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Effects of Mg^{2+} on the Stimulation-Induced Changes in Transmitter Release at the Frog Neuromuscular Junction

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ABSTRACT—Four components of stimulation-induced changes in neurotransmitter release are known as the synaptic plasticity at the frog neuromuscular junction. These are: fast and slow facilitation, augmentation and potentiation, classified by their decay time constants after repetitive nerve stimulation. Most experiments support the view that fast facilitation is caused by residual Ca^{2+}. However, the causes of the other three components are not clear. We have studied electrophysiologically the effect of Mg^{2+} on these three components. Transmitter release was estimated by the amplitudes of endplate potential (EPP) and by the frequencies of miniature endplate potential (MEPP). The increase in the transmitter release by nerve stimulation is described as the product of four components. The magnitude of potentiation of MEPP frequencies after a tetanic nerve stimulation (100 Hz, 5000 times) increased markedly in high Mg^{2+} concentrations. Conversely, the magnitude of augmentation (MEPP frequencies and EPP amplitude) decreased in the higher Mg^{2+} Ringer solution.

INTRODUCTION

The process of transmitter release from the nerve terminal follows a sequential occurrence [1, 2] from the uptake into the vesicles of the transmitter to the exocytosis. Many proteins are reported to participate in the transmitter release at every step of the sequential occurrence.

The transmitter release from the frog neuromuscular junction is modulated after the repetitive nerve stimulations by four components (fast and slow facilitation, augmentation and potentiation) [10], with different decay time constants. Fast facilitation is generally accepted as originating from the residual Ca^{2+} that enters through Ca channels after nerve stimulation [5, 6, 8]. We reported that fast facilitation is completely diminished in the presence of a Ca^{2+} chelator, bis (O-aminophenoxy)ethane-N,N,N',N'-tetraacetic acid (BAPTA) in the nerve terminal, yet the other three components still occurred in the same degree, regardless of loading with or without BAPTA [8, 9]. This suggests that the latter three occur independently of internal free Ca^{2+} concentration. However, it is not yet known what brings the other three components about.

These three components may be occurred by the modification of some of a series of proteins or the membrane components that participate in the transmitter release. These may be directly or indirectly modulated by chemicals and ions or the physical state of the membrane. To find the direct or indirect modulating factors of transmitter release, we studied the effect of Mg^{2+} on three components.

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MATERIALS AND METHODS

Materials

The nerve sartorius muscle preparations of the frogs (*Rana nigramaculata*) were used throughout in these experiments as described in previous studies [6, 8, 9].

Ringer solution and chemicals

The composition of frog Ringer solution was essentially the same as that in previous experiments [9]. The standard solution contains (in mM) NaCl 112, KCl 2.6, $CaCl_2$ 0.6, $MgCl_2$ 4, HEPES-Na buffer 5 (pH 7.2). The high Mg^{2+} solution contains $MgCl_2$ 10, $CaCl_2$ 1 (or 2) and the low Mg^{2+} solution contains $MgCl_2$ 1, $CaCl_2$ 0.2. To study the frequency of miniature endplate potentials (MEPPs), the Ca free Ringer solution with 1 mM EGTA was also used. To keep the solution isotonic as the 10 mM Mg^{2+} Ringer solution, sucrose was added to 1 mM and 4 mM Mg^{2+} Ringer solutions.

MEPP frequency

The measurements of MEPP frequency were made by a conventional intracellular glass microelectrode method [6]. These were collected by a differential amplifier, amplified, fed into a microcomputer and processed. Stimulation patterns were generated by another microcomputer and the nerve trunk was stimulated by isolated signals. Most experiments were carried out by perfusing Ca^{2+} free solution containing 1 mM EGTA. A repetitive stimulation of 100 Hz, 5000 times was delivered. Because of Ca^{2+}-free external solution, no EPP was evoked. However, in some experiments, a repetitive stimulation of 20 Hz, 450 times was delivered in the presence of Ca^{2+}. MEPP frequencies were shown by the moving bin method [7]. The bin size was 9 sec and the step size was 3 sec. We estimated decay time constants and the magnitude of augmentation and potentiation after the repetitive stimulation by curve-fitting the multiplicative experimental terms with the nonlinear least square methods [8, 9].

