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AN ANALYSIS OF ACETYLCHOLINE RESPONSES OF JUNCTIONAL AND EXTRAJUNCTIONAL RECEPTORS OF FROG MUSCLE FIBRES

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SUMMARY

1. An analysis has been made of the distribution and rise time of the responses induced by electrophoretic application of ACh on junctional and extrajunctional receptors of the neuromuscular junction of the frog.

2. The junctional and the extrajunctional responses appear to constitute two independent groups with no overlapping characteristics: (*a*) the responses evoked from junctional receptors show always a high 'sensitivity' (30–100 mV/nC) and a rise time usually shorter than 10 msec that does not lengthen as the ACh dose is increased; (*b*) the responses evoked from extrajunctional area show a lower sensitivity (0·5–10 mV/nC) and a slow rise time that is a linear function of the dose $Q^{\frac{2}{3}}$ of ACh.

3. The lengthening of the rise time of extrajunctional response with increasing doses of ACh is interpreted by supposing that larger doses saturate larger areas of a membrane of low sensitivity uniformly covered with receptors. On the contrary, at junctional spots the dose of ACh would be insufficient to saturate the receptors and rise time will be independent of dose.

4. Relatively unresponsive zones may exist between junctional spots and extrajunctional areas and between neighbouring extrajunctional areas.

5. Extrajunctional areas are subjected to significant seasonal variations; in summer they are practically absent, in autumn they appear as elongated areas of about 20 μ wide and 50 μ long, and late in winter they fuse together and all the 'end-plate' zone becomes sensitive to ACh.

INTRODUCTION

It has been observed by Miledi (1960*a*, *b*, 1962) that the acetylcholine (ACh) sensitive area of muscle fibres extends beyond the synaptic contacts. In the rat, because of the lower sensitivity at increasing distances from the centre of the reactive area, ACh receptors were supposed to be distributed along a density gradient with a maximum at the end-plate (Miledi, 1960*b*). In the frog, although the over-all sensitivity decreases with distance, it has been shown by Miledi (1960*a*) that at a given distance from the centre of the reactive area some spots are more sensitive than others, as can be expected from the morphology of the Kühne arborization (Cajal, 1881).

The time course of ACh potentials has been shown to depend on the regional sensitivity (Miledi, 1960*a*). The slow rise time of extrajunctional responses has been explained by Katz & Miledi (1964) by assuming that at relatively insensitive areas the nearest receptor sites become quickly saturated and the response is built up by the recruitment of more distant receptors.

The present study had the twofold purpose of analysing the variations in time course of responses mediated by junctional and extrajunctional receptors as a function of the dose of ACh, and of obtaining information on the spatial distribution of both types of receptor on the neuromuscular junction of the frog.

In order to delimit with accuracy the boundaries between the areas covered by junctional or extrajunctional receptors of frog muscle fibres, the 'resolving power' of the electrophoretic technique needs to be increased; this was achieved by using short ACh pulses.

The results reported here will show that the extrajunctional receptive area of the frog is made of sensitive patches or strips whose size is subjected to important seasonal variations and that the time course of their responses can be described assuming a diffusion of ACh from an instantaneous point source over a relatively large area of low sensitivity uniformly covered with receptors.

A short report of some of the findings has already been published (Mallart & Feltz, 1969).

METHODS

Experiments were made on frogs (*Rana esculenta*) kept in a vivarium at 12–20° C. The sartorius muscle was dissected and immersed in a Ringer solution of the following ionic composition: (in mM) Na, 117·6; K, 2·5; Cl, 121·1; Ca, 1·8; H_2PO_4, 0·4; HPO_4, 1·12. To prevent contractions with depolarizations above spike threshold, tetrodotoxin at a concentration of 5×10^{-8} g/ml. was added to the bath; this drug has been shown not to act on synaptic channels (Katz & Miledi, 1967). The experiments were conducted at a temperature of 15–18° C. An intracellular electrode was

inserted at a junctional area where miniature end-plate potentials with a fast rise time could be recorded.

Iontophoretic application of drugs. A micropipette filled with 0·9 M-ACh and attached to a Leitz micromanipulator was used for electrophoretic application of the drug. Current pulses of 2–10 msec duration and of 5×10^{-11} to 1×10^{-9} C were applied to the pipette. Receptor sensitivity was expressed in mV/nC (see Katz & Miledi, 1964). The 'braking' current was adjusted at the minimum level required to prevent the depolarization of the 'end-plate' by steady diffusion of the drug.

ACh receptive areas were searched by trial and error using as a guide the changes in latency of the ACh potential and displacing the pipette until responses with no appreciable latency were found. This search was made easy by displaying successive responses on the screen of a Tektronix type 564 B storage oscilloscope.

Several assumptions have been made in order to describe the effects of electrophoretic application of ACh in terms of the diffusion laws.

