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THE EFFECTS OF PRESYNAPTIC POLARIZATION ON THE SPONTANEOUS ACTIVITY AT THE MAMMALIAN NEUROMUSCULAR JUNCTION

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Recent investigations (Boyd & Martin, 1956*b*; Liley, 1956*b*) have shown that the mammalian end-plate potential (e.p.p.) is generated by the synchronous release of quanta of transmitter whose individual spontaneous liberation gives rise to the miniature potentials. These observations immediately raise the question of the mechanism by which a co-ordinated discharge of the quanta is produced by an impulse arriving at the motor nerve terminals. Del Castillo & Katz (1954*b*) have investigated a similar problem at the frog myoneural junction.

In the present investigation a study has been made by intracellular recordings in the rat diaphragm of the relation between the frequency of the miniature discharge and electrotonic polarization of the motor nerve terminals. A study has been made also of the effects of potassium concentration on the frequency of the miniature potentials.

METHODS

The technique of intracellular recording and details of the preparation, solutions and apparatus employed have been described in a previous paper (Liley, 1956*a*). For experiments involving polarization of the motor nerve terminals the dissection and mounting of the preparation were modified as follows. The terminal portion of the left phrenic nerve and its posterior branch were dissected free from adherent tissue (pericardium and pericardial fat). The anterior branch of the nerve was cut close to its origin. The hemi-diaphragm was now divided by a cut parallel to the fibres and immediately anterior to the point of entry of the posterior nerve branch. In a suitable dissection the muscle fibres on the cut edge of the preparation obtained their nerve supply immediately from the entering posterior nerve branch. However, the origin of the nerve supply of the central fibres of the hemi-diaphragm was very variable. In about 50% of preparations a recurrent twig from the (cut) anterior branch not only supplied the central muscle fibres but also frequently encroached considerably on the territory of the posterior branch. Such preparations were discarded as also were preparations in which the nerve twigs ran some distance radially before innervating the muscle fibres.

Suitable preparations were mounted as shown in Fig. 1, the bordering fibres being placed as close as possible to the Perspex partition. This partition was 1·5 mm thick and beneath it, through a notch sealed with soft paraffin, the posterior branch of the phrenic nerve was led. With non-polarizable electrodes arranged as shown to pass direct current, the lines of current flow converge at this notch. For a given current the polarization produced at a given nerve terminal is a function of the space constant of the nerve fibres, the proximity of the terminal to the notch (normally less than 200 μ) and the relative volume of tissue, including other nerve fibres, passing through the notch. A disadvantage of this arrangement was that a considerable potential difference was produced by current flow in the bath solution. Where necessary this potential was compensated either by adjusting the operating condition of the input cathode follower to bring its output back to the original voltage level, or else by applying a compensating potential to the other input of the differential amplifier.

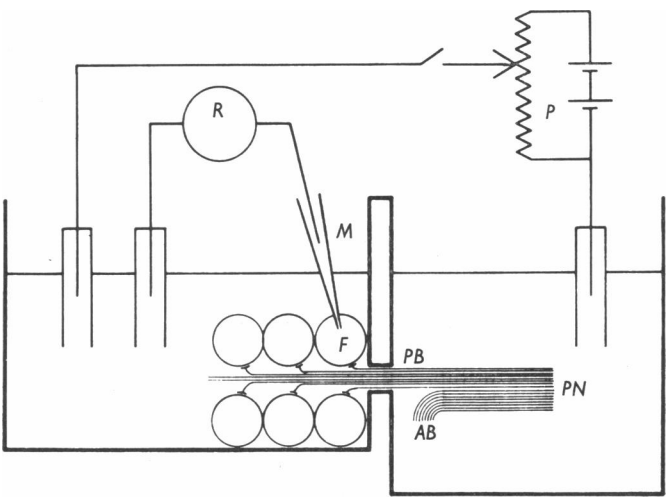

Fig. 1. Arrangement of polarizing (P) and recording (R) electrodes. Not to scale. Heavy lines indicate Perspex. Microelectrode (M) shown penetrating a muscle fibre (F). PB indicates posterior branch of phrenic nerve (PN) of which anterior branch (AB) has been cut.

Normally the polarizing current was applied by manual operation of a potentiometer. A monitoring resistor was placed in series with the nerve and the p.d. developed across it was read on a d.c. voltmeter. For brief rectangular pulses the potentiometer was present and the pulses were applied by the action of a Carpenter relay which was driven from the sweep potential via a Schmitt trigger circuit. By manual operation the polarizing current could be gradually increased over several seconds, so utilizing the accommodation process to prevent the intense repetitive discharge which would have been produced by the sudden onset of such a current.

