Facilitation, Augmentation, and Potentiation of Synaptic Transmission at the Superior Cervical Ganglion of the Rabbit

J. E. ZENGEL, K. L. MAGLEBY, J. P. HORN, D. A. McAFEE, and P. J. YAROWSKY

From the Department of Physiology and Biophysics, University of Miami School of Medicine, Miami, Florida 33101 (Zengel and Magleby), and the Division of Neurosciences, City of Hope Research Institute, Duarte, California 91010 (Horn, McAfee, and Yarowsky). J. E. Zengel's present address is the Department of Anatomy, Emory University School of Medicine, Atlanta, Georgia 30322. J. P. Horn's present address is the Department of Neurobiology, Harvard Medical School, Boston, Massachusetts 02115. P. J. Yarowsky's present address is the Laboratory of Cerebral Metabolism, National Institutes of Health, Bethesda, Maryland 20205.

ABSTRACT The effect of repetitive stimulation on synaptic transmission was studied in the isolated superior cervical ganglion of the rabbit under conditions of reduced quantal content. Excitatory postsynaptic potentials (EPSP) were recorded with the sucrose gap technique to obtain estimates of transmitter release. Four components of increased transmitter release, with time constants of decay similar to those observed at the frog neuromuscular junction at 20°C, were found in the ganglion at 34°C: a first component of facilitation, which decayed with a time constant of 59 ± 14 ms (mean ± SD); a second component of facilitation, which decayed with a time constant of 388 ± 97 ms; augmentation, which decayed with a time constant of 7.2 ± 1 s; and potentiation, which decayed with a time constant of 88 ± 25 s. The addition of 0.1–0.2 mM Ba^{2+} to the Locke solution increased the magnitude but not the time constant of decay of augmentation. Ba^{2+} had little effect on potentiation. The addition of 0.2–0.8 mM Sr^{2+} to the Locke solution appeared to increase the magnitude of the second component of facilitation. Sr^{2+} had little effect on augmentation or potentiation. These selective effects of Ba^{2+} and Sr^{2+} on the components of increased transmitter release in the rabbit ganglion are similar to the effects of these ions at the frog neuromuscular junction. Although the effects of Ba^{2+} and Sr^{2+} are similar in the two preparations, the magnitudes of augmentation and the second component of facilitation after a single impulse were about 6–10 times greater in the rabbit ganglion than at the frog neuromuscular junction. These results suggest that the underlying mechanisms in the nerve terminal that give rise to the components of increased transmitter release in the rabbit ganglion and frog neuromuscular junction are similar but not identical.

INTRODUCTION

The amount of transmitter released by each nerve impulse changes as a function of previous activity at a variety of synapses (Liley, 1956; Kuno, 1964;

J. GEN. PHYSIOL. © The Rockefeller University Press · 0022-1295/80/08/0213/19 $1.00 213
Volume 76 August 1980 213–231

Zucker, 1974; Linder, 1974; McNaughton, 1977; Woodson et al., 1978). At the frog neuromuscular junction, where use-dependent changes in transmitter release have been studied most extensively, four components of increased transmitter release have been identified on the basis of their kinetic and pharmacological properties. These components are: the first and second components of facilitation, which decay with time constants of ~50 and 300 ms, respectively (Mallart and Martin, 1967; Magleby, 1973; Younkin, 1974); augmentation, which decays with a time constant of ~7 s (Magleby and Zengel, 1976; Erulkar and Rahamimoff, 1978); and potentiation, which decays with a time constant that ranges from tens of seconds to minutes (Hubbard, 1963; Rosenthal, 1969; Magleby and Zengel, 1975 *a* and *b*). Components of increased transmitter release have also been observed in autonomic ganglia (Larrabee and Bronk, 1947; Martin and Pilar, 1964; Brimble and Wallis, 1974; Tashiro et al., 1976; McLachlan, 1977). From these studies, however, it is not clear how many components are present in ganglia, nor is it known what the relationship is between the components that have been observed in ganglia and those that have been characterized at the frog neuromuscular junction.

In this paper we examine the effect of repetitive stimulation on synaptic transmission in the rabbit superior cervical ganglion to determine how many components of increased transmitter release are present in this preparation and to characterize their properties. We find that under conditions of low quantal contents EPSP amplitudes increase progressively during repetitive stimulation, just as reported by Libet (1967). After repetitive stimulation, the amplitudes of EPSPs elicited by testing impulses return to the control level with four apparent components of decay with time constants of about 60 ms, 400 ms, 7 s, and 90 s (34°C). On the basis of kinetic and pharmacological data, we suggest that these four components in the rabbit ganglion are analogous to the first component of facilitation, the second component of facilitation, augmentation, and potentiation, respectively, at the frog neuromuscular junction. A preliminary report of some of this work has appeared (Magleby et al., 1978).

METHODS

Preparation and Recording

Experiments were performed on superior cervical sympathetic ganglia isolated from New Zealand white rabbits (~2 kg). The rabbits were sacrificed by an air embolus, and the ganglia were removed and decapsulated in oxygenated Locke solution. A ganglion was then mounted in a sucrose-gap apparatus with the internal carotid nerve spanning the gap and continuously superfused with Locke solution (McAfee and Greengard, 1972). Supramaximal preganglionic stimuli (0.5-ms) were applied to the cervical sympathetic nerve, and postganglionic responses recorded differentially (frequency response, 0.1–1,000 Hz) between the solution flowing over the ganglion and the solution flowing over the internal carotid nerve. A modified Locke solution low in Ca^{2+} (0.4–0.8 mM) and high in Mg^{2+} (10 mM) was used to reduce neurotransmitter release to considerably below that required to generate action potentials in the

postganglionic neuron (Libet, 1967). In this manner, stable monophasic EPSPs electrotonically conducted in the internal carotid nerve across the sucrose gap were recorded for up to 12 h.

The modified Locke solution referred to as "low Ca^{2+} Locke" in this paper had the composition (mM): NaCl, 136; KCl, 5.6; $NaHCO_3$, 20.0; NaH_2PO_4, 1.2; $CaCl_2$, 0.4–0.8; $MgCl_2$, 10; and glucose, 8.3. This solution was equilibrated with 95% O_2 and 5% CO_2 in the reservoirs leading to the sucrose-gap apparatus. To examine the effects of Ba^{2+} and Sr^{2+}, the low Ca^{2+} Locke was modified by the addition of 0.1–0.2 mM Ba^{2+} (low Ca^{2+} + Ba^{2+} Locke), by the addition of 0.2–0.4 mM Sr^{2+} (low Ca^{2+} + Sr^{2+} Locke), or by the replacement of the Ca^{2+} with 0.8 mM Sr^{2+} (Sr^{2+} Locke).

In a few preliminary experiments, EPSPs were recorded with intracellular techniques (McAfee and Yarowsky, 1979) from postganglionic neurons in the rat superior cervical ganglion. Because of the quantal fluctuations in the response (del Castillo and Katz, 1954 *a*) in low Ca^{2+} Locke solution, it was usually necessary to average 20–100 intracellular trials. The sucrose-gap technique used in this work gives a good measure of the average response in one or a few trials because this technique sums the postsynaptic responses from many cells at once.

