen in this experiment, in addition to the effect of Ba\(^{2+}\).
Figure 3. Effect of Ba$^{2+}$ on augmentation and facilitation after 10-impulse conditioning trains delivered at 20 impulses/s. Single testing impulses were applied from 40 ms to 10 s after each conditioning train, with 30 s between conditioning-testing trials. (A) EPPs recorded with a surface electrode in 0.5 mM Ca$^{2+}$ Ringer and in 0.5 mM Ca$^{2+}$ plus 0.15 mM Ba$^{2+}$ Ringer; testing impulses were applied 400 ms after the conditioning train. (B) Increases in EPP amplitude during (bar) and after a series of conditioning-testing trials are plotted against time. The average response of >2,500 conditioning-testing trials was obtained from three preparations. The Ca$^{2+}$ Ringer contained 0.45-0.55 mM Ca$^{2+}$ to which 0.15-0.25 mM Ba$^{2+}$ was added for the Ca$^{2+}$ + Ba$^{2+}$ Ringer. (C) Decay of $A^*(t)$ in Ca$^{2+}$ Ringer (continuous line, $A^*(T) = 0.26$, $\tau_a^0 = 6.0$ s) and Ca$^{2+}$ + Ba$^{2+}$ Ringer (broken line, $A^*(T) = 0.47$, $\tau_a^0 = 6.8$ s). (D and E) Decay of $F_1^*(t)$ (broken lines) and $F_2^*(t)$ (continuous lines) for two models of transmitter release. For the following data the first value of each pair is for Ca$^{2+}$ Ringer and the second for Ca$^{2+}$ + Ba$^{2+}$ Ringer. (D) Additive model: $F_1^*(T) = 1.2$ and 1.4; $\tau_{F_1} = 75$ and 85 ms; $F_2^*(T) = 0.73$ and 0.88; $\tau_{F_2} = 395$ and 400 ms. (E) Multiplicative model: $F_1^*(T) = 0.25$ and 0.25; $\tau_{F_1} = 70$ and 80 ms; $F_2^*(T) = 0.16$ and 0.16; $\tau_{F_2} = 440$ and 440 ms.
In a few experiments we placed both sartorius nerve-muscle preparations from one frog in the same preparation chamber at the same time and stimulated one and not the other. After the preparations had spent several hours in the presence of Ba\(^{2+}\), we tested the unstimulated preparation and found that the Ba\(^{2+}\) effect was evident, although not always as pronounced as in the stimulated preparation. Notice also in Fig. 2 that the Ba\(^{2+}\) effect was still partially evident at a time when most of the Ba\(^{2+}\) should have been washed out of the muscle. Apparently, direct entry of Ba\(^{2+}\) through nerve terminal channels activated by action potentials is not necessary for the slow cumulative effect of Ba\(^{2+}\).

The Ba\(^{2+}\)-induced Increase in the Magnitude of Augmentation Is Evident after 10 Conditioning Impulses

The previous sections establish that Ba\(^{2+}\) increases the magnitude of augmentation after long conditioning trains. In this section it is shown that Ba\(^{2+}\) also increases the magnitude of augmentation after short conditioning trains. For the experiment shown in Fig. 3, the nerve was stimulated with a series of conditioning-testing trials, each consisting of a conditioning train of 10 impulses delivered at 20 impulses/s, followed by a single testing impulse placed at an interval of from 40 ms to 10 s after each train. Fig. 3 A presents examples of EPPs recorded in Ca\(^{2+}\) Ringer and in Ca\(^{2+}\) + Ba\(^{2+}\) Ringer during trials in which the testing interval was 400 ms. The EPPs increased to a greater amplitude during the 10-impulse conditioning train in Ca\(^{2+}\) + Ba\(^{2+}\) Ringer than in Ca\(^{2+}\) Ringer, and the response to the testing impulse was also greater in Ca\(^{2+}\) + Ba\(^{2+}\) Ringer. This is more clearly seen in Fig. 3 B, which presents composite plots of the increase in EPP amplitudes against time during and after the conditioning trains for the two experimental conditions. The decays of the EPP amplitudes after the conditioning trains in Fig 3 B are plotted semilogarithmically against time in Fig. 3 C. The lines indicate the decay of \(A^*(t)\). Notice that Ba\(^{2+}\) increased \(A^*(T)\) 1.8 times from 0.26 to 0.47.
but had little effect on $\tau_A$ (multiplicative model; similar conclusions were reached with the additive and power models).

The data in Fig. 3 have not been corrected for possible contributions from potentiation. The magnitude of potentiation would be expected to be small after 10-impulse conditioning trains (Magleby and Zengel, 1975 a, and b), and to obtain reliable estimates of potentiation would have required a large number of additional trials, which was not practical within the time constraints of this experiment. Correcting the data for potentiation using calculated estimates based on previous studies (Magleby and Zengel, 1975 a and b) led to similar conclusions.

$\text{Ba}^{2+}$ Has Little or No Effect on Facilitation

Since facilitation falls on the more slowly decaying augmentation and potentiation, estimates of facilitation depend on the assumed relationship of facilitation, augmentation, potentiation, and transmitter release. Estimates of the two components of facilitation for the data shown in Fig. 3 A–C are presented for the additive model in Fig. 3 D and for the multiplicative model in Fig. 3 E. The broken lines in these figures indicate the decay of $F_1^*(t)$, the fractional increase in the factor in the nerve terminal that gives rise to the first component of facilitation, and the continuous lines represent the decay of $F_2^*(t)$, the fractional increase in the factor that gives rise to the second component of facilitation. If the multiplicative model is assumed (Fig. 3 E), $\text{Ba}^{2+}$ had little effect on either component of facilitation. If the additive (Fig. 3 D) or the power model (not shown) is assumed, $\text{Ba}^{2+}$ increased $F_1^*(T)$ and $F_2^*(T)$ ~ 1.1–1.3 times, as compared with the 1.8-fold increase in the magnitude of $A^*(T)$ shown in Fig. 1 C.

Similar results were obtained in four experiments in which impulses were applied to test for facilitation after longer (100–300-impulse) conditioning trains. As was observed after 10-impulse conditioning trains, $\text{Ca}^{2+} + \text{Ba}^{2+}$ Ringer increased $A^*(T)$ but had little or no effect on either component of facilitation when a multiplicative model was assumed. When a linear or power model was assumed, $\text{Ba}^{2+}$ increased $F_1^*(T)$ and $F_2^*(T)$ somewhat, but the increase was always less than the increase in $A^*(T)$.

**Dose Dependence of the $\text{Ba}^{2+}$ Effect**

$\text{Ba}^{2+}$ increased $A^*(T)$ in a dose-dependent manner. For example, 0.8 mM $\text{Ba}^{2+}$ increased $A^*(T)$ after 10-impulse conditioning trains about five times (Fig. 4 A), whereas 0.15–0.25 mM $\text{Ba}^{2+}$ increased $A^*(T)$ only about 1.8 times in a similar type of experiment (Fig. 3 C). The dose-dependent effect of $\text{Ba}^{2+}$ is more clearly shown in Fig. 4 B, which plots (filled squares) the ratio of the estimates of $A^*(T)$ obtained in $\text{Ca}^{2+} + \text{Ba}^{2+}$ Ringer to the estimates of $A^*(T)$ obtained in $\text{Ca}^{2+}$ Ringer against the concentration of $\text{Ba}^{2+}$ for four experiments of the type presented in Figs. 3 and 4 A. The filled triangles and diamonds in Fig. 4 B show the ratios of estimates of $F_1^*(T)$ and $F_2^*(T)$ obtained in the presence and absence of $\text{Ba}^{2+}$ from these same experiments, assuming a multiplicative model of transmitter release. As was the case for the experiment presented in Fig. 3, $\text{Ba}^{2+}$ had little effect on facilitation. Estimates of the effect
of Ba\(^{2+}\) on \(F_1^*(T)\) and \(F_2^*(T)\), assuming an additive model, are plotted as open triangles and diamonds. With this model, Ba\(^{2+}\) increased the magnitude of facilitation somewhat, but the effect was small when compared with the effect of Ba\(^{2+}\) on \(A^*(T)\). A power model gave results similar to those obtained with the additive model. The data in Fig. 4 are for 10-impulse conditioning trains. A dose-dependent effect of Ba\(^{2+}\) on increasing \(A^*(T)\) was also observed after longer (200-300-impulse) conditioning trains.