EPP amplitude

The endplate potentials (EPPs) were recorded extracellularly

from the muscle surface using a glass pipette with large pore size (100–200 μm) as described in a previous paper [8], or these were recorded intracellularly by conventional methods. To study slow facilitation, a train of repetitive stimulation of 50 times 33 Hz was given, and test stimulations were delivered 5 times at 1.5 sec intervals and 60 times at 4 sec intervals. One special test stimulation was applied at 100, 150, 200, 300, 500, 700 or 900 ms after the end of the repetitive stimulation. To study augmentation and potentiation, a train of repetitive stimulation of 20 Hz, 450 times and test stimulations at every 1.5 sec, 5 times and at 4 sec, 60 times, were delivered. The magnitude and decay time constants after repetitive stimulation were calculated by curve fitting in the same way as in the analysis of the components of MEPP frequencies.

RESULTS

The effect of Mg^{2+} on augmentation and potentiation of MEPP frequencies under Ca^{2+} free conditions

Augmentation and potentiation of MEPP frequencies after a tetanic nerve stimulation (100 Hz, 5000 times) were studied under the various Mg^{2+} concentrations, perfusing the Ca^{2+} free Ringer solution containing 1 mM EGTA.

Figure 1 shows MEPP frequencies during and after tetanic stimulation in the perfusion solutions containing 1 mM (Fig. 1A), 4 mM (B) or 10 mM (C) Mg^{2+}; the perfusion solution was adjusted by sucrose so as to be as isotonic as the Ringer solution containing 10 mM Mg^{2+}. The magnitude of augmentation tended to decrease with increase in Mg^{2+} concentrations (5.47 at 1 mM, 2.17 at 4 mM and 0.99 at 10 mM in Fig. 1 and 5.24 at 1 mM, 3.65 at 4 mM and 1.02 at 10 mM on average, Table 1). Time constants of augmentation were lengthened with the increase in Mg^{2+}.

Magnitudes of potentiation increased markedly (11.5 at 1 mM, 24.8 at 4 mM and 28.8 at 10 mM for Fig. 1) with increase of Mg^{2+} concentrations. The average values were 11.4 at 1 mM, 33.0 at 4 mM and 31.9 at 10 mM. No significant changes were observed in decay time constants of potentiation, as shown in Table 1. Figure 2 shows the relationships between Mg ion concentration and magnitude of augmentation and potentiation. This shows that magnitude of potentiation increase with Mg^{2+} concentration, and magnitude of augmentation decrease with Mg^{2+} concentration.

In order to check whether the effect of Mg^{2+} observed above was different, under various stimulating conditions, we examined the effect of Mg^{2+} at another stimulating condition: 20 Hz, 450 times. The results are shown in Table 2. Under these stimulating conditions, magnitude of augmenta-

