EFFECT OF MAGNESIUM AND CALCIUM IONS ON THE POSTTETANIC ACCELERATION OF MINIATURE END-PLATE POTENTIALS IN FROG SKELETAL MUSCLE

M. A. Kamenskaya and T. D. Puskaeva

UDC 612.743.014.46 : /546.46+546.41

In experiments on the isolated frog sartorius muscle Mg ions (16 mM) reduced the degree of posttetanic acceleration of miniature end-plate potentials (MEPPs) after application of 100 stimuli to the nerve at 200 or 50 Hz. With an increase in the Ca ion concentration (7.2 mM) the degree of acceleration of MEPPs after the first tetanic stimulation remained unchanged. Repeated tetanic stimulation at 200 Hz was accompanied by a more marked decrease in the degree of MEPP acceleration in the case of an increase in the Ca concentration and during the action of Mg ions.

The mechanism of poststimulation phenomena in the neuromuscular synapse is an important aspect of the general problem of the principles governing rhythmic activity of the neuromuscular apparatus [2, 3]. An exceptionally important role in poststimulation phenomena is played by processes connected with liberation of the mediator from nerve endings [5, 8, 10, 14-16, 19]. The onset of facilitation or depression of neuromuscular transmission with the regular arrival of nervous impulses depends on the relationship between changes in the probability (P) of liberation of each quantum and the size of the pool of available mediator [8, 10, 13, 15, 19]. It is known that Mg ions decrease and Ca ions increase P for liberation of mediator in response to a nervous impulse [6, 7] and also during depolarization of the presynaptic membrane by an electric current [1, 9, 14]. As regards the frequency of spontaneous liberation of mediator, there are facts which suggest either that no change takes place in response to an increase in the Ca ion concentration [6, 7] or that it is increased [17]. If the degree of increase in frequency of miniature end-plate potentials (MEPPs) in the poststimulation period is determined by the increase in P [15] and if the mechanisms of spontaneous and evoked liberation of mediator are identical in nature [1, 11, 12, 16, 18], a lesser degree of posttetanic acceleration of the MEPPs would be expected in the presence of a magnesium block, in connection with the decrease in P for evoked liberation. In fact, when a muscle was kept in calcium-free solution prolonged (for 21 sec) tetanization of the nerve was accompanied by a smaller increase in MEPP frequency than when Ca ion concentration was normal [18].

In the investigation described below the effect of Mg and Ca ions on posttetanic acceleration of MEPPs during brief repetitive stimulation of the motor nerve was studied.

EXPERIMENTAL METHOD

MEPPs were recorded intracellularly from an isolated sartorius muscle preparation of Rana temporaria. During continuous recording of MEPPs on a loop oscillograph 100 stimuli (0.5 msec, 200 or 50 Hz) were applied to the nerve. The first series of stimuli was applied when the muscle was in ordinary Ringer's solution, the second series 30 min after replacement of the Ringer's solution by an isotonic solution containing 16 mM $MgCl_2$ and 0.9 mM $CaCl_2$. Development of a block was assessed from disappearance

Department of Human and Animal Physiology, Faculty of Biology and Soil Science, Moscow State University. (Presented by Academician of the Academy of Medical Sciences of the USSR, S. E. Severin.) Translated from Byulleten' Eksperimental'noi Biologii i Meditsiny, Vol. 73, No. 6, pp. 3-6, June, 1972. Original article submitted May 14, 1971.

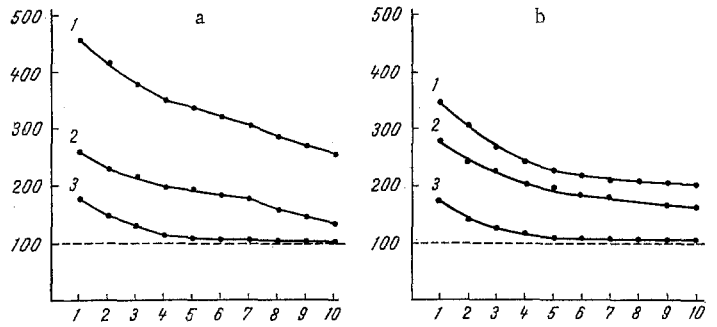

Fig. 1. Mean MEPP frequency after tetanic stimulation under normal conditions and during magnesium block: a) after application of 100 stimuli at 200 Hz (results of five experiments); b) after application of 100 stimuli at 50 Hz (results of five experiments); 1) normal; 2) MEPP frequency after application of first series of stimuli during magnesium block. Here and in Figs. 2 and 3: ordinate, MEPP frequency (in percent of MEPP frequency before corresponding stimulation), abscissa, time (in sec) after end of stimulation.

of the action potentials and a decrease in amplitude of the end-plate potentials photographed from the screen of a cathode-ray oscilloscope simultaneously with recording of the MEPPs. In the control series of experiments the Ringer's solution was replaced after the first series of stimuli by the same solution without Mg ions. Analysis of the MEPPs on motion picture film began 2 sec after the end of stimulation. For each series of experiments curves of mean MEPP frequencies were plotted, and the curves were balanced by the weighted sliding mean method [4].