The ACh released during a brief current pulse will rapidly distribute under the action of the electric field into a restricted volume where its concentration is nearly uniform. This volume will define the instantaneous source from which the drug diffuses away through the surrounding medium. The receptors are regarded as situated on a plane surface at a variable distance from the source. In practice, the instantaneous source is likely to be a sphere or a hemisphere (depending on the distance from the pipette to membrane) whose radius is likely to be a function of the strength of the electrophoretic pulse (see Crank, 1956).

When receptors are situated within the source radius, they will be activated before the end of the current pulse and the ACh potentials will have no detectable latency. On the contrary, if the source is far away from the receptive area, both the onset and the peak time of responses will be delayed by an amount that can be predicted from the diffusion laws (Castillo & Katz, 1955). In practice, the effective area of diffusion is limited by the fact that the concentration of a substance that diffuses from an instantaneous source falls to about 1 % at a distance equal to three times the source radius (Crank, 1956).

These considerations are of practical importance when mapping the reactive areas of the 'end-plate' region. The smaller the source radius, the better will be the discrimination between two closely located sensitive spots and the better the accuracy in delimiting the boundaries of the extrajunctional areas. An estimation of the source radius in our experimental conditions has been made by delivering ACh pulses from one barrel of a 'twin pipette' at a synaptic spot, identified by extracellular recording of spontaneous miniature potentials by the second barrel, and then displacing the pipette away from the spot. The minimum displacement required for recording responses with a detectable latency indicates approximately the value of the source radius. The measure of the displacement can be obtained from the increase in peak time T according to eqn. (1) (Castillo & Katz, 1955):

$$6DT = r^2, \tag{1}$$

where D is the constant of diffusion of ACh (approximately 10^{-5} cm^2 sec^{-1}, according to Krnjević & Mitchell, 1960) and r the distance between source and receptor. Source radius was estimated to be about 5–10 μ for pulses of 5×10^{-10} C.

RESULTS

Bimodal distribution of the rise time of responses

While exploring the surface of the neuromuscular junction with electrophoretic pulses of ACh, it soon became apparent that the sensitive membrane is made of spots or patches of variable size and sensitivity whose responses showed big differences in time course (Fig. 1). The histogram of

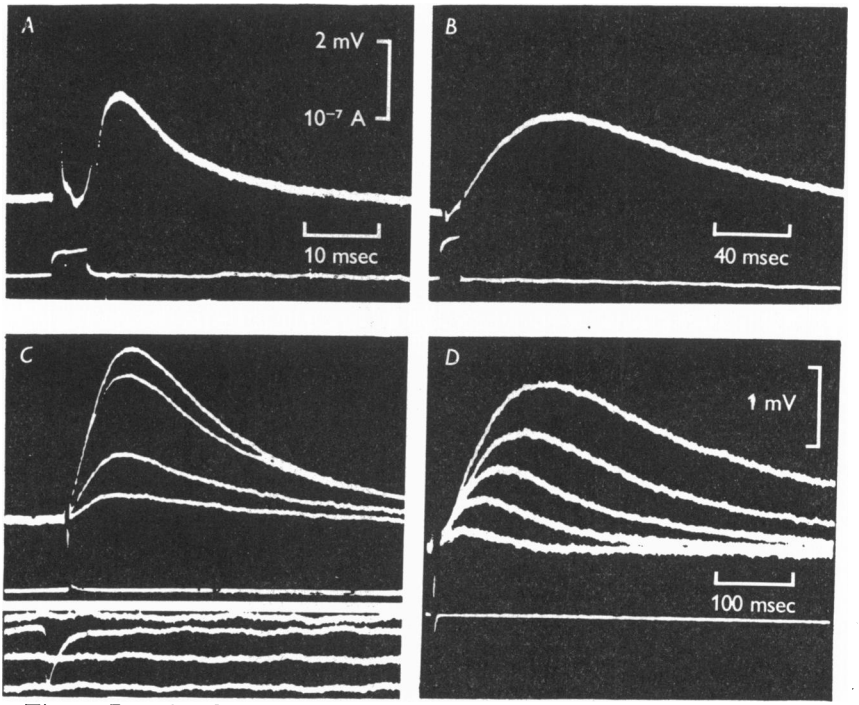

Fig. 1. Junctional (*A*, *C*) and extrajunctional (*B*, *D*) responses to ACh. *A* and *B* are from the same muscle fibre. In *C* ACh was delivered by one barrel of a double micropipette while the other barrel was used for extracellular recording of the spontaneous activity (lower traces). The variations in rise time with increasing doses of ACh observed at junctional and extrajunctional responses are shown in *C* and *D*. Same voltage calibration for *A*, *B*, and *C*. Same time calibration for *A* and *C*.

the rise time of responses of 3–6 mV of peak amplitude shows in fact a bimodal distribution (Fig. 2). The most sensitive spots respond with a mean rise time of 13 ± 6 msec (S.D.) but responses with a rise time as short as 4 msec have been observed (Fig. 1*A*). These fast responses have been regarded as arising from junctional spots, since an application of ACh at spots identified by extracellular recording of spontaneous activity induced depolarizations of similar rise time (Fig. 1*C*). Elsewhere the sensitivity

to electrophoretic pulses of ACh was much lower: the strength of ACh pulses necessary to get responses of comparable amplitude was 10–100 times higher. Using drug applications of 10 msec duration the rise time had a mean value of 120 msec, but individual responses showed a considerable degree of scatter. The depolarizations cannot be attributed to the activation of distant junctional receptors since the responses did not display a detectable latency. Receptors inducing these responses were regarded as extrajunctional.