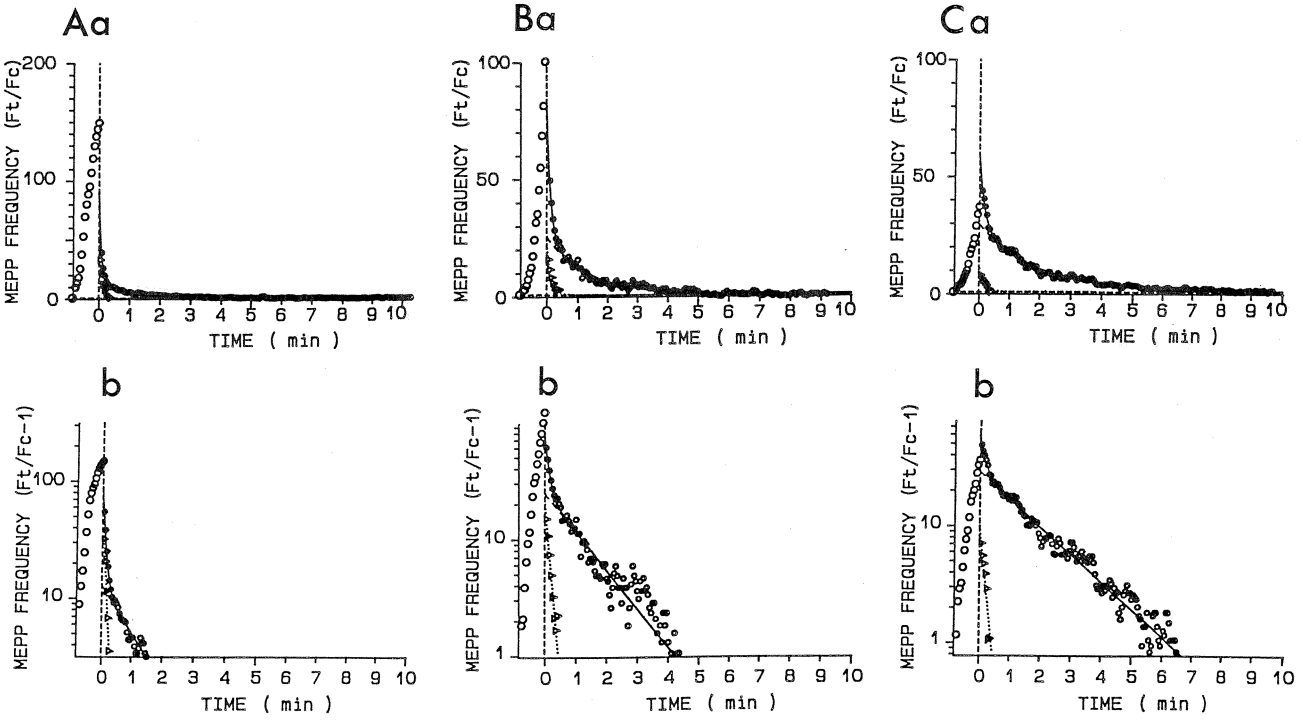

FIG. 1. Effects of Mg^{2+} concentration on augmentation and potentiation of MEPP frequency after repetitive stimulation (100 Hz, 5000 times). A, B and C show MEPP frequency during and after tetanic stimulation at 1, 4 and 10 mM Mg^{2+} Ringer solution, respectively. These show normal plots of MEPP frequencies (○) during ($t<0$) and after ($t>0$) tetanic stimulation. The control MEPP frequencies before stimulation (sampled for 1 min) were set at unity. A moving bin display was used, with a bin size of 9 sec and a step size of 3 sec. After tetanic stimulation ($t>0$), the best fit-curves [9] of (A+1) (P+1) (—), the best-fit potentiation curves, (P+1), (---), experimental points of augmentation obtained by dividing the measured frequencies by the best-fit values of (P+1), (▷) the best-fit curves of augmentation (···) and the control level (---) are shown. The magnitudes of augmentation of A, B and C are 5.47, 2.17 and .99, respectively. The time constants are 5.90, 8.29 and 10.4 sec, respectively. The magnitudes of potentiation of A, B and C are 11.5, 24.8 and 28.8, respectively. The time constants are 62.5, 79.0 and 110.7 sec, respectively. b; semilogarithmic plots of the decay of MEPP frequencies. Symbols are the same as in a. Tenfold values are plotted for augmentation. Methods of analysis followed previous reports [8, 9].

TABLE 1. Parameters of augmentation and potentiation of MEPP frequencies by 100 Hz 5000 times stimulation

$[Mg^{2+}]$	Augmentation A(0)	τ	Potentiation P(0)	τ	Frequency Fq(0)	n
1 mM	5.24±0.73	5.07±0.79	11.35± 1.86	68.8±7.5	0.49±0.29	6
4 mM	3.65±0.96	6.89±0.29	33.00± 6.30	85.3±4.7	0.66±0.16	6
10 mM	1.02±0.12	13.41±2.23	31.87±11.14	123.7±6.6	0.72±0.18	3

Means±S.E.M.
A(0); the magnitude of augmentaion, P(0); the magnitude of potentiation Fq(0); MEPP frequencies before tetanic stimulation, τ; time constant n; number of experiments

