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A COMPARATIVE ELECTROPHYSIOLOGICAL STUDY OF MOTOR END-PLATE DISEASED SKELETAL MUSCLE IN THE MOUSE

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SUMMARY

1. Experiments using intracellular recording, stimulation, and microionophoretic techniques were performed on extensor digitorum longus nerve-muscle preparations excised from mice having hereditary 'motor end-plate disease'. Control experiments were performed on normal innervated and chronically denervated nerve-muscle preparations.

2. Two physiologically distinct groups of muscle fibres were found in the diseased muscles. Group I is similar to normal innervated muscle with respect to resting potentials, cable properties, neuromuscular transmission, miniature end-plate potentials, and extrajunctional acetylcholine sensitivity. Group II is similar to denervated muscle in the above respects except that (i) neuromuscular transmission, though abnormal, was present, and (ii) miniature end-plate potentials (m.e.p.p.s), often having large amplitudes, were found in these muscle fibres.

3. Large m.e.p.p.s appear to be due to an increase in muscle fibre input resistance and to the quantal release of abnormally large amounts of acetylcholine from motor nerve terminals.

4. Nerve stimulation of Group II muscle fibres evoked action potentials with a delayed repolarization phase, suggesting that a prolonged acetylcholine-induced conductance change occurs at motor end-plates.

5. Neuromuscular physiology in motor end-plate disease is similar to that reported for frog nerve-muscle preparations which have been incubated in high Ca^{2+} Ringer.

INTRODUCTION

Investigations have been carried out to identify the site and mode of action of 'motor end-plate disease' of the mouse (Duchen & Stefani, 1971; Harris & Ward, 1974; Zacks & Sheff, 1972, 1974, 1975; Zacks, Sheff, Roades & Saito, 1969). This disease is a hereditary (autosomal recessive) neuromuscular disorder which is characterized by a progressive muscular weakness appearing from 8 to 10 days after birth (Duchen, 1970). Affected mice usually die at about 20 days of age. The results of electrophysiological studies (Duchen & Stefani, 1971; Harris & Ward, 1974) which utilized intracellular recording techniques on biceps brachii nerve–muscle

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preparations, preparations which had previously been shown in a histological survey to be the most severely affected in motor end-plate diseased mice (Duchen, 1970), suggested that this malady is caused by a failure of peripheral motor nerve action potentials to invade motor nerve terminals. Low (depolarized) resting potentials, tetrodotoxin resistant action potentials, spontaneous fibrillations, and extrajunctional supersensitivity to acetylcholine were found in diseased muscle. Since these phenomena have been seen consistently in chronically denervated skeletal muscle (Albuquerque & McIsaac, 1970; Miledi, 1960; Purves & Sakmann, 1974; Redfern & Thesleff, 1971*b*), the authors described the pathology of motor end-plate disease as a 'functional denervation'. In addition, very large miniature end-plate potentials (m.e.p.p.s) were found in diseased muscle fibres.

The present study was begun in order to provide a detailed description of the electrophysiological characteristics of the diseased neuromuscular system in a different, less affected nerve-muscle preparation, to determine the extent to which motor end-plate diseased skeletal muscle and chronically denervated muscle are similar, and to shed some light on the lesion responsible for this pathology. To these ends, I measured the resting potentials, cable properties, m.e.p.p.s, action potentials, and extrajunctional acetylcholine (ACh) sensitivities of motor end-plate diseased extensor digitorum longus (e.d.l.) muscle fibres. The e.d.l. was chosen because it has been shown in the rat that the e.d.l. is essentially homogenous with respect to the contractile properties of its motor units (all fast, Close, 1967). The parameters measured from diseased muscle fibres were compared to those obtained from normal innervated and chronically denervated e.d.l. muscle fibres.

METHODS

Mice. Motor end-plate diseased breeding stock was obtained from Dr S. I. Zacks, Department of Neurology, Pennsylvania Hospital, Philadelphia, Pennsylvania, U.S.A. Seventeen to 19-day-old mice with this disease were generally used since, at those ages, they were very weak but not immobile. Presumably, the effects of the disease were almost maximal. Normal control mice, either heterozygous for the disease gene or homozygous normal, were used at 17–19 days of age, and denervates were used at 17–24 days of age.

Denervation procedure. Denervations were performed on 10–11 day old normal mice since weakness in diseased mice appears at the same age. Mice were injected with atropine sulphate (3.3 mg/ml. I.P., 20 mg/kg) in order to reduce bronchial secretions. After 30 min, they were anaesthetized with ether. The left e.d.l. was denervated by removing a small length (1–2 mm) of the deep peroneal nerve at the knee.

