Miniature end-plate potentials in neuromuscular disease: an electrophysiological investigation of motor-point muscle biopsies

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SUMMARY A technique is described for the electrophysiological investigation of motor-point muscle biopsies and the data from 13 biopsies are reported. It was not possible to demonstrate any influence of disease upon the resting potentials of individual biopsies, but resting potentials were in general lower than those reported for limb muscle *in vivo*. Miniature end-plate potentials (MEPPs) in clinically normal limb muscle were smaller and more frequent than those previously described in isolated intercostal muscle. In two biopsies from myasthenic patients very small amplitude MEPPs were recorded and, in one of these, MEPPs were also of very low frequency, possibly associated with the severity of the disease. The frequency and amplitude of MEPPs in muscular dystrophy and neurogenic disease have been reported and the possible significance of these findings is discussed.

The motor-point muscle biopsy provides a means of demonstrating pathological changes which occur not only in muscle but also in the lower motor neurone, and consequently it has become well established as an aid to the diagnosis of neuromuscular disease. In the course of disease, morphological changes in the motor end-plate and terminal nerve branches have been revealed by vital staining with methylene blue (Woolf, 1966; Woolf, Alberca-Serrano, and Johnson, 1969; Allen, Johnson, and Woolf, 1969) and changes in the muscle enzymes by histochemical techniques (Morris, 1969, 1970). The present investigation examines the frequency and amplitude of miniature end-plate potentials (MEPPs) in motor-point muscle biopsies in the hope that these might characterize particular disease states. At the same time this information might reveal a possible basis for any functional defects at the neuromuscular junction. It is important to this investigation, therefore, that the selection of a particular muscle for motor-point biopsy is made on the basis of clinical examination and electromyography, so that the muscle is known to be involved in the disease process without being grossly atrophic.

METHODS

Motor-point biopsies were usually obtained from fore-

arm muscles using the technique of Coërs and Woolf (1959). A length of muscle about 4 cm by 0.5 cm diameter was taken and transported to the laboratory immersed in Ringer solution bubbled with 4% CO₂ in O₂ (for the composition of the Ringer solution see Elmquist, Hoffmann, Kugelberg and Quastel, 1964). In the laboratory it was transferred to a Perspex bath (volume 10 ml.) and secured by threads tied to both ends, care being taken not to stretch the muscle. The Ringer solution in the bath was exchanged with fresh medium (again aerated with 4% CO₂ in O₂) at the rate of 4 ml./minute.

A standard arrangement for microelectrode recordings was employed, potentials being displayed on a Tektronix 502A dual beam oscilloscope and a Devices M2 pen recorder. Glass microelectrodes were filled with 3M KCl using the vacuum boiling technique and had tip resistances of 8-15 MΩ, with tip potentials less than 5 mV. All the recordings referred to in this study were taken from the outer one or two layers of muscle fibres and, unless otherwise stated, at least 1 cm from where the muscle was tied. The muscle bath was allowed to follow room temperature (20-24°C), at which temperature the muscle continued to contract in response to direct stimulation for the duration of the recording session (four to 10 hours).

It is a feature of each of the muscles biopsied in this study that the motor end-plates lie in a discrete band, and the position of this innervation zone was revealed by taking a thin strip of muscle from the length of the biopsy and staining it for cholinesterase (Buckley and Nowell, 1966).

RESULTS

The findings from 13 biopsies are reported (see Table), two of which were from a patient with right hemiparesis caused by a meningioma. He consented to muscle biopsies from both forearms.

During the first minute after microelectrode penetration of a muscle fibre the resting potential often fell by up to 5 mV but this usually then stabilized and remained constant over the recording period (about five to 10 minutes). Resting potentials rarely showed any improvement after such an initial decline and occasionally they continued to fall slowly. In the two biopsies taken from patients suffering from dystrophia myotonica (biopsies 6 and 7), a slow fall in resting potential sometimes terminated abruptly in a spontaneous burst of muscle action potentials accompanied by the contraction of the muscle fibre and the rejection of the microelectrode tip. Spontaneous action potentials were not observed in any of the other biopsies.

