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ON THE FACTORS WHICH DETERMINE THE AMPLITUDE OF THE 'MINIATURE END-PLATE POTENTIAL'

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Recent studies of neuromuscular transmission have made it probable that the release of acetylcholine (ACh) from the nerve endings occurs in discrete 'quanta', each of which gives rise to a 'miniature end-plate potential' (see del Castillo & Katz, 1956*a*). One of the remarkable properties of these ACh-quanta is their constancy during a variety of experimental changes: the size of the 'unit-parcel' of ACh remains the same whether the release takes place spontaneously at infrequent intervals, or in a momentary synchronous burst after arrival of the nerve impulse, or at graded frequencies during electrotonic polarization of the nerve endings (del Castillo & Katz, 1956*a*; Liley, 1956*a*, *b*). Many chemical agents are known which alter the size of the end-plate potential (e.p.p.). These substances act either by increasing or reducing the number of ACh-quanta released per nerve impulse, e.g. calcium, magnesium, botulinum toxin (Brooks, 1956), and glucose (Liley, 1956*b*), or by modifying the post-synaptic efficacy of the transmitter substance, e.g. curare, neostigmine. No agent has yet been found which has a demonstrable effect on the size of the transmitter quantum.

This apparent constancy has given rise to the suggestion (del Castillo & Katz, 1955, 1956*a*; Liley, 1956*b*) that ACh may be contained, within the nerve endings, in structural parcels (possibly the terminal vesicles of de Robertis & Bennett (1955), Palade (1954), Palay (1954) and Robertson (1956)) from each of which it is discharged in all-or-none fashion. The size of the quantum would thus depend on the statistical properties of the intracellular population of ACh-carriers and not necessarily be influenced by agents which exert their effect, principally or solely, on the cell surface.

This view would be strengthened if it could be shown that constancy of the transmitter quantum is observed not only during experimental alterations at any one end-plate, but also if one uses different end-plates and different muscles.

It was noted by Fatt & Katz (1952) that the mean size of miniature e.p.p.'s varied considerably in different fibres, and there appeared to be a systematic discrepancy between the foot muscle (ext. l. dig. IV) and the sartorius of the frog, the former having, on the whole, larger miniature e.p.p.'s than the latter. This discrepancy is relevant, and far from invalidating our present surmise, lends it considerable support. For there is a systematic difference in fibre size between the two muscles (average diameters being about 45μ in the toe muscle and 80μ in the sartorius (Mayeda, 1890; Katz, 1948)), and it can be shown on a simple dimensional argument that a given quantum of transmitter substance will produce a larger depolarization in smaller muscle fibres (see Theoretical section). This argument is quite general and applies not only to chemical but to any other form of stimulation, provided a given quantity of the stimulus is

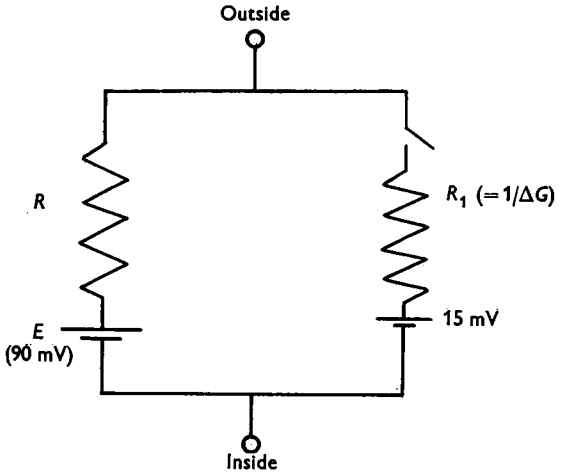

Fig. 1. Schematic diagram of end-plate membrane. Action of a transmitter quantum is equivalent to closure of key. For explanation of symbols see text.

allowed to act on each fibre. For the present case we have assumed, in accordance with the available evidence, that the miniature potential arises from a small, localized increment of conductance, ΔG (Fig. 1), in the post-synaptic membrane. The value of ΔG depends only on the number of reacting transmitter and receptor molecules, that is on the size of the ACh-quantum and the local density of receptor sites. If we are right in supposing that ΔG is the same at all end-plates, then the amplitude of the miniature e.p.p. should be proportional to the 'input resistance' R measured between inside and outside of the muscle fibre, and inversely proportional to the 3/2 power of the fibre size (see below).