![Graph showing the effect of Ba\(^{2+}\) on \(A^*(T)\) and \(F_1^*(T), F_2^*(T)\).](image)

**Figure 4.** Dose dependence of Ba\(^{2+}\) effect. (A) Decay of \(A^*(t)\) after 10-impulse conditioning trains recorded in 0.4 mM Ca\(^{2+}\) Ringer (○, continuous line, \(A^*(T) = 0.17, \tau_{A^*} = 7.9\) s) and 0.4 mM Ca\(^{2+}\) plus 0.8 mM Ba\(^{2+}\) Ringer (□, broken line, \(A^*(T) = 0.86, \tau_{A^*} = 7.1\) s). The data are from >600 conditioning-testing trials from one preparation. The experiment is similar to that shown in Fig. 3 C, to which this figure should be compared to see dose effect. (B) Ba\(^{2+}\)-induced effect on \(F_1^*(T), F_2^*(T)\), and \(A^*(T)\) plotted against the concentration of Ba\(^{2+}\) added to 0.4 mM Ca\(^{2+}\) Ringer. Facilitation was estimated for both the multiplicative and additive release models.
**Ba**

**Does Not Maintain Evoked Transmitter Release**

In agreement with the findings of other investigators (Miledi, 1966; Silinsky, 1977; McLachlan, 1977; Illes and Thesleff, 1978), we found that **Ba**

![Image of graphs showing EPP amplitudes during and after repetitive stimulation.](image)

**Figure 5.** Effect of high concentrations of **Ba**

on EPP amplitudes during and after repetitive stimulation. (A and B) Plots of EPP amplitudes against time. The conditioning trains were 300 impulses delivered at 20 impulses/s (horizontal bar). Control and testing impulses were delivered once every 5 s except for the six testing impulses immediately after the train, which were delivered at one impulse every 1.5 s. The data in A represent the average response of four consecutive conditioning-testing trials recorded in 0.6 mM Ca

+ Ringer. The data in B represent the average response of the first three consecutive conditioning-testing trials after the bathing solution had been changed to 2.5 mM **Ba**

+ Ringer. **Ca**

+ was washing from the preparation during these trials. (The conditioning train is off scale in B). (C) Decay of **P***(t) in Ca

+ Ringer (continuous line, **P***(T) = 1.1 **t**_\_p_ = 51 s) and **Ba**

+ Ringer (broken line, **P***(T) = 3.6, **t**_\_p_ = 48 s). (D) Decay of **A***(t) in Ca

+ Ringer (continuous line, **A***(T) = 1.1, **t**_\_A_ = 7.9 s and **Ba**

+ Ringer (broken line, **A***(T) = 13, **t**_\_A_ = 7.7 s). The multiplicative model was used for the data plotted in C and D. Additive model (data not shown): Ca

+ Ringer, **A***(T) = 2.3, **t**_\_A_ = 7.3 s. **Ba**

+ Ringer, **A***(T) = 60, **t**_\_A_ = 6.9 s. **P***(T) and **t**_\_p_ were the same as for multiplicative model.

nous release by Silinsky and phasic release by McLachlan). For these experiments the **Ca**

+ in the bathing solution was replaced with 2.5–5 mM **Ba**

+. Within a few minutes after the solution change the magnitude of augmentation increased dramatically and continued to increase for several consecutive conditioning-testing trials. The evoked response then decreased to the noise
level over the next trial or two. Replacing the Ba\(^{2+}\) Ringer with Ca\(^{2+}\) Ringer restored the response, and the magnitude of augmentation was reduced, as compared with its highest level in Ba\(^{2+}\) Ringer, but remained above the control level for several trials. One interpretation of these results is that the large increase in \(A^*(T)\) occurred when Ba\(^{2+}\) first reached the nerve terminal and before the Ca\(^{2+}\) had been washed out; the decrease and eventual absence of evoked release then occurred as Ca\(^{2+}\) was washed from the preparation.

An interesting feature of these experiments was the large magnitude of augmentation in the high concentrations of Ba\(^{2+}\) before the response failed. Fig. 5 A and B shows the rise and decay of EPP amplitudes during and after

![Figure 5](image)

300-impulse conditioning trains recorded in 0.6 mM Ca\(^{2+}\) Ringer and in 2.5 mM Ba\(^{2+}\) Ringer before the Ca\(^{2+}\) was washed out. Semilogarithmic plots of the decays after these trains are presented in Fig. 5 C. In this experiment, \(A^*(T)\) was 12 times greater in the Ba\(^{2+}\) Ringer than in the Ca\(^{2+}\) Ringer, assuming a multiplicative model (Fig. 5 D), and 26 times greater, assuming an additive model (not shown). Ba\(^{2+}\) also increased \(P^*(T)\) about three times, from 1.1 to 3.6 (Fig. 5 C), but had little effect on \(\tau_{A^*}\) (7.9 s in Ca\(^{2+}\) Ringer compared with 7.7 s in Ba\(^{2+}\) Ringer) or on \(\tau_{P^*}\) (51 s for Ca\(^{2+}\) Ringer and 48 s for Ba\(^{2+}\) Ringer). Similar results were seen in other experiments of this type. High concentrations of Ba\(^{2+}\) dramatically increased the magnitude of augmentation, and this increase was 4–30 times greater than the increase in the magnitude of potentiation. (It is important to remember that Fig. 5 C is a semi-logarithmic plot, which tends to obscure the increase in \(A^*(T)\), which is much greater than the increase in \(P^*(T)\). The greater increase in augmenta-
tion is clearly seen in Fig. 5 A and B. Notice the much larger increase in EPP amplitudes in Ba\(^{2+}\) Ringer immediately after the conditioning train when augmentation is present and the similarities of the decays 40–150 s after the train when augmentation would have decayed and only potentiation is present.}

\textit{Sr}\(^{2+}\) \textbf{Increases EPP Amplitudes during Repetitive Stimulation}

The addition of small amounts of Sr\(^{2+}\) to the bathing solution or the replacement of Ca\(^{2+}\) with Sr\(^{2+}\) leads to a greater increase in EPP amplitude during repetitive stimulation. Fig. 6 shows the effect of replacing the 0.5 mM Ca\(^{2+}\) in the bathing solution with 1 mM Sr\(^{2+}\). EPP amplitudes increased more rapidly and reached a greater magnitude during the 200-impulse conditioning trains recorded in Sr\(^{2+}\) Ringer. In experiments of this type, substituting 1 mM Sr\(^{2+}\) for 0.5 mM Ca\(^{2+}\) typically led to little change in the control EPP amplitudes. Decreasing or increasing the concentration of Sr\(^{2+}\) from this level led to decreases or increases in the control EPP amplitudes. The addition of 0.1–0.2 mM Sr\(^{2+}\) to bathing solutions containing 0.4 mM Ca\(^{2+}\) increased the control EPP amplitudes 5–20%, whereas the addition of 1 mM Sr\(^{2+}\) increased control EPP amplitudes 200–400%. These effects of Sr\(^{2+}\) are consistent with those reported by Meiri and Rahamimoff (1971), whose paper should be consulted for further details on the effects of Ca\(^{2+}\), Sr\(^{2+}\), and Mg\(^{2+}\) on quantal content. The important point is that regardless of whether the control EPP amplitudes increased, decreased, or remained unchanged, the presence of Sr\(^{2+}\) in the Ringer’s solution always led to a greater rate of increase in EPP amplitudes during the conditioning trains.