Physiological preparation. The e.d.l. muscle with its nerve stump (in innervated preparations) was quickly removed from mice after cervical dislocation, and secured in a Sylgard (Dow Corning) bathing dish at slightly over resting length. A standard oxygenated mammalian Ringer solution (or experimental solution) continuously perfused the preparation.

Solutions and drugs. The standard mammalian Ringer solution had the following composition (in m-mole/l.): NaCl, 113; KCl, 5·4; $MgSO_4$, 1·2; KH_2PO_4, 1·0; $NaHCO_3$, 25; $CaCl_2$, 1·9; glucose, 11. Tetrodotoxin (TTX), tubocurarine, and edrophonium chloride (Tensilon), when used, were at a concentration of 1×10^{-6} M in the standard Ringer. High Mg^{2+}, low-Ca^{2+} Ringer solutions were made by adjusting the concentrations of $MgSO_4$ and $CaCl_2$ in the standard Ringer to 9·5 and 0·95 mM, respectively, or to 2·1 and 0·2 mM, respectively. Na dantrolene was used in saturating concentrations ($\sim$ 10 mg/l.) in Ringer solutions to reduce the spontaneous contractile activity of muscles with motor end-plate disease without altering the electrical characteristics of the muscle fibres (Ellis & Bryant, 1972; Honkomp, Halliday & Wessels, 1970); this drug was a gift from Norwich Pharmacal Co., Norwich, New York. The above solutions were saturated with a 95% O_2–5% CO_2 gas mixture, had pHs of 7·3–7·4, and were used at room temperature (19–21 °C).

Basic electrophysiological techniques. Standard electrophysiological techniques were used for intracellular recording, current injection, and nerve stimulation. Conventional glass microelectrodes filled with 3 M-KCl (10–50 MΩ DC resistance) were used for recording and passing currents. Nerve stimulation was accomplished with a snugly fitting suction electrode pulled from polyethylene tubing. Direct stimulation of muscle fibre action potentials was done using an intracellular current-passing electrode. When recording action potentials, resting potentials were locally set to a constant potential with a current-passing electrode (within 100 μm of the recording electrode) in order to control for potential-dependent changes in muscle fiber excitability. All action potentials and m.e.p.p.s were recorded in the region of the motor end-plate.

Ionophoretic technique. Acetylcholine microionophoretic electrodes were filled with 2 M-ACh chloride, and had DC tip resistances of 100–200 MΩ. Iontophoresis of ACh was performed using the method of del Castillo & Katz (1955). Extrajunctional ACh sensitivity was measured by placing the ACh micro-electrode directly over a muscle fibre about 500 μm from the end-plate region. The same muscle fibre was impaled with the recording electrode within 100 μm of the ACh electrode. Between 1·5 and 7·5 nA of negative 'bucking' current were constantly applied to the ACh electrode to prevent diffusion of ACh out of the tip. The ACh electrode was positioned close to the muscle fibre membrane using a technique similar to that of Fischbach & Robbins (1971). Ionophoretic pulses were of 10 msec duration and varying amplitude. ACh sensitivities are expressed as millivolts of depolarization per nanocoulomb of charge passed through the ACh electrode (Miledi, 1960). The ACh-induced depolarizations (and, where indicated, m.e.p.p.s) were corrected for changes in 'driving force' (Katz & Thesleff, 1957; Martin, 1955). The ACh reversal potential was taken to be −15 mV (del Castillo & Katz, 1954; Takeuchi & Takeuchi, 1960; author's observations). ACh responses were corrected to a resting potential of −70 mV. When possible, linear regression analyses were performed on the corrected ACh responses versus nanocoulombs of charge passed. The ACh sensitivity of each muscle fibre was calculated as the slope of its regression line. In cases where this analysis could not be done (e.g. on fibres with very low ACh sensitivities), the ACh sensitivity was calculated at each ACh dose, and the largest value was chosen.

Square pulse analyses. Electrical cable properties were determined from square pulse analyses (Albuquerque & McIsaac, 1970; Fatt & Katz, 1951). Hyperpolarizing current pulses were passed from the stimulating electrode which was placed 400, 150 to 250, and 10 to 25 μm away from the recording electrode. Interelectrode distances, as well as muscle fibre radii (ρ), were measured with a calibrated micrometer eyepiece in the microscope through which the preparation was viewed. A graph of $\log_{10}(V/I)$ vs. the interelectrode distance was plotted for each muscle fibre; data were accepted only if the points were colinear (correlation coefficients $\leqslant -0{\cdot}95$). A linear regression analysis was used to compute the slope and intercept of each line. These parameters were used to compute the input resistance (R_{in}) and the specific membrane resistance (R_m) of each muscle fibre.