The object of the experiments was, therefore, to determine whether the predicted correlation between the amplitude of the miniature e.p.p.'s at different junctions and the diameter or the 'input resistance' of the muscle fibre does, in

fact, exist. We did not attempt to measure fibre sizes directly which would be difficult and inaccurate on an intact muscle, but measured the electric resistance between inside and outside with the help of a second internal electrode. The value of this resistance is of more immediate relevance to our present problem than the fibre diameter as such, of which it provides, however, an approximate electrical correlate.

THEORETICAL

A simplified circuit equivalent to a motor end-plate is shown in Fig. 1, where the resting e.m.f. of the muscle fibre is given as 90 mV and the normal 'input resistance' between inside and outside as R. The equilibrium potential of the 'active' end-plate is taken to be 15 mV, of the same sign as the resting potential (del Castillo & Katz, 1954*b*), $1/\Delta G = R_1$ being the leakage resistance produced by the action of a single 'quantum' of transmitter substance. Reactance and time constant of the fibre have been disregarded.

The amplitude V_1 of the miniature e.p.p. is (90–15) mV $\times R/(R_1+R)$, and provided $R_1 \gg R$, which is usually the case,

$$V_1 = 75 \text{ mV} \times R/R_1 = 75 \text{ mV} \times R\Delta G. \tag{1}$$

If ΔG is constant at all end-plates, then the amplitude of the miniature e.p.p. will be proportional to R.

$$R = \frac{1}{\pi}\sqrt{(R_m R_i/d^3)} \tag{2}$$

(see Fatt & Katz, 1951, 1952), where R_m is the specific membrane resistance of the muscle fibre (ohms $\times$ cm^2), R_i the specific intracellular resistance (ohms $\times$ cm), and d the fibre diameter. Hence, *ceteris paribus*, the amplitude of the miniature e.p.p. is proportional to $d^{-3/2}$, and more directly to R, which was determined experimentally.

METHODS

Most experiments were made, at about 20° C, on sartorius muscles of English *Rana temporaria*, though in several cases the m. ext. l. dig. IV was also used. All preparations were soaked in Ringer's solution (116 mM-NaCl, 2 mM-KCl, 1·8 mM-$CaCl_2$) to which neostigmine methylsulphate, 10^{-6} w/v, had been added. This served to increase the amplitude and duration of the miniature potentials and so to improve the accuracy of measurement. Indirectly, the use of the anti-esterase diminished the spatial attenuation of the miniature e.p.p. (cf. del Castillo & Katz, 1956*b*) and so helped to reduce random errors arising from inaccurate localization of the end-plate foci.

Localization of end-plates. This was done by following fine superficial nerve branches and inserting the recording micro-electrode at a few points on a fibre until a spot had been found at which the miniature potentials displayed the largest amplitude and shortest rising phase. Focally recorded e.p.p.'s (and their unit components) have such a characteristic shape that it is often possible to tell from the time course alone whether the right spot has been hit, without having to impale the fibre more than once or twice. When the localization appeared to be in doubt, the fibre was either discarded or series of miniature potentials were recorded at two different places, some 200–300 μ apart.

Measurement of 'input resistance' R. When a focus had been located with one micro-electrode, another electrode was inserted within less than 100 μ. This electrode was connected through a 100 MΩ resistance to the negative terminal of a rectangular current pulse generator. The entry of the electrode was signalled by the appearance of anelectrotonic potentials in the recording channel, while the current pulse was monitored across a 15 kΩ resistance and registered on the second beam of the oscilloscope. The voltage/current ratio (i.e. the 'input resistance' R of the muscle fibre) was measured with pulses of varying intensities, covering a range of anelectrotonic potentials between about 5 and 20 mV. The average result of two series, made before and after the recording of miniature e.p.p.'s, was used. The pulse duration was approximately 160 msec. In many cases the electrotonic potential did not attain a steady plateau during the current pulse, but showed a prolonged 'creep' (cf. Fig. 2). This made the selection of a time at which the voltage/current ratio was measured somewhat arbitrary. The final deflexion (at 160 msec) as well as the '50 msec deflexion' was measured in all cases; but when plotted, the relations were much the same with either of these values. For the statistical analyses presented below, the final '160 msec deflexion' alone was used.