\textit{The Sr}\(^{2+}\)-\textit{induced Increase in EPP Amplitude during Repetitive Stimulation Results from Increased Transmitter Release}

Intracellular recordings of MEPPs during experiments of the type shown in Fig. 6 B established that estimates of mean MEPP amplitude (determined in each case from 10–70 MEPPs) made before, during, and after the conditioning trains differed from each other by <6%, indicating that postsynaptic sensitivity and quantal size remained constant during and after the conditioning trains. The Sr\(^{2+}\)-induced increase in EPP amplitudes in these experiments with intracellular recording was comparable to that observed with surface recording. Thus, as with Ba\(^{2+}\), the Sr\(^{2+}\)-induced increase in EPP amplitudes during repetitive stimulation results from an increase in the number of quanta released from the nerve terminal by each impulse.

At concentrations of Ba\(^{2+}\) and Sr\(^{2+}\) >1–2 mM, some change in postsynaptic sensitivity might be expected since increased concentrations of other divalent ions affect single channel properties (Lewis, 1979; Magleby and Weinstock, 1980). Any changes of this type would have a scaling effect on the data, changing all EPP amplitudes in a conditioning-testing trial a constant percentage. Inasmuch as the data are expressed in terms of the control EPP amplitudes for analysis (Eq. 5), changes of this type would not affect the results.
Figure 6. Effect of Sr\(^{2+}\) on EPP amplitudes during and after repetitive stimulation. (A and B) Plots of EPP amplitudes against time for conditioning-testing trials recorded in 0.5 mM Ca\(^{2+}\) Ringer (A) and 1 mM Sr\(^{2+}\) Ringer (B). Bars indicate time of 200-impulse conditioning trains (20/s). Control and testing impulses were delivered once every 5 s except for the three testing impulses immediately after the trains, which were delivered at one impulse every 2 s. The data in A represent the average response of 22 trials recorded before and after exposure to Sr\(^{2+}\); B is the average response of 19 trials recorded in Sr\(^{2+}\) Ringer. C is the superimposed plots of the EPP amplitudes after the conditioning trains in A and B plotted semilogarithmically against time. The line is the decay of \(P^*(T)\), with \(P^*(T) = 0.65\) and \(\tau_p = 82\) s. (O) Sr\(^{2+}\). (●) Ca\(^{2+}\).
**Time-course and Dose Dependence of the Sr\(^{2+}\) Effect**

The Sr\(^{2+}\)-induced increase in EPP amplitude during repetitive stimulation always occurred, came on rapidly, and was readily reversible. The time-course of the effect appeared to parallel the time required for the new solution to soak into the muscle. The rapid (5–10-min) reversal of the Sr\(^{2+}\) effect can be contrasted with the slower (30–60-min) reversal of the Ba\(^{2+}\) effect. The Sr\(^{2+}\)-induced increase in EPP amplitudes during repetitive stimulation became progressively greater when increasing concentrations of Sr\(^{2+}\) (explored range, 0.15–0.6 mM) were added to a Ca\(^{2+}\)-containing bathing solution. (An example is shown in Fig. 10.) In the absence of Ca\(^{2+}\), increasing the concentration of Sr\(^{2+}\) in the explored range of 0.8–1.4 mM sometimes, but not always, increased the magnitude of the already present Sr\(^{2+}\) effect. The higher concentrations of Sr\(^{2+}\) often led to a smaller increase in EPP amplitude at the end of 200–300-impulse conditioning trains when compared with those in lower concentrations of Sr\(^{2+}\).

**Sr\(^{2+}\) has Little Effect on Augmentation or Potentiation**

Fig. 6 C presents semilogarithmic plots of the decay of EPP amplitudes after the conditioning trains shown in Fig. 6 A and B. Notice that the decays in Ca\(^{2+}\) Ringer (filled circles) and Sr\(^{2+}\) Ringer (open circles) are superimposed, indicating that Sr\(^{2+}\) had little effect on augmentation and potentiation in this experiment. This suggests that the Sr\(^{2+}\)-induced increase in EPP amplitudes during the conditioning train shown in Fig. 6 B does not result from an increase in the magnitudes or the time constants of decay of augmentation or potentiation.

Ten additional experiments of the type shown in Fig. 6 were performed with conditioning trains of 200 or 300 impulses. In four experiments, 0.4–0.5 mM Ca\(^{2+}\) was replaced with 0.6–1.4 mM Sr\(^{2+}\); in the other six experiments, 0.15–1 mM Sr\(^{2+}\) was added to a bathing solution containing 0.4–0.45 mM Ca\(^{2+}\). In each experiment there was a greater increase in EPP amplitudes during repetitive stimulation in the presence of Sr\(^{2+}\), with no increase in the magnitudes of augmentation or potentiation. In fact, the presence of Sr\(^{2+}\) often led to a slight decrease in the magnitudes of potentiation and augmentation. The mean value of \(P^\ast(T)\) in these experiments decreased 21% from 0.76 ± 0.18 in Ca\(^{2+}\) Ringer to 0.60 ± 0.13 in the presence of Sr\(^{2+}\) (\(P < 0.01\), \(t\) test, paired data), whereas \(\tau_p\) remained unchanged at 76 ± 8 s in Ca\(^{2+}\) Ringer and 78 ± 15 s in the presence of Sr\(^{2+}\) (\(P > 0.5\)). \(A^\ast(T)\) decreased an average of 24% from 1.1 ± 0.81 in Ca\(^{2+}\) Ringer to 0.84 ± 0.52 in the presence of Sr\(^{2+}\), but this effect was not significant (\(P > 0.2\)); \(\tau_{A^\ast}\) remained unchanged at 7.8 ± 1.7 s in Ca\(^{2+}\) Ringer and 7.4 ± 1.8 s in the presence of Sr\(^{2+}\) (\(P > 0.5\)). (These results were obtained with the multiplicative model. Similar conclusions were reached with the additive and power models.)