The time constant (τ) of each fibre was determined by measuring the time taken for an anodal potential to reach 84% of its steady-state value with the recording and current-injecting electrode placed 30 μm or less apart in the fibre (Hodgkin & Rushton, 1946). In experiments where only input resistances were determined, a recording and current-passing electrode were inserted into the same muscle fibre less than 30 μm apart, and a 100 msec hyperpolarizing current was injected. The input resistance was then calculated as V/I.

RESULTS

Resting potentials. Table 1 shows resting potentials pooled from many 17–19 day old normal and motor end-plate diseased e.d.l.s and 17–20 day old chronically denervated e.d.l.s. As will be shown later, many diseased e.d.l. muscle fibres exhibited abnormally large m.e.p.p.s. In the diseased e.d.l., the resting potential of a muscle fibre appeared to depend strongly on the fibre's m.e.p.p. amplitudes. Note that diseased e.d.l. muscle fibres with large m.e.p.p.s had resting potentials which were very similar to those of chronically denervated muscle fibres.

Electrical cable properties. The results of square-pulse analyses of normal, motor

end-plate diseased, and chronically denervated e.d.l. muscle fibres are shown in Table 2. Seventeen to 19 day old diseased lateral e.d.l. muscle fibres which exhibited large m.e.p.p.s had significantly higher R_{in}s than did normal muscle fibres ($P < 0{\cdot}001$, one-tailed Student's t test). This increase reflects both a decrease in muscle fibre radius, and a striking increase in R_m ($P < 0{\cdot}003$ and $P < 0{\cdot}01$, respectively, one-tailed Student's t test). These changes are very similar to those which occur after denervation.

TABLE 1. Resting potentials of normal, motor end-plate diseased, and denervated e.d.l. muscle fibres

17–19 day old normal	17–19 day old diseased with normal m.e.p.p.s	17–19 day old** diseased with large m.e.p.p.s	17–19 day old denervated
*$-69 \pm 0{\cdot}3$ (456, 12)	$-69 \pm 0{\cdot}4$ (275,30)	$-54 \pm 0{\cdot}5$ (328,30)	$-53 \pm 0{\cdot}3$ (250,7)

* Values are in millivolts, mean ± S.E. (no. of fibres, no. of muscles).

** In normal e.d.l. muscle fibres, the average m.e.p.p. amplitude was approximately 1 mV. The fibres of motor end-plate diseased e.d.l.s presented a much larger spectrum of mean m.e.p.p. amplitudes (1–8 mV). A criterion for m.e.p.p. classification was established: M.e.p.p.s less than 2 mV were considered to be normal; all others were considered to be large.

TABLE 2. Electrical cable properties of normal, motor end-plate diseased, and denervated e.d.l. muscle fibres

	17–19 day old normal medial ($n=5$)	17–19 day old normal lateral ($n=6$)	17–19 day old diseased medial ($n=5$)‡	17–19 day old diseased lateral ($n=10$)†	17–24 day old denervated medial ($n=7$)	20 day old denervated lateral ($n=9$)
R_{in} (kΩ)	*460 ± 79	560 ± 67	650 ± 130	1600 ± 100	1100 ± 110	1500 ± 130
R_m (Ωcm²)	410 ± 72	640 ± 29	600 ± 190	1400 ± 220	870 ± 140	1700 ± 650
τ (msec)	2·0 ± 0·2	1·6 ± 0·2	1·3 ± 0·2	2·4 ± 0·1	2·5 ± 0·1	2·8 ± 0·4
ρ (μm)	11 ± 0·4	11 ± 0·7	10 ± 0·4	7 ± 0·3	6 ± 0·3	7 ± 0·4

* Mean values ± S.E.

† This sample consists mainly of muscle fibres with large m.e.p.p.s.

‡ Muscle fibres with normal m.e.p.p.s.

Miniature end-plate potentials. Miniature end-plate potentials recorded from an 18 day old normal lateral e.d.l. muscle fibre are illustrated in Fig. 1*A*. Seventeen to 19 day old motor end-plate diseased lateral e.d.l. muscle fibres with normal resting potentials exhibited m.e.p.p.s which were essentially identical to those in Fig. 1*A*. However, as previously noted, a large population of muscle fibres was found which exhibited large m.e.p.p.s. A photograph of these m.e.p.p.s is shown in Fig. 1*A*.