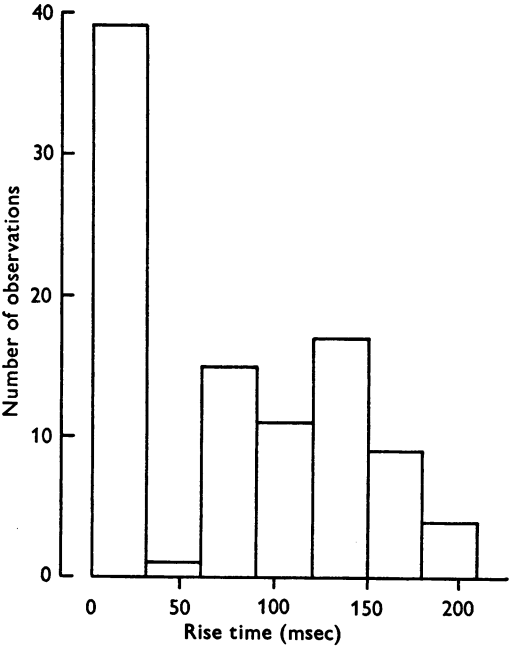

Fig. 2. Histogram of the distribution of the rise time of ACh potentials of less than 5 msec latency and amplitudes comprised between 3 and 6 mV, recorded from eight neuromuscular junctions.

Fig. 3 shows the relation between amplitude and rise time of responses observed at a junctional and an extrajunctional area from the same neuromuscular junction and illustrates that the bimodal distribution of the rise time of responses is more apparent for responses of large amplitude. This is due to the fact that, as will be shown below, the rise time of responses elicited at extrajunctional areas, unlike that of junctional responses, increases with the amplitude of the depolarization.

Variations in the rise time of responses

Junctional responses. Responses evoked at junctional spots show always a fast rise time that does not lengthen when the strength of the ACh pulse

is increased (Fig. 1*C*). The positioning of the micropipette is very critical: a slight lateral displacement results in a drop in amplitude, a lengthening of the rise time and in an appreciable latency of the responses (Fig. 4) (see Castillo & Katz, 1955). These facts suggest that the area covered by a junctional spot is very small. Assuming that the amplitude V of the depolarization is a function of the instantaneous concentration of ACh, $V = \phi(C)$, and ignoring cholinesterase action, peak time of junctional

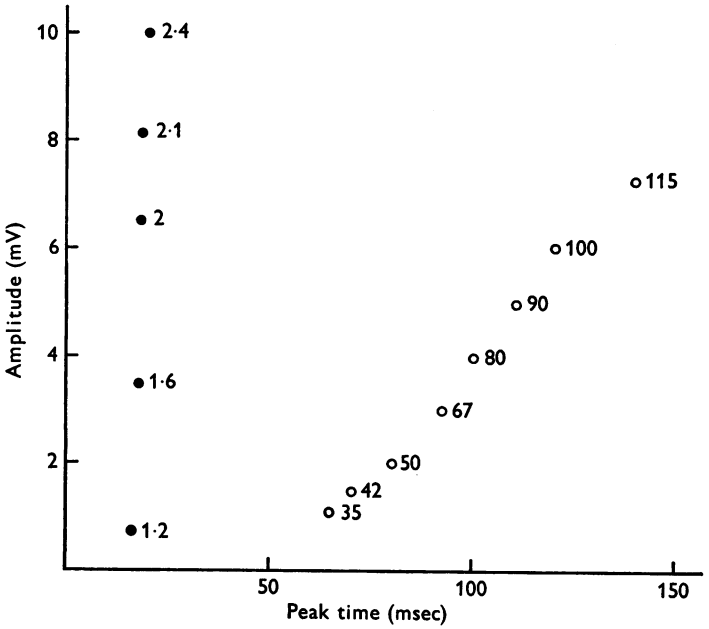

Fig. 3. Variation of the peak time as a function of the amplitude of junctional (filled circles) and extrajunctional (open circles) responses. The coulombic strength ($\times 10^{-11}$ C) of ACh pulses is indicated for each experimental point. Data from one experiment.

responses can be accurately described by the diffusion eqn. (1) (Castillo & Katz, 1955). Peak time T of responses appears to be dependent only on the distance between the source and the receptors but not on the amount of ACh released (Figs. 3 and 4).