The temperature of the preparation was monitored by a thermister probe mounted near the ganglion within the sucrose-gap apparatus. Most experiments were performed at room temperature. Temperatures above room temperature were achieved by immersing the tubing carrying the perfusion solutions in a warm water bath.

Data Collection and Analysis

A PDP-11 computer (Digital Equipment Corp., Maynard, Mass.) was used to generate the stimulation patterns and to collect and analyze the data. The data acquisition and analysis techniques have been described in considerable detail (Magleby and Zengel, 1976). For most experiments, the nerve was conditioned with a train of impulses; testing impulses were then applied to examine the effect of the conditioning stimulation. The conditioning stimuli were usually applied at a rate of 5 impulses/s because this gave the most consistent results; but in some experiments, in particular those performed at higher temperatures, the conditioning impulses were applied at a rate of 10/s. For experiments like those shown in Figs. 1, 2, and 4, amplitudes of the EPSPs elicited by the conditioning and testing stimuli were measured and stored by the computer during each trial. Data from three to five conditioning-testing trials of each type were usually averaged for analysis. Because the EPSPs fell upon the tails of preceding EPSPs during the conditioning stimulation, the amplitudes of EPSPs during the train were underestimated by ~10%. In experiments like that shown in Fig. 3, the conditioning-testing interval was often short enough that testing EPSPs fell on the tail of the preceding conditioning EPSP. Therefore, in experiments of this type, the entire response during each conditioning-testing trial was sampled by the computer at a rate of 1 point/5 ms and stored. In a typical experiment of this type, the preparation was presented with 18 different conditioning-testing intervals until >60–80 trials were collected. After the experiment, the averaged response for each conditioning-testing interval was displayed on an oscilloscope and photographed, and the EPSP amplitudes were then measured by hand. When a testing EPSP fell on the tail of a conditioning EPSP, the amplitude was measured from the "projected tail" of the preceding conditioning EPSP. The projected tail was determined directly in a separate trial in which the testing impulse was not present.

Potentiation, $P(t)$, augmentation, $A(t)$, and the first and second components of facilitation, $F_1(t)$ and $F_2(t)$, are all defined in a similar manner; each one is given by

the fractional increase in a test EPSP amplitude over a control in the absence of the other processes (Magleby and Zengel, 1976). Experimentally, it is not always possible to measure one component in the absence of the others. Consequently, the magnitude and time-course of the individual components are derived from the fractional change in EPSP amplitude, $V(t)$, which is given by

$$V(t) = \frac{v(t)}{v_0} - 1, \tag{1}$$

where $v(t)$ is the testing EPSP amplitude at time t and v_0 is the control EPSP 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 various processes have distinct and nonoverlapping time constants which characterize their decays. Potentiation, the slowest decaying component, can be estimated in the absence of augmentation and facilitation using data points collected after these faster components have decayed. Since 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 which defines the relationship between the appropriate components and transmitter release. The data in this paper were analyzed by assuming:

$$V(t) = [F_1^*(t) + F_2^*(t) + A^*(t) + 1][P^*(t) + 1] - 1, \tag{2}$$

where $V(t)$ is the fractional increase in transmitter release as defined by Eq. 1, 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 the first component of facilitation, the second component of facilitation, augmentation, and potentiation, respectively (see Zengel and Magleby, 1980). The methods used to derive estimates of the components with Eq. 2 are presented in Magleby and Zengel (1976) and Zengel and Magleby (1980). It should be noted that estimates of the various components depend on the model assumed for transmitter release (Zengel and Magleby, 1980). Eq. 2 was used in this study because it permits comparison with data obtained previously in the frog. The time constants of decay of the various components and the conclusions reached in this study were relatively independent of which of several commonly proposed models of transmitter release were used to analyze the data. However, the absolute magnitudes of the components can be model dependent, so that a comparison of magnitudes between studies requires that similar models be used for data analysis.

Time

Time is indicated in two ways: $A^*(T)$, for example, refers specifically to the magnitude of augmentation at the end of a conditioning train of T s duration, while $A^*(t)$ refers to the decay of augmentation, where t is the time after the end of the conditioning train.

Solution Changes

In experiments in which we looked at the effects of Ba^{2+} or Sr^{2+} on the response, conditioning-testing trials were first collected in low Ca^{2+} Locke. The superfusion solution was then changed to the Ba^{2+}- or Sr^{2+}-containing solution, and additional conditioning-testing trials were collected, followed once again by trials in low Ca^{2+} Locke. The data obtained under each set of conditions were then averaged and analyzed.

Effect of Temperature

The Q_{10}s of the decays of the components were determined as described in Magleby and Zengel (1976).

Data are presented as mean ± SD.

RESULTS

The Rise and Decay of EPSP Amplitudes during and after Long Conditioning Trains

In low Ca^{2+} Locke (0.4–0.8 mM Ca^{2+}, 10mM Mg^{2+}) transmitter release is greatly reduced so that it is posssible to record fast EPSPs (*inset*, Fig. 1 *A*) uncontaminated by propagated action potentials and slow potentials (Libet, 1967; McLachlan, 1975). The effect of repetitive stimulation on EPSP amplitude is shown in Fig. 1 *A*, where EPSP amplitude is plotted against time for a conditioning-testing trial. The preganglionic nerve was first stimulated once every 15 s to establish a control response. The preparation was then conditioned by stimulating with 100 impulses delivered at a rate of 5/s, and the effect of this conditioning stimulation was followed by applying testing impulses. The EPSP amplitudes rapidly increase during the conditioning train (horizontal bar) and then decay back to the control level in the posttetanic period. When the EPSP amplitudes folowing the conditioning train in Fig. 1 *A* are plotted semilogarithmically against time (Fig. 1 *D*), a dual exponential decay becomes readily apparent. The line through the filled circles in Fig. 1 *D*, determined by a least squares fit to the data points beyond 60 s, describes the exponential decay of the slower component, which had a time constant of 157 s. The initial magnitude of this component of decay, obtained from the intercept of the line with the ordinate at 0 time (the time at the end of the conditioning train) was 0.63. A magnitude of 0.63 would increase EPSP amplitude 1.63 times.

Superimposed on the slower component of decay of EPSP amplitudes is a faster component. The filled circles in Fig. 1 *F* show the decay of this faster component, which had a time constant of 17.4 s and an initial magnitude of 2.4.

The effect of long conditioning trains on EPSP amplitudes in the sympathetic ganglion appears to be similar to the effect of long conditioning trains on EPP amplitude at the frog neuromuscular junction (cf. Fig. 1 and Fig. 1 in Zengel and Magleby, 1980). This similarity suggests that the fast and slow components of the decay of EPSP amplitude observed in the ganglion after long conditioning trains might correspond to the decay of augmentation and potentiation described for the frog neuromuscular junction. Further evidence for this proposal will be presented in the following sections.

Two components of decay were seen in 18 additional experiments of the type shown in Fig. 1 (25–36.5°C) in which the nerve was conditioned with 200 or 600 impulses. The initial magnitudes and times constants of decay of the components in these experiments are presented in Table I, where the faster component is referred to as augmentation (A^*) and the slower as potentiation (P^*).