Limitation of the usable range of fibres. In following a relation between amplitude of miniature potentials and fibre size, there are two limitations: (*a*) when the average miniature e.p.p. falls below 200 μV (presumably in very large fibres), its signal : noise ratio becomes too small for reasonably accurate measurement; (*b*) as the fibre diameter decreases, not only the effect of the transmitter quantum, but also that of the membrane leakage produced by the insertion of the two micro-electrodes becomes more and more serious and will cause the membrane potential and the amplitude of the miniature e.p.p.'s to fall well below their normal values. To some extent, this will be reflected in a simultaneous lowering of the measured 'input resistance', and by using a voltage-correction described below, insertion artifacts can be partly allowed for. But in practice it was difficult to work with fibres whose 'input resistance' exceeded 1 MΩ, and almost impossible to use fibres of more than 2 MΩ resistance. Even with these restrictions, however, a tenfold range of 'input resistances' and miniature e.p.p.'s was covered in the present experiments, and some additional indirect evidence will be described relating to the behaviour of smaller fibres.

Sources of error. The two main sources of error in the present investigation were (*a*) inaccuracies in locating end-plate 'foci', and (*b*) the possibility of a faulty insertion of the current-passing electrode. Errors arising from the first cause were minimized by searching for points with sharply rising potentials, by using neostigmine (cf. del Castillo & Katz, 1956*b*) and rejecting fibres in which several insertions did not lead to a satisfactory localization. Imperfect insertion of the current electrode could allow an appreciable fraction of the registered current to by-pass the fibre and result in an underestimate of its 'input resistance'. To counter this, the amplitude of the electrotonic potential was watched while fine adjustments were made on the micromanipulator carrying the intracellular current electrode, and only stable electrotonic potentials were accepted. An alternative procedure was tried, viz. to connect the current electrode temporarily to the voltage amplifier and check the value of the membrane potential recorded with this electrode. However, the switching procedure introduced a number of other difficulties and was discarded. In spite of the precautions taken, it is probable that these two sources of inaccuracy were responsible for a large part of the observed scatter and for the occurrence of occasional 'odd points' which deviated markedly from the behaviour of the rest.

Other inaccuracies of relatively minor importance were inherent in the measurement of the resting potential (see del Castillo & Katz, 1955; Adrian, 1956) and of the mean amplitude of the miniature e.p.p.'s. The usual procedure was to record a series of 50–100 spontaneous potentials, preceded and followed by a series of anelectrotonic potentials at different current strengths from which the 'input resistance' was determined. Finally the resting potential was checked on withdrawing the recording electrode. In measuring miniature e.p.p.'s, account had to be taken of coincident 'multiple' discharges (Fatt & Katz, 1952), i.e. of occasional large potentials well outside the normal range of amplitude dispersion. These were very infrequent, and it made little difference to the result whether such potentials were included in, or rejected from, the determination of mean

amplitudes. It was more important to eliminate small, slow deflexions arising from a distant end-plate which were seen in some fibres. Some of these 'bi-focal' fibres had to be discarded; others could still be used because the two sets of spontaneous potentials were sufficiently discrete in size and shape to be readily distinguished in the records.

Comparison of fibres with different resting potentials. In comparing miniature e.p.p.'s from different fibres an allowance must be made for differences in the level of the resting potential E, for there is reason to believe that the driving force of the miniature e.p.p. is $(E - 15)$ mV (see Fig. 1, Theoretical section; also Fatt & Katz, 1951; del Castillo & Katz, 1954*b*). We have adopted 90 mV as a standard value of E and multiplied all miniature e.p.p.'s by the factor $75/(E - 15)$. The majority of fibres had resting potentials between 80 and 100 mV (mean of 121 fibres was 87·5 mV, S.E. $\pm$ 1 mV), so that usually the correction was not large. The allowance enables one, however, to include, at least tentatively, some of the fibres whose membrane potential had fallen considerably, after the insertion of both electrodes.

RESULTS

The first few trials confirmed that a general correlation of the predicted kind does exist. Examples from four fibres are illustrated in Fig. 2. The left part of the figure shows records from two fibres of high 'input resistance', each set containing both miniature e.p.p.'s and voltage and current pulses across the fibre membrane. On the right, similar records from fibres of about five times lower 'input resistance' are shown. It is clear that the miniature potentials at these end-plates are several times smaller than in the high-resistance fibres, and this is further shown in the histograms of Fig. 3.