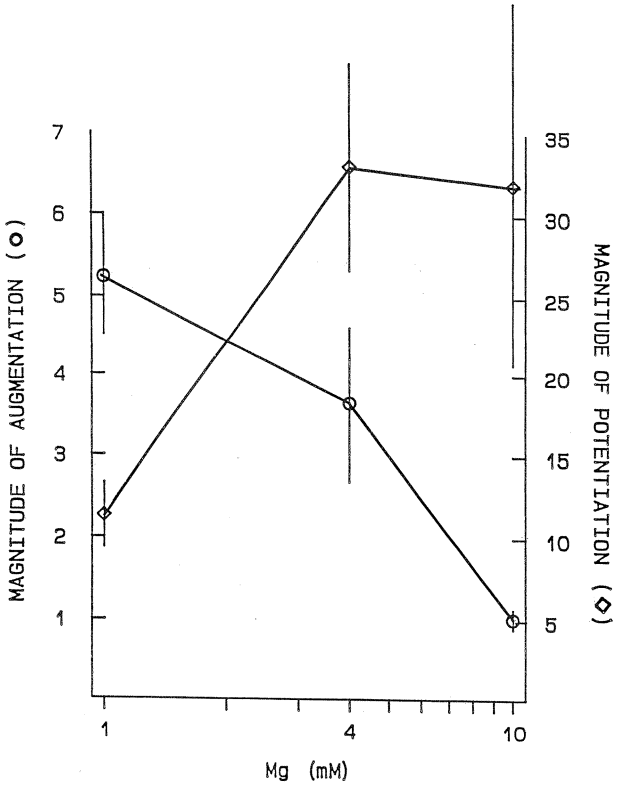

FIG. 2. The relations between Mg^{2+} concentration and the magnitude of augmentation and potentiation of MEPP frequency. It is clear that the magnitude of augmentation is suppressed by high Mg^{2+} concentration, but the magnitude of potentiation is promoted. (○), augmentation and (◇), potentiation. Bars show S.E.M. (n=6 at 1 and 4 mM and n=3 at 10 mM)

tion showed the same tendency, but magnitude of potentiation were not significantly different at Mg^{2+} concentrations of 1, 4 and 10 mM.

As a result, augmentation and potentiation occurred independently of external Ca^{2+}, and the magnitude of potentiation increased with external Mg^{2+} concentration (Fig 2 and Table 1) but the time constants of potentiation were not significantly different. The magnitude of augmentation decreased with the increase of external Mg^{2+} concentration.

The effect of Mg^{2+} on three components by the study of EPP amplitude

Slow facilitation

The effects of Mg^{2+} on slow facilitation of EPP amplitude were studied with the surface recoding method. When the nerve trunk was stimulated by mild stimulating conditions (33 Hz 50 times), fast and slow facilitation increased but augmentation and potentiation did not do so significantly. Under these conditions, we studied the magnitude and time constants of slow facilitation under the various Mg^{2+} concentrations. The different Mg^{2+} concentrations did not make differences in magnitude, nor in time constants. That is, magnitude in the Ringer solution containing 4 mM Mg^{2+} and 10 mM are 1.14±0.18 (mean±S.E.M.), (n=5) and 1.07± 0.24 (n=5) and time constants were 0.31±0.09 and 0.33± 0.09 (Fig. 3A and B), respectively. These results show that Mg^{2+} has no effect on slow facilitation of EPP under mild stimulating conditions.

Augmentation and potentiation

The nerve trunk was stimulated by a train of 450

TABLE 2. Parameters of augmentation and potentiation of MEPP frequency by 20 Hz 450 times stimulation

$[Mg^{2+}]$	Augmentation A(0)	τ	Potentiation P(0)	τ	Frequency Fq(0)	n
1 mM	3.61±0.43	6.57±0.85	1.66±0.18	88.0±13.7	0.41±0.06	6
4 mM	2.63±0.25	7.59±0.46	1.93±0.26	70.0± 9.5	0.49±0.05	8
10 mM	2.08±0.32	6.97±1.16	1.89±0.28	69.4±14.3	0.76±0.09	8

Means±S.E.M.
A(0); the magnitude of augmentaion, P(0); the magnitude of potentiation Fq(0); MEPP frequencies before tetanic stimulation, τ; time constant n; number of experiments