Identification of Augmentation in the Sympathetic Ganglion

At the frog neuromuscular junction, the addition of small amounts of Ba^{2+} to low Ca^{2+} Ringer's solution selectively increases the magnitude of augmentation during and after a conditioning train but has little effect on its time constant of decay or on potentiation (Zengel and Magleby, 1977 and 1980). If Ba^{2+} has the same effect on synaptic transmission at the ganglion as at the

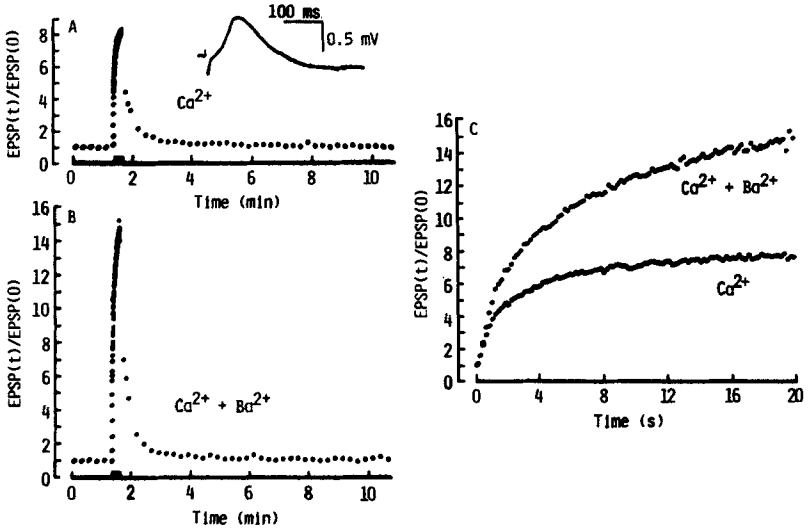

FIGURE 1. Effect of repetitive stimulation on EPSP amplitudes in low Ca^{2+} (0.4 mM) Locke and in low Ca^{2+} Locke containing 0.1 mM Ba^{2+}. (*Inset* in *A*) EPSP recorded in low Ca^{2+} Locke. (*A* and *B*) Plots of EPSP amplitudes against time. Horizontal bars indicate times of 100-impulse conditioning stimulation delivered at 5 impulses/s. Control and testing impulses before and after the conditioning stimulation were delivered once every 15 s, except for the three testing impulses immediately after the train, which were delivered at one impulse every 5 s. (*A*) Ca^{2+}. (*B*) Ca^{2+} + Ba^{2+}. (*C*) EPSP amplitudes recorded during the conditioning trains shown in *A* and *B* plotted on an expanded time scale (upper train, Ca^{2+} + Ba^{2+}; lower train, Ca^{2+}). (*D* and *E*) Decay of EPSP amplitudes after the conditioning trains in *A* and *B* plotted semilogarithmically against time. The line in *D* is the decay of potentiation, $P^*(t)$, in low Ca^{2+} Locke, and the line in *E* is the decay of potentiation in low Ca^{2+} + Ba^{2+} Locke. (*F*) Decay of augmentation, $A^*(t)$, in low Ca^{2+} Locke (continuous line) and low Ca^{2+} + Ba^{2+} Locke (broken line). See Methods for details on the derivation of the components of decay. Temperature, 26°C.

frog neuromuscular junction, it should be possible to identify augmentation in the ganglion with Ba^{2+}.

Fig. 1 *B* and *C* shows the effect of adding 0.1 mM Ba^{2+} to the low Ca^{2+} Locke solution perfusing the ganglion. Control data from the same preparation in the absence of Ba^{2+} is shown in in Fig. 1 *A* and *C*. EPSP amplitudes increase more rapidly and reach a greater final magnitude at the end of the conditioning train in the presence of Ba^{2+} than in its absence. The amplitudes of the

first few testing EPSPs after the conditioning train are also increased more in the presence of Ba^{2+}, suggesting that there is an increase in the magnitude of the faster component of decay. This is more clearly shown by the semilogarithmic plots of the decay in Fig. 1 *E* and *F*. Although the slower component of decay (broken line in Fig. 1 *E*) was not affected by the addition of Ba^{2+} (cf. Fig. 1 *D*), the initial magnitude of the faster component (broken line in Fig. 1 *F*) was increased about two times, from 2.4 to 4.5. When Ba^{2+} was further increased to 0.2 mM in this experiment the initial magnitude of the faster component was increased even more to 7.3, a threefold increase (data not shown). Although Ba^{2+} increased the magnitude of the faster component, Ba^{2+} had little effect on its time constant of decay (Fig. 1 *F*). This increase in the magnitude of the faster component, with little effect on its time constant or on the slower component, is similar to the effect of Ba^{2+} on augmentation

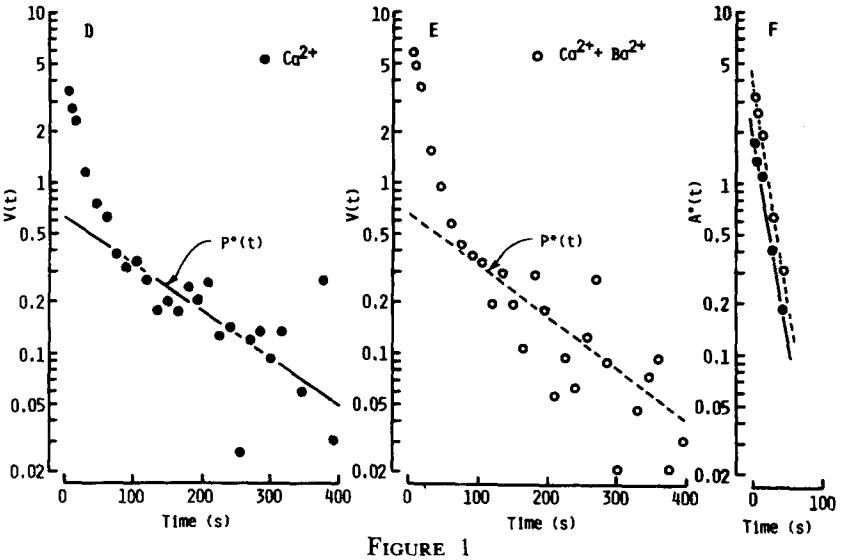

FIGURE 1

and potentiation, respectively, at the frog neuromuscular junction. (Cf. Fig. 1 and Figs. 1 and 10 in Zengel and Magleby [1980].) Our interpretation of these results is that the faster component of decay in Fig. 1 is analogous to augmentation at the frog neuromuscular junction and that the slower component is analogous to potentiation.

In six experiments like that presented in Fig. 1 in which the nerve was conditioned with 100–600 impulses at a rate of 5/s, the addition of 0.1–0.2 mM Ba^{2+} increased the magnitude of augmentation an average of 2.8 times, from 3.8 ± 2.2 to 10.8 ± 5.2 ($P < 0.01$, t test, paired data). In these experiments, Ba^{2+} had little effect on the time constant of decay of augmentation (14.5 ± 2.7 s in Ca^{2+} Locke compared with 15.2 ± 1.8 s in Ca^{2+} + Ba^{2+} Locke) or on the magnitude (1.0 ± 0.6 compared with 1.3 ± 0.6) or time constant of decay (144 ± 27 s compared with 138 ± 34 s) of potentiation. The Ba^{2+}-induced increase in the magnitude of augmentation was slowly reversible (20–40 min) after the removal of Ba^{2+} from the perfusion solution. Complete

substitution of Ba^{2+} for Ca^{2+} was not tried since Ba^{2+} alone cannot support evoked release (Miledi, 1966; McLachlan, 1977; Silinsky, 1977; Illes and Thesleff, 1978).