Extrajunctional responses. At extrajunctional sensitive areas, a lateral displacement of the ACh pipette by several micra did not produce a visible change either in latency or in peak time of responses. On the other hand, when the strength of the ACh pulse was increased, the responses displayed both an increase in amplitude and in peak time.

The changes in peak time of responses when the dose of ACh was varied have been analysed in conditions where the latency was zero. It was

observed that the initial slope of a family of responses is fairly constant but the rise time depends on the amount of ACh released (Figs. 1*D*, 3 and 5). In Fig. 6 the rise time T of responses recorded from two 'end-plates' is plotted as a function of the coulombic strength Q of ACh pulses in double logarithmic co-ordinates. As can be seen, a linear relation exists between

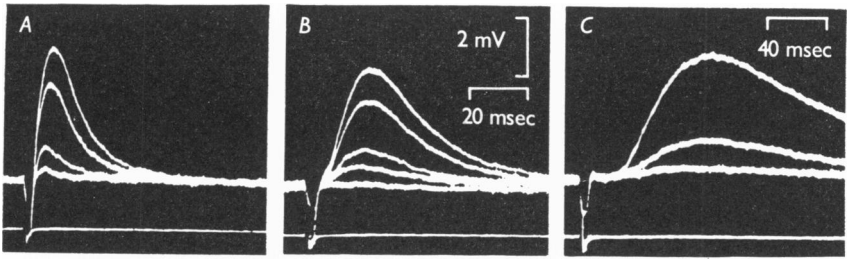

Fig. 4. Responses of a junctional sensitive spot to ACh pulses of variable strength delivered at different positions of the pipette. In *A* the pulse duration varied from 1 to 2 msec; in *B*, from 2 to 3 msec and in *C*, from 3 to 5 msec. The distance from source to receptors was increased from *A* to *C*: in *A* the receptors were located within the source radius, in *B* and *C* the calculated distances were 14 μ and 21 μ respectively. Notice that the time to peak depends on the position of the pipette but not on the pulse strength. Same time calibration for *A* and *B*.

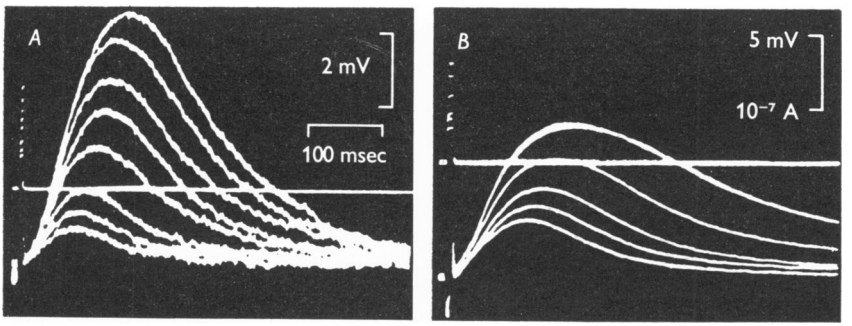

Fig. 5. Response of an extrajunctional area to increasing doses of ACh. This type of recording was used for the calculation of the relationship between peak time of responses and amount of ACh delivered. The pulse duration was 5 msec in *A* and 10 msec in *B*; same time and intensity calibration for *A* and *B*.

log T and log Q. The slope of the function was evaluated as $0{\cdot}67 \pm 0{\cdot}12$ (S.D.) using data obtained in six experiments, where T varied in the range of 70–500 msec. Thus T is a linear function of $Q^{2/3}$. As will be shown in the Appendix, this relation can be predicted from theoretical considerations.

The peak amplitude of extrajunctional responses is also closely related to the coulombic strength of ACh pulses. If a plot is made of the logarithm of the maximum amplitude V against the logarithm of the pulse strength

Q, it appears that V is a function of $Q^{2\cdot2}$. An obvious discrepancy exists between this finding and the expected value, since the calculations developed in the Appendix predict that both the peak amplitude and the rise time must be a function of $Q^{2/3}$.

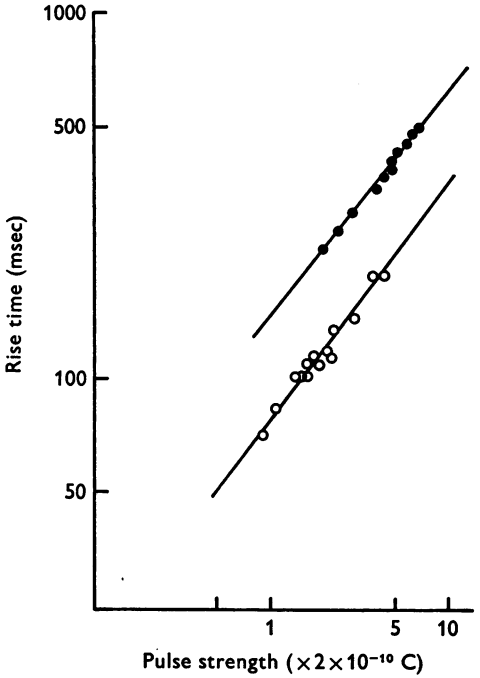

Fig. 6. The peak time T of responses, evoked at two extrajunctional areas from different fibres, is plotted versus the coulombic strength Q of ACh pulses on double logarithmic co-ordinates. Peak time appears to be a function of $Q^{0\cdot66}$.

