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THE EFFECT OF MAGNESIUM ON THE ACTIVITY OF MOTOR NERVE ENDINGS

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It has been shown by Castillo & Engbaek (1954) that the effects of calcium and magnesium on the neuromuscular junction are mutually antagonistic. The end-plate potential (e.p.p.) can be reduced and transmission blocked by either lowering Ca or increasing Mg concentration, and the Mg block can be cancelled by raising Ca above its normal level. There is evidence that both ions influence, in an opposite manner, the release of acetylcholine (ACh) from nerve endings, the amount liberated per impulse varying directly with Ca, and inversely with Mg, concentration. These observations suggest that some calcium compound may act as an intermediary during the release of ACh from motor nerve endings, and that magnesium inhibits the process by displacing calcium from its strategic position.

This idea links up with a different line of speculation coming from a study of the spontaneous miniature e.p.p.'s (Fatt & Katz, 1952*a*, *b*, 1953). The miniature e.p.p. appears to be due to a synchronous release of a large number of ACh ions from discrete spots of the nerve terminals. Its origin is as yet unknown, but it has been suggested that the primary event could be the activation of a specific carrier molecule which is responsible for the rapid transfer of a large number of ACh ions (Fatt & Katz, 1953). During nerve-muscle transmission, it has similarly been proposed (see Fatt & Katz, 1952*b*; also Hodgkin, Huxley & Katz, 1949; and Gordon & Welsh, 1948) that the nerve impulse acts in the first instance by releasing such carrier molecules (X') from an inactive calcium compound (CaX) giving rise to an e.p.p. which is a statistical fusion of miniature potentials. Ca deficiency or Mg excess would, therefore, inhibit ACh release by depleting the store of an essential precursor CaX and thereby reducing the number of available 'carriers'. Moreover, this would explain why the e.p.p. is diminished in a quantal 'stepwise' fashion, corresponding to the dropping out of whole miniature units (Fatt & Katz, 1952*a*; Castillo & Engbaek, 1954; Castillo & Katz, 1954).

An obstacle to this hypothesis was that Ca had no effect on spontaneous

activity. One would expect that an agent like Ca lack, which reduces the number of available responding units, would also diminish the probability of spontaneous firing, but no consistent change of the firing rate was found (Fatt & Katz, 1952*a*). There remained the possibility that, in this instance, two opposite effects of Ca might have cancelled out (see Fatt & Katz, 1952*b*): Ca lack is known to raise electric excitability; lowering the Ca concentration, therefore, although reducing the number of available carrier-units, might somehow lower their activation threshold, so that the total spontaneous firing rate remained unaltered. The use of magnesium, however, enables one to decide this matter; for while the actions of Mg and Ca on the e.p.p. are opposite, their actions on electric excitability are the same (e.g. Gordon & Welsh, 1948). Hence if we increase Mg, rather than lower Ca, then instead of cancellation we should get enhancement of the two effects: namely (i) reducing the number of responding units, and (ii) raising their activation threshold. On this view, the net result should be a drastic reduction by Mg of the spontaneous firing rate, at least as great as the reduction in size of the e.p.p. response.

METHODS

The effect of Mg on amplitude and frequency of spontaneous miniature e.p.p.'s was determined using the procedure of Fatt & Katz (1951, 1952*a*). The m. ext. l. dig. IV of the frog (English *Rana temporaria*) was employed in which miniature potentials are relatively large and easy to locate. Prostigmine was used in a concentration of 10^{-6} (w/v) to increase the size of the potentials further and improve the accuracy of measurement. Mg was substituted for osmotically equivalent amounts of Na (84 mM-$MgCl_2$ being taken as isotonic with 120 mM-NaCl).

A recording microelectrode was inserted into muscle fibres at different points until spots with suitably large spontaneous potentials had been located. Records were taken from the same spots, before and after the muscle had been immersed in a Mg-rich Ringer and again after the Mg had been washed out. During the Mg treatment, nerve-muscle transmission was blocked and, in addition to the spontaneous activity, the end-plate response to a nerve impulse could be recorded without risk of damage. The characteristics of the response will be described in the following paper.