The resting potentials were usually measured when the electrode tip was removed from the muscle fibre. However, in those fibres where the resting potential continued falling, an average value was taken over that period in which the frequency and amplitude of the MEPPs were assessed. In the case of one biopsy from a myasthenic patient (biopsy 4) data on the resting potential were not recorded. The mean resting potentials of all the

other biopsies, except that from the affected arm of the hemiparetic patient (biopsy 1a; mean resting potential $\pm$ SD, 55.6 ± 5.4 mV), were between 60 to 70 mV.

MEPPs were usually found after a few preliminary impalements in that region indicated by the staining of end-plate cholinesterase in the parallel strip of muscle taken for this purpose (Fig. 1). Further exploration clearly defined that band of muscle from which MEPPs of the greatest amplitude could be recorded and subsequent impalements were then made by moving the electrode transversely to a new fibre after each recording. If there was an obvious reduction in MEPP amplitude after a change of microelectrode position, then more exploratory impalements were made and, if necessary, the microelectrode moved slightly along the length of the muscle. This procedure was adopted because end-plates on adjacent muscle fibres are rarely separated by more than 100 μ m and commonly much less (Fig. 2). Attempts to localize the end-plate very accurately by multiple impalements of individual fibres were unsatisfactory, nearly always resulting in a deterioration of the muscle fibre resting potential. The rise time of MEPPs assumed to have been recorded focally was usually 2 to 3 msec (cf. Boyd and Martin, 1956: rise time of MEPPs in cat tenuissimus at 20°C, 2.0 ± 0.4 msec).

The MEPP frequency was often depressed immediately after the penetration of the microelectrode

TABLE
SUMMARY OF FINDINGS RELATING TO EACH BIOPSY (MEAN VALUES GIVEN $\pm$ SD)

Biopsy no.	Age (yr.)	Sex	Diagnosis or symptoms	Muscle biopsied	Fibres (no.)	Mean resting potential (mV)	Mean MEPP frequency (no./sec)	Mean MEPP amplitude (mV)
1a	44	M	Right hemiparesis caused by meningioma	R. palmaris longus	5	55.6 ± 5.4	0.41 ± 0.17	0.26 ± 0.08
b	Same patient as above		Clinically normal muscle	L. palmaris longus	7	66.1 ± 6.7	0.39 ± 0.26	0.58 ± 0.07
2*	14	M	Osteoma of R. humerus; clin. normal muscle	R. deltoid	5	61.2 ± 3.0	0.28 ± 0.15	0.39 ± 0.07
3	21	M	Myasthenia gravis	R. palmaris longus	7	69.1 ± 2.9	0.18 ± 0.09	0.16 ± 0.02
4	33	F	Myasthenia gravis	R. flexor carpi rad.	8	No data obtained	0.08 ± 0.04	0.16 ± 0.06
5	21	M	Facioscapulohumeral dystrophy	R. deltoid	6	65.5 ± 4.6	0.12 ± 0.06	0.47 ± 0.15
6	30	F	Dystrophia myotonica	R. palmaris longus	8	61.8 ± 9.0	0.30 ± 0.13	0.61 ± 0.20
7	34	M	Dystrophia myotonica	R. flexor carpi rad.	13	66.0 ± 7.6	0.34 ± 0.13	0.42 ± 0.14
8	26	M	Guillain-Barré syndrome	R. flexor carpi rad.	11	63.5 ± 4.7	0.25 ± 0.07	0.32 ± 0.06
9	34	M	Peroneal muscular atrophy	L. palmaris longus	11	65.3 ± 6.3	0.19 ± 0.06	0.35 ± 0.08
10	15	F	Exertional cramp	R. deltoid	13	65.8 ± 7.3	0.19 ± 0.07	0.41 ± 0.12
11	8	M	Slight generalized weakness	R. palmaris longus	8	63.6 ± 9.7	0.30 ± 0.13	0.70 ± 0.18
12	12	M	Muscle pain	R. vastus medialis	9	65.6 ± 5.7	0.22 ± 0.10	0.45 ± 0.19

*Muscle obtained during an orthopaedic operation.

FIG. 1. A pen recording of membrane resting potential (upper trace) and miniature end-plate potentials in muscle taken from a patient suffering from exertional cramps (biopsy 10). Upper vertical scale 50 mV; lower vertical scale 1 mV; time scale 10 sec.

