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THE NATURE OF THE NEUROMUSCULAR BLOCK PRODUCED BY MAGNESIUM

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Magnesium is a physiologically occurring cation which blocks neuromuscular transmission in concentrations which do not prevent either nerve conduction or the muscle response to direct stimulation. This block is counteracted by increasing the calcium concentration (for a review of the literature see Engbaek, 1952).

Magnesium, like curarine, reduces the stimulating effect of acetylcholine on muscle; it prevents the contractions which follow local application of that substance to the end-plate region (Engbaek, 1948). Some differences between the action of magnesium and that of curarine have however been described; magnesium, for example, prevents the contracture caused by potassium more readily than that produced by acetylcholine.

The object of this work was to provide information on the mechanism of action of magnesium at the neuromuscular junction, by studying separately, as far as was possible, its effect on each of the different stages of the process of impulse transmission from nerve to muscle.

METHODS

The experiments were performed on the sartorius nerve-muscle preparation of the frog (*Rana temporaria*) during the months October–November, at a room temperature of about 21° C.

External fluid electrodes. The end-plate potential (e.p.p.) which follows supramaximal stimulation of the nerve in preparations blocked by magnesium was recorded in most experiments with external fluid electrodes. The muscle was placed vertically in a bath provided with two Ag/AgCl electrodes. One electrode was placed on the uppermost, pelvic end of the muscle, and the other was immersed in the fluid below the tibial end, the surface of the bathing solution acting as a moving electrode. The fluid level was varied gradually until the locus of maximum e.p.p. was established. This point could easily be found again in the course of the experiment. The nerve was lifted out of the fluid and attached to two stimulating platinum electrodes. The frequency of the supramaximal shocks applied to the nerve has never been higher than 0·5–1 per sec. The same arrangement was employed to measure the depolarizing action of acetylcholine by recording

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the difference in surface potential between the nerve-free pelvic end of the sartorius and the region containing end-plates (Fatt, 1950). In this type of experiment the fluid was run out at an approximately constant rate and the potential distribution along the muscle surface was recorded continuously. The muscle had to be prepared with care to prevent injury; muscles which showed potential differences greater than 1 mV in the absence of acetylcholine were discarded. After addition of acetylcholine to the bathing solution a depolarization of the muscle surface could be observed the distribution of which depended on the location of the end-plates. The depolarization reached a maximum value within about 2 min, if no anticholinesterase was present, and then declined slowly. Several records were taken after addition of acetylcholine and only maximum values were used for comparison.

Intracellular recording. Resting potentials and e.p.p.'s were also recorded with intracellular capillary microelectrodes of external tip diameter less than about $0{\cdot}5\mu$, filled with 3 M-KCl (see for details of technique Nastuk & Hodgkin, 1950; and Fatt & Katz, 1951).

Measurement of the electrical threshold of the muscle membrane. Two microelectrodes, separated by an earthed electrostatic shield, were cemented together so that their tips were less than 50μ apart, and inserted into a muscle fibre. The experimental arrangement was the same as that described by Fatt & Katz (1951); one of the electrodes was connected to a square pulse generator and rectangular pulses of outward current were made to flow through the fibre membrane. The second microelectrode was used to record the resulting potential changes.

Recording apparatus. An input stage (cathode followers) of high impedance similar to that described by Huxley & Stämpfli (1949) and a d.c. amplifier were used for work both with external and internal electrodes, the final recording being from a cathode-ray tube. E.p.p.'s were recorded with single sweeps, whereas the surface potential distribution was recorded on slowly moving film.

Solutions. The Ringer's fluid had the following ionic composition, expressed in m.mole/l.: Na, 115; K, 2·1; Ca, 1·8; all the salts were used as chlorides. Mg and Ca were added by exchange of NaCl for isotonic amounts of the salts. The conductivity of a solution containing 16·8 m.mole/l. of Mg ions was measured and found to be only about 3% higher than that of normal Ringer's fluid. D-Tubocurarine chloride (Burroughs Wellcome and Co.) was used in a concentration of 3×10^{-6}. Acetylcholine chloride (Hoffmann-La Roche and Co.) was used in a concentration of $2\text{–}4 \times 10^{-6}$, and of 3×10^{-5} in curarized preparations. Neostigmine bromide (Prostigmine, Roche) was used in a concentration of 1×10^{-6} in some of the depolarization experiments; in this case the acetylcholine concentration was only $2\text{–}4 \times 10^{-7}$.