**Sr\(^{2+}\) Increases the Magnitude and Time Constant of Decay of the Second Component of Facilitation**

In Fig. 6 it was shown that Sr\(^{2+}\) led to a greater increase in EPP amplitudes during the conditioning train but had little effect on augmentation or potentiation. Because facilitation also contributes to the increase in EPP amplitudes...
Effects of Ba$^{2+}$, Sr$^{2+}$, and Ca$^{2+}$ on Transmitter Release

during repetitive stimulation, Sr$^{2+}$ may act by increasing facilitation. To determine whether Sr$^{2+}$ increases facilitation, tests were made for facilitation after short conditioning trains. Sr$^{2+}$ was found to increase the magnitude and time constant of decay of the second component of facilitation. An experiment of this type in which 0.55 mM Ca$^{2+}$ was replaced with 1.2 mM Sr$^{2+}$ is shown in Fig. 7. The EPP amplitudes increased to a greater magnitude during the 10-impulse train in Sr$^{2+}$ Ringer than in Ca$^{2+}$ Ringer, and after the conditioning train the EPP amplitudes decayed more slowly in Sr$^{2+}$ Ringer than in Ca$^{2+}$ Ringer. In contrast to the Ba$^{2+}$ induced increase in EPP amplitudes, which took ~20 s to decay to the level observed in Ca$^{2+}$ Ringer, the Sr$^{2+}$-induced increase decayed in about 2 s (cf. Figs. 3 and 7). As might be expected from this rapid decay of the Sr$^{2+}$-induced increase in EPP amplitudes, Sr$^{2+}$ had little effect on augmentation (line, Fig. 7 C). This observation that Sr$^{2+}$ has little effect on augmentation after 10-impulse conditioning trains agrees with the observation shown in Fig. 6 C that Sr$^{2+}$ has little effect on augmentation after longer conditioning trains.

Estimates of $F_1^*(T)$ (broken lines) and $F_2^*(T)$ (continuous lines) after the 10-impulse conditioning trains shown in Fig. 7 B are presented in Fig. 7 D and E. For the additive model of transmitter release (Fig. 7 D), Sr$^{2+}$ increased $F_2^*(T)$ about two times, from 0.73 to 1.5, and increased the time constant of decay about 1.5 times, from 395 to 570 ms. Sr$^{2+}$ had a smaller effect on the first component of facilitation, increasing $F_1^*(T)$ about 1.4 times but had little or no effect on $\tau_{F_1}$. Similar results were obtained with a power model. For the multiplicative model (Fig. 7 E) Sr$^{2+}$ increased $F_2^*(T)$ about two times, from 0.16 to 0.30, and increased $\tau_{F_2}$ about 1.5 times, from 440 to 650 ms. Sr$^{2+}$ had little or no effect on the first component of facilitation for the multiplicative model. Similar results were obtained in several additional experiments like the one shown in Fig. 7. Sr$^{2+}$ increased the magnitude and time constant of decay of the second component of facilitation but had a smaller effect (additive and power models) or no effect (multiplicative model) on the first component of facilitation. These results were obtained whether the solution changes led to a slight increase or decrease in the control EPP amplitudes, or had no effect on them.

Our observation that Sr$^{2+}$ slows the decay of the second component of facilitation generally agrees with the observation of Balnave and Gage (1974) that the amplitudes of testing EPPs placed 10–100 ms after a conditioning impulse decay slower in the presence of Sr$^{2+}$. Balnave and Gage assumed that Sr$^{2+}$ slowed the decay of the first component of facilitation; they did not test for the second component.

As was the case for the experiments shown in Fig. 3, the data presented in this section were not corrected for potentiation. Similar conclusions were reached after correcting the data for potentiation using calculated values based on previous studies (Magleby and Zengel, 1975 a and b).

Sr$^{2+}$ Leads to a Greater Increase in EPP Amplitudes after One Impulse

In a few experiments testing impulses were applied at various intervals after a single conditioning impulse to determine whether Sr$^{2+}$ has an effect after one impulse. Fig. 8 presents results from such an experiment. Replacing the
Figure 7. Effect of Sr\(^{2+}\) on augmentation and facilitation after 10-impulse conditioning trains delivered at 20 impulses/s. Single testing impulses were applied from 40 ms to 10 s after each conditioning train, with 30 s between conditioning-testing trials. (A) EPPs recorded with a surface electrode in 0.55 mM Ca\(^{2+}\) Ringer and 1.2 mM Sr\(^{2+}\) Ringer; testing impulses were applied 400 ms after the conditioning train. (B) Increases in EPP amplitude during (bar) and after a series of conditioning-testing trials are plotted against time. The average response of >5,000 trials was obtained from six preparations. The Ca\(^{2+}\) Ringer contained 0.45–0.55 mM Ca\(^{2+}\), which was replaced with 1–1.5 mM Sr\(^{2+}\) for the Sr\(^{2+}\) Ringer. The Ca\(^{2+}\) Ringer data from this series of experiments were almost identical to those obtained in the series of experiments presented in Fig. 3. For this reason all the Ca\(^{2+}\) Ringer data were combined for these figures. (C) Decay of \(A^*(t)\) in both Ca\(^{2+}\) Ringer and Sr\(^{2+}\) Ringer (line, \(A^*(T) = 0.26, \tau_{A^*} = 6.0\) s). (D and E) Decay of \(F_1^*(t)\) (broken lines) and \(F_2^*(t)\) (continuous lines) for two models of transmitter release. For the following data, the first value of each pair is for Ca\(^{2+}\) Ringer and the second for Sr\(^{2+}\) Ringer. (D), Additive model: \(F_1^*(T) = 1.2\) and 1.8; \(\tau_{F_1^*} = 75\) and 75 ms; \(F_2^*(T) = 0.73\) and 1.5; \(\tau_{F_2^*} = 395\) and 570 ms. (E) Multiplicative model: \(F_1^*(T) = 0.27\) and 0.27; \(\tau_{F_1^*} = 70\) and 75 ms; \(F_2^*(T) = 0.16\) and 0.30; \(\tau_{F_2^*} = 440\) and 650 ms.
0.6 mM Ca\(^{2+}\) in the bathing solution with 1.2 mM Sr\(^{2+}\) led to an approximately twofold increase in EPP amplitudes at intervals >100 ms after the conditioning impulse. This increase is most likely due to an increase in the second component of facilitation since the first component of facilitation would have decayed to insignificant levels by 200 ms and since Sr\(^{2+}\) does not increase the magnitude of augmentation or potentiation (Fig. 6).

The data in Fig. 8 are consistent with the suggestion that 1.2 mM Sr\(^{2+}\) Ringer approximately doubles the increment of the second component of facilitation added by a single impulse. If Sr\(^{2+}\) has the same effect for each impulse in a train, then, in terms of our working hypothesis, 1.2 mM Sr\(^{2+}\) Ringer should also lead to a doubling of \(F_2^*(T)\) at the end of the conditioning train since the increased increments added by each impulse in Sr\(^{2+}\) Ringer would build up to a higher level during repetitive stimulation (see Mallart and Martin, 1967). Notice in Fig. 7 that 1.2 mM Sr\(^{2+}\) Ringer does lead to an approximate doubling of \(F_2^*(T)\) at the end of a 10-impulse conditioning train, supporting the proposal that Sr\(^{2+}\) increases the increment of the second component of facilitation added by each impulse.

In experiments of the type shown in Fig. 8, the amplitudes of the testing EPPs placed closer than \(\sim 40\) ms after the single conditioning impulse were often decreased in Sr\(^{2+}\) Ringer when compared with Ca\(^{2+}\) Ringer. A similar decrease in amplitude in the presence of Sr\(^{2+}\) was observed by Balnave and Gage (1974). A delayed onset of the Sr\(^{2+}\) effect or a delayed onset of the second component of facilitation (Mallart and Martin, 1967) could give rise to this initial decrease.

**Selective Effects of Sr\(^{2+}\) and Ba\(^{2+}\) during Repetitive Stimulation**

The results reported in the previous sections led to the proposal that the Sr\(^{2+}\)-induced increase in EPP amplitudes during repetitive stimulation results from an increase in the magnitude and time constant of decay of the second component of facilitation, whereas the Ba\(^{2+}\)-induced increase in EPP amplitudes...
tudes results from an increase in the magnitude of augmentation. Most of the data leading to these proposals were obtained by analyzing the decay of EPP amplitudes after repetitive stimulation. If the proposed actions of Ba$^{2+}$ and Sr$^{2+}$ are correct, and if our working hypothesis for the effect of repetitive stimulation on transmitter release is valid, then it should be possible to predict the effects of Ba$^{2+}$ and Sr$^{2+}$ on transmitter release during repetitive stimulation using our working hypothesis and the conclusions drawn from the data obtained after repetitive stimulation.