FIG. 2. A methylene blue squash preparation of a motor-point muscle biopsy showing the intramuscular nerve bundle and the distribution of motor end-plates.

into the muscle fibre, but in nearly every case it had recovered and assumed a relatively constant level after about one minute. For this reason, the measurement of MEPP frequency and amplitude was delayed until after the first minute. The figures obtained for the mean MEPP frequency of individual biopsies varied from $0.08 \pm 0.04/\text{sec}$ in a case of myasthenia gravis (biopsy 4) to $0.41 \pm 0.17/\text{sec}$ for the biopsy from the affected arm of the hemiparetic patient (biopsy 1a). The highest sustained frequency found for any fibre, $0.90/\text{sec}$, was recorded from 'normal' muscle (biopsy 1b; from the clinically unaffected arm of the hemiparetic patient), although in two fibres from a case of dystrophia myotonica (biopsy 7) frequencies of over $3/\text{sec}$ were recorded in the first minute. One of these subsided subsequently to a relatively steady $0.3/\text{sec}$; the other fibre was lost after about one minute and the figure for this fibre was therefore rejected.

The amplitude of the recorded MEPPs varied from about $50 \mu\text{V}$, the smallest which could be resolved on the pen recorder, to about 2 mV . In the two biopsies from myasthenic patients (biopsies 3 and 4) the distribution of MEPP amplitudes suggested that possibly some MEPPs were not recorded because they were too small to be resolved. The values obtained for mean MEPP amplitude in these two cases, $0.16 \pm 0.02 \text{ mV}$ and $0.16 \pm 0.06 \text{ mV}$, were the lowest recorded, the highest mean value being $0.70 \pm 0.18 \text{ mV}$ for a young patient with generalized weakness, suggestive of myasthenia gravis (Fig. 3).

The data on the paretic muscle from the patient with a meningioma (biopsy 1a) were difficult to

evaluate because recordings were made within 0.5 cm of where the muscle was tied. However, the findings on the other biopsies revealed the variation of MEPP frequency and amplitude to be highly significant (for both, $P < 0.001$). The variation of membrane resting potentials was not significant ($P > 0.20$).

DISCUSSION

The membrane resting potentials recorded in this study are in general somewhat lower than those reported for normal human limb muscle *in vivo* (for example, Creutzfeldt, Abbot, Fowler, and Pearson (1963) $87.4 \pm 8.9 \text{ mV}$; Goodgold and Eberstein (1966) $77.5 \pm 11.3 \text{ mV}$; McComas, Mrozek, and Bradley (1968) $83.6 \pm 7.8 \text{ mV}$). This discrepancy may be no more than a reflection of the different experimental methods employed, for it is known that the resting potential of normal mouse muscle is significantly lower *in vitro* than *in vivo* (McComas and Mossaw, 1965). Whatever the effect of maintaining the muscle in isolation there is no indication that the resting potentials of any of the biopsies were altered by disease. A reduction in membrane resting potential has been reported in dystrophia myotonica (Hofmann, Alston, and Rowe, 1967; McComas and Mrozek, 1968), but the biopsies from the two patients with this disease included in the present study were distinguished only by the bursts of spontaneous action potentials which were recorded from a few fibres. Clearly small differences in resting potential as a result of pathological changes might have been obscured by other sources of variance.

FIG. 3. Pen recordings of miniature end-plate potentials from (1) clinically normal muscle (biopsy 1b), (2) muscle from a patient with myasthenia gravis (biopsy 3), (3) muscle from a patient with generalized weakness (biopsy 8). Vertical scale for each recording 1 mV . (NB: the gain is increased in the second trace); time scale 30 sec (same for all traces).

MEPPs in isolated limb muscle would appear to be smaller and somewhat more frequent than those recorded from intercostal muscle taken from patients without evidence of neuromuscular disease (see Elmquist *et al.*, 1964). Such differences may be characteristic of these particular muscles or again they could result from variations in the experimental conditions under which the recordings were made. There is some evidence (unpublished data) that normal female rectus abdominis muscle obtained during abdominal operations and maintained at room temperature may have a very low MEPP frequency (mean frequency below 0.1/sec), indicating that MEPP frequency might be lower in muscles which are not normally very active. Unfortunately, the findings on paretic limb muscle (biopsy 1a), which showed a relatively high MEPP frequency, do not support this hypothesis.