Cholinesterase activity. This was determined by continuous electrometric titration to avoid influence of bicarbonate or other buffers. Each sample contained 100 mg of homogenized frog muscle in 10 ml. Ringer's fluid, titrated at pH 7·3 and 25° C with 0·203 N-NaOH. Acetylcholine (0·011 M) was used as substrate; its spontaneous hydrolysis was negligible. No attempt was made to isolate the effect of Ca and Mg on the specific cholinesterase with acetyl-β-methylcholine as substrate, since the hydrolysis of this substrate, in contrast to that of acetylcholine, is not activated by calcium (van der Meer, 1953). The enzymic activity was expressed as μmole acetylcholine hydrolysed per g muscle per min. Each titration was continued for 60 min, the magnesium effect not being constant before 20–30 min had elapsed after the addition of acetylcholine.

RESULTS

Effect of magnesium on the amplitude of the end-plate potential

The minimum concentration of magnesium which produces a block, as shown by the absence of muscle contraction with indirect stimulation, is 5 m.mole/l. The sensitivity of the individual preparations to the blocking action of magnesium varied sometimes by a factor of nearly 2; a concentration of 10 m.mole/l. regularly produced block in less than 30 min. Immersion in normal Ringer's fluid reverses the block within a few minutes.

An e.p.p. can be recorded in the magnesium-blocked preparations when the nerve is stimulated. The shape and time course of the magnesium-e.p.p. are similar to those recorded from curarized muscle. Similar e.p.p.'s were also recorded from single neuromuscular junctions with internal electrodes.

When the magnesium concentration is increased above the initial 10 m.mole/l., the amplitude of the e.p.p. decreases. The results of four experiments are given in Fig. 1. With 15 m.mole/l. magnesium the amplitude of the e.p.p. is less than half the initial value, and with 20 m.mole/l. is only about 10% of that measured in 10 m.mole/l.

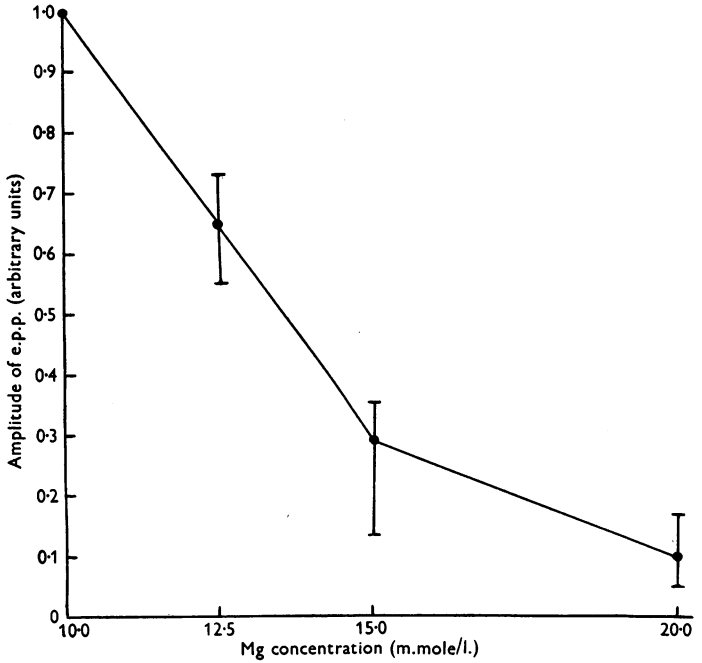

Fig. 1. Effect of an increasing magnesium concentration on the amplitude of the e.p.p. in 'magnesium-blocked' preparations. Ordinate: amplitude of the e.p.p. in relative units; its height in preparations blocked by a solution containing 10 m.mole/l. magnesium is taken as unit. Vertical lines drawn through the points (mean values) show the range of the observations. Abscissa: magnesium concentration.

The effect of low magnesium concentrations on the size of the e.p.p. could be investigated in muscles previously blocked with D-tubocurarine. The results obtained in three experiments are expressed in Fig. 2. A marked reduction in the amplitude of the e.p.p., to about 60%, was observed after adding 1 m.mole/l. magnesium to the bathing fluid. The slope of the curve relating magnesium concentration and size of the e.p.p. is very steep at the low concentrations. These experiments show not only that magnesium con-

centrations such as those which have been found in frog's plasma (cf. Fenn, 1936; Boyle & Conway, 1941) have a great influence on the size of the e.p.p., but also that small variations, such as those which are likely to occur in physiological conditions, result in a marked effect on its amplitude.