Sources of error and corrections. When comparing amplitudes of miniature e.p.p.'s, an allowance should be made for adventitious changes in resting potential, for the e.p.p. is known to vary in approximate proportion to the resting potential. In Table 2 below, the results have been shown with and without this correction.

A systematic error must be considered when small rapid signals, like miniature potentials, are measured on a slowly moving 'noisy' base-line. The usual method of measuring a deflexion from the upper edge of the base-line to the top leads to an underestimate which may in extreme cases amount to half the thickness of the apparent base-line. This is due to the fact that the thickness of the base-line is exaggerated by random fluctuations which are too fast to be resolved photographically and too slow to affect the height of the signal. The possible effect of this error will be considered when comparing magnitudes of miniature potentials in normal and Mg-treated muscles.

RESULTS

Frequency. In Table 1, the results of nine experiments are summarized showing that a magnesium concentration of 16 mM had no significant effect on the frequency of the spontaneous discharge (mean ratio of frequencies

normal/Mg treated was 0·97, S.E. ±0·12). The firing rate at individual junctions is unstable and subject to considerable progressive change (cf. Fatt & Katz, 1952*a*); it is clear, however, that Mg failed to produce the predicted drastic reduction.

In addition to the tabulated results many other experiments were made in which the e.p.p. response had been reduced to a small fraction of its normal amplitude by increasing Mg and lowering Ca: spontaneous miniature potentials were present in all these cases, with frequencies scattered over the wide range observed in normal muscles.

TABLE 1. Effect of Mg on frequency of spontaneous miniature e.p.p.'s

In each experiment, successive observations were made on the same end-plate, when 23 mM-NaCl of the Ringer solution was replaced by 16·2 mM-$MgCl_2$. In Expt. 6, five runs were made (three in Na alternating with two in Mg). The number of discharges in any one series varied between 20 and 350 (average 80).

	Discharge rates (per sec)			Frequency ratio,
Expt.	Na	Mg	Na	Na/Mg
1	2·7	0·87	0·55	1·87
2	0·26	0·4	0·32	0·73
3	1·0	0·61	0·25	1·03
4	0·63	0·89	—	0·71
5	1·7	1·75	1·3	0·86
6	2·51	4·48	—	0·56
7	0·95	0·95	—	1·0
8	1·2	0·94	0·65	0·99
9	0·62	0·54	0·46	1·0
Mean and S.E.	—	—	—	0·97±0·12

TABLE 2. Effect of Mg on amplitude of miniature e.p.p.'s

Mean sizes of miniature potentials were measured in eight of the experiments summarized in Table 1. The ratio of amplitudes in Mg and Na is shown, with and without a correction for variation in resting potential (see Methods).

Expt.	Ratio, Mg/Na	Corrected for changes in R.P.
1	0·51	0·54
2	0·61	0·57
3	0·54	0·53
4	0·62	0·62
5	0·68	0·71
6	0·45	0·53
7	0·74	0·78
8	0·72	0·68
Mean	0·61	0·62 (S.E. ±0·03)

Size. In Table 2, the results of eight experiments are summarized showing that Mg, at 16 mM reduced the amplitude of the miniature potentials to about 60%. With a correction for observed variations of the resting potential (see Methods), the reduction factor became 0·62 (S.E. ±0·03). This value is likely to be too small because of a systematic error in estimating the thickness of the base-line (cf. Methods): an extreme correction would change the factor to 0·68 (±0·03), the correct value lying presumably between these limits.

E.p.p. response. When the response was recorded at the Mg-treated junction small e.p.p.'s of the order of 1 mV were found which fluctuated in amplitude during successive trials in the characteristic manner already described by Fatt & Katz (1952*a*) and Castillo & Engbaek (1954). It will be shown in the following paper that these fluctuations are due to random changes in the *number* of responding units, each contributing its miniature potential in an all-or-none fashion.

DISCUSSION

In conjunction with previous work (Fatt & Katz, 1952*a*; Castillo & Engbaek, 1954) our experiments indicate that Mg and Ca ions, while controlling the release of ACh by motor nerve impulses, have little or no effect on the spontaneous release mechanism which is responsible for the appearance of miniature potentials. The failure of Mg and Ca to influence the frequency of the spontaneous discharge is all the more surprising as both ions have a powerful action on the excitability of nerve and muscle. With Ca, it seemed conceivable that a balance of two opposite effects may have occurred (see the argument on p. 554), but the present results with Mg eliminate this possibility.