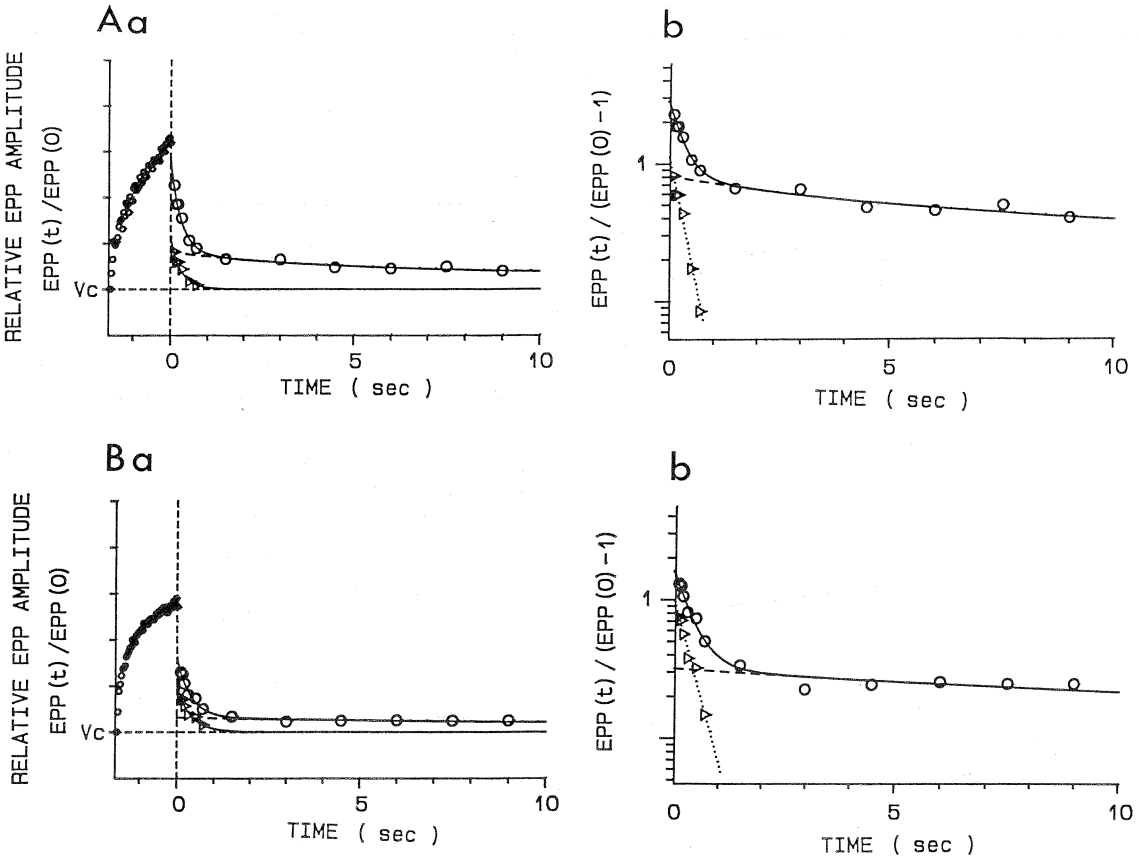

FIG. 3. Effects of Mg^{2+} concentration on slow facilitation of EPP amplitude after repetitive stimulation (33 Hz, 50 times). A; EPP amplitudes in 4 mM Mg^{2+} Ringer solution. B; 10 mM Mg^{2+}. a; relative EPP amplitudes (○) during ($t<0$) and after ($t>0$) the train. The best-fit curves [9] of (F_2+1) $(A+1)$ $(P+1)$ (—), the best-fit curves of $(A+1)$ $(P+1)$ (---), experimental points of slow facilitation obtained by dividing measured EPP amplitudes by the estimated values of $(A+1)$ $(P+1)$, (▷), and the best-fit exponential curves to slow facilitation (···) are shown. Mg^{2+} has no significant effect on slow facilitation. The magnitudes of slow facilitation of A and B are 0.98 and 1.11, respectively. The time constants are 0.38 and 0.29 sec, respectively. b; semilogarithmic plots of the decay of EPP amplitudes. Symbols are the same as in a.

stimulation at 20 Hz in the Ringer solution containing 1, 4 mM or 10 mM Mg^{2+} and these data were recorded by the intracellular electrode. The decay processes of augmentation and potentiation were studied. Under these conditions, the magnitude of augmentation decreased with increase of Mg^{2+} concentration. However, there were no significant differences in magnitude and in time constants of potentiation, as shown in Fig. 4 (A, B and C) and in Table 3. These results show that the magnitude of augmentation was depressed by high Mg^{2+} concentration, as it was with MEPP measurements. On the other hand, the magnitude of potentiation did not change unless Mg concentrations were also different.

DISCUSSION

We have studied the modification of transmitter release after repetitive nerve stimulation. For this purpose, Ringer solution containing Mg^{2+} was used to block muscle contraction. Therefore, we checked whether Mg^{2+} affected transmitter release or not. Mg^{2+} did not have a marked effect, equivalent to Ca^{2+} for fast facilitation, but it affected transmitter release after repetitive nerve stimulation.