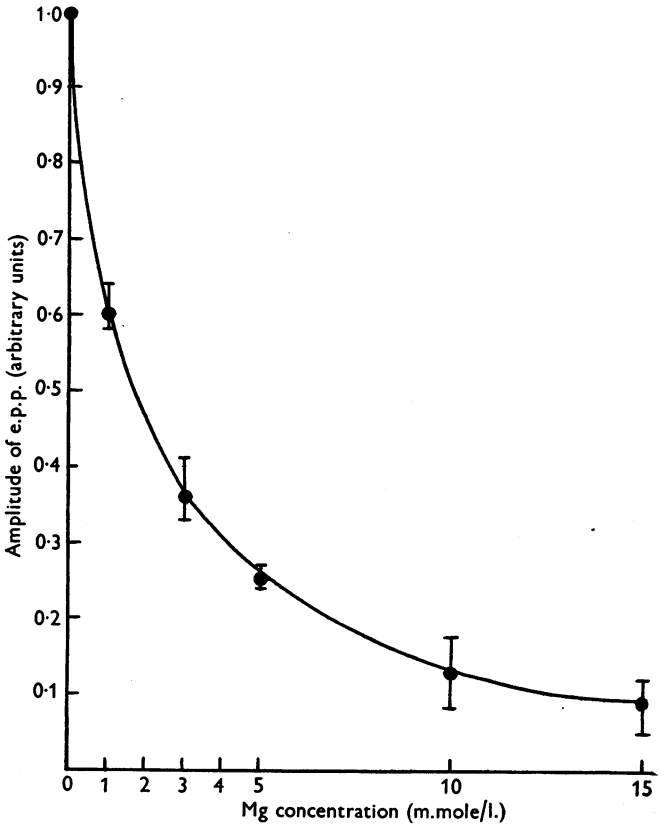

Fig. 2. Effect of magnesium on the 'curare-e.p.p.'. Ordinate: amplitude of the e.p.p. in relative units. The height of the e.p.p. recorded in curarized preparations (soaked in 3×10^{-6} D-tubocurarine) is taken as unit. An increasing amount of Mg ions as indicated by the abscissa, was then added to the bathing fluid while keeping constant the concentration of tubocurarine. Points represent mean values obtained in experiments. Vertical lines show the range.

The decrease of the e.p.p. caused by raising the magnesium concentration might be produced by the following mechanisms: (1) a permanent depolarization of the end-plate region of the muscle fibre, as exemplified by the action of decamethonium (Burns & Paton, 1951); (2) a decreased depolarizing action of acetylcholine at the end-plate region, due either to a curare-like effect or to an activation of the enzymatic breakdown of the transmitter; and (3) a decrease

in the amount of the transmitter liberated; this is, for example, the effect of low calcium and sodium concentrations (del Castillo & Stark, 1952; Fatt & Katz, 1952*b*) and possibly of botulinum toxin (cf. Burgen, Dickens & Zatman, 1949, and Brooks, 1953).

In the following experiments an attempt has been made to decide between these possibilities.

Lack of action of magnesium on the muscle resting potential

A depolarizing action of magnesium ions confined to the end-plate region was excluded by experiments in which the surface potential difference of the sartorius muscle was recorded. Magnesium in concentrations of 10 and 15 m.mole/l. caused no significant alteration of the surface potential in any region of the muscle, either in the curarized or in the non-curarized preparation.

An action of magnesium on the resting potential of the muscle fibre as a whole could also be discounted. The average resting potential measured with an intracellular electrode with the muscle in normal Ringer's fluid was $88{\cdot}1 \pm 0{\cdot}6$ mV (S.E. of mean; 50 measurements). Thirty-six impalements in the same muscle soaked for more than 30 min in a solution containing 15 m.mole/l. magnesium gave an average resting potential of $88{\cdot}3 \pm 0{\cdot}9$ mV.