In the following sections we first predict the expected effects of Ba$^{2+}$ and Sr$^{2+}$ on EPP amplitudes during repetitive stimulation. The patterned stimulation used to test the predictions is then presented, and the observed variations in EPP amplitudes resulting from the patterned stimulation are explained in terms of our working hypothesis. Finally, the observed effects of Ba$^{2+}$ and Sr$^{2+}$

![Figure 8](image_url)

**Figure 8.** Effect of Sr$^{2+}$ on the increase in EPP amplitudes after a single conditioning impulse. A single testing impulse was applied from 40 ms to 2 s after each conditioning impulse; 15–20 s elapsed between trials. The increase in the testing EPP amplitudes is plotted against the conditioning-testing interval for data obtained with 0.6 mM Ca$^{2+}$ Ringer (○) and 1.2 mM Sr$^{2+}$ Ringer (●). The data were obtained from >2,000 conditioning-testing trials from three preparations.

on EPP amplitudes during the patterned stimulation are examined to determine whether the predictions are met.

**Predictions** If Sr$^{2+}$ selectively increases the magnitude and time constant of decay of the second component of facilitation, Sr$^{2+}$ should lead to a greater rate of increase in EPP amplitudes when the second component of facilitation is increasing, displace EPP amplitudes when the second component is present but not increasing, and have no effect when the second component is not present. Similarly, if Ba$^{2+}$ selectively increases the magnitude of augmentation, Ba$^{2+}$ should increase EPP amplitudes when augmentation is present and have no effect when augmentation is not present.

**Patterned Stimulation** Examples of data used to test the predicted effects of Ba$^{2+}$ and Sr$^{2+}$ during repetitive stimulation are shown in Fig. 9 A
and B. In these experiments the conditioning train consisted of five blocks of 41 impulses delivered at 20 impulses/s with a 2-s interval between blocks. Testing impulses were applied following the conditioning trains to test for the decay of augmentation and potentiation. Fig. 9 A presents superimposed plots

![Figure 9 A](image_url)

**FIGURE 9.** Effect of Sr$^{2+}$ and Ba$^{2+}$ on the increase in EPP amplitudes during repetitive stimulation consisting of five blocks of 41 impulses delivered at 20 impulses/s with a 2-sec interval between blocks. (A) Superimposed plots of EPP amplitudes during stimulation in 0.7 mM Ca$^{2+}$ Ringer (lower points) and 1.4 mM Sr$^{2+}$ Ringer (upper points, displaced slightly to the left for clarity), representing the average response of >20 trials in each solution. The continuous arrows indicate the first EPP amplitude in each 2-s stimulation block in Ca$^{2+}$ Ringer and the broken arrows indicate the corresponding EPP amplitude in Sr$^{2+}$ Ringer. Testing impulses applied after the trains (not shown) gave: Ca$^{2+}$ Ringer, $P^*(T) = 0.54$, $\tau_p = 65$ s, $A^*(T) = 1.1$, $\tau_A = 8.3$ s; Sr$^{2+}$ Ringer, $P^*(T) = 0.39$, $\tau_p = 55$ s, $A^*(T) = 1.1$, $\tau_A = 6.0$ s. (B) Superimposed plots of EPP amplitudes in 0.4 mM Ca$^{2+}$ Ringer (lower points) and 0.4 mM Ca$^{2+}$ plus 0.3 mM Ba$^{2+}$ Ringer (upper points, displaced slightly to the left for clarity), representing the average response of 20 trials in Ca$^{2+}$ Ringer and six trials in Ca$^{2+}$ + Ba$^{2+}$ Ringer. The continuous arrows indicate the first EPP amplitude in each 2-s stimulation block in Ca$^{2+}$ Ringer and the broken arrows indicate the corresponding EPP amplitude in Ca$^{2+}$ + Ba$^{2+}$ Ringer. Testing impulses after the trains (not shown) gave: Ca$^{2+}$ Ringer, $P^*(T) = 0.64$, $\tau_p = 80$ s, $A^*(T) = 0.73$, $\tau_A = 5.9$ s; Ca$^{2+}$ + Ba$^{2+}$ Ringer, $P^*(T) = 0.66$, $\tau_p = 81$ s, $A^*(T) = 2.6$, $\tau_A = 5.8$ s.
of EPP amplitudes obtained from one preparation during conditioning trains recorded in 0.7 mM Ca^{2+} Ringer and in 1.4 mM Sr^{2+} Ringer. Fig. 9B presents data recorded from another preparation in 0.4 mM Ca^{2+} Ringer and in 0.4 mM Ca^{2+} plus 0.3 mM Ba^{2+} Ringer. In each figure the lower curves, which have been displaced slightly for clarity, represent the response in Ca^{2+} Ringer. Notice in Fig. 9 that the effects of Sr^{2+} and Ba^{2+} are distinctly different. Before examining the effects of these ions in greater detail it is first necessary to understand the response in the absence of these ions.

**RESPONSE IN Ca^{2+}** In terms of our working hypothesis, the increase in EPP amplitudes during repetitive stimulation results from the addition by each nerve impulse of increments of potentiation, augmentation, and the two components of facilitation (see working hypothesis section). Previous studies have established that the amount of the first and second components of facilitation added by each impulse (additive model) is about 1 and 0.1, respectively (Mallart and Martin, 1967; Magleby, 1973a). The amount of augmentation is ~0.01 (Zengel and Magleby, 1977a), and the amount of potentiation is ~0.01 (Magleby and Zengel, 1975a and b). The two components of facilitation decay with time constants of ~70 and 400 ms (Figs. 3 and 7), augmentation decays with a time constant of ~7 s (Figs. 1 and 3), and potentiation decays with a time constant of ~50 s after 300 impulses (Figs. 1 and 6).

On the basis of the kinetic properties of facilitation, augmentation, and potentiation, the rapid increase in EPP amplitudes during each 2-s stimulation block in Fig. 9 is mainly due to the rapid increase in facilitation, which then decays to insignificant levels during the 2-s periods between stimulation blocks. These rapid changes in facilitation are superimposed on a more gradual increase in augmentation and potentiation during the conditioning train, as indicated by the general increase in EPP amplitudes in successive stimulation blocks (see Magleby, 1973b).

More specifically, the rapid increase in amplitudes of the first few EPPs in each of the 2-s stimulation blocks in the conditioning trains results mainly from the rapid increase in the first component of facilitation; this component dominates during this time since the increment of the first component of facilitation added by each impulse is 10–100 times larger than the increments added for the other components. The first component of facilitation contributes little to the increase in EPP amplitudes after ~200 ms (the first few impulses) in each stimulation block since it has approached a steady state level at this time due to its 70-ms time constant of decay. (See Mallart and Martin [1967] for a detailed discussion of the properties of facilitation and the relationship between the time constant of decay of a component and its steady-state value during repetitive stimulation.)

The increase in EPP amplitude during the next 15 impulses of each stimulation block is mainly due to the increase in the second component of facilitation; the first component of facilitation is no longer increasing, and the increment of the second component of facilitation added by each impulse is about 10 times larger than the increments of augmentation and potentiation.
The second component of facilitation contributes little to the increase in EPP amplitudes after ~1 s (the first 20 impulses) of stimulation in each stimulation block since it has approached a steady-state level by this time due to its 400-ms time constant of decay.