From the experiment with mild stimulating conditions (33 Hz, 50 times repetitive nerve stimulation), the magnitude and the time constants of slow facilitation did not change with Mg^{2+} concentrations. That is, the change in Mg^{2+} concentrations did not affect slow facilitation.

Mg^{2+} had no effect on potentiation in EPP amplitudes and MEPP frequencies at 20 Hz, 450 times, but under more extensive stimulating conditions (the study of MEPP frequency at 100 Hz, 5000 times), the magnitude of potentiation increased with increase of Mg^{2+} concentrations. On the other hand, the magnitude of augmentation decreased with increase in Mg^{2+} concentrations under all experimental conditions. The same tendency was observed when the experiment was carried out in Ringer solution without both Ca^{2+} and Mg^{2+}. That is, under no divalent cation in the perfusion solution and with EDTA, the frequency of MEPP during a tetanic stimulation increased by more than ten times for the first ten seconds and then decreased suddenly to near the resting frequency level (data not shown). The rate of

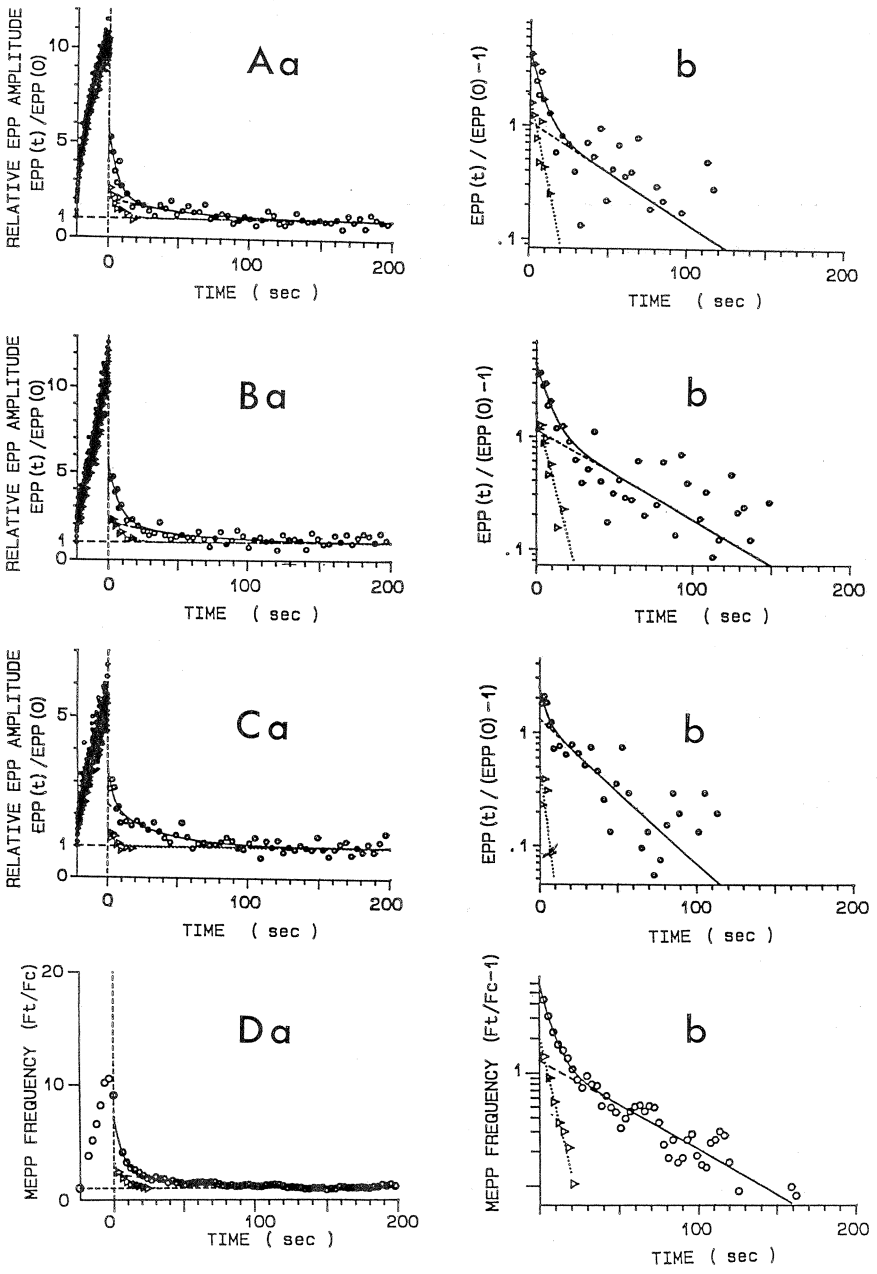

FIG. 4. Effects of Mg^{2+} concentration on augmentation and potentiation of EPP amplitude after repetitive stimulation (20 Hz, 450 times). This data was recorded by intracellular methods. A, B and C show EPP amplitudes during and after tetanic stimulation in 1, 4 and 10 mM Mg^{2+} Ringer, respectively. a, normal plots of EPP amplitudes (○) during ($t<0$) and after ($t>0$) tetanic stimulation. After tetanic stimulation ($t>0$), the best fit-curves of (A+1) (P+1) (—), the best fit potentiation curves, (P+1), (---), experimental points of augmentation obtained by dividing the counted frequencies by the best-fit values of (P+1), (▷) the best-fit curves of augmentation (···). These three graphs do not show any significant differences at their magnitudes of potentiation. However, the magnitudes of augmentation decreased at high Mg^{2+} concentration: these values of A, B and C are 1.89, 1.59 and 0.52, respectively. The time constants are 6.51, 7.82 and 3.87 sec, respectively. The magnitudes of potentiation of A, B and C are 1.07, 1.14 and 1.31, respectively. The time constants are 49.1, 54.3 and 33.9 sec, respectively. b; semilogarithmic plots of the decay of EPP amplitudes. D ; MEPP frequency obtained from the same experiment as the data in C. Its magnitude of potentiation is a good fit with C, but its magnitude of augmentation is not.