The experiments suggest that a number of discrete steps intervene between the arrival of a nerve impulse and the release of ACh, and it will be worth while discussing them with the help of a simple scheme.

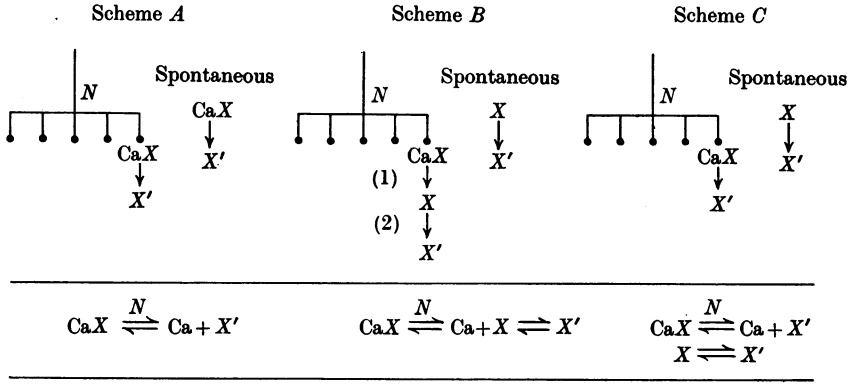

Fig. 1. Three alternative ways of explaining origin of e.p.p. and of spontaneous miniature discharges. *N*, nerve impulse; *X*, inactive, and *X'*, active 'ACh-carrier molecules'. For further explanation see text.

Scheme *A* (Fig. 1) represents the initial hypothesis outlined on p. 553. The impulse *N* releases, at each junction, numerous carrier molecules *X'* from inactive precursors Ca*X*. Each *X'* is responsible for the release of a large number of ACh molecules. The reaction $CaX \rightarrow Ca + X'$ also takes place spontaneously leading to a miniature potential, but is in some way catalysed by *N*, i.e. its probability is enormously increased. Block by deficiency of Ca

or addition of Mg is due to a progressive reduction of available precursor molecules. This simple hypothesis must now be abandoned because it requires that the reduction of the e.p.p. be associated with a reduction in spontaneous activity. The fact that one type of activity can be altered without affecting the other is accommodated in Scheme *B* which contains an additional step. The nerve impulse releases, in the first place, inactive carrier molecules X; they are spontaneously transformed to the active state X' in which they release ACh and give rise to miniature potentials. In the absence of nerve impulses, spontaneous firing arises from the random activity of a pool of precursor molecules X, whose concentration is independent of Ca or Mg. Lack of Ca or excess of Mg blocks transmission during the first step without interfering with the spontaneous second step.

Scheme *B* implies that a nerve impulse momentarily increases the rate of the spontaneous reaction ($X \rightarrow X'$) by liberating a large number of molecules X, in excess of those present at rest. But as pointed out below this would require the release of some millions of carrier molecules at a single junction, an excessive amount which is in the estimated order of magnitude of released ACh molecules (Acheson, 1948).

An alternative suggestion is that instead of two successive steps postulated in Scheme *B*, there are two different pathways, both leading to the appearance of active carriers X'. This is indicated in Scheme *C*: the reaction started by the impulse is the same as in Scheme *A*, except that it does not take place spontaneously. Spontaneous activation of X is a different event which does not involve Ca or Mg, nor depend on the electric excitability of the membrane.