The more gradual increase in EPP amplitudes during the last second (20 impulses) of each stimulation block is due to the progressive increase in augmentation and potentiation. Because these components decay slowly, they do not reach a steady-state level during the individual 2-s stimulation blocks as facilitation does, nor do they decay to insignificant levels between stimulation blocks, as facilitation does, but they gradually increase during the course of the conditioning trains, as indicated by the increase in amplitude of the first EPP of each stimulation block (continuous arrows in Fig. 9).

**EFFECT OF Sr²⁺** Notice in Fig. 9 A that Sr²⁺ had no effect on the amplitude of the first EPP of each successive 2-s stimulation block (broken arrows) when facilitation was not present but when augmentation and potentiation were. Notice also that Sr²⁺ had little effect on the first few impulses in each stimulation block when the first component of facilitation is increasing most rapidly. Sr²⁺ did have the pronounced effect of increasing the rate of rise of EPP amplitudes during the first second (the first 20 impulses) of each stimulation block when the second component of facilitation is increasing rapidly, and Sr²⁺ displaced the EPP amplitudes parallel to the control EPP amplitudes during the last second (the last 20 impulses) of each stimulation block when the second component of facilitation was present but no longer increasing with time.

These effects of Sr²⁺ on EPP amplitudes during repetitive stimulation are just what would be expected if Sr²⁺ selectively increases the increment of the second component of facilitation added by each impulse while having little effect on the first component of facilitation, augmentation, or potentiation. An increase in the increment of the second component of facilitation (and a 50% increase in the time constant of decay; see Fig. 7) would lead to a greater rate of increase in the second component of facilitation during repetitive stimulation and hence to a greater increase in EPP amplitudes during the first second of each stimulation block when the second component of facilitation is increasing. As expected from previous experiments (Fig. 6), Sr²⁺ had little effect on estimates of augmentation and potentiation obtained from semilogarithmic plots of the decays of EPP amplitudes after the conditioning trains in Fig. 9 A.

**EFFECT OF Ba²⁺** The form of the Ba²⁺-induced increase in EPP amplitudes during the patterned conditioning trains (upper curves, Fig. 9 B) is different from that of Sr²⁺ (Fig. 9 A). Ba²⁺ led to a greater rate of increase in EPP amplitudes during the entire duration of each 2-s stimulation block, in contrast to Sr²⁺, which led to a greater rate of increase only during the first part of each stimulation block. In addition, Ba²⁺ led to a greater increase in the amplitude of the first EPP (broken arrows) of each successive stimulation block during the conditioning train, in contrast to Sr²⁺, which had no effect on the first EPP amplitudes.
These results are just what would be expected if Ba$^{2+}$ increased the increment of augmentation added by each nerve impulse during repetitive stimulation. A larger increment would lead to a more rapid increase in augmentation and hence to a more rapid and greater increase in EPP amplitudes. The more rapid increase would continue throughout each 2-s stimulation block and throughout the conditioning train (as indicated by the increased amplitude of the first EPP of each stimulation block) because augmentation, with its long time-course, would not reach a steady-state level during the conditioning stimulation. As expected from previous experiments (Fig. 1), the presence of Ba$^{2+}$ led to an increase in the magnitude of augmentation after the conditioning train shown in Fig. 9 B but had little effect on potentiation. ($A^* [T]$ increased from 0.73 to 2.63 [multiplicative model] in this experiment.)

The results presented in this section suggest that the selective effects of Ba$^{2+}$ and Sr$^{2+}$ on the components of transmitter release develop during the conditioning train in a manner that is consistent with our working hypothesis for transmitter release.

Sr$^{2+}$ and Ba$^{2+}$ Retain Their Selective Effects in the Presence of the Other Ion

The selective effects of Sr$^{2+}$ on the second component of facilitation and of Ba$^{2+}$ on augmentation suggest that the underlying factors that give rise to the components of increased release are not necessarily tightly coupled and that there are some differences in underlying mechanisms. Further support for this suggestion is presented in this section, where it is shown that Sr$^{2+}$ and Ba$^{2+}$ retain their selective effects in the presence of the other ion.

Fig. 10 presents superimposed plots of EPP amplitudes against time obtained during conditioning trains of 200 impulses delivered at 20 impulses/s. Train A represents the response in 0.4 mM Ca$^{2+}$ Ringer. The addition of 0.1 mM Sr$^{2+}$ to the bathing solution containing 0.4 mM Ca$^{2+}$ led to a greater rate of increase in EPP amplitudes during the first second of stimulation (train B), after which the response paralleled the control response. Increasing the concentration of Sr$^{2+}$ to 0.2 mM led to a further increase in EPP amplitudes (train C) during the first second of stimulation but did not change the shape of the response. Adding 0.1 mM Ba$^{2+}$ to the Ca$^{2+}$ + Sr$^{2+}$ Ringer led to a still greater increase in EPP amplitudes (train D), but in this case the increased rate of rise continued for the duration of the conditioning train.

The estimates of augmentation and potentiation after the conditioning trains shown in Fig. 10 were obtained from the decays of EPP amplitudes. As expected, Sr$^{2+}$ had little effect on the magnitude or time-course of decay of augmentation or potentiation. The addition of Ba$^{2+}$ to the Ca$^{2+}$ + Sr$^{2+}$ Ringer increased the magnitude of augmentation about three times (multiplicative model) but had little effect on potentiation or the time constant of decay of augmentation. The Ba$^{2+}$-induced increase in EPP amplitudes during the conditioning train and the increase in the magnitude of augmentation after the train were similar to those observed in other experiments in the absence of Sr$^{2+}$. Thus, Ba$^{2+}$ retains its effect on augmentation in the presence of Sr$^{2+}$. 
The Sr\(^{2+}\)-induced increase in EPP amplitudes during the first second of stimulation was still apparent after the addition of Ba\(^{2+}\). Thus, Sr\(^{2+}\) appears to retain its specific effect on the second component of facilitation in the presence of Ba\(^{2+}\).

In a series of experiments with stimulation patterns like those shown in Figs. 1, 3, and 9, Sr\(^{2+}\) and Ba\(^{2+}\) retained their selective effects in the presence of the other ion. The effects of each ion were reversible and independent of the order of application of the ions. The effect of Ba\(^{2+}\) could also be obtained in the presence of Ca\(^{2+}\) in the bathing solution. However, to reverse the Ba\(^{2+}\) effect under these conditions it was necessary to wash the preparation with a Ca\(^{2+}\)-containing solution. Sr\(^{2+}\) Ringer alone would not reverse the Ba\(^{2+}\) effect. Perhaps Ca\(^{2+}\) is needed to displace or remove Ba\(^{2+}\) from its site of action. Since all the solutions contained 5 mM Mg\(^{2+}\), it appears that Mg\(^{2+}\), like Sr\(^{2+}\), is not capable of reversing the Ba\(^{2+}\) effect in the absence of Ca\(^{2+}\).