Decay of EPSP Amplitudes after Five Conditioning Impulses

In addition to augmentation and potentiation, first and second components of facilitation, with time constants of decay of about 50 and 300 ms, respectively, have been described at the frog neuromuscular junction (Mallart and Martin, 1967; Magleby, 1973). Note in Fig. 1 *A* and *B* the sudden drop in EPSP amplitudes between the end of the conditioning train and the first

TABLE I

MAGNITUDES AND TIME CONSTANTS OF DECAY OF THE FIRST COMPONENT OF FACILITATION, THE SECOND COMPONENT OF FACILITATION, AUGMENTATION, AND POTENTIATION IN THE RABBIT SUPERIOR CERVICAL GANGLION

Number of conditioning impulses	Temperature	Sample size	$F_1^*(T)$	τ_{F_1}	$F_2^*(T)$	τ_{F_2}	$A^*(T)$	τ_A	$P^*(T)$	τ_P
	°C			ms		ms		s		s
1	25–26	4	‡	‡	0.90 ±0.24	464 ±102	0.13 ±0.06	17.3 ±1.2	§	§
1	32–34	5	1.43 ±1.02	59 ±14	0.77 ±0.49	388 ±97	0.18 ±0.10	8.9 ±1.2	§	§
5	26.5–28	6	§	§	4.30 ±2.42	496 ±54	0.82 ±0.28	18.6 ±3.7	§	§
200	25–27.5	10	§	§	§	§	2.30 ±0.70	16.4 ±2.5	0.63 ±0.25	150 ±45
200	34.5–36.5	5	§	§	§	§	2.00 ±1.05	7.2 ±1.0	0.46 ±0.18	88 ±25
600	27	4	§	§	§	§	2.11 ±0.59	16.0 ±2.5	1.05 ±0.64	177 ±34

‡ The first component of facilitation was not evident at these temperatures.
§ Testing impulses were not applied to test for these parameters.
Results are shown as mean ± SD.

testing impulse at 5 s. A similar rapid drop at the frog neuromuscular junction results from the decay of facilitation (Zengel and Magleby, 1980), suggesting that facilitation may also be present in the sympathetic ganglion. To look for facilitation in the ganglion, we tested for a component of release that decays faster than augmentation, by using short rather than long conditioning trains since short trains can be applied more often, making it possible to perform the large number of conditioning-testing trials necessary to map the decay of facilitation.

For the experiment shown in Fig. 2, the nerve was presented with a series of conditioning trains consisting of five impulses at a rate of 5/s. A single testing impulse was applied from 330 ms to 60 s after each conditioning train. The inset in Fig. 2 *A* shows a trial in which the conditioning-testing interval was 330 ms. Fig. 2 *A* presents a semilogarithmic plot of the decay of the EPSP amplitudes after the five-impulse conditioning trains. Two components of

decay are clearly present. The slower component in Fig. 2 *A* (continuous line) decays with a time constant of 18 s. This is similar to the decay rate of augmentation after the long conditioning trains in Fig. 1, suggesting that this component is augmentation; both at the frog neuromuscular junction (Magleby and Zengel, 1976) and in the sympathetic ganglion (Table I), the time constant of decay of augmentation is relatively independent of the number of conditioning impulses.

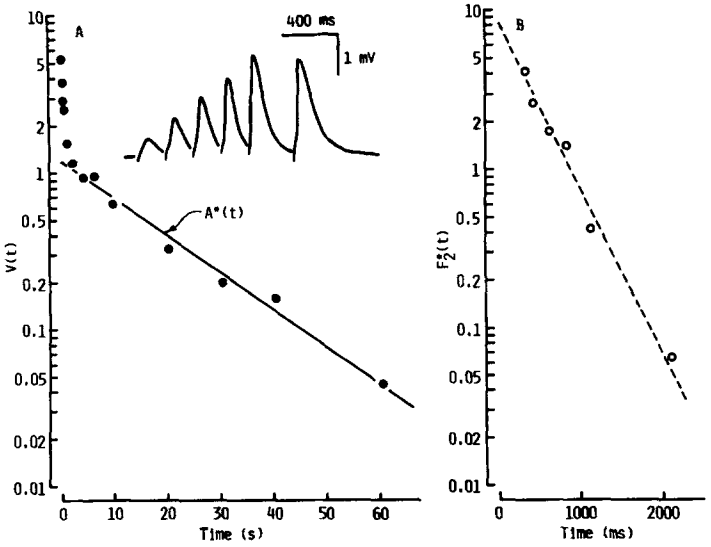

FIGURE 2. Decay of EPSP amplitudes after conditioning trains of five impulses at a rate of 5/s. A single testing impulse was applied from 330 ms to 60 s after each conditioning train. Conditioning-testing trials were applied about once every 3 min. The inset in *A* presents a drawing of the recorded response based on a computer-measured conditioning-testing trial with a 330-ms testing interval. (*A*) Semilogarithmic plot of the decay of the testing EPSP amplitudes. Each filled circle represents the average response from four to eight conditioning-testing trials. The line in *A* represents the decay of augmentation, $A^*(t)$. (*B*) Decay of the second component of facilitation. $[Ca^{2+}]_o = 0.4$ mM. Temperature, 27°C.

Superimposed on augmentation is a faster component of decay that presumably represents facilitation. The decay of this faster component is plotted in Fig. 2 *B*. The broken line represents the exponential decay of this component, which had a time constant of 425 ms. At the frog neuromuscular junction, the faster component of decay that immediately precedes augmentation is the second component of facilitation. If facilitation in the ganglion is similar to facilitation at the frog neuromuscular junction, then the faster component of decay plotted in Fig. 2 *B* would be the second component of facilitation. To obtain enough data to define this component and augmentation in the same experiment, we applied testing impulses only after intervals >300 ms after the train. Consequently, it was not possible to determine whether the first component of facilitation was present in this experiment.

In five additional experiments of the type shown in Fig. 2, augmentation and a faster decaying component, presumably the second component of facilitation, were clearly present. The parameters describing the decays of these components are presented in Table I. (In analyzing these additional experiments, or the one in Fig. 2, we have not corrected the data for any possible contributions from potentiation. Other experiments established that the magnitude of potentiation after a 5-impulse train was sufficiently small to have little effect on the results.)

Evidence for Two Components of Facilitation

To look for the first component of facilitation in the ganglion we used the method initially employed by Mallart and Martin (1967) to describe the two components of facilitation at the frog neuromuscular junction; a single testing impulse was applied at various intervals after a single conditioning impulse. The inset in Fig. 3 *A* shows a trial in which the conditioning-testing interval was 400 ms. The decay of the fractional increase in EPSP amplitudes from a series of two-pulse conditioning-testing trials is plotted semilogarithmically against time in Fig. 3 *A*. The EPSP amplitudes are greatest at the time of the testing impulse applied at 50 ms and then decay with time. The line represents an estimate of the decay of augmentation, which had an initial magnitude of 0.28 and a time constant of decay of 7.7 s. The faster decay of augmentation in this experiment, when compared with those shown in Figs. 1 and 2, is due to the higher temperature in this experiment (32.5°C) compared with the previous experiments (26–27°C). The effect of temperature will be considered in detail in a later section.