Special problems were encountered in attempting to determine the mean frequency of the miniature discharge when frequencies were very high or very low. For an accurate determination of the low discharge frequencies that occurred with hyperpolarization of the terminals, it was necessary to extend observations over many minutes at each setting of the polarizing current. Beside expending photographic film, such long periods of recording greatly increased the risk of encountering either progressive change or transitory disturbances of the discharge frequency due to extraneous factors. Progressive effects could be detected by checking the value of the resting frequency at intervals during a series. More serious problems were encountered with very high discharge frequencies. Minimal base-line 'noise' was essential. Further it was necessary to localize the junction precisely so that potentials would be recorded with maximum amplitude and

briefest time-course. Anticholinesterases were undesirable since they involved a prolongation of time-course and an increased liability to the generation of action potentials. Under favourable conditions and with a very high sweep velocity, frequencies as high as 600–700/sec were readily determined, but above this level there was an increasing uncertainty in the recognition of individual potentials and counts were very likely to underestimate the true values. The effective frequencies during very brief intervals after a rectangular pulse were determined by photographing several thousand pulses and counting the number of discharges occurring within the corresponding intervals on each sweep. In all records interspersed large miniature potentials were assumed to result from the fortuitous coincident discharge of quanta and hence the quantal content of such potentials was estimated for the frequency count. Such a procedure ignores the tendency of the mammalian miniature discharges to 'couple' or 'snowball', thereby producing a greater number of coincident discharges than would be expected by stochastic theory (Liley, 1956*a*).

In the appropriate experiments any alteration in potassium concentration was accompanied by an iso-molar change (of opposite sign) in sodium concentration. In such experiments the following technique was adopted. The frequency of the miniature discharge was recorded at some twenty junctions in the normal solution. The altered solution was now introduced and the preparation allowed 15–20 min for equilibration, after which the discharge frequencies at another twenty junctions were determined. Finally the preparation was re-immersed in normal solution and after 15–20 min the discharge frequency in a further twenty fibres was determined. Simultaneously with the recordings of the miniature potentials, at each junction, the muscle resting membrane potential was noted, and an experiment was discarded unless the initial and final mean values of both discharge frequency and muscle membrane potential agreed satisfactorily.

According to Creese (1955) a rat diaphragm oxygenated by the method employed in the present experiments is far removed from an ionic steady state. Unfortunately Creese's technique for optimal oxygenation, involving the direction of a fine jet of gas on to the under surface of the preparation, is quite incompatible with intracellular recording. It is therefore of interest that, by the present method of oxygenation, it was possible for several hours to obtain good agreement with control values of mean muscle membrane potential and mean frequency of the miniature discharge.

RESULTS

Effect of electrotonic polarization of motor terminals on miniature discharge frequency

Cathodic polarization of the terminals produced an increase and anodic polarization a decrease in the frequency of the miniature discharge (Fig. 2). These effects were graded according to the intensity of the polarizing current and at a given junction were readily reproducible. The mean amplitude of the miniature potentials was unaffected by the polarization of the terminals.

No change in miniature discharge activity could be detected at junctions whose axons were severed close to the terminals nor could any effect be obtained at junctions situated more than a few millimetres from the sealed notch transmitting the nerve. These observations establish that the changes in frequency were dependent on electrotonic polarization of the nerve terminals and that the current flow in the bath solution played no part in their causation.

Experiments involving the application of rectangular current pulses to the terminals showed that the alteration in discharge frequency developed very rapidly (Fig. 3). By 2 msec after the onset of the polarizing current the

frequency had attained a value from which no statistically significant deviation occurred by 16, 50 or 150 msec. With more prolonged applications of current the discharge was stable at its new frequency over the observed range from 1 sec to 5 min. Thus when the membrane potential of the nerve terminals is altered, the frequency of the miniature potentials faithfully parallels the potential, there being no appreciable delay at the onset nor decline during a prolonged change.

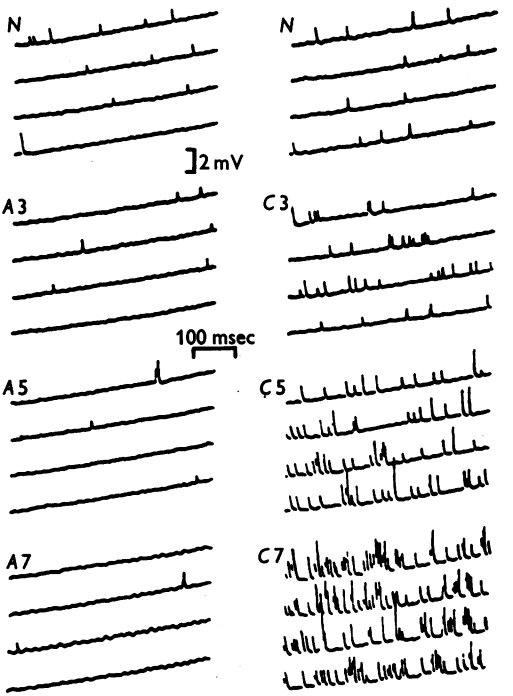

Fig. 2. Electrotonic effects on the miniature discharge frequency. *N*, initial and check recordings of resting discharge; *A*, anodic and *C*, cathodic polarization of nerve terminals. The numbers give current intensity in relative units.