Spatial distribution of ACh receptors on the neuromuscular junction

The topography of junctional and extrajunctional receptors has been studied by systematic displacement of the ACh micropipette on the 'end-plate' region. The muscle fibre was examined with a binocular dissecting microscope of 80×. One eyepiece was fitted with a grid giving a calibration of 25 μ. The position of the preterminal branches of the motor nerve and the outlines of the muscle fibre were used as landmarks for drawing a map of the region. The position of the points responding without latency to the electrophoretic application of ACh is indicated in the maps shown in Fig. 7.

The time course of responses was used as a criterion for identifying junctional or extrajunctional receptors. The former were found in sharply localized spots that could be traced along irregular lines. At the end of

several experiments a methylene blue staining was made and it was observed that the points where junctional responses could be induced coincided fairly well with the fine terminal branches of the motor nerve (Fig. 7*B*).

The extrajunctional areas of a given fibre may vary in number, size, shape and sensitivity. Their limits were determined by applying ACh pulses of 5×10^{-10} to 1×10^{-9} C delivered in 10 msec and by displacing

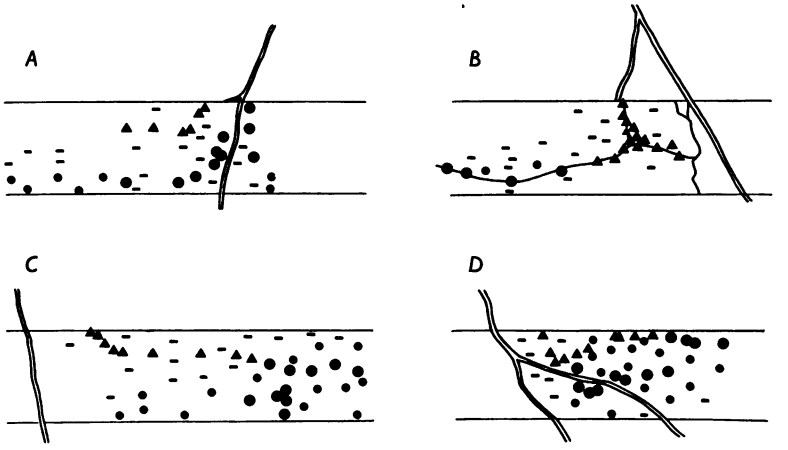

Fig. 7. Schematic diagram of the responsive area of four neuromuscular junctions. The motor nerve is represented by an irregular double line. Positions of the pipette giving a junctional response are indicated by triangles; those giving extrajunctional responses, by small dots. Larger dots indicate a sensitivity 5 times higher than smaller ones. Places not responding to ACh pulses of 1×10^{-9} C are marked by dashes. In *B* a drawing of the fine nerve branches stained with methylene blue is shown. *A* and *B* were mapped in October, *C* in January and *D* in February.

the micropipette on the surface of the muscle fibre. When responses displayed a detectable latency, it indicated that the point source of ACh was off the limits of the sensitive area. Sensitivities of 4–8 mV/nC were usually found at the centre of most of the extrajunctional areas. The sensitivity appeared to be lower at the edges of each area, and areas of lower sensitivity (about 0·5 mV/nC) were always found towards the limits of the sensitive region. Unresponsive spaces were found between junctional spots and extrajunctional areas and between neighbouring extrajunctional areas. Probably some degree of sensitivity could be detected at these 'empty' spaces if larger ACh doses were delivered, but this could not be done without increasing the diameter of the source and losing the possibility of two point discrimination (see Methods). Sometimes responses of extrajunctional type could be recorded as strips of 20–30 μ wide that seemed to prolong

rows of junctional spots. This finding was confirmed by a methylene blue staining of the motor nerve terminals (Fig. 7*B*).

An interesting finding was the observation of important seasonal changes in the extension of the reactive area. No responses of extrajunctional type were found in summer. They became apparent and could be elicited in strips of about 20 μ wide and 50 μ long in autumn; they increased in size during the winter and in February there was almost no discontinuity between junctional and extrajunctional areas. At this time the 'end-plate' area showed a widespread sensitivity with the junctional type of responses restricted to its central part. Synaptic spots were harder to find in winter than in summer.