Most of the diseased muscle fibres with large m.e.p.p.s were located on the lateral side of the e.d.l. Studies in which m.e.p.p. amplitudes were systematically surveyed showed that 70% of 224 muscle fibres from thirteen diseased lateral e.d.l.s exhibited abnormally large m.e.p.p.s, whereas only 13% of 153 muscle fibres from eight diseased medial e.d.l.s exhibited large m.e.p.p.s. If the percentage from these diseased

lateral e.d.l.s is calculated on a 'per muscle' basis, however, it becomes 61 % with a standard deviation of 23 %, indicating that the percentage of fibres per muscle which exhibited large m.e.p.p.s varied greatly from muscle to muscle.

Mean m.e.p.p. amplitudes from normal e.d.l. muscle fibres and from most diseased medial e.d.l. muscle fibres were similar (1·1 ± 0·06 mV, twenty-three fibres, and 1·2 ± 0·1 mV, seventeen fibres, respectively. These values are the mean ± S.E.).

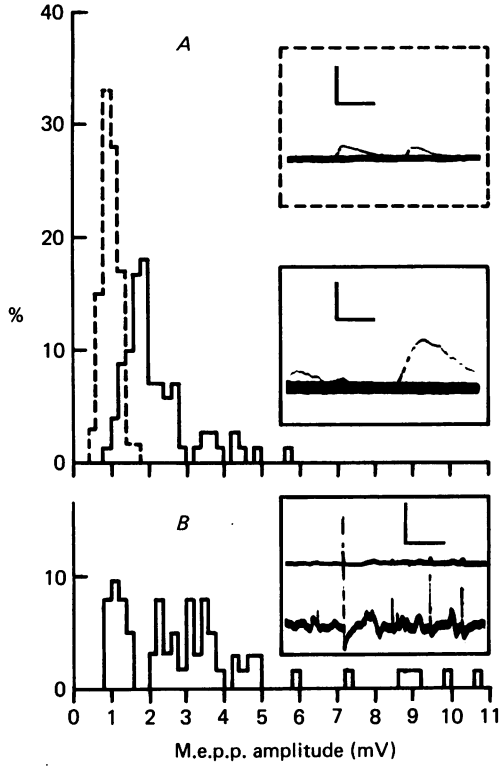

Fig. 1. M.e.p.p. amplitude distributions from normal and muscle fibres with motor end-plate disease. (*A*) Distributions from an 18 day old normal lateral e.d.l. muscle fibre (dashed line, resting potential = − 67 mV, sample of 60 m.e.p.p.s) and from an age-matched diseased lateral e.d.l. muscle fibre with large m.e.p.p.s (continuous line, resting potential = − 50 mV, sample of seventy-two m.e.p.p.s). The insets illustrate m.e.p.p.s recorded from the same muscle fibres (dashed and continuous borders) that are described by the histograms. The calibration bars are 4 mV vertically and 4 msec horizontally for both insets. *B*, m.e.p.p. amplitude distribution from a 20 day old diseased lateral e.d.l. muscle fibre with large m.e.p.p.s (resting potential = − 38 mV, sample of sixty-two m.e.p.p.s). The inset illustrates m.e.p.p.s recorded from this fibre. The calibration bar represents 40 mV vertically for the upper trace, 4 mV vertically for the lower trace, and 1 sec horizontally for both traces. The resting potential is somewhat unsteady due to mechanical artifact from fibrillatory activity.

The relationship between modal m.e.p.p. amplitude and muscle fibre input resistance was examined in order to determine whether the increase in m.e.p.p. amplitudes in the diseased lateral e.d.l. muscle fibres was due solely to changes in the muscle fibres' electrical characteristics (Katz & Thesleff, 1957). Modal m.e.p.p. amplitudes were used because, as will be shown later, their amplitude distribution in muscle

fibres with large m.e.p.p.s is positively skewed. I reasoned that by using the modal m.e.p.p. amplitude instead of the mean m.e.p.p. amplitude, I would select for m.e.p.p.s resulting from the release of uniquantal packets of ACh, i.e., packets consisting of the normal quantal complement of ACh. Fig. 2 shows a linear correlation (correlation coefficient $= 0{\cdot}925$, $P < 0{\cdot}01$) between the modal m.e.p.p. amplitude and input resistance of 19 day old motor end-plate diseased lateral muscle fibres. The increase in the electrical response of diseased muscle fibres to uniquantal packets of ACh, therefore, results from changes in the electrical characteristics of the muscle fibres.