EXPERIMENTAL RESULTS

The mean MEPP frequency in Ringer's solution was 2 ± 0.5/sec, falling after replacement of the Ringer's solution by solutions containing 16 mM Mg and 0.9 nM Ca or 7.2 mM Ca to 1.5 ± 0.7 and 1.8 ± 0.7/sec respectively. Since Mg ions reduced the MEPP amplitude by 30–40%, there is reason to suppose that the decrease in the mean MEPP frequency evoked by Mg ions took place primarily because of the decrease in amplitude of some MEPPs to values comparable with the noise level of the amplifier. Accordingly, the effect of Mg ions on the degree of poststimulation acceleration of the MEPPs was assessed only for those ten experiments in which the initial MEPP frequency was unchanged after the addition of Mg ions.

After stimulation of the nerve at 200 Hz the MEPP frequency in ordinary Ringer's solution was about 455% of its initial value (Fig. 1a, curve 1). During magnesium block the MEPP frequency increased after stimulation only to 258% (Fig. 1a, curve 2). By 10 sec after the end of stimulation the difference between the MEPP frequencies before and after Mg block was less marked than at the beginning of the 2nd second. In the case of stimulation at 50 Hz (Fig. 1b), Mg ions also reduced the degree of posttetanic acceleration of MEPPs, but only by 65%, i.e. less than in the case of stimulation at 200 Hz. The difference between the MEPP frequency before and after the block 10 sec after the end of stimulation was only 40%. Determination of the criterion of the excess of curve 1 over curve 2 in Fig. 1a and b [4] showed that the difference is significant.

Replacement of the Ringer's solution in the control series of experiments by the same solution without Mg ions was not followed by any significant changes in the degree of acceleration of the MEPPs.

After replacement of the solution containing 16 mM Mg and 0.9 mM Ca by Ringer's solution without Mg ions and with an increased concentration of Ca ions (7.2 nM) the degree of acceleration of MEPPs again increased. As Fig. 2 shows, acceleration of the MEPPs, which was reduced by the action of Mg ions from 500% (Fig. 2, curve 1) to 290% (Fig. 2, curve 1 Mg), was again increased to 410% by the action of Ca ions (Fig. 2, curve 1 Ca). The reason why the acceleration of MEPPs did not reach the normal level under these circumstances was that at the time of replacement of the solution with Mg ions by the solution with Ca ions the preparation was receiving series of ten stimuli at 200 Hz, applied every 15 sec. It is interesting to note

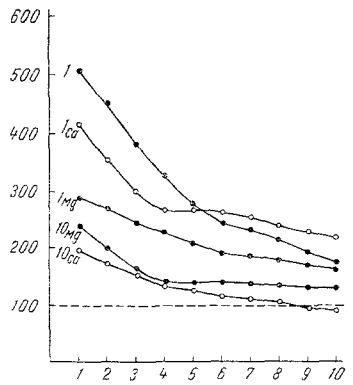

Fig. 2. Effect of Mg and Ca ions on MEPP frequency after application of 100 stimuli to the nerve at 200 Hz (results of five experiments): 1) normal, 1 Mg) MEPP frequency after stimulation during magnesium block; 10 Mg) after tenth tetanization during magnesium block; 1 Ca) after first tetanization with an increased Ca ion concentration; 10 Ca) after tenth tetanization with an increased Ca ion concentration.

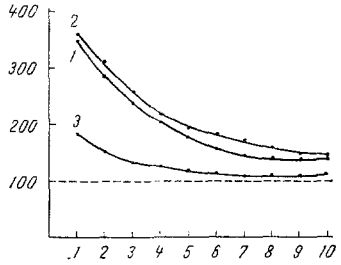

Fig. 3. Curves of mean values of MEPP frequency after application of 100 stimuli to nerve at 200 Hz under normal conditions and with an increase in the Ca ion concentration in the solution to 7.2 mM (results of five experiments): 1) normal; 2) MEPP frequency after first tetanization with an increased Ca ion concentration; 3) after tenth tetanization.

that a more marked decrease in the degree of acceleration of the MEPPs at the time of the tenth tetanization took place in the solution with an increased Ca ion concentration than in the solution containing Mg ions (Fig. 2, curves 10 Ca, 10 Mg).