It may be asked whether the differences in resistance are due mainly to variations in fibre size, or in 'leakiness' of the fibre membrane. It is relevant in this respect that the time course of the electrotonic potential was approximately the same in the four fibres (slightly faster in the high-resistance fibres). This is consistent with the view that the differences in resistance are determined by the fibre diameter, and not by the membrane conductance. If the latter were the controlling factor, the time course of the electrotonic potentials in C and D should have been some 25 times faster than in A and B. (The membrane time constant is proportional to R_m, the input resistance to $R_m^{\frac{1}{2}}$.)

It may be assumed, therefore, that the records in A and B represent the behaviour of small, and those in C and D of large, muscle fibres. From a more detailed analysis of the electrotonic potential, Fatt & Katz (1951) concluded that an input resistance of 200,000 Ω corresponds to a fibre diameter of about 140μ (near the upper end of the range in the frog's sartorius muscle, cf. Mayeda, 1890). Using this information and equation (2) one can estimate the fibre diameters in the present experiments, and arrives at values of 100–130μ for C and D, and 36–40μ for A and B. Both are within the range of sizes seen in transverse sections on the inner surface of the sartorius muscle.

Experiments of the kind illustrated in Fig. 2 were made on 121 end-plates from twelve different muscles. The results have been plotted in Figs. 4–6, which reveal a strong correlation between the two variables examined in these experiments. There is a good deal of scatter which may be attributed partly to

experimental error (see Methods), and partly to variability of other factors besides the resistance and diameter of the muscle fibres (see Discussion).

Different statistical treatments were applied, two of which are shown in Figs. 5 and 6. The logarithmic plot (Fig. 6) gives probably a fairer presentation of the results than the linear scatter diagram (Fig. 5), though the correlation coefficient was approximately the same (between 0·9 and 0·95 in all cases).

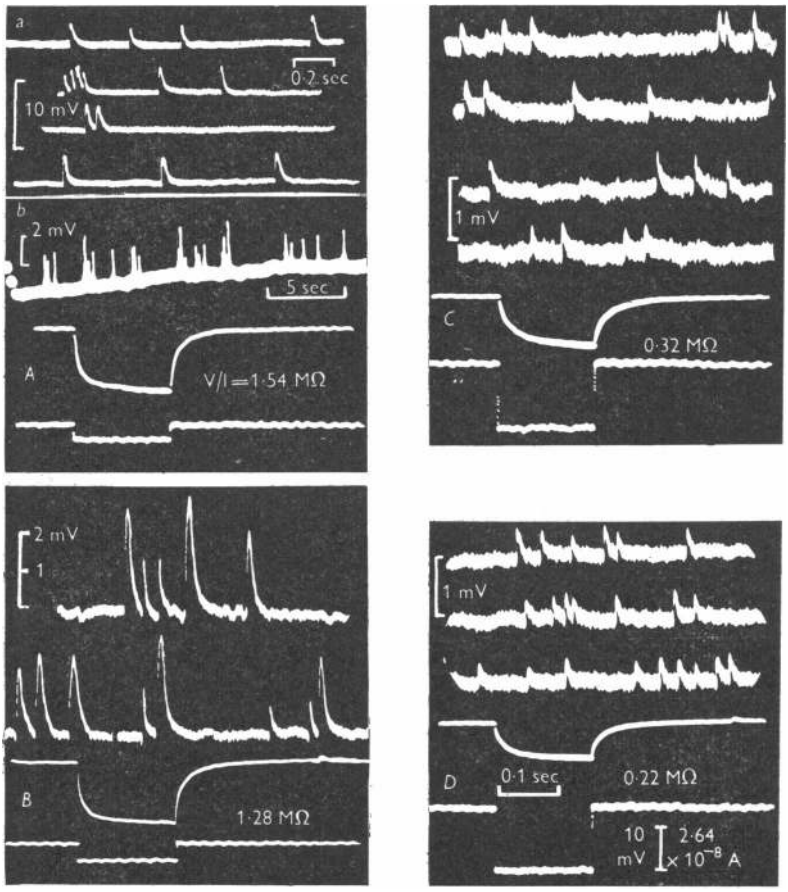

Fig. 2. Sample records of miniature e.p.p.'s and electrotonic potentials from four different muscle fibres (*A* to *D*). In each case upper part shows spontaneous miniature potentials (separate voltage calibrations; the 0·2 sec time calibration in *A a* applies also to the min. potentials in *B–D*). The lower portions show 'anelectrotonic' potentials and the applied rectangular current pulses. Time, voltage and current calibrations for the lower parts are given in *D*. The 'input resistance' (the voltage/current ratio) is shown for each fibre. In *A*, two series of miniature potentials are illustrated; *a* was recorded before, *b* after insertion of the second micro-electrode which, in this fibre, caused an appreciable lowering of the membrane potential. In the other fibres, miniature potentials were recorded after the second insertion. Final levels of membrane potential were: *A*, 67 mV; *B*, 80 mV; *C*, 98 mV; *D*, 95 mV.