Superimposed on the more slowly decaying augmentation in Fig. 3 *A* is what we presume to be facilitation. The decay of facilitation is plotted on an expanded time scale in Fig. 3 *B*. First and second components of facilitation similar to those reported at the frog neuromuscular junction (Mallart and Martin, 1967) are clearly evident. The line through the open circles in Fig. 3 *B* represents the decay of the second component of facilitation, which had an initial magnitude of 1.5 and a time constant of decay of 356 ms. The line through the filled squares in Fig. 3 *B* represents the decay of the first component of facilitation, which had an initial magnitude of 2.8 and a time constant of decay of 61 ms.

Five additional experiments like that shown in Fig. 3 were performed at temperatures ranging from 29 to 34°C. In each of these experiments there was a clear indication of the two components of facilitation and of augmentation. The mean magnitudes and time constants of decay for these components from the experiments performed at 32–34°C are presented in Table I. Surprisingly, in four experiments performed at a lower temperature (25–26°C), a first component of facilitation was not observed, even though the second component of facilitation and augmentation were present. The data from these experiments are also summarized in Table I. More experiments over a range of temperatures are necessary to determine whether the first component of facilitation is only observable at higher temperatures.

Sr^{2+} Appears to Increase Facilitation Selectively

At the frog neuromuscular junction, the addition of small amounts of Sr^{2+} to the bathing solution or the replacement of Ca^{2+} with Sr^{2+} increases the magnitude and time-course of the second component of facilitation but has little effect on the first component of facilitation, augmentation, or potentiation (Zengel and Magleby, 1977 and 1980). To determine whether Sr^{2+} has a similar effect in the ganglion we examined the effect of Sr^{2+} on the increase in EPSP amplitude during and after repetitive stimulation.

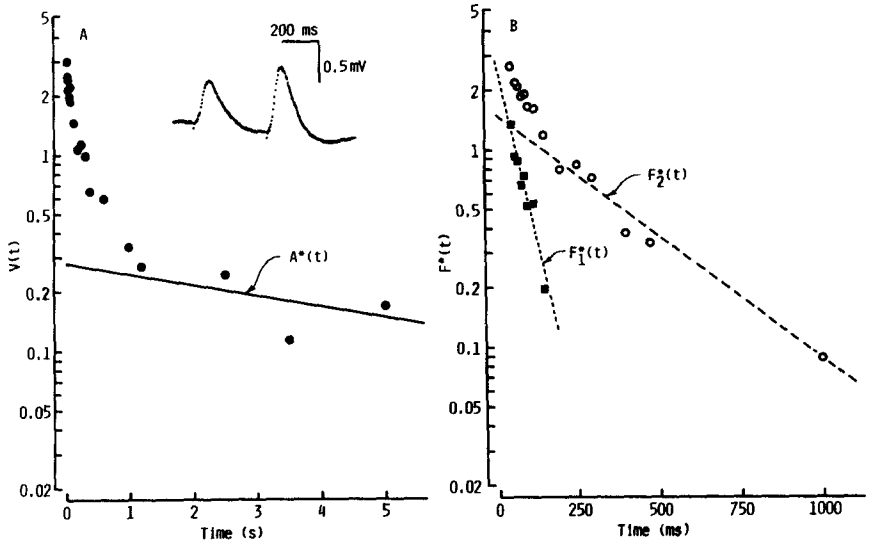

FIGURE 3. Decay of EPSP amplitudes after single conditioning impulses. A single testing impulse was applied from 50 ms to 5 s after each conditioning impulse. Conditioning-testing trials were applied about once every 30 s. Data from two preparations are averaged. The inset in *A* presents a computer-recorded conditioning-testing trial with a 400-ms testing interval. The undershoot following the EPSPs results because the preamplifier was a-c coupled at 1 Hz for this figure, compared with 0.1 Hz for the previous figures. (*A*) Semilogarithmic plot of decay of testing EPSP amplitudes. Each filled circle represents the average response to six conditioning-testing trials. The continuous line in *A* represents the decay of augmentation, $A^*(t)$. (*B*) Decay of first, $F_1^*(t)$ (■), and second, $F_2^*(t)$ (○), components of facilitation. $[Ca^{2+}]_o$ = 0.6 mM. Temperature, 32.5°C.

Fig. 4 *A* and *B* shows the rise and decay of EPSP amplitudes during and after 200-impulse conditioning trains in low Ca^{2+} Locke and after the addition of 0.2 mM Sr^{2+} to the solution. EPSP amplitudes reached a greater magnitude during the conditioning train in the presence of Sr^{2+}. Fig. 4 *C* shows on an expanded time scale the rise of EPSP amplitudes during the conditioning stimulation. Notice that this greater rate of increase in the presence of Sr^{2+} occurs mainly during the first 2 s of stimulation, after which EPSP amplitudes