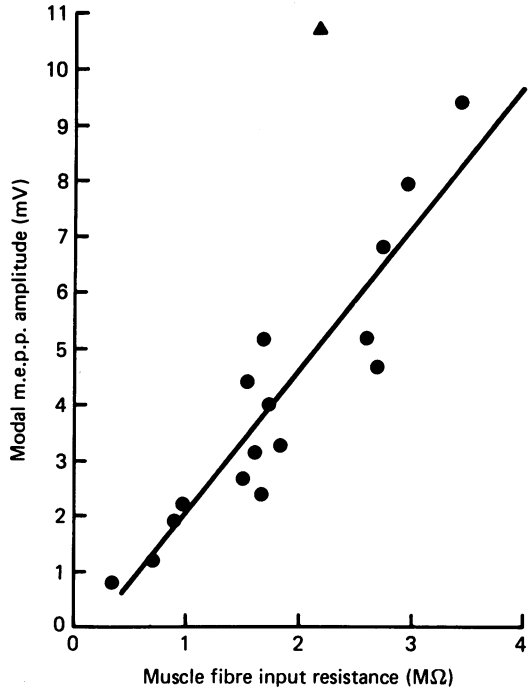

Fig. 2. A linear plot of modal m.e.p.p. amplitude versus muscle fibre input resistance. Modal m.e.p.p. amplitudes were corrected to a resting potential of -70 mV (see Methods). The correction procedure allows one to compare m.e.p.p.s from muscle fibres with different resting potentials and different mean (or modal m.e.p.p. amplitudes. (▲) This value was not included in the regression analysis. Note that its amplitude is approximately twice that predicted by the regression line.

Certain changes in the amplitude distribution of m.e.p.p.s from muscle fibres exhibiting large m.e.p.p.s can not be explained entirely by post-synaptic changes. Fig. 1*A* shows amplitude distributions of m.e.p.p.s from an 18 day old normal muscle fibre and from an age-matched diseased muscle fibre with large m.e.p.p.s. Fig. 1*B* illustrates the same type of data from a 20 day old diseased muscle fibre with large m.e.p.p.s. It is evident that the m.e.p.p. amplitude distributions from the diseased muscle fibres and from the normal muscle fibre have different shapes; m.e.p.p. amplitude distributions from diseased muscle fibres with large m.e.p.p.s are positively skewed. The standard deviations of the m.e.p.p. amplitude distributions from 19 day old diseased muscle fibres with large m.e.p.p.s, and from 20 day old diseased fibres

with large m.e.p.p.s are significantly greater than the standard deviations of the distributions from pooled 18 and 19 day old normal muscle fibres ($P < 0{\cdot}0005$ and $P < 0{\cdot}005$, respectively; one-tailed Student's t test). This increase is not simply due to the general increase in m.e.p.p. amplitudes. Indeed, even after compensating for the increase in spread which normally occurs when a distribution's mean is increased by a multiplicative process (which is analogous to the way in which an increase in muscle fibre input resistance causes an increase in m.e.p.p. amplitudes), the differences between the standard deviations are still found ($P < 0{\cdot}01$ and $P < 0{\cdot}025$, respectively,

TABLE 3. Action potentials elicited from innervated normal, motor end-plate diseased (MED) and chronically denervated normal lateral e.d.l. muscle fibres by direct and nerve stimulation

	Direct stimulation action potentials		Nerve stimulation action potentials	
	†Overshoot (mV)	Maximum rate of rise (V/sec)	Overshoot (mV)	Maximum rate of rise (V/sec)
Normal	*$+34 \pm 1{\cdot}2$ (27,3)	319 ± 11 (27,3)	$+25 \pm 1{\cdot}1$ (24,3)	346 ± 11 (24,3)
MED**	$+12 \pm 1{\cdot}7$ (20,3)	192 ± 10 (20,3)	$+4 \pm 1{\cdot}4$ (20,3)	191 ± 14 (20,3)
Denervate (resting potential set to -80 mV)	$+19 \pm 1{\cdot}7$ (48,4)	163 ± 9 (48,4)		
Denervate (resting potential set to -110 mV)	$+29 \pm 1{\cdot}3$ (29,2)	296 ± 10 (29,2)		

* Mean values ± S.E. (no. of fibres, no. of muscles).