Unfortunately, events within the initial 2 msec after the onset of the current were obscured by artifacts. With electrotonic polarization of the terminals it would not be expected that the frequency would attain its final value instantaneously with the onset of current. Although the generating circuit delivered a rectangular pulse, the wave-front of a pulse as it affected a nerve terminal would be distorted in a manner determined by the time and length constants of the axonal membrane. No reliable estimates of these constants in mammalian axon are available. The distortion may be minimized by selecting a junction as close as possible to the orifice admitting the nerve, but unless the time constant of the axon lies in the range 0·6–1·1 msec, it cannot be

concluded that the discharge frequency instantaneously follows the changing membrane potential.

The frequency of the miniature discharge was not linearly related to the current intensity (Fig. 2). However, in eleven out of thirteen experiments on normal junctions, when the logarithms of the frequencies were plotted against the respective current intensities, a relation was obtained which, statistically, was strongly suggestive of linearity (Fig. 4A).

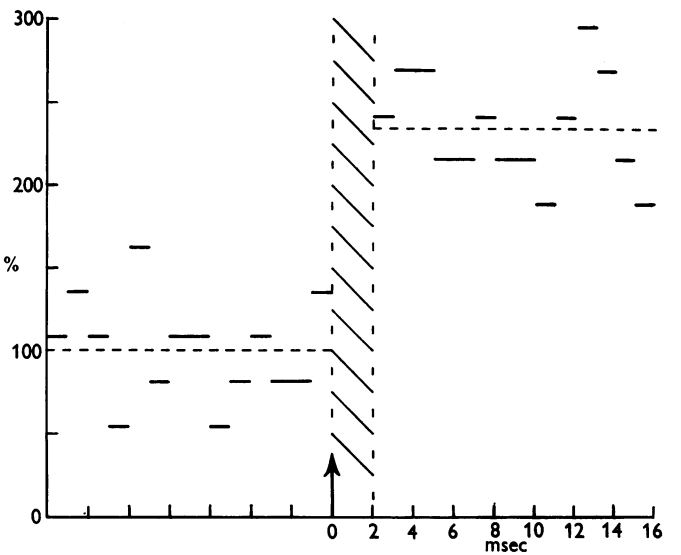

Fig. 3. Cathodic polarization of nerve terminals. Effect on miniature discharge frequency of a rectangular pulse. Onset of pulse and origin of time scale marked by an arrow. Ordinate: effective discharge frequency (see Methods) as a percentage of mean resting frequency (1·53/sec). Horizontal broken lines show mean resting frequency and mean frequency during pulse. Hatched area represents record obscured by artifact.

In the deviating series the depression of discharge frequency with anodic polarization was less than expected, the larger deviation being illustrated in Fig. 4B. In each experiment redetermination of the resting frequency showed that no progressive change had occurred. At these very low frequencies, even when series are recorded over several minutes, there may be considerable error in the determination of the frequency, and further, any errors may be magnified in a logarithmic plot, but it is not to be expected that all the determinations would be overestimates of the true values. The significance of these two experiments is difficult to assess. The records showed no bursts of high-frequency activity, and the effect does not remotely resemble the triggered 'breakdown' discharge encountered with strong hyperpolarization of frog nerve terminals (del Castillo & Katz, 1954*b*).

In calcium-deficient or magnesium-enriched solutions the effects of both depolarizing and hyperpolarizing currents on the miniature discharge were markedly reduced. Thus with a calcium concentration of 0·25 mM (normal 2 mM) or a magnesium concentration of 12 mM (normal 1 mM) polarizing currents of either sign were virtually unable to displace the discharge frequency from its resting value (Fig. 4B). However, in two experiments with 12 mM magnesium, intense depolarizing currents were able to produce a small increase in the frequency. When the effects of polarization had been suppressed by 12 mM magnesium, almost complete restoration was obtained by raising the calcium concentration to 8 mM.

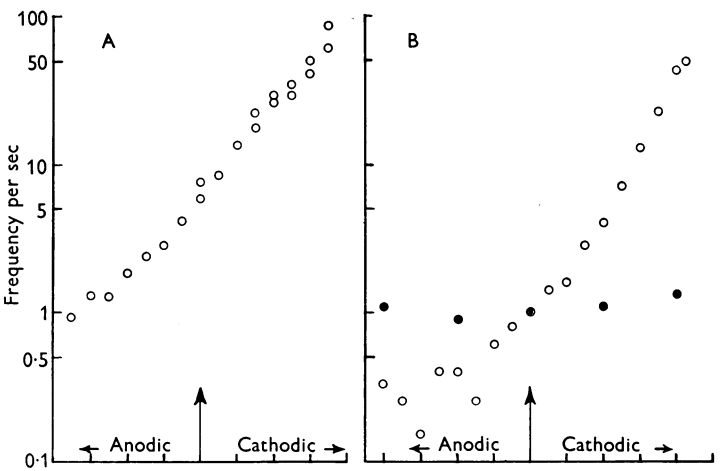

Fig. 4. Electrotonic effects on miniature discharge frequency. Note logarithmic ordinate scales. Abscissae: polarizing current intensity in relative units. A: muscle in normal solution showing linear relation between log discharge frequency and polarizing current intensity (product moment correlation coefficient, $r\left(=\frac{\text{covariance of } x \text{ and } y}{\sqrt{[\text{var}\,(x) \times \text{var}\,(y)]}}\right) = 0{\cdot}99$). B: experiment showing deviation from this empirical relationship, see text. ○, muscle in normal solution; ●, 12·5 mM magnesium.