Effect of magnesium on the sensitivity of the end-plate to applied acetylcholine

The influence of magnesium on the sensitivity of the end-plate to the depolarizing action of acetylcholine was studied by measuring the surface potential distribution in muscles immersed in a fluid containing a constant concentration of acetylcholine and varying amounts of magnesium ions. The magnitude of the depolarization elicited by a constant concentration of acetylcholine in normal Ringer's fluid decreases slowly in successive trials in the course of an experiment. The depolarization produced in presence of magnesium must therefore be compared with the mean of that measured in normal Ringer's fluid before and after the test in Mg-Ringer. Fig. 3 shows one of the experiments. In five experiments of this type 10 m.mole/l. magnesium reduced the depolarization produced by the same amount of acetylcholine in normal Ringer by $18 \pm 7\%$ (S.E. of mean), and 15 m.mole/l. magnesium decreased it by $40 \pm 4\%$.

The decreased depolarizing action of acetylcholine in the presence of magnesium might be attributed to either of two different factors; on the one hand to a curare-like effect, on the other to an increased destruction of acetylcholine, since magnesium is known to be an activator of cholinesterase as Nachmansohn (1940), working on the electric organ of *Torpedo*, has observed. Mg ions in concentrations which block neuromuscular transmission also increase cholinesterase activity in frog muscle homogenate (see Methods). The results

are given in Table 1 (Expts. 1–5, 4th column). The addition of 15 m.mole/l. magnesium ion increases the activity of cholinesterase by a factor of 2·5–4. However, we have no evidence indicating that a similar effect might occur in the intact muscle; a number of factors, not yet fully understood, such as substrate concentration and ionic activity at the site of cholinesterase in the end-plate etc., influence the enzyme activity *in vivo*.

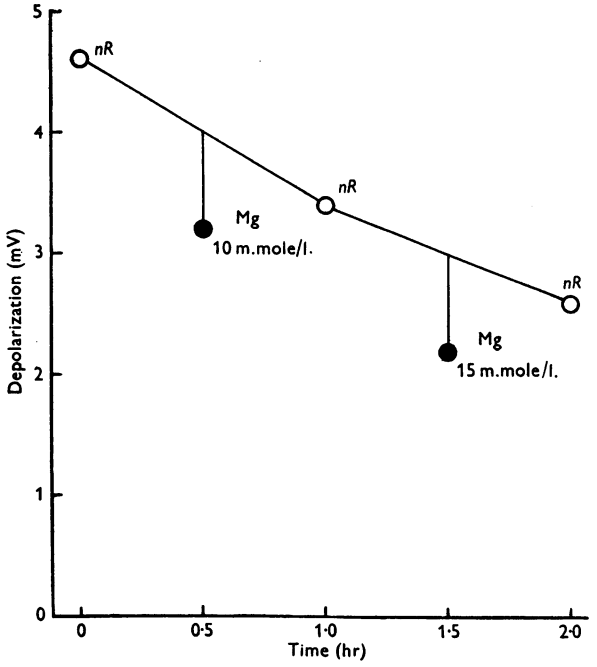

Fig. 3. Action of magnesium ions on the depolarization elicited by acetylcholine. Ordinate: amplitude of the depolarization in mV. Hollow circles, in Ringer's fluid; full circles, in fluid containing magnesium, as indicated. Abscissa: time in hours from the beginning of the experiment.

No attempt has been made to study the relative contribution of each factor, as we were primarily concerned in estimating the role played by the reduced depolarizing action of acetylcholine in the establishment of the 'magnesium-block'. This can be done by comparing the influence of equally effective blocking concentrations of Mg ions and tubocurarine on the sensitivity of the end-plate.

In a non-curarized muscle, and under the same experimental conditions, a 3 mV depolarization is produced by about 4×10^{-6} acetylcholine; if tubocurarine is added to the Ringer's fluid in the concentration necessary to produce neuromuscular block (3×10^{-6}), 15 times as much acetylcholine is now

TABLE 1. Effect of magnesium and calcium on hydrolysis of acetylcholine by frog muscle homogenate

(The figures in columns 1, 2, 3, 5 and 7 indicate μmoles acetylcholine hydrolysed per g (wet weight) per minute in various solutions: see Methods.)