Our reason for rejecting Scheme *B* is that it requires the liberation of an improbably large number of carriers. We arrived at this conclusion by comparing the probability of a spontaneous discharge, with that of a response to a nerve impulse. The normal e.p.p. at a single junction may be regarded as a statistical fusion of at least 100, probably a few hundred, active miniature units (Fatt & Katz, 1952*a*). Suppose we have a population of 500 units at each junction, and the average probability of a single unit being activated by one impulse is of the order of 1/3. Activation occurs at a fixed time interval after the arrival of the impulse; judging from the constant latency of the e.p.p. and its brief rising phase most of, or all, the contributing units must be activated synchronously within a fraction, say 0·5, of a millisecond. The average chance of any one unit discharging *spontaneously* during such a brief interval is $p = \frac{1}{500} \times 0{\cdot}5/T$, T being the observed mean interval, in msec, of the spontaneous firing. If the firing rate is 1 per sec, $p = 10^{-6}$. The nerve impulse increases this probability by a factor of about 3×10^5. On Scheme *B* this would need a liberation of molecules X exceeding in numbers those normally present by a factor of 3×10^5. This is hardly feasible on our present picture if X are to be carrier molecules, and not individual ACh ions. The latter possibility would imply that the spontaneous miniature e.p.p. is due to the collision of a single ACh ion with the motor end-plate, and this has already been dismissed on experimental grounds (Fatt & Katz, 1952*a*). It was incompatible with the finding that application of ACh did not change the frequency of the spontaneous potentials, though it must have raised the molecular collision frequency by several orders of magnitude. Nor did it fit the observations that curarine and prostigmine produce graded changes in size and shape of the miniature potentials, without significantly altering their frequency.

The results might, of course, be interpreted in an entirely different way without linking them to any carrier hypothesis. It could, for instance, be argued that block by Mg or lack of Ca signifies failure of propagation into the terminal axon branches, the impulse being stopped at individual bifurcation points, while the spontaneous activity at the terminal is unaffected. The idea of a 'bifurcation block' is based on the reasonable assumption that the safety margin of an impulse would be reduced when it arrives at a region of membrane expansion. A hypothesis of this kind has been proposed for various situations (cf. Eccles, 1953; Katz, 1950), but in the present case it seems unattractive for the following reasons: (*a*) It is not clear why Ca and Mg should have antagonistic action at points of dichotomy, but not at other parts of a nerve fibre. (*b*) The reduction of safety factor at a bifurcation is not great, unless the surface expansion is very large. Suppose the axon diameter is reduced at each point of branching to $1/\sqrt{2}$; then the 'input resistance' at the dichotomy falls to $2^{-0.25}$ times, about 0·84, of the characteristic resistance of the undivided line, requiring a correspondingly larger current for a threshold potential change. If there are, say, seven successive divisions the input resistance would fall to 0·3. Opposed to this is the effect of the 'closed end' of the line which approximately doubles the 'input resistance'. The net result is that the nerve impulse does not lose very much of its safety margin as it enters the terminal arborization. Moreover, the safety factor would be lowest at the first point of division and increase again as the impulse passes on to the finer branches, for progressively fewer resting branches remain to be stimulated. If blockage does occur under abnormal conditions, it would probably be all-or-none and located at the first point of dichotomy, not in the terminal structures. (*c*) Finally, there is evidence (Castillo & Katz, 1954) that even under *normal* conditions only a fraction of the terminal apparatus is activated by a motor impulse and that the number of active units can be increased by raising the Ca concentration above the normal level. All this seems difficult to explain on 'geometrical' grounds, and to support a more specific explanation along the lines of the carrier hypothesis.

The effect of Mg on the size of the miniature potential (30–40% reduction by 16 mM-Mg) requires some comment. It does not seem to be specific, for a small reduction is also observed with high concentrations of Ca (Fatt & Katz, 1952*a*). There is evidence that this action is largely, if not entirely, due to a 'curarizing' (i.e. post-synaptic) effect of Mg, for Castillo & Engbaek (1954) have shown that the depolarization produced by a constant dose of applied ACh is similarly reduced by Mg.

SUMMARY

1. Magnesium ions, in concentrations which nearly abolish the end-plate potential, have little or no effect on the frequency of spontaneously firing miniature e.p.p.'s and cause their amplitudes to be reduced to about 60%.

2. It is concluded, from these and earlier experiments, that calcium and magnesium ions which influence in an antagonistic manner the release of acetylcholine by a motor nerve impulse, have no effect on the spontaneous release of acetylcholine which gives rise to a miniature e.p.p.

3. Several hypotheses are discussed concerning the origin of the spontaneous potential and its relation to the normal end-plate response.

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