increase was faster than in the experiments with Ringer solution containing Mg^{2+}. This may mean that MEPP frequencies increased more rapidly than under Ringer solution with Mg^{2+}, but also that they decreased suddenly through other causes. Mg^{2+} may therefore affect these two components by suppressing augmentation and by promoting potentiation.

The four modulating components of transmitter release after repetitive stimulation may be caused by the different processes of transmitter release. From these experiments

TABLE 3. Parameters of augmentation and potentiation of EPP amplitude by 20 Hz 450 times stimulation

$[Mg^{2+}]$	Augmentation A(0)	τ	Potentiation P(0)	τ	n
1 mM	2.44±0.37	6.68±1.50	1.69±0.26	92.5±47.5	5
4 mM	1.58±0.53	5.57±1.37	1.69±0.21	49.2± 8.5	6
10 mM	0.72±0.07	7.29±1.25	1.64±0.17	55.4± 9.4	8

Means±S.E.M.
A(0); the magnitude of augmentaion, P(0); the magnitude of potentiation τ; time constant, n; number of experiments

and previous studies, we know that Ca^{2+} and Mg^{2+} affect different processes of transmitter release. Fast facilitation is promoted by Ca^{2+} [5, 6] and slow facilitation is promoted by Sr^{2+} [10]. Augmentation is promoted by Ba^{2+} [10] and suppressed by Mg^{2+}, while potentiation is promoted by Mg^{2+}. It is well known that Ca^{2+} triggers transmitter release from the nerve ending [4] and also modulates it after repetitive nerve stimulation (fast facilitation) [5, 6]. Probably, Ca^{2+} will act directly on the transmitter release by binding with some exocytosis relating protein. Mg^{2+} will act on transmitter release indirectly or directly, and negatively for augmentation, and positively for potentiation, but will have no effect on slow facilitation. We know there are many kinds of protein relating to transmitter release or exocytosis. It may have some effects on some enzymes or some proteins relating to a sequential process of transmitter release. Mg^{2+} may affect some steps of this process and may reflect the modification of augmentation and potentiation.

The results of EPP and MEPP of Table 2 and 3, and Figure 4 (C and D) were obtained at simultaneously from the same neuromuscular junction. The magnitude of potentiation had similar results with EPP and MEPP. However, the magnitude of augmentation had different results from each other. The magnitude of MEPP are larger than those of EPP by about 1.2 at all Mg^{2+} concentrations. These results may suggest that augmentation contains another factor that it does not change by Mg^{2+} concentrations when tested by MEPP frequency.

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