Homogenate no.	Normal Ringer	15 mM-Mg	Relative change in activity	15 mM-Mg + 3 mM-Ca	Relative change in activity	3 mM-Ca	Relative change in activity
1	0·81	2·74	3·38	2·74	3·38	—	—
2	1·32	3·49	2·64	3·24	2·46	—	—
3	1·30	3·94	3·03	3·48	2·68	—	—
4	0·79	3·28	4·15	3·28	4·15	—	—
5	0·81	1·93	2·38	2·78	3·43	—	—
6	1·26	—	—	—	—	1·16	0·92
7	0·75	—	—	—	—	0·73	0·97
8*	—	3·96, 3·86, 3·35	—	—	—	—	—
9*	—	—	—	2·94, 2·84, 2·44	—	—	—

* Three determinations with identical electrolyte concentrations to give a measure of the reproducibility.

needed to produce a depolarization of the same magnitude (del Castillo & Stark, 1952). In view of the quantitative difference existing between the effects of tubocurarine and Mg ions on the depolarizing action of acetylcholine, it seems unlikely that a reduced end-plate sensitivity is the main cause of the magnesium block.

The effect of magnesium on the sensitivity of the end-plate in curarized muscles differs appreciably from that observed in non-curarized muscle. In the presence of tubocurarine, magnesium produces a slight increase in the depolarizing action of acetylcholine. In four experiments on curarized muscle 5 m.mole/l. magnesium increased the depolarization produced by acetylcholine by an average of $11 \pm 8\%$ (S.E. of mean), whereas 10 m.mole/l. increased it by $25 \pm 10\%$.

Action of magnesium on acetylcholine liberation

Direct determinations of the amount of acetylcholine liberated per motor nerve volley in presence of different magnesium concentrations have not been made. Indirect evidence can be obtained, however, by comparing the influence of magnesium on the e.p.p. with its effect on the sensitivity of the end-plate, a procedure which has been applied to the effect of calcium and sodium ions (del Castillo & Stark, 1952; Fatt & Katz, 1952*b*).

As mentioned above, in the non-curarized muscle an increase in magnesium concentration from 10 to 15 m.mole/l. caused an additional decrease in the end-plate sensitivity of about 25%. The same increment in the magnesium concentration had a much more striking effect on the size of the e.p.p., its amplitude being diminished by 70%. This difference could be explained on the assumption that magnesium reduces the amount of transmitter released.

In the curarized muscle the difference between the effect of magnesium on the end-plate sensitivity and the size of the e.p.p. is even clearer; 5 m.mole/l. magnesium reduced the e.p.p. by about 75%, and 10 m.mole/l. by about 90%, while there was no decrease, and even an increase, in the depolarization caused by acetylcholine in the same ionic environment. A decreased acetylcholine output seems to be the only satisfactory explanation for these experimental findings.

Action of magnesium on the muscle membrane

The two actions of magnesium which have so far been described, viz. a reduction in the amount of released acetylcholine and a decreased sensitivity of the end-plate region, can certainly account for the neuromuscular block produced by this cation. Apart, however, from the abolition of the responses to nerve stimulation, a decrease in direct excitability has also been observed in muscles treated with magnesium, even in denervated and curarized preparations (Ashkenaz, 1938; Maaske & Gibson, 1939; Engbaek, 1948). This is

probably due to an effect of magnesium ions on the electrical threshold of the muscle membrane; an effect of this type is produced by increasing the concentration of calcium (Katz, 1942). In order to obtain quantitative information on this action two intracellular electrodes inserted into the same muscle fibre were employed (see Methods). One of the electrodes was used to deliver a rectangular pulse of outward current of variable strength through the membrane; the resulting potential changes were recorded with the other. By gradually increasing the current strength, a level of depolarization was reached at which the membrane became unstable and an action potential was generated. The largest potential change which just failed to flare up into an action potential was taken as a measure of 'threshold'. In some cases, however, the inflexion point of the potential, i.e. the point of minimum slope, was taken as an indication of the critical level of depolarization (see Fig. 4).

In normal Ringer's fluid the mean threshold obtained in thirty-one measurements in three muscles was $34{\cdot}5 \pm 0{\cdot}67$ mV (S.E. of mean). In twenty-one measurements on two muscles soaked in a solution containing 15 m.mole/l. magnesium the average threshold was $53{\cdot}7 \pm 0{\cdot}8$ mV. This effect was fully reversible when the preparation was brought back to normal Ringer's fluid.

Calcium antagonism of magnesium block

It has been known for a long time that calcium ions antagonize the blocking action of magnesium on the neuromuscular junction (Bryant, Lehmann & Knoefel, 1939). In the following experiments this was studied in some detail and an attempt was made to locate the site of interaction of these cations.