** Muscle fibres with large m.e.p.p.s.

† Innervated and diseased e.d.l.s were from 17–19 day old mice. Denervated e.d.l.s were from 17–20 day old mice. In the case of innervated preparations, action potentials were elicited by direct and nerve stimulation from the same muscle fibres.

one-tailed Student's t test). Therefore, the very large m.e.p.p.s (those at the far right of the amplitude distributions) in diseased muscle fibres are due to both pre- and post-synaptic changes.

Very large m.e.p.p.s generally did not arise from the random temporal summation of smaller ones occurring at a high frequency. Motor end-plate diseased muscle fibres whose amplitude distributions exhibited the greatest amount of spread (20 day old diseased muscle fibres) had m.e.p.p. frequencies which were within the normal range of frequencies (normal range: 0·4–1·0 sec^{-1}).

The effects of low-Ca^{2+}, high-Mg^{2+} Ringer solutions and various pharmacological agents on the m.e.p.p. amplitudes in diseased lateral e.d.l. muscle fibres with large m.e.p.p.s were investigated in order to determine the mechanism subserving the generation of the exceptionally large m.e.p.p.s. Not surprisingly, tubocurarine blocked all m.e.p.p.s, and Tensilon increased m.e.p.p. amplitudes. The entire range of m.e.p.p. amplitudes was seen in Ringer containing TTX. The effect of high-Mg^{2+}, low-Ca^{2+} Ringer on the appearance of very large m.e.p.p.s was investigated using two experimental solutions (see Methods). In most experiments, very large m.e.p.p.s were recorded in both types of solution.

It was of interest to determine whether large m.e.p.p.s in motor end-plate disease were unique to fast twitch muscle fibres. Therefore, the soleus muscle, a slow twitch muscle (Close, 1967), was briefly examined for the presence of large m.e.p.p.s; large m.e.p.p.s were easily found.

Action potentials. Table 3 presents action potential characteristics, and Fig. 3 illustrates typical responses. The mean overshoot of directly evoked action potentials

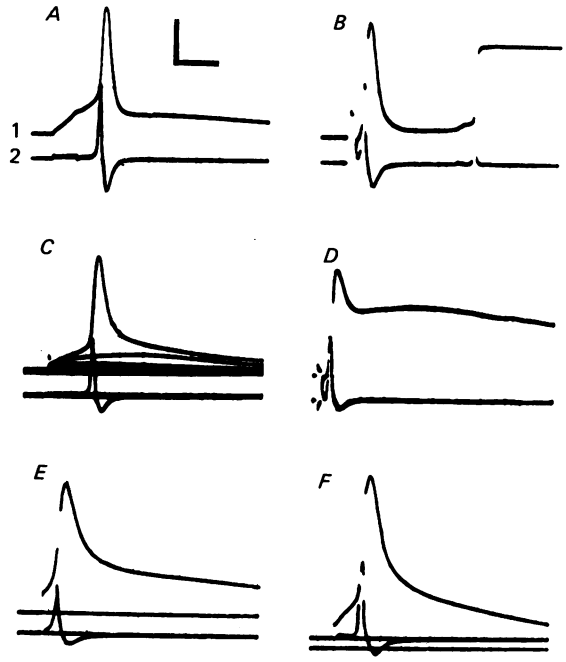

Fig. 3. A composite photograph (retouched) of oscilloscope traces showing directly stimulated action potentials (*A*, *C*, *E*, and *F*) and nerve-stimulated action potentials (*B* and *D*) from innervated normal, motor end-plate diseased and denervated normal lateral e.d.l. muscle fibres. Resting potentials were locally set to -80 mV in all cases except in *F*, which was set to -110 mV. *A*, a directly stimulated action potential and *B*, a nerve-stimulated action potential from a normal innervated muscle fibre. *C*, a directly stimulated action potential and *D*, a nerve-stimulated action potential from a diseased muscle fibre with large m.e.p.p.s. (*E* and *F*) Directly stimulated action potentials from a chronically denervated muscle fibre. The horizontal calibration bar represents 4 msec. (1) DC-coupled trace of the membrane potential. The vertical calibration bar represents 40 mV. (2) The first derivative of the action potentials. The calibration bar represents 200 V/sec. A delayed repolarization phase is seen in *D*, but not in *C*. In *C*, depolarizing stimuli were slowly increased until an action potential was recorded.