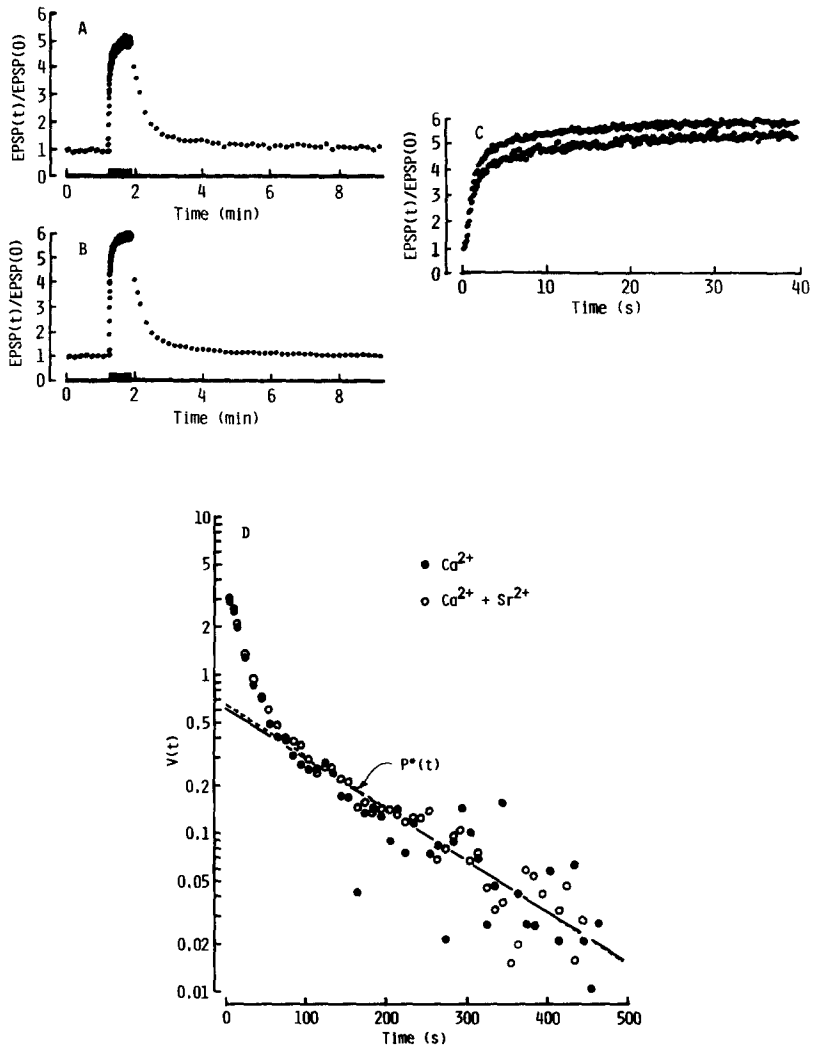

FIGURE 4. Effect of Sr^{2+} on EPSP amplitudes during and after repetitive stimulation. (*A* and *B*) Plots of EPSP amplitudes against time for conditioning-testing trials recorded in 0.4 mM Ca^{2+} Locke (*A*) and 0.4 mM Ca^{2+} plus 0.2 mM Sr^{2+} Locke (*B*). Bars indicate time of conditioning trains of 200 impulses delivered at 5 impulses/s. Control and testing impulses were delivered once every 10 s, except for the three testing impulses immediately after the trains, which were delivered at one impulse every 5 s. (*C*) Amplitudes of EPSP recorded during the conditioning trains shown in *A* and *B* plotted on an expanded time scale. (*D*) Decay of EPSP amplitudes after the conditioning trains shown in *A* and *B* plotted semilogarithmically against time. The continuous line through the filled circles is the decay of $P^*(t)$ in low Ca^{2+} Locke and the broken line through the open circles is the decay of $P^*(t)$ in low Ca^{2+} + Sr^{2+} Locke. Temperature, 25°C.

in the presence and absence of Sr^{2+} rise parallel to each other. This observation is consistent with the suggestion that Sr^{2+} selectively increases the magnitude of facilitation, since facilitation, with its rapid time-course, would be expected to reach a steady-state level during the first few seconds of repetitive stimulation (see Mallart and Martin, 1967). Supporting the suggestion that the increase in EPSP amplitudes in the presence of Sr^{2+} results from an increase in facilitation is the observation that Sr^{2+} had little effect on augmentation or potentiation after the conditioning train (Fig. 4 *D*). These effects of Sr^{2+}, which were also observed in two additional ganglion preparations, were reversible and are similar to the effects of Sr^{2+} at the frog neuromuscular junction (See Figs. 6 and 10 in Zengel and Magleby, 1980).

The effect of Sr^{2+} on the decay of the increased EPSP amplitude after five-impulse conditioning trains was examined to see if Sr^{2+} acts mainly on the second component of facilitation, as it does at the frog neuromuscular junction. In several experiments, replacing the Ca^{2+} in the low Ca^{2+} Locke with 0.8 mM Sr^{2+}, or reducing Ca^{2+} to 0.1 mM and adding 0.8 mM Sr^{2+}, led to a 20–40% greater increase in the testing EPSP amplitudes during the first 1–2 s of the decay after the five-impulse conditioning trains ($P < 0.01$, t test, paired data, $n = 10$). Because the first component of facilitation would decay within several hundred milliseconds after the train, and because Sr^{2+} has little effect on augmentation or potentiation (Fig. 4 *D*), the increase in EPSP amplitudes during the first 1–2 s after the conditioning trains in Sr^{2+} most likely arises from an increase in the second component of facilitation. The effect of Sr^{2+} in the ganglion thus appears to be similar to the effect of Sr^{2+} at the frog neuromuscular junction. Because we had difficulty maintaining the preparation in the presence of Sr^{2+}, it was not possible to collect sufficient data to estimate the two components of facilitation and augmentation in a single preparation in the presence and absence of Sr^{2+}. Therefore, a more direct investigation of the proposed effect of Sr^{2+} on the second component of facilitation in the ganglion is necessary.

Effect of Ca^{2+}

Changing the average level of transmitter release severalfold by changing the concentration of Ca^{2+} had little effect on estimates of facilitation, augmentation, or potentiation when compared with the dramatic and selective effects produced by Sr^{2+} and Ba^{2+}, suggesting that the effects of Sr^{2+} and Ba^{2+} are quite specific and do not arise from general changes in the concentrations of divalent cations or from changes in the average level of transmitter release.

Effect of Temperature on the Decay of Facilitation, Augmentation, and Potentiation

The rates of decay of potentiation (Rosenthal, 1969), augmentation (Magleby and Zengel, 1976), and facilitation (Balnave and Gage, 1974) are temperature sensitive at the frog neuromuscular junction. We found that the decays of these components are also temperature sensitive in the ganglion. (In doing these studies we found it convenient to analyze the data in terms of time constants which are the reciprocal values of the rate constants used to calculate

Q_{10}s.) In four preparations in which we were able to obtain estimates of the decays of augmentation and potentiation at two different temperatures in the same preparation, the mean Q_{10} for the decay of potentiation was 2.1 ± 0.6 and for the decay of augmentation, 3.0 ± 0.5. For example, in one experiment (200-impulse conditioning trains), increasing the temperature from 27.5 to 34°C decreased the time constant of decay of potentiation from 153 to 92 s (Q_{10} = 2.1) and decreased the time constant of decay of augmentation from 16.0 to 7.7 s (Q_{10} = 2.8). In another experiment (one conditioning impulse), increasing the temperature from 29 to 32.5°C decreased the time constant of decay of the second component of facilitation from 500 to 361 ms (Q_{10}= 2.5). We were not able to estimate the decay of the first component of facilitation at two different temperatures from a single preparation. Data obtained from six preparations, each examined at only one temperature, do suggest, however, that the decay of the first component of facilitation is also temperature sensitive. This is shown in Fig. 5, where the time constants of decay of potentiation, augmentation, and the two components of facilitation are plotted as a function of temperature. Because the data were obtained from 20 preparations using a variety of stimulation patterns, there is considerable scatter in the data. The data do show, however, that the time constants of decay of all four components are temperature sensitive and do not overlap. For comparison, the time constants for the decay of the four components at the frog neuromuscular junction at 20°C are also included in Fig. 5. The temperature sensitivity of the decays of the pooled ganglion data, as indicated by the lines in Fig. 5, is most likely an underestimate since the more reliable estimates of Q_{10}s obtained from data from single preparations were 15–40% higher than the Q_{10}s that can be obtained from the averaged data.

DISCUSSION

The marked similarity in the kinetic and pharmacological properties of synaptic transmission in the rabbit ganglion and frog neuromuscular junction suggest similarities in the basic underlying mechanisms of transmitter release in the two preparations. On this basis, the four components of increased transmitter release in the ganglion, which had time constants of decay at 34°C of about 60 ms, 400 ms, 7 s, and 90 s, would be analogous to the first component of facilitation, the second component of facilitation, augmentation, and potentiation, respectively, at the frog neuromuscular junction. Unlike the frog neuromuscular junction, the superior cervical ganglion of the rabbit is not a simple synapse. In addition to the fast EPSP studied in this paper, slow synaptic potentials in the ganglion can result from the activation of postsynaptic muscarinic receptors. Under appropriate experimental conditions these slow synaptic potentials could modify synaptic transmission postsynaptically (see Libet, 1964; Volle, 1969; Libet, 1970; and Brimble and Wallis, 1974). Under the conditions of greatly reduced transmitter release used in our study, however, slow synaptic potentials are greatly reduced or absent (Libet, 1967) and would not be expected to have any appreciable effect on the fast EPSPs that we studied.