Neuromuscular transmission was easily restored in preparations blocked by Ringer's fluid containing 15 m.mole/l. magnesium if the calcium content of the solution was increased by 3 or 4 m.mole/l., *without* removing the magnesium ions. Electrical recording revealed that this process was accompanied by, and probably due to, a large increase in the amplitude of the e.p.p. Fig. 5 shows one experiment of this type. It can be seen how an added m.mole/l. calcium increased the amplitude of the e.p.p. by about 4 times, and 2 m.mole/l. calcium by about 8 times. Transmission was restored by 3–4 m.mole/l. A further increase to 5 m.mole/l. or more caused, in this and other similar experiments, a depression of the amplitude of the e.p.p.

The amount of transmitter liberated per impulse at the nerve terminals is known to vary directly with the logarithm of the calcium concentration, whereas the sensitivity of the end-plate region to acetylcholine seems to be unaffected by this cation (del Castillo & Stark, 1952). One would expect therefore that the increase in the amplitude of the e.p.p. is due to an increased liberation of transmitter rather than to a change in the sensitivity of the end-plate. This second possibility has been excluded by direct experiment.

The sensitivity of the end-plate as measured by the depolarization caused by

acetylcholine was reduced by magnesium, and remained unaffected by an excess of calcium sufficient to increase the amplitude of the e.p.p. and relieve

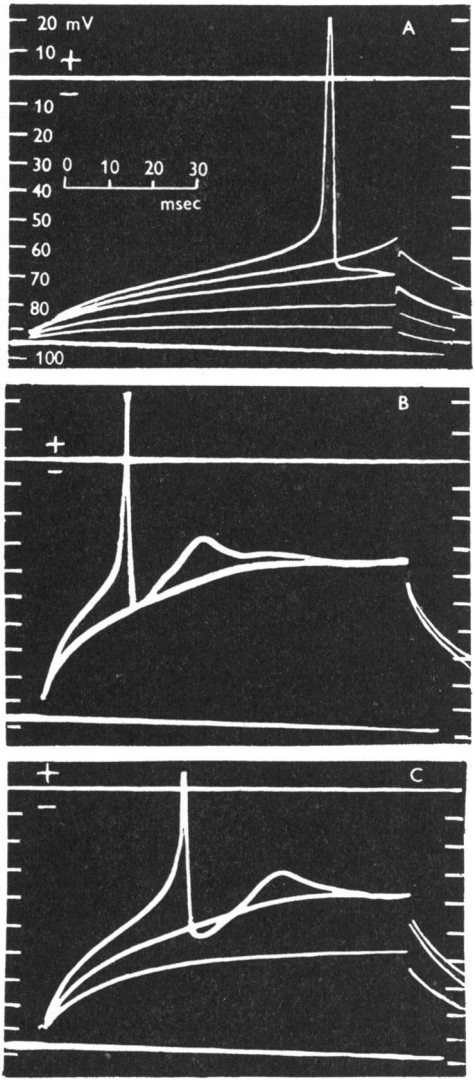

Fig. 4. Effect of magnesium on the electric threshold of the muscle fibre membrane. A, muscle soaked in normal Ringer's fluid. B and C, muscle soaked in a solution containing 15 m.mole/l. magnesium. (See text.)

the block. In two experiments in which 15 m.mole/l. magnesium caused a decrease of nearly 50% in the end-plate sensitivity, measurements performed in a bathing solution containing 15 m.mole/l. magnesium + 3 m.mole/l.

calcium did not reveal any significant change in the depolarization produced by the same concentration of acetylcholine, although the muscle response to nerve stimulation was restored in the second solution. In another experiment a preparation blocked by 10 m.mole/l. magnesium, showing a decrease of about 28% in the end-plate sensitivity, was transferred to a solution containing 10 m.mole/l. magnesium + 5 m.mole/l. calcium. A further reduction in the sensitivity of the end-plate was observed, which became now 44% lower than in normal Ringer, although the neuromuscular block was again relieved.