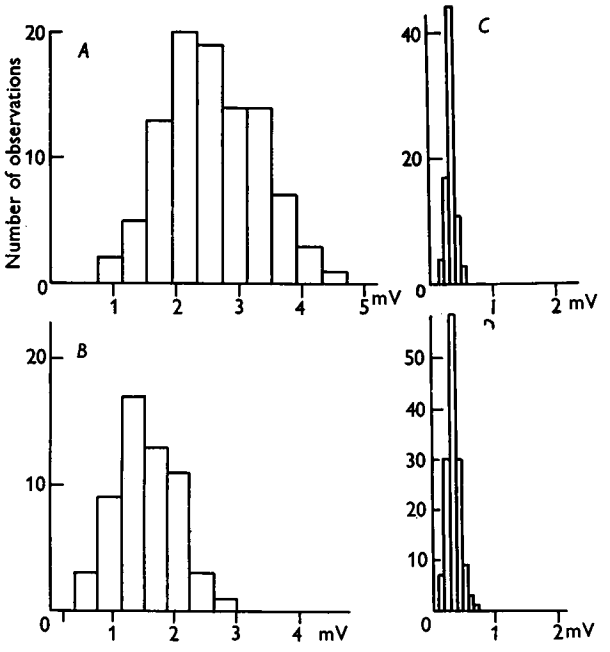

Fig. 3. Histograms from the experiments illustrated in Fig. 2, showing distribution of amplitudes of miniature e.p.p.'s in the four fibres, *A* and *B* being of high, *C* and *D* of low resistance. (The histogram in *A* was obtained from records taken before the insertion of the second electrode.)

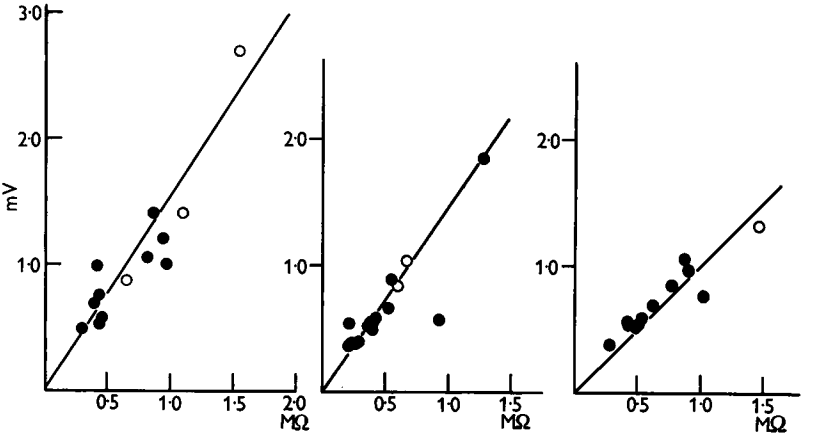

Fig. 4. Results obtained from three sartorius muscles, each circle representing a different fibre. Abscissae, 'input resistance'; ordinates, mean amplitude of miniature e.p.p.'s, corrected for differences in resting potentials (see p. 271). ●, muscle fibres whose final resting potential was between 80 and 100 mV; ○, other fibres.