An investigation was made of the ability of polarizing currents to modify the miniature frequency when this had been artificially displaced from its resting value. At three junctions an increase in the discharge frequency to the order of 100/sec was produced by small (3–8 μ) lateral movements of the microelectrode. Although the frequency was not very stable following this procedure, in each case it was readily influenced by both anodic and cathodic polarization (Fig. 5A) following the same empirical linear relation between current intensity and logarithm of discharge frequency. Similarly, when the discharge frequency was increased from 2/sec to 43/sec by the application of

15 mM potassium chloride (q.v.), it was still affected in the same manner by polarizing currents (Fig. 5B).

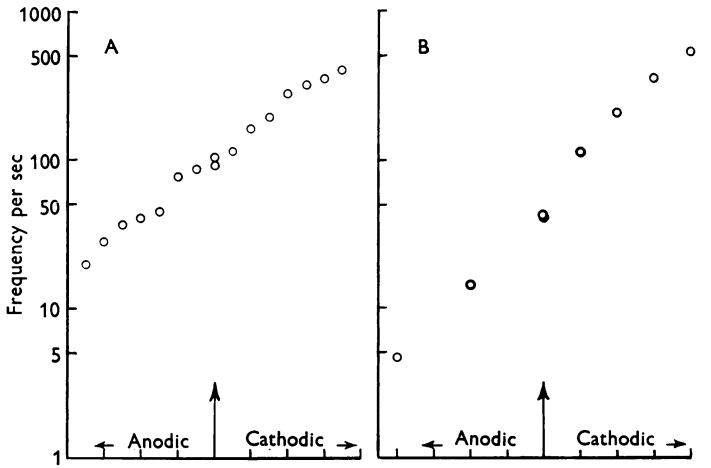

Fig. 5. Electrotonic effects on miniature discharge frequency. Conventions of scales as in Fig. 4. A: muscle in normal solution but initially discharge frequency was increased from resting rate of 1·6 to 100/ sec by trauma to nerve terminals. B: muscle in solution with potassium concentration of 15 mM. Discharge frequency in normal solution was 2/sec.

Effect of potassium concentration on the miniature potentials

Alteration in potassium concentration affected both the amplitude and frequency of the miniature discharge. Reduction in potassium concentration below the normal level of 5 mM produced no significant change in the amplitude of the discharge, but increasing the concentration to 30 mM reduced the amplitude to approximately one-third. This effect would appear to be post-synaptic in origin, and to result from the fall in muscle membrane potential which is observed in high potassium concentrations. It may be assumed that the action of acetylcholine on the mammalian post-synaptic membrane is similar to that demonstrated in the frog (Fatt & Katz, 1951); hence it would be expected that the amplitude of the miniature potentials would vary with the level of the muscle membrane potential.

Much more striking was the effect of potassium on the frequency of the miniature potentials (Fig. 6, open circles). Reduction of the potassium concentration from 5 to 2 mM produced a small but statistically significant reduction in the discharge frequency. On the other hand, the frequency was more than doubled when the concentration was increased from 5 to 10 mM. At 30 mM the frequency was of the order of 700/sec. There was a further increase at higher concentrations, but an accurate count was no longer possible. Over the range of 10–30 mM the mean frequency appeared to vary

linearly with potassium concentration. When any single junction was followed after a change in the potassium concentration, it was observed that some 1–4 min had elapsed before the alteration in discharge frequency was fully developed. Thereafter the discharge rate was stable for at least 20 min even when it was above 500/sec.

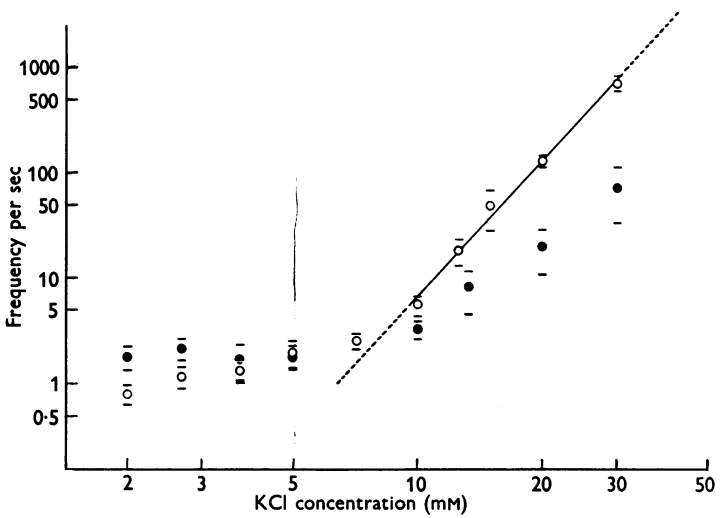

Fig. 6. Effect of potassium concentration on miniature discharge frequency. Normal potassium concentration was 5 mM. Note that both scales are logarithmic. Circles represent mean frequency at approximately twenty junctions with bars placed at ±2 S.E. of mean. ○, muscles in solution with normal magnesium (1 mM) and calcium (2 mM). For the five points beyond 10 mM potassium ($r = 0{\cdot}98$) the regression line for log frequency on log potassium concentration has been drawn. ●, muscles in solutions containing 12·5 mM magnesium, normal calcium.