Spontaneous activity

In a few experiments the spontaneous activity of the neuromuscular junction was recorded late in winter, when the extrajunctional areas constitute the largest part of the sensitive region.

The general pattern of discharge was in agreement with the classical description and most of the miniature end-plate potentials showed the typical time course with a rise time of 1–2 msec. Occasionally a different type of activity was observed: very slow depolarizations reaching 1 mV amplitude in 20 msec were recorded from several junctions where the spontaneous activity looked otherwise normal (Fig. 8*B*, *C*, *D*). Obviously spatial attenuation cannot account for such a slow rise time since this would imply that giant potentials of about 20 mV were occurring at long distance (see Fatt & Katz, 1951). Sometimes these slow depolarizations looked like the summation of a burst of spontaneous miniature potentials released far from the recording electrode (Fig. 8*D*), but this does not seem to be always the rule. A likely explanation may be that ACh is released and diffuses away from the nerve endings over a large sensitive area with low cholinesterase activity, as has been proposed by Eccles, Katz & Kuffler (1942) to account for the slow potential that develops at the end-plates of eserinized muscle (see Miledi, 1960*b*).

DISCUSSION

Some of the results reported in this paper deserve special comment.

Rise time of ACh potentials. An interesting result obtained in this series of experiments was the observation of a difference in rise time between junctional and extrajunctional responses, and a variation of the rise time of the latter with the amount of ACh released.

Both types of response were analysed in conditions where they did not present a detectable latency, i.e. when at least some receptors were located within the source radius. This situation cannot be treated by the ordinary

diffusion equations because here we are not dealing with the diffusion of a substance from an instantaneous point source to an idealized point receptor but, rather, with a diffusion over a reactive area.

In the Appendix, calculations have been developed according to one of the many possible models that can be considered. The basic assumptions were

(1) the receptors of junctional spots as well as those of extrajunctional areas are uniformly distributed with a density σ on a plane surface A,

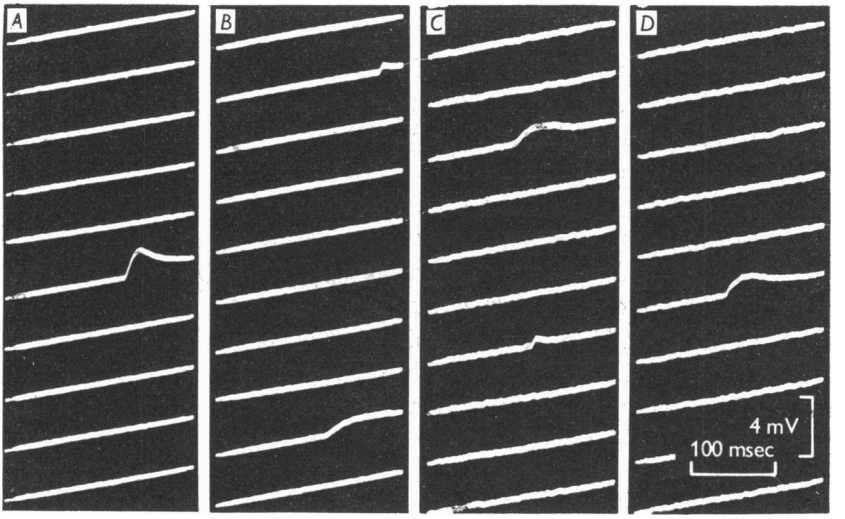

Fig. 8. Spontaneous activity recorded from three neuromuscular junctions of winter frogs. The slow depolarization shown in *D* seems to be composed of at least two units. The recording electrode was not positioned at the end-plate focus as can be inferred from the rise time of normal m.e.p.p.s in *B* and *C*.

(2) the source of ACh can be considered as an instantaneous point source,

(3) the depolarization of a unit area ($\mathrm{d}A$) of the membrane is a function of σ and of the concentration C of ACh on this area, so that the total depolarization will be given by

$$V = \sigma \int_0^\infty \phi(C)\,\mathrm{d}A, \tag{2}$$

(4) $\phi(C)$ is supposed to increase until it reaches a plateau for $C \geqslant C_0$.

These hypotheses lead to the following conclusions.

(1) If the concentration of ACh over the whole reactive area is smaller than C_0, the peak time will be independent of the ACh dose. This is what is usually observed at junctional spots.

(2) On the contrary, if a large amount of ACh is released at a region of low receptor density, those receptors situated in the vicinity of the ACh source will be rapidly saturated ($C \geqslant C_0$), and the peak time will depend mainly on the time at which the drug has saturated a maximal surface of the reactive area. This situation is likely to occur at extrajunctional level and rise time T is related to the dose Q of ACh according to eqn. (9) derived from the calculations developed in the Appendix:

$$T = \frac{Q^{2/3}}{4\pi D X_0^{2/3}}$$

where X_0 is a constant which depends on the function $\phi(C)$.