There is a significant departure from the predicted direct proportionality between fibre resistance and miniature potential. This is shown by the facts that the regression line in the linear plot does not pass through the origin, and the slope of the regression line in the logarithmic plot is less than unity. The discrepancy appeared to be larger for the foot muscle than for the sartorius; e.g. it was quite small in Fig. 5, where the sartorius experiments have been treated separately. A departure from direct proportionality would be expected for

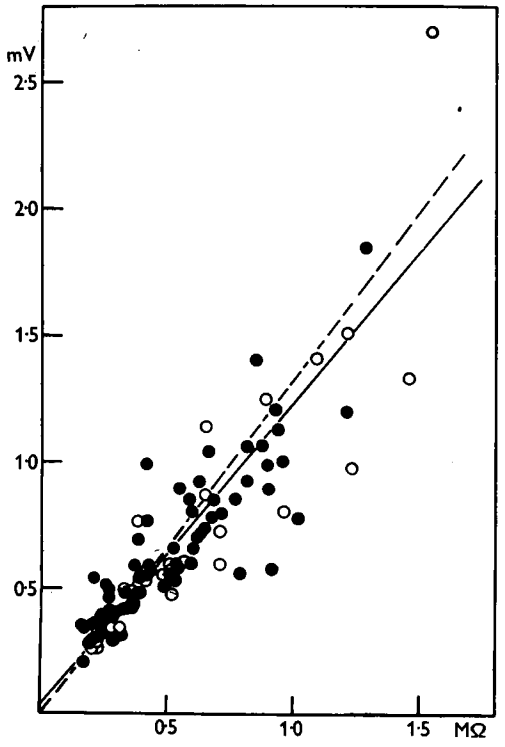

Fig. 5. Results from ten sartorius muscles (103 fibres) plotted together. Symbols as in Fig. 4. The correlation coefficient is 0·954. A regression line is shown (full line) following the equation $y=a+bx$, where $a=0{\cdot}032$ mV and $b=1{\cdot}2$ mV/MΩ. The average slope through the origin (broken line) is 1·33 mV/MΩ (S.E. $\pm 0{\cdot}034$).

very small fibres, when the amplitude of the miniature potential exceeds a few millivolts (and the ratio R/R_1 becomes an appreciable fraction; see p. 269), but this explanation cannot be applied to the range of the present results.

This discrepancy, however, seems of minor interest compared with the main finding, namely that, among different muscle fibres, a tenfold change in the amplitude of the miniature e.p.p.'s is associated with an even larger change of the 'input resistance' of the fibres, and can be explained by the known

variation in fibre size without any change in the size of the transmitter quantum.

Because of the difficulties mentioned on p. 270, it was not possible to extend the range of observations to the smallest muscle fibres (to diameters of the order of 10μ). Nevertheless, there are indications that the amplitude of the miniature e.p.p.'s continues to increase as the fibre size is reduced. Bundles of thin fibres can be recognized in the m. ext. l. dig. IV, which sometimes allow

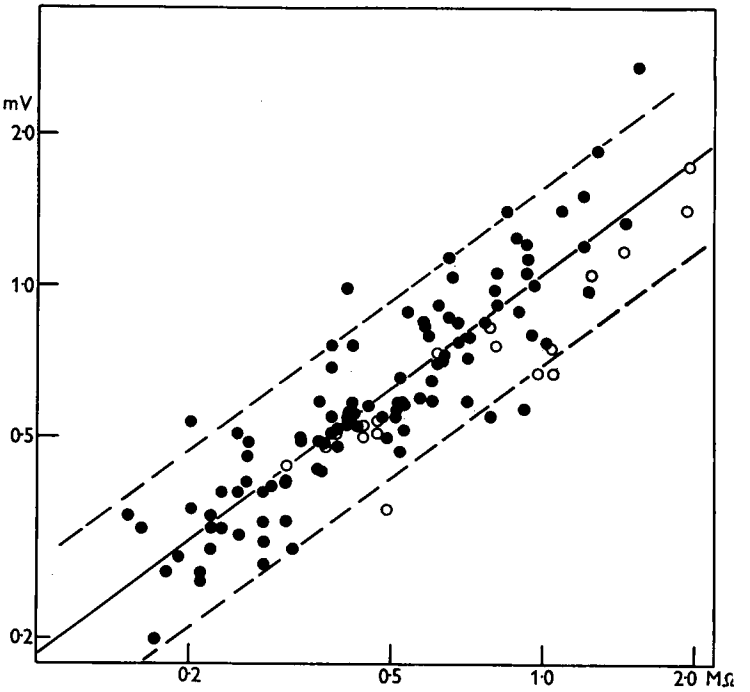

Fig. 6. Results from 121 fibres (twelve muscles) plotted on logarithmic scales. Full circles represent sartorius fibres, hollow circles fibres from m. ext. l. dig. IV. The regression line (full line) has a slope of 0·735. Parallel interrupted lines have been drawn at a distance of $\pm 2S_y$.

the insertion of a single microelectrode without excessive damage. Mean amplitudes of miniature e.p.p.'s of over 3 mV were found in some of these fibres, and a correction for their low membrane potentials (see p. 271) would bring these values up to 4–5 mV.