The effects of potassium on the discharge frequency were modified considerably by high concentrations of magnesium. As had been observed previously, magnesium was without effect on the frequency at junctions in solutions of normal potassium content (Boyd & Martin, 1956*a*; Liley, 1956*b*; see also del Castillo & Katz, 1954*a*). However, in the presence of 12·5 mM magnesium, reduction in potassium concentration exerted no significant effect on the discharge frequency (Fig. 6, filled circles), while the influence of raised concentrations of potassium was markedly depressed. Thus 30 mM potassium in the presence of (normal) 1 mM magnesium increased the mean frequency to 715/sec, whereas in 12·5 mM magnesium the increase was limited to 74/sec. Nevertheless, as seen in Fig. 6, in high concentrations of magnesium there was still a linear relation between the discharge frequencies and potassium concentrations greater than 10 mM.

DISCUSSION

The present results show that the effects of presynaptic polarization on the miniature discharge are not the same in mammals and amphibia (del Castillo & Katz, 1954*b*). Although depolarizing currents produced comparable effects, moderate hyperpolarization was without influence on the discharge in the frog, whereas in the rat the discharge was readily depressed well below the resting value in all of thirteen experiments. It might possibly be argued that the motor nerve terminals of the isolated rat diaphragm are already depolarized in their abnormal environment and that hyperpolarizing currents are merely restoring the membrane potential and hence reducing the discharge frequency toward a true basal level. This is unlikely, however, for experiments on the gracilis muscle *in vivo* (Liley, 1956*a*) indicated that the mean frequency of the miniature discharge was of the same order as that observed in the isolated diaphragm. Furthermore, an increase in magnesium (or a reduction in calcium) concentration offsets the increase in frequency produced by depolarizing currents, but is without effect on the resting discharge frequency in the isolated diaphragm.

The sudden bursts of high-frequency activity observed in the frog with strong anodic polarization (del Castillo & Katz, 1954*b*) were not encountered in the rat diaphragm despite the fact that, as judged by cathodic effects, comparable current intensities across the terminal membrane were obtained in the two investigations (compare fig. 5 of del Castillo & Katz, 1954*b*, with Fig. 4 of the present paper). If this phenomenon in the frog results, as suggested, from an event resembling a dielectric breakdown, the absence of any comparable effect in the rat indicates that the mammalian membrane is more resistant to electrical stress.

The effects of polarizing currents suggest that the discharge frequency should be influenced when the membrane potential is altered by variations in extracellular potassium concentration. Unfortunately the membrane potential of the motor nerve terminals cannot be directly measured. Further, the membrane potential will not be linearly related to the logarithm of potassium concentration as predicted by the Nernst equation. Rather, as shown by Hodgkin & Katz (1949), an equation derived from the constant field theory of Goldmann would apply. This equation predicts that a low concentration of potassium will exert little influence on the membrane potential, the effect of other ions being dominant, but as the concentration of potassium is progressively raised above normal, the membrane potential will more nearly be related linearly to the logarithm of the potassium concentration as predicted by the Nernst equation.

Observations with polarizing currents at junctions covering a wide range of resting frequencies have indicated a linear relationship between the logarithm

of the discharge frequency and the polarization of the motor nerve terminals (Fig. 4). Therefore it may be predicted that, as the potassium concentration is increased, a value will be reached beyond which the logarithm of the discharge frequency will be related linearly to the logarithm of potassium concentration. Alternatively, dispensing with logarithms, the frequency would be expected to vary directly with the potassium concentration. The experiments illustrated in Fig. 6 show that this prediction was fulfilled for potassium concentrations beyond 10 mM. Unfortunately, investigations could not be carried beyond 30 mM since the discharges became too frequent for an accurate count. However, accepting the risks inherent in extrapolation, the straight line in Fig. 6 indicates that, beyond a concentration of 10 mM, a tenfold increase in potassium concentration augments the discharge frequency by a factor of 10^4.

If it be assumed that beyond 10 mM the potassium concentration determines the membrane potential of the motor nerve terminals in the manner predicted by the Nernst equation, it follows that the miniature discharge frequency is increased by a factor of 10^4 for a decrease in the membrane potential of approximately 60 mV. Hence from the logarithmic relationship between discharge frequency and displacement of the membrane potential (Figs. 4A, 5A, B) the discharge frequency corresponding to any change of the membrane potential may be predicted. Similarly, it may be calculated that, for a muscle in 12·5 mM magnesium the discharge frequency would be altered by a factor of $10^{2.5}$ for a change in the membrane potential of approximately 60 mV.