Replacement of the ordinary Ringer's solution by a solution with an increased Ca ion concentration (7.2 mM compared with the normal 1.8 mM) did not produce a significant change in the degree of posttetanic acceleration of the MEPPs (Fig. 3). In the course of repeated tetanizations (every 15 sec) a decrease in the degree of acceleration of the MEPPs was observed [3]; with the same frequency of stimulation (200 Hz) the decrease by the tenth tetanization was much more marked during exposure to the action of Ca ions (Fig. 3, curve 3) than during magnesium block (Fig. 1a, curve 3); whereas the degree of acceleration of the MEPPs in the presence of an increased Ca ion concentration was reduced by 180% at the time of the tenth tetanization, during magnesium block it was reduced by only 81%.

Hence, during magnesium block, when the value of P of evoked liberation of mediator is reduced [6, 7], the degree of posttetanic acceleration of the MEPPs is lower than normal. This fact is evidence that poststimulation acceleration of the MEPPs is connected with an increase in P. However, the Ca ions, which increase P of evoked liberation of the mediator [6, 7], did not affect the degree of acceleration of the MEPPs. According to some observations the Ca ion concentration for the system responsible for spontaneous liberation of the mediator is close to saturation even if the Ca ion concentration in the solution is normal [11, 12]. In that case an increase in the Ca ion concentration in the medium changes only the level of evoked liberation, which requires the participation of more Ca ions than spontaneous liberation [11, 12], and it does not add to the acceleration of the MEPPs.

Repeated tetanic stimulation of the nerve led to a decrease in the degree of MEPP acceleration both in Ringer's solution of normal composition [3] and during magnesium block (see above), i.e., they are evidently associated with a decrease in P. Under these conditions additional utilization of the pool of accessible mediator on account of the increase in Ca ion concentration leads to a more rapid decrease in the degree of MEPP acceleration. Consequently, with the same reduced value of P, the smaller the pool of accessible mediator the lower the degree of MEPP acceleration.

LITERATURE CITED

1. Zh. L. Bliokh, A Study of the Role of Cations in the Mechanism of Liberation of Quanta of Mediator From Motor Nerve Endings. Candidate's Dissertation, Moscow (1970).
2. M. A. Kamenskaya, M. V. Kirzon, Fiziol. Zh. SSSR, No. 11, 1369 (1969).
3. M. A. Kamenskaya, M. V. Kirzon, and E. V. Migulina, Fiziol. Zh. SSSR, No. 1, 52 (1971).
4. N. A. Plokhinskii, Biometrics [in Russian], Moscow (1970).
5. M. Braun, R. F. Schmidt, and M. Zimmerman, Pflug. Arch. ges. Physiol., 287, 41 (1966).
6. J. Castillo and L. Engbaek, J. Physiol. (London), 124, 370 (1954).
7. J. Castillo and B. Katz, J. Physiol. (London), 124, 553 (1954).
8. J. Castillo and B. Katz, J. Physiol. (London), 124, 574 (1954).
9. J. Castillo and B. Katz, J. Physiol. (London), 124, 586 (1954).

10. D. Elmquist and D. Quastel, J. Physiol. (London), 178, 505 (1965).
11. J. I. Hubbard, J. Physiol. (London), 169, 641 (1963).
12. J. I. Hubbard, S. F. Jones, and E. M. Landau, J. Physiol. (London), 194, 355 (1968).
13. J. I. Hubbard, S. F. Jones, and E. M. Landau, J. Physiol. (London), 196, 75 (1968).
14. P. Kruckenberg, Arch. Kybernetik., 4, 113 (1968).
15. E. M. Landau, J. Physiol. (London), 203, 281 (1969).
16. A. W. Liley, J. Physiol. (London), 133, 571 (1956).
17. A. W. Liley, J. Physiol. (London), 134, 427 (1956).
18. J. Mambrini and P. Benoit, C. R. Soc. Biol., 158, 1454 (1964).
19. R. Miledi and R. Thies, J. Physiol. (London), 212, 245 (1971).
20. J. Rosenthal, J. Physiol. (London), 203, 121 (1969).