Small Changes in [Ca\(^{2+}\)]\(_{o}\) Have Marked Effects on Transmitter Release but Little Effect on Facilitation, Augmentation, or Potentiation

The selective effects of Sr\(^{2+}\) and Ba\(^{2+}\) reported in the previous sections were observed whether the Sr\(^{2+}\)- or Ba\(^{2+}\)-containing Ringer solutions increased, decreased, or had little effect on the control EPP amplitudes in the absence of repetitive stimulation. Both Sr\(^{2+}\) and Ba\(^{2+}\) did lead to greater increases in EPP amplitudes during repetitive stimulation, however, and the possibility arises that some of the effects of these ions may result indirectly from the greater amounts of transmitter released during the conditioning trains in the presence...
of these ions. To investigate this possibility, we changed transmitter release by changing the concentration of Ca$^{2+}$ in the bathing solution and looked for possible effects on facilitation, augmentation, and potentiation. It was found that changes in [Ca$^{2+}$]$_o$, which changed transmitter release severalfold, had little effect on facilitation, augmentation, or potentiation.

An example of such an experiment is shown in Fig. 11. Plots of EPP amplitudes during the conditioning stimulation for two [Ca$^{2+}$]$_o$ are shown in Fig. 11 A. The conditioning stimulation consisted of six stimulation blocks of 41 impulses delivered at 20 impulses/s with 2-s intervals between blocks. Increasing Ca$^{2+}$ from 0.4 to 0.5 mM approximately doubled the amount of evoked transmitter release in the absence of repetitive stimulation, as indicated by the increased amplitude of the first (control) EPP of the train. This increase

![Graph showing the effect of Ca$^{2+}$ on EPP amplitudes during repetitive stimulation.](image-url)
is about what would be expected assuming a fourth power relationship between \([\text{Ca}^{2+}]_0\) and evoked transmitter release (Dodge and Rahamimoff, 1967).

In addition to doubling the control EPP amplitude in the absence of repetitive stimulation, increasing the \([\text{Ca}^{2+}]_0\) from 0.4 to 0.5 mM approximately doubled the EPP amplitudes throughout the conditioning train. When the two conditioning trains in Fig. 11A are scaled (normalized) so that the control EPP amplitudes are the same, the conditioning trains virtually superimpose during the first three stimulation blocks and then deviate from each other only slightly, reaching a maximum difference of about 8% at the end of the conditioning trains, as shown in Fig. 11B. Notice especially that the normalized EPP amplitudes usually superimpose during the rising phase of each stimulation block when facilitation is increasing most rapidly. Since facilitation, augmentation, and potentiation are all expressed in terms of the control EPP amplitude (Eqs. 1–4), the virtual superposition of the normalized EPP amplitudes during the conditioning trains suggests that a change in the \([\text{Ca}^{2+}]_0\) sufficient to double transmitter release has little effect on facilitation, augmentation, or potentiation during the conditioning stimulation. Fig. 11C shows that changing the \([\text{Ca}^{2+}]_0\) also has little effect on augmentation or potentiation after the conditioning trains. Similar results were observed in five additional experiments employing a variety of stimulation patterns.

These observations that changes in \([\text{Ca}^{2+}]_0\) sufficient to change transmitter release severalfold have little effect on facilitation, augmentation, and potentiation are consistent with the observations by Linder (1973 and 1974) and Zuker (1974) that the magnitude and time-course of facilitation in the crab and crayfish are unaffected by varying \([\text{Ca}^{2+}]_0\).

As shown in Fig. 11B, normalized EPP amplitudes near the end of the conditioning trains tended to be slightly lower in solutions containing increased \([\text{Ca}^{2+}]_0\). This decrease may be the result of a slight depletion of transmitter due to the greater transmitter release during the trains recorded in increased \([\text{Ca}^{2+}]_0\) or of nonlinear summation of unit potentials (Martin, 1955) due to the greater absolute EPP amplitudes during the trains recorded in increased \([\text{Ca}^{2+}]_0\).

Katz and Miledi (1968) have shown that \(\text{Ca}^{2+}\) is required during the conditioning impulse for facilitation of transmitter release to a following testing impulse. Thus, it might be expected that the relative increase in EPP amplitudes during short trains of repetitive stimulation would be increased in increased concentrations of \(\text{Ca}^{2+}\). Although experiments like the one presented in Fig. 11 show that small changes in \([\text{Ca}^{2+}]_0\) have little effect on facilitation, augmentation, and potentiation, they do not exclude the possibility that there may be small effects. In a series of six experiments undertaken to look for possible small effects, it was found that increasing \([\text{Ca}^{2+}]_0\) an average of 0.24 mM led to a small (4%) but significant \((P < 0.05, t\ test, \ paired\ data)\) increase in the amplitudes of normalized testing EPPs placed 50 ms after single conditioning impulses. These results agree in general with those of Rahamimoff (1968), who observed an increase of \(\sim 10\%\) under slightly different
exp[perimental conditions. We also observed small but significant increases in
normalized EPP amplitudes during 10-impulse conditioning trains in in-
creased [Ca\textsuperscript{2+}]\textsubscript{o}. These effects would be too small to become readily apparent
during the first 10 impulses of experiments such as those presented in Fig. 11.

Our observation that the addition of 0.1-0.3 mM Ca\textsuperscript{2+} to the bathing
solution increased transmitter release severalfold but had little effect on
normalized EPP amplitudes during and after repetitive stimulation when
compared with the dramatic and selective effects that were obtained by the
addition of similar amounts of Ba\textsuperscript{2+} and Sr\textsuperscript{2+} suggests that the effects of Ba\textsuperscript{2+}
and Sr\textsuperscript{2+} are specific and do not arise from general changes in divalent ion
concentration or from possible changes in the total amount of transmitter
released. The specific effects of Sr\textsuperscript{2+} do, however, require the presence of Mg\textsuperscript{2+}
in the bathing solution.\textsuperscript{1}

DISCUSSION

The major findings of this study are summarized as follows. The presence of
Sr\textsuperscript{2+} in the bathing solution led to an increase in the magnitude, \( F_2 (T) \), and
time constant of decay, \( \tau_{F_2} \), of the second component of facilitation. The
presence of Ba\textsuperscript{2+} in the bathing solution led to an increase in the magnitude
of augmentation, \( A (T) \) but had little effect on its time constant of decay \( \tau_A \).
In contrast to these specific effects, Sr\textsuperscript{2+} had little effect on \( \tau_A \) and \( \tau_P \), and,
depending on the experiment, Sr\textsuperscript{2+} had either little effect on \( A (T) \) and \( P (T) \)
or decreased them slightly (~20%). At low concentrations (0.1-0.5 mM), Ba\textsuperscript{2+}
had little effect on \( P (T) \) and \( \tau_P \). At higher concentrations (2.5-5 mM), Ba\textsuperscript{2+}
increased \( P (T) \), but the increase was always considerably less than the increase
in \( A (T) \).

Estimates of augmentation and the two components of facilitation depend
on the relationships between these components. Because these relationships
are not known, it is not possible to exclude the possibility that Sr\textsuperscript{2+} affects the
first component of facilitation and that Ba\textsuperscript{2+} affects one or both components
of facilitation. However, for the three models of transmitter release considered
in this paper there was no effect of Sr\textsuperscript{2+} on the first component and of Ba\textsuperscript{2+} on
both components of facilitation, or the effects were small when compared with
the specific effects of Sr\textsuperscript{2+} and Ba\textsuperscript{2+}. The specific effects were always independent
of the model used to analyze the data, were reversible, were present both
during and after repetitive stimulation, and were obtained in the presence of the
other ion.