Since the mammalian end-plate potential consists of summated miniature potentials (Boyd & Martin, 1956*b*; Liley, 1956*b*) it is reasonable to interpret the increase in miniature discharge frequency associated with depolarization as a prolonged and attenuated version of the events occurring when the nerve terminals are briefly and intensely depolarized by an action potential. This interpretation is supported by the observation that neuromuscular transmission and the effects of depolarizing current are both depressed by increase in magnesium concentration, an action which in turn is antagonized by calcium. Several predictions capable of simple experimental test can be immediately derived from this hypothesis that the e.p.p. or liberation of quanta by an impulse is simply the random release of quanta accelerated by depolarization of the terminal membrane.

First, on the tacit assumption that the action potential is of the same amplitude in all the motor nerve terminals, the quantal content of the e.p.p. should be proportional to the resting discharge frequency. This relationship should apply not only for normal neuromuscular transmission but also for conditions where the effect of an impulse has been attenuated by an excess of magnesium and/or a deficiency of calcium ions. In the latter case, where the quantal content of the responses is small and easily measurable, the predicted relationship may be readily tested.

Fig. 7 illustrates the results obtained when twenty-three junctions in one preparation were investigated. The points in this graph show a considerable scatter. Indeed, previous observations on a smaller number of junctions prompted the statement that the quantal content of a response and the miniature discharge frequency were not directly related—although both were increased following indirect stimulation (Liley, 1956*b*). However, as shown by a larger series of observations in Fig. 7, the quantal content of a response and

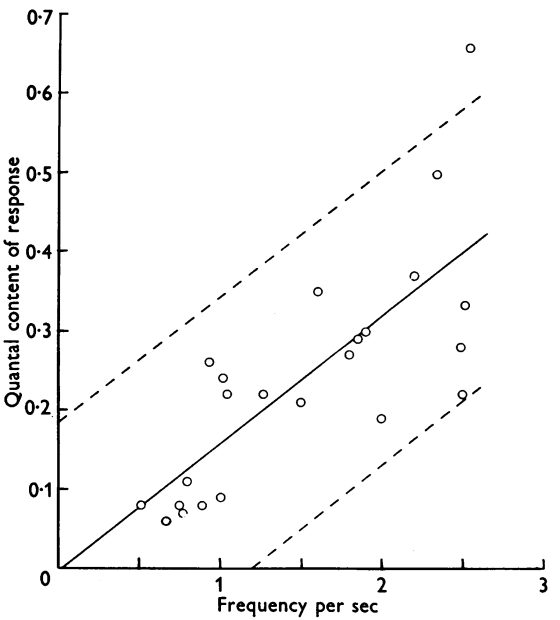

Fig. 7. Relation between quantal content of response and resting frequency of miniature discharge at twenty-three junctions. Full line is regression line for quantal content on frequency with broken lines at ± 2 S.E. of estimate.

the resting discharge frequency show a positive correlation which, statistically, is highly significant ($r=0{\cdot}76$, Student's $t=5{\cdot}36$, $f=21$). Boyd & Martin (1956*b*), on the contrary, observed no consistent relationship between the miniature potential frequency and the e.p.p. quantal content in the cat tenuissimus muscle.

A second prediction concerns the derivation of the absolute quantal content of a response, or, essentially, the calibration of an action potential in terms of the number of quanta which it should liberate under defined conditions. This calibration is made possible by the relationship (deduced from Fig. 6) between discharge frequency and displacement of membrane potential (Fig. 8, inset). Now the action potential of a motor nerve terminal cannot be fully and directly recorded, but as an approximation the action potential of a mammalian

(cat) motor axon may be investigated (Fig. 8, *R*). If such an action potential, corrected for distortion in recording (Fig. 8, *C*), is divided into a series of very brief (0·02 msec) steps, the miniature discharge frequency corresponding to each potential step may be determined, and hence the theoretical quantal content of a response may be estimated. Implicit in this procedure are two assumptions—first, that the discharge frequency follows faithfully and instantaneously any change in membrane potential, and secondly that there is no serious depletion of available quanta during an impulse even though the discharge frequency attains very high values. The former assumption would