As shown by eqn. (10) of the Appendix, the maximum amplitude V of responses at the time T defined by eqn. (9) must be also a function of $Q^{2/3}$. But, in fact, the experimental data show that V is related to $Q^{2\cdot2}$. No satisfactory explanation can be proposed to account for this discrepancy.

Spatial distribution of reactive areas. The results reported in this paper complete and extend previous observations by Miledi (1960*a*, 1962) on the distribution of junctional and extrajunctional receptors at the neuromuscular junction of the frog. The picture that emerges from our experiments is that, in the frog muscle fibre, the receptive area is made of a number of highly sensitive spots, readily identified as synaptic contacts, and of wider and less sensitive areas. These two kinds of receptive areas are not necessarily contiguous, but one cannot exclude the possibility that some degree of sensitivity exists between them.

The observed seasonal variations in the extent of the extrajunctional sensitive area may be dependent on the intensity of the restraining action of a neural factor (Miledi, 1960*a*). This action might be less powerful in hibernating frogs. Differences in the function of motor nerve endings between summer and winter frogs have been observed: Otsuka, Endo & Nonomura (1962) and Maeno (1969) reported a marked facilitation of transmitter release by repetitive stimulation in winter frogs; Braun, Schmidt & Zimmermann (1966) found that the quantal content of the end-plate potential is more sensitive to Mg in winter than in summer frogs.

On the other hand, Barker & Ip (1965) produced some evidence of a process of partial degeneration and sprouting in mammalian motor nerve endings. Such a process may occur in winter frogs, although electron micrographs showing signs of degeneration in non-denervated neuromuscular junctions have not been produced so far. More evidence is necessary before one can decide whether the induction of extrajunctional receptors in normal as well as in denervated muscles is initiated by the presence of degenerating nervous tissue (Vrbová, 1967) or by the lack of a 'receptor-controlling factor' (Miledi, 1962).

We are indebted to Dr Ph. Ascher for his many helpful suggestions and advice in the preparation of the manuscript. This work was aided by grants from the Délégation Générale á la Recherche Scientifique et Technique.

APPENDIX

By R. Kahn and A. Le Yaouanc

We will examine here what relation may be expected, according to the diffusion law, between the time course of responses and the quantity of drug delivered from an instantaneous point source, when the receptors are uniformly distributed over a sensitive area.

At time $t = 0$ an amount Q of ACh is released from an instantaneous point source. The ACh receptors are assumed to be distributed with an uniform density σ on a plane surface ($z = -b$). The source is taken as the origin of the cylindrical co-ordinates (ρ, θ, z).

The concentration C of ACh at any point of the membrane is a function of time and distance and might be expressed, in cylindrical co-ordinates, by the following equation (cf. Carslaw & Jaeger, 1959):

$$C(\rho, \theta, z) = \frac{Q}{8(\pi Dt)^{\frac{3}{2}}} \exp - \left(\frac{\rho^2+z^2}{4Dt}\right) + \exp - \left(\frac{\rho^2+(2b+z)^2}{4Dt}\right) \qquad (1)$$

where D is the constant of diffusion of ACh (approx. 10^{-5} cm^2 sec^{-1}, Krnjević & Mitchell, 1960) and the second exponential term accounts for reflexion of ACh at the membrane. But, since an appreciable amount of drug is being hydrolysed there, and both actions tend, in part, to cancel each other, this term may be disregarded. Then an estimate of the ACh concentration at a given point of the membrane can reasonably be made by considering that the drug is released from an instantaneous point source and diffuses into a large fluid volume.

The depolarization of a unit area of the membrane is assumed to be a function of the ACh concentration at that point:

$$V(\rho, \theta, t) = \sigma\phi[\mathrm{C}(\rho, \theta, -b, t)] \qquad (2)$$

where $\phi(C)$ is an increasing function of C, until a plateau is reached for $C \geqslant C_0$ (saturation of receptors, for instance). Then, integrating over the whole sensitive area, the membrane depolarization at time t will be:

$$V(t) = \iint \rho \, \mathrm{d}\rho \, \mathrm{d}\theta V(\rho, \theta, t),$$

$$V(t) = 2\pi\sigma \int_0^\infty \rho \, \mathrm{d}\rho \phi \left[\frac{Q}{8(\pi Dt)^{\frac{3}{2}}} \exp - \left(\frac{\rho^2+b^2}{4Dt}\right)\right].$$

If we define

$$U = \frac{Q}{8(\pi Dt)^{\frac{3}{2}}} \exp - \left(\frac{\rho^2+b^2}{4Dt}\right)$$

then,

$$V(t) = 4\pi\sigma Dt \int_0^{Q/8(\pi Dt)^{\frac{3}{2}} \exp -(b^2/4Dt)} \phi(u) \frac{du}{u}. \tag{3}$$