At the frog neuromuscular junction the first component of facilitation, the second component of facilitation, augmentation, and potentiation all result from an increase in the number of quanta of transmitter released from the nerve terminal by each impulse (see del Castillo and Katz, 1954 *b*; Liley, 1956;

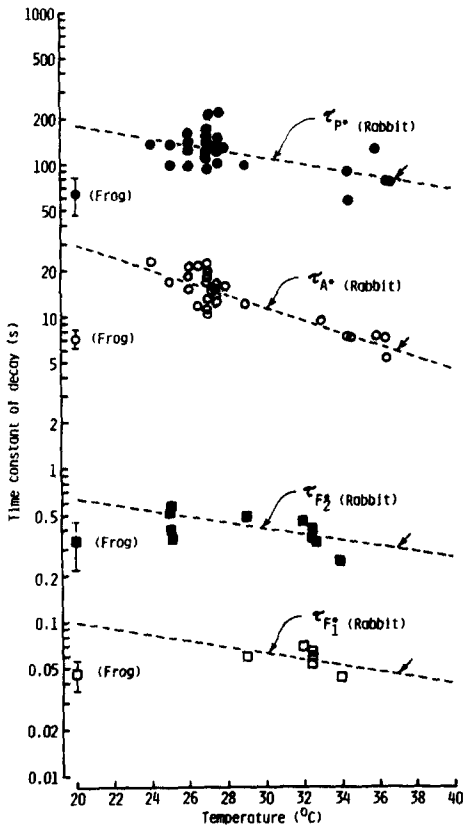

FIGURE 5. Effect of temperature on the time constants of decay of potentiation, augmentation, and the first and second components of facilitation. The broken lines are least squares fits to the data points. The arrows indicate projected estimates of the time constants of decay of the four components at rabbit body temperature (37°C). Data obtained from 20 ganglion preparations. The mean ± SD of time constants of decay of the four components obtained at 20°C for the frog neuromuscular junction are also plotted. Estimates for the frog were obtained from Mallart and Martin (1967), Magleby (1973), and Magleby and Zengel (1976). (●) τ_{P°. (○) τ_{A°. (■) $\tau_{F_2^\circ}$. (□) $\tau_{F_1^\circ}$.

Magleby and Zengel, 1976). It seems likely, therefore, that the facilitation, augmentation, and potentiation of EPSP amplitudes that we observed in the ganglion also arise from changes in transmitter release (see Larrabee and Bronk, 1947; Martin and Pilar, 1964; McLachlan, 1975, for related experiments in other ganglion preparations).

For experiments performed at 20°C, corresponding components of increased transmitter release would be expected to decay two to five times slower in the rabbit ganglion than at the frog neuromuscular junction, as shown in Fig. 5. These differences in decay rates would be expected to persist over a range of temperatures since the decay rates of the components are temperature sensitive both in the rabbit ganglion (as indicated by the slope of the lines in Fig. 5) and at the frog neuromuscular junction (Balnave and Gage, 1974; Rosenthal, 1969; Magleby and Zengel, 1976). Fig. 5 also shows that the projected time constants of decay of the four components in the rabbit ganglion at rabbit body temperature (37°C, arrows) would be similar to the time constants of decay of the corresponding components at the frog neuromuscular junction at frog body temperature (~20°C). Thus, it appears that the corresponding components would decay at about the same rates in vivo in the frog and rabbit, despite the large difference in body temperature.

Because the time constants of decay for the various components of increased transmitter release are temperature and species dependent (Fig. 5), it is necessary to identify the components in different preparations using criteria in addition to their time constants of decay. In this study we made use of the observations of Zengel and Magleby (1980) that Ba^{2+} increases the magnitude of augmentation and that Sr^{2+} increases the magnitude of the second component of facilitation to identify these two components in the ganglion. We then identified the first component of facilitation and potentiation in the ganglion by assuming that the component that decayed faster than the second component of facilitation was the first component of facilitation, and that the component that decayed slower than augmentation was potentiation.

The magnitude of the first component of facilitation after a single conditioning impulse was ~1.4 in the rabbit superior cervical ganglion (Table I). This estimate is not markedly different from those of 1.3 (Mallart and Martin, 1967) and 0.8 (Magleby, 1973) for the frog neuromuscular junction. The magnitude of potentiation after 200 conditioning impulses was also similar in the ganglion (~0.55; averaged from Table I) and at the frog neuromuscular junction (~0.6; Magleby and Zengel, 1976). The magnitudes of the second component of facilitation and augmentation after a single impulse were markedly greater, however, in the rabbit ganglion. The magnitude of the second component of facilitation after a single impulse in the ganglion was ~0.85 (averaged from Table I). This value is about six times greater than the values of 0.15 (Mallart and Martin, 1967) and 0.12 (Magleby, 1973) found at the frog neuromuscular junction. The magnitude of augmentation after a single impulse in the rabbit ganglion was ~0.15 (averaged from Table I). This value is about 10 times greater than the values of 0.01–0.03 calculated for the frog neuromuscular junction (Zengel and Magleby, 1977, and unpublished observations). Unexpectedly, even though the magnitude of augmentation after a single impulse is greater in the rabbit ganglion, the magnitude of augmentation after 200-impulse conditioning trains in the ganglion (~2.15; averaged from Table I) is similar to that after 200-impulse conditioning trains at the frog neuromuscular junction (1.8; Magleby and Zengel, 1976).

Because of the large magnitudes of augmentation and the second component of facilitation in rabbit superior cervical ganglion, these two components are the easiest to detect in this preparation. It appears that they are the two components present in Fig. 13 of Larrabee and Bronk (1947; cat stellate ganglion) and in Fig. 1 of McLachlan (1975; guinea pig superior cervical ganglion).

One or more components of increased transmitter release have been observed in a variety of other preparations, including cat spinal cord (Curtis and Eccles, 1960; Kuno, 1964; Porter, 1970), rat hippocampus (McNaughton, 1977), abdominal ganglion in *Aplysia* (Woodson et al., 1978), and the squid giant synapse (Charlton and Bittner, 1978). Studies of the similarities and differences among the components of increased transmitter release in different preparations when correlated with studies on the similarities and differences in the structural and biochemical aspects of the synapses may give further insight into the mechanism of transmitter release.

This work was supported by National Institutes of Health grants NS 10277, NS 07044, NS 05820, and NS 05363, and by a Scottish Rite Fellowship to J. P. Horn.

Received for publication 10 December 1979.

REFERENCES

BALNAVE, R. J. and P. W. GAGE. 1974. On facilitation of transmitter release at the toad neuromuscular junction. *J. Physiol. (Lond.)*. **239:**657–675.