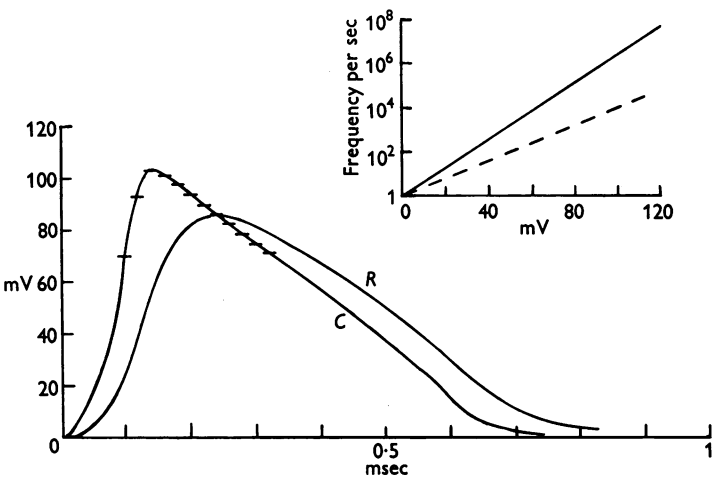

Fig. 8. Derivation of quantal content of an e.p.p. Curve *R*: intracellularly recorded action potential of an intramedullary motor axon of a cat (Eccles, unpublished observations). Curve *C*: same action potential corrected for distortion by recording system. Bars indicate 0·02 msec intervals for which the expected frequency of the miniature discharge was calculated, see text.

Inset: theoretical relationship between discharge frequency and displacement of membrane potential (from Fig. 6) used in calculation. Strictly, the ordinate scale as labelled represents the frequency which would be attained by a terminal with a resting discharge rate of 1/sec when the membrane was depolarized by an amount shown in the abscissal scale. In general, the ordinate scale represents the factor by which the frequency would be altered for a given displacement of membrane potential, depolarization raising the frequency and hyperpolarization reducing it. Full line: relation in normal solution. Broken line: relation in solution containing 12·5 mM magnesium.

appear reasonable, for as shown in Fig. 3, the discharge frequency adapts very rapidly to electrotonic polarization of the terminals. On the other hand, the latter assumption is open to question for immediately after an impulse in normal solutions there appears to be a considerable depletion of the available transmitter (Liley & North, 1953). However, ignoring this possible restriction, it is possible to compute the number and temporal distribution of the quanta

which would be liberated by an action potential, of the amplitude and time-course shown in Fig. 8, from a nerve terminal having a resting discharge frequency in the modal range of 1–2/sec. This liberation would comprise 250 to 500 quanta and would be produced almost entirely by that portion of the action potential exceeding 60 mV. Hence it would be spread over some 0·3 msec.

The quantal content of the e.p.p. in normal solutions cannot be measured precisely. However, on indirect evidence it was concluded that the normal e.p.p. resulted from the summation of at least some 80–100 miniature potentials and possibly more (Liley, 1956*b*). Hence the derived figure of 250–500 appears to be of the correct order. Further it is in very good agreement with figures of 220 and 310 which Boyd & Martin (1956*b*) deduced for the quantal content of two e.p.p.'s in the cat tenuissimus muscle.

A more precise check is possible when the calculation is repeated for the same action potential but using the discharge frequency–membrane potential relationship derived in the presence of 12·5 mM magnesium. In such a solution the quantal content of responses can be determined accurately. Furthermore, depletion of available quanta is no longer a theoretical hazard compromising the calculation. For a solution containing 12·5 mM magnesium the predicted quantal content of a response is 1·2 for a junction with a resting discharge frequency of 1/sec. Measurements on nine junctions gave a mean discharge frequency of 2·7/sec and therefore a theoretical mean quantal content of the response of 3·24. The observed mean value was 3·4. This very close agreement of predicted and observed values is probably fortuitous, for it is not reasonable to assume that the action potentials of rat motor nerve terminals and cat motor axons are identical. Nevertheless, the agreement of order supports the hypothesis that the e.p.p. is merely the random miniature discharge accelerated by the changes in membrane potential which constitute the propagated impulse.

The derivation of the quantal content of a response automatically leads to a prediction of the time-course of release of the quanta or, essentially, the frequency distribution of stimulus-response intervals for the quanta in a large series of responses, provided it be assumed that the quanta all take the same time to diffuse from the liberating terminal membrane to the responsive muscle junctional membrane. Fig. 9A shows this theoretical distribution for the quanta involved in a large series of responses at a junction in 12·5 mM magnesium. Fig. 9B, C illustrates the distributions of stimulus-response intervals observed at two junctions in such a solution. These junctions both had responses of low quantal content, the majority of stimuli being followed by a failure of response or the discharge of a single quantum. However, since a number of responses in each series (about 15% in Fig. 9B and 25% in Fig. 9C) involved two or three quanta and in such responses only the latency

of the earliest quantum could be measured, the distribution of Fig. 9 B and C will be skewed in favour of the briefer latencies. No accurate correction for this distortion can be applied. Nevertheless, although the test lacks precision, the predicted and observed distributions agree reasonably both in form and range.