Another interesting observation was made incidentally several years ago during experiments (unpublished) on isolated muscle spindles. It was found that miniature e.p.p.'s can be recorded from intrafusal muscle bundles, with external electrodes, and that even in the absence of prostigmine, spikes arise occasionally from the peak of summed spontaneous potentials. No accurate information is available on the size of the unit-potentials or the

threshold of the fibres, but the observation indicates that the spontaneous potentials are much larger in the small intrafusal, than in the ordinary, muscle fibres.

DISCUSSION

It appears from these results that despite the wide range of miniature e.p.p.'s which have been recorded from different end-plates, the amount of transmitter substance which produces them is relatively constant. At any rate, a more than tenfold variation in the size of the potentials can be attributed to a corresponding variation in the post-synaptic 'input resistances' arising from the wide dispersion of fibre diameters. In the frog's sartorius fibre sizes range from about 150 to 10μ; the corresponding 'input resistances' would range from about 180,000 Ω to 10 MΩ (using equation (2) and data from Fatt & Katz, 1951). It is clear that only the lower part of this range has been investigated with the present technique.

TABLE 1

Fibre diameter (μ)	140	76	48	16	10
Input resistance (MΩ)	0·2	0·5	1·0	5·0	10·0
Mean size of min. e.p.p. (neostigmine present) (mV)	0·26	0·62	1·05	3·4	5·6

If one tries to extrapolate, one may assume direct proportionality between R and miniature e.p.p., with an average slope, in neostigmine-treated muscle, of about 1 mV/MΩ. On this calculation, the mean amplitude in a 10μ fibre would be 10 mV, so that unit-discharges could occasionally, and summed discharges would frequently, lead to twitching. The assumption of direct proportionality, however, is not justified on theoretical or empirical grounds (see del Castillo & Katz, 1954*a*; Martin, 1956), and it seems more reasonable to use the slope of the regression line in Fig. 6 for such extrapolations. This gives the values shown in Table 1. Even this calculation leads probably to a slight overestimate for the largest values to which a correction of the kind used by Martin (1956) could be applied. It appears, in any case, that in a frog muscle fibre of 90 mV resting potential, requiring some 40 mV depolarization for the initiation of a spike, fibrillation is not likely to arise from individual miniature discharges, even in the presence of neostigmine, though a high-frequency burst or a synchronized discharge of multiple units (cf. Liley, 1956*b*) could elicit a twitch. In the absence of an anti-esterase, the amplitudes are all reduced by a factor of two to three (cf. Fatt & Katz, 1952), and unless the membrane potential has been lowered to near-threshold by other means, and the rate of the spontaneous discharge is abnormally high, there is clearly a wide margin of safety, even in the smallest muscle fibres, between the miniature potentials and the firing level. The occasional twitching which was seen in isolated intrafusal bundles was probably an abnormal event.

The residual scatter shown in Figs. 5 and 6 can be attributed to several causes. A large part may simply be due to the various causes of experimental

error which have been mentioned above. But there are also physiological factors which may vary in different fibres and different muscles, and which have been deliberately ignored in the present study. Among such possible sources are variations in the local density of receptor molecules and, of course, any real differences in the mean size of the transmitter quantum. All these factors together, however, did not cause a dispersion of more than about 2:1 (Fig. 6, $2S_y = 0{\cdot}177$ log. units), a small range compared with the total observed variation.

SUMMARY

1. When miniature end-plate potentials are recorded from different frog muscle fibres, the mean amplitudes are found to vary over a range of more than 10:1.

2. By determining also the 'input conductance' of the muscle fibres (which is related to their diameter), a highly significant correlation was found (miniature e.p.p. and resistance across fibre surface varying in the same direction). It appears that the large differences in the mean amplitudes of miniature potentials can be attributed to the electrical changes associated with differences in fibre size.

3. Previous evidence indicated that the mean 'quantum' of acetylcholine which is released from motor nerve endings remains constant under changing experimental conditions. The present results indicate that this constancy is obtained even if one compares myoneural junctions from different fibres of widely varying diameters.

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