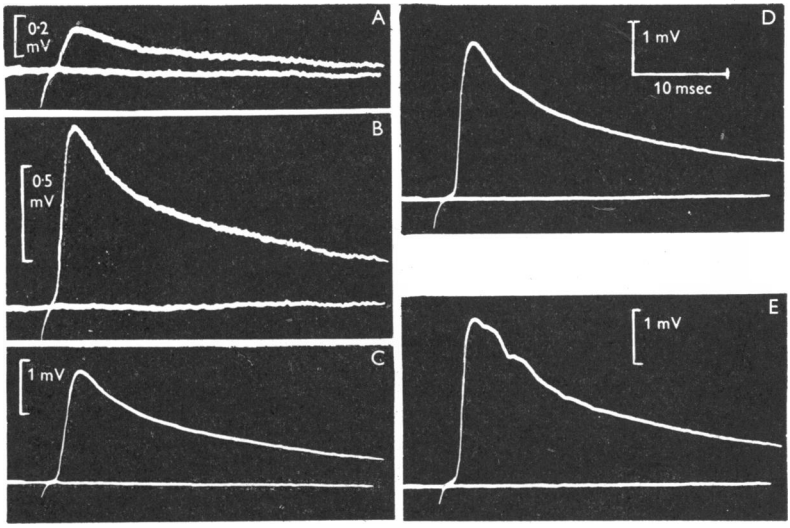

Fig. 5. Effect of calcium ions on the amplitude of the e.p.p. in a magnesium-treated muscle. A, e.p.p. in a preparation soaked in fluid containing 15 m.mole/l. magnesium (external electrodes). This concentration is kept constant in successive records while the calcium concentration was increased as follows: B, 1; C, 2; D, 3; E, 4 m.mole/l. Note that the gain was reduced between records B and C. In record E action potentials can be observed. (See text.)

The action of calcium on the electric excitability of the muscle membrane in presence of magnesium was also investigated. In one experiment the average threshold in seven muscle fibres, the muscle being immersed in normal Ringer's fluid, was 34·6 mV ± 1·1 (S.E. of mean). The muscle was then soaked in a solution containing 15 m.mole/l. magnesium and the mean threshold in six fibres rose to 52 mV ± 0·91. 3 m.mole/l. calcium was now added to the magnesium Ringer; the block was thereby relieved but the average threshold found in 6 fibres was now 54·1 mV ± 0·76.

The combined effect of magnesium and calcium on the cholinesterase

activity of muscle homogenate has also been studied with the same order of concentrations as used in the other experiments described in this paragraph. It has already been mentioned that 15 m.mole/l. magnesium increased the hydrolysis of acetylcholine by a factor of 2·5–4. However, 3 m.mole/l. calcium had no effect on cholinesterase activity under the same experimental conditions, either alone or combined with magnesium (see Table 1, columns 6–8). The absence of a calcium-magnesium antagonism in the activation of the cholinesterase is also a proof that this action does not play a significant part in the establishment of the neuromuscular block.

It seems clear from these experiments that the antagonism between calcium and magnesium must be solely due to their opposite actions on the release of acetylcholine at the motor nerve terminals. The other effects of magnesium are unaffected or even reinforced by the addition of calcium in the quantities necessary to relieve the block.

Quantal fragmentation of the e.p.p. in high magnesium concentrations

If a nerve-muscle preparation is immersed in a Ringer's solution containing only one-quarter of the normal calcium concentration the size of the e.p.p. is greatly reduced through a diminished acetylcholine output at the nerve terminals. Fatt & Katz (1952*a*) have examined in detail this effect at single junctions, recording the e.p.p. with an intracellular electrode. They found that when the amplitude of the e.p.p. is approaching zero, successive nerve stimuli elicit e.p.p.'s whose size changes at random in a step-wise fashion. Furthermore, they found that a relationship exists between the magnitude of these 'steps' and the size of the miniature e.p.p.'s which are generated spontaneously at the end-plate. This suggests that the fluctuating size of the e.p.p. is due to the activity of a varying number of units or groups concerned with acetylcholine liberation, the amplitude of the smallest steps representing the individual contribution of one of such unit to the e.p.p.

As the effect of magnesium excess appears to be similar to that of low calcium, one would expect analogous changes in the end-plate potential. The effect of high magnesium concentrations on the response of single neuromuscular junctions was therefore studied with intracellular electrodes. In muscles blocked with relatively low magnesium concentrations the position of the end-plate was first determined. The magnesium concentration was then increased until the amplitude of the e.p.p. fell to about 1–3 mV. The amount of magnesium necessary for this varied with the individual junctions, but in general, was between 15 and 20 m.mole/l. When the e.p.p. was thus decreased, striking 'quantal' fluctuations of its size were observed (Fig. 6), similar to those demonstrated by Fatt & Katz (1952*a*). This suggests that in fact calcium and magnesium act in an exactly opposite manner on the quantal liberation of acetylcholine at the nerve terminals.