from motor end-plate diseased muscle fibres with large m.e.p.p.s was found to be lower than those of both denervated and innervated normal lateral e.d.l. muscle fibres (resting potentials preset to -80 mV, $P < 0{\cdot}005$ and $P < 0{\cdot}0005$, respectively; one-tailed Student's t test). The same is true of the mean maximum rise rate of directly stimulated action potentials from the diseased muscle fibres ($P < 0{\cdot}025$ and $P < 0{\cdot}0005$, respectively; one-tailed Student's t test). In normal and diseased muscle

fibres, nerve stimulation evoked action potentials which had lower overshoots than those when directly stimulated ($P < 0{\cdot}0005$ and $P < 0{\cdot}0005$, respectively; one-tailed Student's t test for matched pairs). Most striking was the difference between the directly-stimulated and the nerve-stimulated action potentials recorded from motor end-plate diseased muscle fibres with large m.e.p.p.s (Fig. 3*C* and *D*). Direct stimulation of these fibres evoked action potentials whose wave forms were very similar to those from normal muscle fibres. Nerve stimulation on the other hand, elicited action potentials whose falling phases were very prolonged. This delayed repolarization

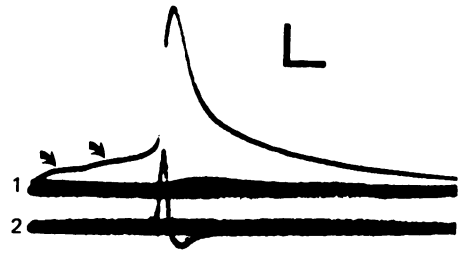

Fig. 4. A photograph (retouched) of oscilloscope traces showing an action potential being triggered from the temporally-summed potential of two very large m.e.p.p.s (arrows) in a 19 day old motor end-plate diseased lateral e.d.l. muscle fibre. The resting potential of the fibre was locally polarized to -80 mV. The horizontal calibration bar represents 2 msec. (1) A low gain, DC coupled trace of the membrane potential. The vertical calibration bar represents 20 mV. (2) The first derivative of the action potential. The vertical calibration bar represents 100 V/sec.

TABLE 4. Extrajunctional ACh sensitivities of normal, motor end-plate diseased, and denervated lateral e.d.l. muscle fibres

	Normal fibres	MED fibres without large m.e.p.p.s	MED fibres with large m.e.p.p.s	Denervated fibres
ACh sensitivity (mV/nC)	*$<0{\cdot}09$ ($<0{\cdot}04$ to $<0{\cdot}27$) ($n=5$)	$<1{\cdot}0$ (0·7 to 17) ($n=6$)	490 (310 to 5100) ($n=6$)	640 (58 to 4200) ($n=12$)

* Median value, (range), (no. of fibres tested).
19 day old normal e.d.l.s and 19–20 day old diseased and denervated e.d.l.s were used.

was not an artifact of contraction. Contraction either caused the recording electrode to be pulled out of the muscle fibre (e.g. see Fig. 3*B*), or introduced a small 'glitch' in the membrane potential trace. More convincing is the fact that the delayed repolarization always occurred with a latency which was much shorter than that of contraction (compare Fig. 3*B* and *D*). Delayed repolarization was not seen in diseased muscle fibres which did not exhibit large m.e.p.p.s.

Spontaneous fibrillatory activity. Spontaneous fibrillations in e.d.l.s with motor end-plate disease were blocked by TTX and by tubocurarine, and reappeared after washing, as was shown previously by Harris & Ward (1974). This suggested that the m.e.p.p.s in some muscle fibres were large enough to elicit action potentials. Fig. 4 illustrates an action potential being triggered by the temporally summed potential of

two very large m.e.p.p.s in a diseased muscle fibre which began to twitch when its resting potential was locally set to − 80 mV.

Extrajunctional ACh sensitivity. The extrajunctional ACh sensitivities of normal, motor end-plate diseased and denervated muscle fibres are presented in Table 4. Innervated normal and diseased muscle fibres with normal m.e.p.p.s had low extrajunctional ACh sensitivities, whereas denervated normal and diseased muscle fibres with large m.e.p.p.s had very high extrajunctional ACh sensitivities.