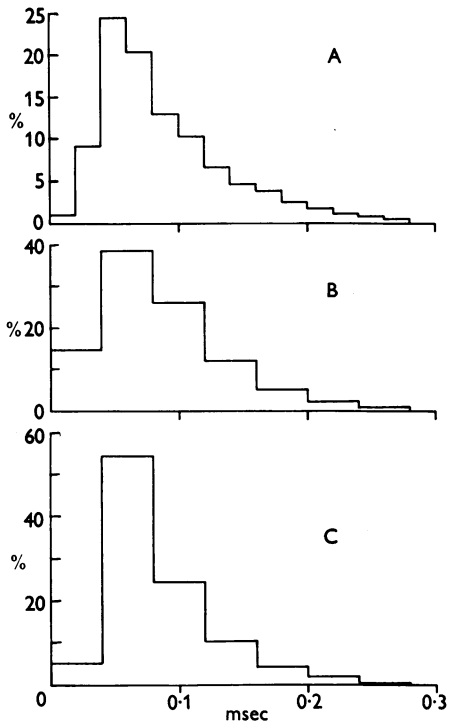

Fig. 9. A: theoretical frequency distribution of stimulus-response intervals for the quanta involved in a large series of responses in 12·5 mM magnesium. B and C: observed distributions of latencies of responses at two junctions in 12·5 mM magnesium. 766 responses in B, 895 in C: see text. Ordinate: percentage of total number of latencies falling in a given class interval. (Note class interval for A is 0·02 msec and for B and C, 0·04 msec.) Common abscissal scale has an arbitrary origin corresponding to briefest latency. Mean stimulus-response interval in B was 1·8 msec and in C 1·2 msec.

Thus the evidence so far adduced suggests that, in so far as it affects the release of transmitter, an impulse does not differ from a depolarization of the terminal membrane produced electrotonically or by excess potassium. It is not necessary to postulate any 'triggering' or threshold effect or to associate chemically the release of acetylcholine with the activity of a special transport mechanism such as the sodium or potassium carriers.

The observation that, in the mammal, the miniature discharge frequency may be reduced by hyperpolarization and increased by depolarization of the

terminal membrane suggests initially that the quanta might be positively charged particles whose passage across the nerve membrane is determined simply by potential gradients. According to this concept the resting discharge frequency is merely the release rate corresponding to one particular membrane potential—the resting membrane potential—in a continuum. However, this simple model cannot readily explain the influence of calcium and magnesium concentrations on the release of quanta. If the presence of calcium ions was essential for the liberation of quanta, variation in calcium and magnesium concentrations should affect the resting discharge frequency. In the cat tenuissimus muscle the resting discharge frequency varied with calcium but was uninfluenced by magnesium concentration (Boyd & Martin, 1956*a*),

TABLE 1. Effect of calcium on resting discharge frequency in one preparation. Mean values ±S.D. and range of frequencies in groups of junctions, the number of junctions in each group being indicated in parentheses. Groups were recorded in the order shown in the table, the preparation being allowed 2 hr to equilibrate with each solution. Magnesium 1 mM (normal) throughout

	2 mM-Ca (normal)	Zero Ca	2 mM-Ca
Mean frequency (sec^{-1})	0·72±0·28 (20)	0·61±0·39 (23)	0·78±0·47 (19)
Range (sec^{-1})	0·3–1·4	0·2–1·7	0·2–1·8

despite the fact that magnesium antagonized the effect of calcium on neuromuscular transmission (Boyd & Martin, 1956*b*). On the contrary in the rat diaphragm, as in the frog (Fatt & Katz, 1952; del Castillo & Katz, 1954*a*), neither absolute reduction in calcium concentration, even to zero (Table 1), nor the addition of magnesium has any marked effect on the resting discharge frequency, but this frequency appears to be stabilized so that it cannot be displaced from its resting level by hyperpolarization or depolarization of the terminal membrane.

No explanation can be offered for this stabilization of the discharge frequency in calcium-deficient and/or magnesium-enriched solutions. Further experiments are also necessary to determine the mechanism by which the membrane potential affects the miniature discharge frequency.

Several workers (Brown & Feldberg, 1936; Feldberg & Guimarais, 1936; Hutter & Kostial, 1955) have demonstrated that potassium may initiate release, or augment the spontaneous release, of acetylcholine from cholinergic terminals in the mammalian autonomic nervous system. Although the spontaneous quantal release of transmitter has not been demonstrated at these cholinergic junctions, the effect of potassium would appear analogous to the action observed at the mammalian motor nerve terminals.

SUMMARY

1. By intracellular recording at the rat neuromuscular junction a study has been made of the effects of presynaptic polarization on the discharge of quanta of transmitter.

2. Electrotonic hyperpolarization of the motor nerve terminals reduced the frequency of the miniature discharge and depolarization augmented the discharge frequency.

3. A linear relation was demonstrated between the logarithm of the discharge frequency and electrotonic displacement of the terminal membrane potential.

4. When the calcium concentration was reduced or the magnesium concentration increased the effect of electrotonic polarization of the terminals on the discharge frequency was suppressed.

5. Reduction of potassium concentration produced a slight reduction and increase of potassium concentration a marked increase in the discharge frequency.

6. The effect of potassium concentration on the discharge frequency was greatly reduced in high concentrations of magnesium.

7. In so far as it affects the release of transmitter an impulse does not appear to differ from a depolarization of the terminal membrane produced electrotonically or by excess potassium.

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