If we define

$$X = \frac{Q}{8(\pi Dt)^{\frac{3}{2}}} \exp -\left(\frac{b^2}{4Dt}\right)$$

the slope of the response at time t will be:

$$\frac{dV}{dt} = 4\pi\sigma D \int_0^X \phi(u) \frac{du}{u} + 4\pi\sigma Dt \frac{\phi(X)}{X} \frac{dX}{dt}$$

and rearranging:

$$\frac{dV}{dt} = 4\pi\sigma D \left\{ \int_0^X \phi(u) \frac{du}{u} - \left(\frac{3}{2} - \frac{b^2}{4Dt}\right) \phi(X) \right\}. \tag{4}$$

Variation of the slope when t is small. Let us examine the variation of the slope of the response as a function of the amount Q of ACh released from the pipette when $t_0 < \frac{b^2}{6D}$

$$\text{(1) If } X \leqslant C_0 \quad \frac{dV}{dt} = 4\pi\sigma D \left\{ \int_0^X \phi(u) \frac{du}{u} + \left(\frac{b^2}{4Dt_0} - \frac{3}{2}\right) \phi(X) \right\}.$$

The law of the variation is not defined and will depend on the choice of $\phi(u)$. But

(*a*) if when $X \to 0$ $(Q \to 0)$, $\phi(X) \approx X^\alpha$ then $\left(\frac{dV}{dt}\right) t_0 \approx Q^\alpha$.

(*b*) $\phi(X)$ being a growing function of X, $\int_0^X \phi(u) \frac{du}{u}$ will be also a growing function of X, then it will follow that dV/dt will be a growing function of Q.

(2) If $X > C_0$, $\phi(X) = \phi(C_0)$

$$\left(\frac{dV}{dt}\right)_{t_0} = 4\pi\sigma D \left\{ \int_0^{C_0} \phi(u) \frac{du}{u} + \phi(C_0) \int_{C_0}^X \frac{du}{u} + \left(\frac{b^2}{4Dt_0} - \frac{3}{2}\right) \phi(C_0) \right\},$$

$$\begin{cases} \dfrac{dV}{dt} = 4\pi\sigma D \left\{ A + \phi(C_0) \log \dfrac{X}{C_0} \right\}, \\ A = \displaystyle\int_0^{C_0} \phi(u) \frac{du}{u} + \left(\frac{b^2}{4Dt_0} - \frac{3}{2}\right) \phi(C_0). \end{cases}$$

The slope will vary as $\log Q$, i.e. can be considered as constant.

Variation of the rise time as a function of Q. T is the time when $V(t)$ is maximum:

$$\left(\frac{dV}{dt}\right)_T = 0.$$

According to eqn. (4) T must be consistent with:

$$\begin{cases} \int_0^{X_m} \phi(u)\frac{du}{u} - \left(\frac{3}{2} - \frac{b^2}{4DT}\right)\phi(X_m) = 0, & (5) \\ X_m = \frac{Q}{8(\pi DT)^{\frac{3}{2}}} \exp - \frac{b^2}{4DT}. & (6) \end{cases}$$

(1) If $\frac{b^2}{4DT} \ll 1$, T will be given very closely by

$$\begin{cases} \int_0^{X_m} \phi(u)\frac{du}{u} - \frac{3}{2}\phi(X_m) = 0, & (7) \\ X_m = \frac{Q}{8(\pi DT)^{\frac{3}{2}}}. & (8) \end{cases}$$

Eqn. (7) determines if the condition $X_m = X_0$ for which

$$\int_0^{X_0} \phi(u)\frac{du}{u} = \frac{3}{2}\phi(X_0)$$

exists, and from eqn. (8) the time when the maximum will be reached will be:

$$T = \frac{Q^{\frac{2}{3}}}{4\pi D X_0^{\frac{2}{3}}}. \quad (9)$$

(2) When $Q \to 0$, if $\phi(C) \approx C^\alpha$, solving eqn. (5) leads to

$$T = \frac{b^2}{2D}\frac{\alpha}{3\alpha - 2} \quad (\alpha > 2/3)$$

that is a peak position of $V(t)$ independent of Q.

The situation where Q is small corresponds to cases where the 'sensitivity' below the pipette is high; this can be due to a higher density of receptors and/or to a higher affinity of the receptor molecule.

Value of V at the peak of the response. In the case where

$$b^2/4DT \ll 1$$

the peak time T of the response is given by eqn. (9) and the maximum depolarization V at that time can be derived from eqn. (3):

$$V_{(T)} = 4\pi\sigma DT\int_0^{X_m} \phi u \frac{du}{u} = 4\pi\sigma DT \cdot \tfrac{3}{2}\phi(X_0)$$

and

$$V_{(T)} = \tfrac{3}{2}\sigma \frac{Q X_0}{X_0^{\frac{3}{2}}} Q^{\frac{2}{3}} \quad (10)$$

Thus, the amplitude of responses should also be proportional to $Q^{\frac{2}{3}}$.

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