DISCUSSION

The present study confirms previous work by Duchen & Stefani (1971) and by Harris & Ward (1974) in several respects. In this and the previous studies, resting potentials, action potentials, and extrajunctional ACh sensitivities measured in affected diseased muscle fibres were found to be similar to the respective parameters reported in the literature for denervated muscle fibres (Albuquerque & McIsaac, 1970; Redfern & Thesleff, 1971*a*, *b*). In addition, both affected and unaffected muscle fibres with motor end-plate disease have been found in all studies to date. However, a major difference between this and the previous studies is that the affected diseased muscle fibres used in this work were from a decidedly different population than those used previously: muscle fibres examined in this study did not exhibit an absolute failure of neuromuscular transmission as did the muscle fibres examined previously. This discrepancy may be due to the use of mice with different genetic backgrounds and/or to the use of nerve-muscle preparations which are more or less affected by motor end-plate disease. The abnormal transmission found in this study may be the manifestation of a transitional stage between normal and complete transmission failure in the progressive pathophysiology of motor end-plate disease.

When diseased muscle fibres which exhibit large m.e.p.p.s are made excitable by local hyperpolarization, action potentials can be elicited by nerve stimulation; these have very long repolarization phases. It is likely that the ACh-induced conductance change in these muscle fibres produces a substantial and prolonged 'current sink' at the motor end-plate. This does not appear to be due to low acetylcholinesterase activity at affected diseased neuromuscular junctions since (i) the effect of Tensilon on m.e.p.p. amplitudes revealed the presence of functional acetylcholinesterase, and (ii) the regression line of motor end-plate disease modal m.e.p.p. amplitudes (Fig. 2) accurately predicts the amplitude of m.e.p.p.s from normal muscle fibres. It is possible that the prolonged repolarization is due to altered kinetics of the synaptic ACh receptors; miniature end-plate currents recorded fortuitously and extracellularly from one diseased muscle fibre with large m.e.p.p.s suggest that this is not the case. Conclusive information on receptor kinetics could be obtained by rigorous analysis of miniature end-plate currents recorded from many such fibres using voltage-clamp techniques. Alternatively, the delayed repolarization may result from a prolonged action of ACh at the neuromuscular junction due to oversecretion. This could result, hypothetically, from a pathophysiological condition in which motor nerve terminal action potentials are prolonged (Katz & Miledi, 1969). Prolongation of motor nerve terminal action potentials could eventually lead to intra-terminal accumulation of Na^+ and nerve terminal inexcitability. Prolongation of motor nerve terminal action

potentials could also result in intra-terminal accumulation of Ca^{2+}; pretreatment of frog nerve-muscle preparations in isotonic Ca^{2+} Ringer has been shown to impair neuromuscular transmission (Katz & Miledi, 1969).

Implicit in the latter hypothetical mechanism for motor end-plate disease progression is its dependence upon the presence of motor nerve terminal action potentials. Hence the hypothesis is subject to testing by varying presynaptic activity. Chronic nerve stimulation would be expected to decrease the latency of appearance of those characteristics peculiar to affected diseased e.d.l. muscle fibres. Decreasing motor nerve terminal activity by means of epineural TTX-impregnated cuffs or subperineural TTX injections (Pestronk & Drachman, 1978; Pestronk *et al.* 1976; Lavoie *et al.* 1976) would be expected to delay the appearance of transmission changes. However, several electrophysiological changes which occur in motor end-plate diseased muscle fibres with large m.e.p.p.s would also be expected to occur in normal animals as a result of functional denervation. Hence, such experiments would need to be carefully controlled.

The extremely large m.e.p.p.s which were found in affected diseased muscle fibres appear to result from the quantal release of abnormally large amounts of ACh from motor nerve terminals since very large m.e.p.p.s were recorded in the presence of TTX and low-Ca^{2+}, high-Mg^{2+} Ringer solutions. One diseased muscle fibre with large m.e.p.p.s was found whose modal m.e.p.p. amplitude was twice as large as that predicted by the regression line shown in Fig. 2 (triangle). Since modal m.e.p.p. amplitudes were chosen to select for high probability events, i.e., the release of single packets of ACh, then from a purely probabilistic point of view, it is more satisfying to consider the m.e.p.p.s in this fibre as being due to the release of single packets containing twice the normal amount of ACh as opposed to the repetitive, highly synchronous release of two normal packets from the nerve terminal. Hence, intra-terminal vesicular fusion is implicated in motor end-plate disease. Ca pretreatment is also associated with the appearance of enormous m.e.p.p.s (Katz & Miledi, 1969). Ultrastructural studies have found that this treatment causes an intra-terminal agglutination and fusion of synaptic vesicles (Heuser *et al.* 1971). In view of the physiological similarities between the Ca^{2+} pretreated neuro-muscular system and the abnormal diseased neuromuscular system, it seems reasonable that similar alterations may occur in the motor nerve terminals of both systems.

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