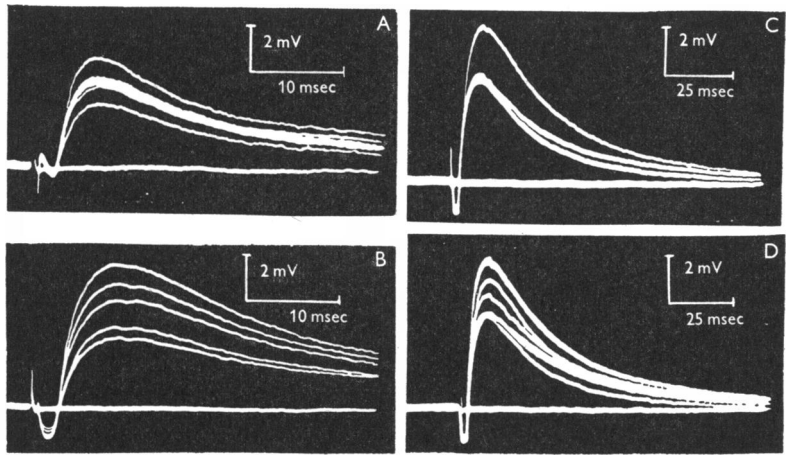

Fig. 6. Effect of high magnesium concentrations on the end-plate potential recorded with an intracellular electrode at single neuromuscular junctions. The preparation was soaked in fluid containing 18 m.mole/l. magnesium. Each record shows several end-plate responses to successive nerve stimuli. A step-wise fluctuation in the amplitude of successive end-plate potentials can be observed. Records B, C and D were taken after the addition of prostigmine to the bathing fluid (10^{-6}).

DISCUSSION

The results of our experiments indicate that an excess of magnesium ions has at least three distinct effects on the neuromuscular junction: (i) decreasing the amount of transmitter liberated at the motor nerve terminals; (ii) diminishing the depolarizing action of acetylcholine at the end-plate; and (iii) depressing the excitability of the muscle fibre membrane.

Although the block of neuromuscular transmission brought about by magnesium ions seems to be due to the addition of these three actions, the most important factor is the reduction in the amount of released transmitter which causes a drastic fall in the amplitude of the end-plate potential. The experiments in which the antagonism betweeen calcium and magnesium ions was studied have shown that transmission of impulses may be restored in spite of a reduced end-plate sensitivity and an increased threshold of the muscle membrane.

From the experiments on curarized preparations it appears that magnesium ions affect acetylcholine liberation particularly within the physiological concentration range, which in the frog plasma is of the order of 1–3 m.mole/l. (Fenn, 1936; Boyle & Conway, 1941). The same applies to the influence of calcium on the e.p.p. amplitude. Very small variations in the calcium concentration, e.g. by adding 1 m.mole/l. in excess of that contained in normal

Ringer's fluid, have also a considerable influence in antagonizing the depression of the e.p.p. produced by high concentration of magnesium.

The amount of transmitter liberated at the nerve terminals and consequently the amplitude of the e.p.p. seem, therefore, to be a function of the relative amounts of calcium and magnesium ions and is probably extremely sensitive to small physiological variations in the concentration of these cations.

SUMMARY

1. An excess of magnesium ions blocks neuromuscular transmission. This effect has been investigated in the sciatic-sartorius preparation of the frog and is due to a drastic reduction in the amplitude of the e.p.p.

2. Magnesium also decreases the sensitivity of the end-plate to the depolarizing action of applied acetylcholine and the direct excitability of the muscle fibres.

3. A quantitative comparison of these different actions indicates that the main effect of magnesium is to decrease the amount of acetylcholine liberated by a motor nerve impulse.

4. An excess of calcium, which is known to increase the amount of transmitter liberated, antagonizes the effect of magnesium on the motor nerve endings, the neuromuscular block being thereby relieved.

5. When the e.p.p. is recorded at single junctions with intracellular electrodes in the presence of high magnesium concentrations the size of successive e.p.p.'s shows 'quantal' fluctuations similar to those previously observed in Ca-deficient preparations.

6. It appears from these results that the amount of acetylcholine liberated by a motor nerve volley is a function of the relative amounts of calcium and magnesium ions present.

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