The differential effects of Ba\textsuperscript{2+} and Sr\textsuperscript{2+} on the components that act to
increase transmitter release suggest that there are some differences in the
underlying mechanisms of action of these ions. Both Sr\textsuperscript{2+} and Ba\textsuperscript{2+} enter nerve
terminals with depolarization (Katz and Miledi, 1969), apparently by passing
through the same channels used by Ca\textsuperscript{2+} (Katz and Miledi, 1969; Mellow et
al., 1978; Silinsky, 1978). Once inside the nerve terminal, whether having
entered through the Ca\textsuperscript{2+} channels or by other means (see Fig. 2 and associated
discussion) Ba\textsuperscript{2+} and Sr\textsuperscript{2+} could modify release by acting differentially on

\textsuperscript{1}Pallotta, B., and K. L. Magleby. Unpublished observations.
various parts of the transmitter releasing system (e.g., release sites, synaptic vesicles) or by acting differentially on various aspects of Ca\(^{2+}\) entry, sequestration, and removal from the nerve terminal. (See Baker and Glitsch [1975] and Blaustein et al. [1978] for a discussion of Ca\(^{2+}\) kinetics.)

Not enough is known about the mechanisms of facilitation, augmentation, and potentiation or of possible effects of Ba\(^{2+}\) and Sr\(^{2+}\) in or on the nerve terminal to suggest specific mechanisms for the selective actions of these ions. However, the results of this study do place a number of restrictions on possible mechanisms:

(a) Whatever the mechanism of potentiation, it will have to display similar kinetic properties in the presence of Ca\(^{2+}\), Sr\(^{2+}\), or small amounts of Ba\(^{2+}\) since replacing Ca\(^{2+}\) with Sr\(^{2+}\) or the addition of small amounts of Ba\(^{2+}\) to the bathing solution had little effect on \(P(T)\) and \(\tau_p\). For example, if potentiation normally results from the accumulation of Ca\(^{2+}\) in one or more compartments in the nerve terminal, then Sr\(^{2+}\) should substitute for Ca\(^{2+}\) in these compartments, with the same kinetics of entry, action, and removal as Ca\(^{2+}\).

(b) Whatever the mechanism of augmentation, it will have to display similar kinetic properties in the presence of Ca\(^{2+}\) or Sr\(^{2+}\) since Sr\(^{2+}\) had little effect on \(A(T)\) and \(\tau_A\).

(c) If the Sr\(^{2+}\)-induced increase in the second component of facilitation results from an increased Ca\(^{2+}\) entry during the conditioning train (see Stinnakre and Tauc, 1973), then augmentation and potentiation could not be triggered by the same increased Ca\(^{2+}\) entry since Sr\(^{2+}\) did not increase the magnitude of either of these components.

(d) If the Ba\(^{2+}\)-induced increase in the magnitude of augmentation results from an increased Ca\(^{2+}\) entry during the conditioning train (Ba\(^{2+}\) does increase action potential duration [McLachlan, 1977; McAfee and Yarowsky, 1979]), then potentiation could not be triggered by the same increased Ca\(^{2+}\) entry inasmuch as concentrations of Ba\(^{2+}\) that increased \(A(T)\) did not increase the magnitude of potentiation.

(e) The factors that determine the magnitude and time constant of decay of augmentation must not be tightly coupled because Ba\(^{2+}\) dramatically increased \(A(T)\) but had little effect on \(\tau_A\). For example, if the decay of augmentation reflects the removal of Ca\(^{2+}\) from a specific compartment in the nerve terminal, then Ba\(^{2+}\) could perhaps act by increasing the effectiveness of the Ca\(^{2+}\) in the compartment or by increasing the rate of Ca\(^{2+}\) entry into the compartment during repetitive stimulation, perhaps by blocking some Ca\(^{2+}\) sequestering systems. Ba\(^{2+}\) could not act in terms of this model by decreasing the rate of removal of Ca\(^{2+}\) from this specific compartment since this would change the observed decay rate of augmentation.

(f) Whatever mechanism is proposed to account for the Ba\(^{2+}\)-induced increase in the magnitude of augmentation, it will most likely also have to be able to account for the increase in the magnitude of augmentation that can occur with repeated stimulation and time inasmuch as the effects of these various experimental procedures on augmentation appear to be similar (see Magleby and Zengel, 1976 b). For example, it is known that the level of ionized Ca\(^{2+}\) in the squid axon resulting from repetitive stimulation can
increase dramatically during a long experiment; perhaps because Ca\(^{2+}\) buffers become saturated (Baker et al., 1971). If \(A(T)\) also increases during the course of a long experiment because certain Ca\(^{2+}\) buffers or Ca\(^{2+}\) sequestering systems in the nerve terminal become saturated or fatigued (Magleby and Zengel, 1976 b and c), then Ba\(^{2+}\) could give the same effect as repeated stimulation and time by blocking these specific Ca\(^{2+}\) buffers or Ca\(^{2+}\) sequestering systems or by not being taken up by them. Once inside the nerve terminal, Ba\(^{2+}\) is probably not as easily removed from the cytoplasm as is Ca\(^{2+}\) (Ahmed and Connor, 1979). It is known that Ba\(^{2+}\) is not taken up by mitochondria in an energy-linked process stoichiometric with electron transport as Sr\(^{2+}\) and Ca\(^{2+}\) are (Lehninger, 1970). (See also Alnaes and Rahamimoff [1975] for a discussion of the possible role of mitochondria in transmitter release and Blaustein et al. [1978] for a discussion of nonmitochondrial Ca\(^{2+}\) buffering systems.)

\((g)\) For changes in [Ca\(^{2+}\)]\(_0\) sufficient to change mean quantal content severalfold, the changes in transmitter release that occur during and after repetitive stimulation must have about the same Ca\(^{2+}\) dependency as evoked transmitter release in the absence of repetitive stimulation since these changes in [Ca\(^{2+}\)]\(_0\) have little effect on facilitation, augmentation, or potentiation. It follows that the changes in [Ca\(^{2+}\)]\(_i\) that result from these changes in [Ca\(^{2+}\)]\(_0\) must have little effect on the magnitudes or the decay rates of facilitation, augmentation, and potentiation.

It is important to note that our observation that small changes in [Ca\(^{2+}\)]\(_0\) have little effect on facilitation, augmentation, and potentiation does not exclude the possibility that these processes are Ca\(^{2+}\) dependent. On the contrary, our data show that increasing [Ca\(^{2+}\)]\(_0\) leads to a greater absolute increase in EPP amplitudes during and after the conditioning train (Fig. 11 A). On this basis, our data agree with the findings of Katz and Miledi (1968), Rosenthal (1969), Weinreich (1971), and Younkin (1974) that increasing [Ca\(^{2+}\)]\(_0\) during the conditioning stimulation leads to a greater absolute increase in EPP amplitudes after the conditioning stimulation. What we have shown by keeping [Ca\(^{2+}\)]\(_i\) constant before, during, and after the conditioning stimulation is that for small changes in [Ca\(^{2+}\)]\(_0\), the Ca\(^{2+}\)-induced increase in EPP amplitudes observed during and after the conditioning stimulation is proportional to the Ca\(^{2+}\)-induced increase in EPP amplitudes observed in the absence of repetitive stimulation (Fig. 11 B).

**Conclusion**

The data in this paper have been analyzed in terms of the working hypothesis that changes in transmitter release during and after repetitive stimulation arise from changes in four kinetically distinguishable components that affect transmitter release. The differential effects of Ba\(^{2+}\) and Sr\(^{2+}\) on these components provide a new way to distinguish among them and suggest that the underlying factors in the nerve terminal that give rise to these components are separable and that they can act relatively independently of one another. The ability of our hypothesis to account for the effects of Ba\(^{2+}\) and Sr\(^{2+}\) on EPP
amplitudes during repetitive stimulation using data obtained after repetitive stimulation lends further support to this working hypothesis.

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