BRIMBLE, M. J., and D. I. WALLIS. 1974. The role of muscarinic receptors in synaptic transmission and its modulation in the rabbit superior cervical ganglion. *Eur. J. Pharmacol.* **29:**117–132.

CHARLTON, M., and G. D. BITTNER. 1978. Facilitation of transmitter release at squid synapses. *J. Gen. Physiol.* **72:**471–486.

CURTIS, D. R., and J. C. ECCLES. 1960. Synaptic action during and after repetitive stimulation. *J. Physiol.* (Lond.). **150:**374–398.

DEL CASTILLO, J., and B. KATZ. 1954 *a*. Quantal components of the end-plate potential. *J. Physiol. (Lond.)*. **124:**560–573.

DEL CASTILLO, J., and B. KATZ. 1954 *b*. Statistical factors involved in neuromuscular facilitation and depression. *J. Physiol. (Lond.)*. **124:**574–585.

ERULKAR, S. D., and R. RAHAMIMOFF. 1978. The role of calcium ions in tetanic and post-tetanic increase of miniature end-plate potential frequency. *J. Physiol. (Lond.)*. **287:**501–511.

HUBBARD, J. I. 1963. Repetitive stimulation at the neuromuscular junction, and the mobilization of transmitter. *J. Physiol. (Lond.)*. **169:**641–662.

ILLES, P., and S. THESLEFF. 1978. 4-Aminopyridine and evoked transmitter release from motor nerve endings. *Br. J. Pharmacol.* **64:**623–629.

KUNO, M. 1964. Mechanism of facilitation and depression of the excitatory synaptic potential in spinal motoneurons. *J. Physiol. (Lond.)*. **175:**100–112.

LARRABEE, M. G., and D. W. BRONK. 1947. Prolonged facilitation of synaptic excitation in sympathetic ganglia. *J. Neurophysiol. (Bethesda)*. **10:**137–154.

LIBET, B. 1964. Slow synaptic responses and excitatory changes in sympathetic ganglia. *J. Physiol. (Lond.)*. **174:**1–25.

LIBET, B. 1967. Long latent periods and further analysis of slow synaptic responses in sympathetic ganglia. *J. Neurophysiol. (Bethesda).* **30:**494–514.

LIBET, B. 1970. Generation of slow inhibitory and excitatory post-synaptic potentials. *Fed. Proc.* **29:**1945–1956.

LILEY, A. W. 1956. The quantal components of the mammalian end-plate potential. *J. Physiol (Lond.).* **133:**571–587.

LINDER, T. M. 1974. The accumulative properties of facilitation at crayfish neuromuscular synapses. *J. Physiol. (Lond.).* **238:**223–234.

MAGLEBY, K. L. 1973. The effect of repetitive stimulation on facilitation of transmitter release at the frog neuromuscular junction. *J. Physiol. (Lond.).* **234:**327–352.

MAGLEBY, K. L., and J. E. ZENGEL. 1975 *a*. A dual effect of repetitive stimulation on post-tetanic potentiation of transmitter release at the frog neuromuscular junction. *J. Physiol. (Lond.).* **245:**163–182.

MAGLEBY, K. L., and J. E. ZENGEL. 1975 *b*. A quantitative description of tetanic and post-tetanic potentiation of transmitter release at the frog neuromuscular junction. *J. Physiol. (Lond.).* **245:**183–208.

MAGLEBY, K. L., and J. E. ZENGEL. 1976. Augmentation: a process that acts to increase transmitter release at the frog neuromuscular junction. *J. Physiol. (Lond.).* **257:**449–470.

MAGLEBY, K. L., J. E. ZENGEL, J. P. HORN, D. A. MCAFEE, and P. J. YAROWSKY. 1978. Synaptic transmission in the rabbit superior cervical ganglion: comparison to the frog neuromuscular junction. *Soc. Neurosci. Abst.* **4:**371.

MALLART, A., AND A. R. MARTIN. 1967. An analysis of facilitation of transmitter release at the neuromuscular junction of the frog. *J. Physiol. (Lond.).* **193:**679–694.

MARTIN, A. R., and G. PILAR. 1964. Presynaptic and postsynaptic events during PTP and facilitation in the avian ciliary ganglion. *J. Physiol. (Lond.).* **175:**17–30.

MCAFEE, D. A., and P. GREENGARD. 1972. Adenosine 3′,5′-monophosphate: Electrophysiological evidence for a role in synaptic transmission. *Science (Wash. D. C.).* **178:**310–312.

MCAFEE, D. A., and P.J. YAROWSKY. 1979. Calcium-dependent potentials in the mammalian sympathetic neurone. *J. Physiol. (Lond.).* **290:**507–523.

MCLACHLAN, E. M. 1975. Changes in statistical release parameters during prolonged stimulation of preganglionic nerve terminals. *J. Physiol. (Lond.).* **253:**477–491.

MCLACHLAN, E. M. 1977. The effects of strontium and barium at synapses in sympathetic ganglia. *J. Physiol. (Lond.).* **267:**497–518.

MCNAUGHTON, B. L. 1977. Dissociation of short- and long-lasting modification of synaptic efficacy at the terminal of the perforant path. *Soc. Neurosci. Abst.* **3:**517.

MILEDI, R. 1966. Strontium as a substitute for calcium in the process of transmitter release at the neuromuscular junction. *Nature (Lond.).* **212:**1233–1234.

PORTER, R. 1970. Early facilitation at corticomotoneuronal synapses. *J. Physiol. (Lond.)* **207:**733–745.

ROSENTHAL, J. 1969. Post-tetanic potentiation at the neuromuscular junction of the frog. *J. Physiol. (Lond.).* **203:**121–133.

SILINSKY, E. M. 1977. Can barium support the release of acetylcholine by nerve impulses? *Br. J. Pharmacol.* **59:**215–217.

TASHIRO, R., J. P. GALLAGHER, and S. NISHI. 1976. Facilitation and depression of synaptic transmission in amphibian sympathetic ganglia. *Brain Res.* **118:**45–62.

VOLLE, R. L. 1969. Ganglionic transmission. *Annu. Rev. Pharmacol.* **9:**135–146.

WOODSON, P. B. J., W. T. SCHLAPFER, and S. H. BARONDES. 1978. Amplitude and rate of decay of post-tetanic potentiation are controlled by different mechanisms. *Brain Res.* **157:**33–46.

YOUNKIN, S. G. 1974. An analysis of the role of calcium in facilitation at the frog neuromuscular junction. *J. Physiol. (Lond.)*. **237**:1–14.

ZENGEL, J. E., and K. L. MAGLEBY. 1977. Transmitter release during repetitive stimulation: selective changes produced by Sr^{++} and Ba^{++}. *Science (Wash. D. C.)* **197**:67–69.

ZENGEL, J. E., and K. L. MAGLEBY. 1980. Differential effects of Ba^{2+}, Sr^{2+}, and Ca^{2+} on stimulation-induced changes in transmitter release at the frog neuromuscular junction. *J. Gen. Physiol.* **76**:175–211.

ZUCKER, R. S. 1974. Characteristics of crayfish neuromuscular facilitation and their calcium dependence. *J. Physiol. (Lond.)*. **241**:91–110.