Elmqvist and co-workers (1964) compared the sub-threshold end-plate activity of 'normal' intercostal muscle with that of muscle obtained from patients with myasthenia gravis. They found the frequency of MEPPs to be the same in both cases (about 0.2/sec), but the MEPP amplitude in myasthenic muscle (mean 0.2 mV) was much smaller than that in the controls (mean 0.98 mV). The two biopsies from myasthenic patients examined here showed similar very small amplitude MEPPs (biopsy 3, mean 0.16 ± 0.02 mV; biopsy 4, mean 0.16 ± 0.06 mV) and, in one, these were very much reduced in frequency (biopsy 4, mean 0.08 ± 0.4 /sec). In this last case the disease followed a rapid and fatal course, suggesting that, whereas small amplitude MEPPs may be a feature common to all myasthenic muscle, a reduced MEPP frequency may be particularly associated with a poor prognosis.

The frequency of MEPPs in a biopsy from a patient with facioscapulohumeral dystrophy (biopsy 5) was considerably lower than in the clinically normal limb muscle, although there was no detectable shift in the mean MEPP amplitude. If this low MEPP frequency was brought about by the disease process, the mechanism is not immediately obvious, but it might be an indirect effect, perhaps a result of disuse of the affected muscles. A more direct effect of the disease upon the motor nerve terminal, even though the sensitivity of the post-junctional membrane was unaltered, could interfere with neuromuscular transmission, so that the muscle weakness in this case might not result solely from the influence of the disease upon the contractile elements of the muscle. MEPPs of low frequency, however, are not a feature of dystrophic muscle in general, for the frequency and amplitude of those recorded in muscle taken from patients with dystrophia myotonica (biopsies 6 and 7) were not

obviously 'abnormal'. It has been reported that intercostal muscle biopsies from patients with dystrophia myotonica also failed to reveal any gross changes in the sub-threshold activity of the neuromuscular junction (Hofmann *et al.*, 1966).

Albuquerque and McIsaac (1970) have shown that after section of the motor nerve to the extensor digitorum longus muscle in rats, changes in MEPP frequency occurred after 10 hours and total failure of spontaneous transmitter release after 24 hours. Before complete failure of transmitter release, MEPPs sometimes appeared in small bursts and in most of these fibres there was also a reduction in MEPP amplitude. It is questionable whether the effects of nerve section can be usefully related to those resulting from denervating disease, especially as some neuropathies may develop rapidly over a few days, while others progress slowly over several decades. None the less it would be very surprising if neurogenic disease was not accompanied by a change in MEPP frequency and, in atrophic muscle, by a shift in mean MEPP amplitude. In fact, in the biopsy from the patient with Guillain-Barré syndrome (biopsy 8) and that from the patient with peroneal muscular atrophy (biopsy 9), examples of both acute and chronic neuropathies, the MEPP frequency and amplitude were marginally lower than in clinically normal muscle. This situation might arise if the motor neurones were affected selectively and at some junctions the spontaneous transmitter release had failed completely, but it is doubtful whether a structural degeneration of the motor nerve terminal could occur sufficiently rapidly to account for such a sudden loss of activity as this would require. The inhibition of synthesis or release of transmitter would appear to be a necessary precursor to gross structural changes if this hypothesis is to be entertained.

The possible presence of newly formed nerve terminals as a result of collateral reinnervation (see Morris, 1969) could bring about a shift in mean MEPP frequency or amplitude in some neuropathies. Such collateral reinnervation might be detectable by examination of the distribution of MEPP amplitude in single muscle fibres.

Two of the patients for whom no diagnosis was reached suffered only mild symptoms; in one case exertional cramp (biopsy 10) and in the other slight generalized weakness (biopsy 11). The patient with muscle pain was severely debilitated (biopsy 12). The mean MEPP amplitude in muscle from the young patient with generalized weakness (biopsy 11) was the highest recorded for any of the biopsies, but the small diameter of muscle fibres in this biopsy (mean fibre diameter about $30 \mu\text{m}$; cf. adult palmaris longus, mean diameter about $60 \mu\text{m}$) may alone be

sufficient to account for this (see Katz and Thesleff, 1957). Clearly the findings contradict the suggestion that this patient could be myasthenic. In both the other biopsies (10 and 12) the mean MEPP frequency was lower than in the 'normal' muscles, but the histochemical and histological studies failed to reveal any evidence of neuromuscular disease.

It is the intercostal muscle biopsy which has so far provided a major contribution to our understanding of the electrophysiology of diseased muscle. Unfortunately, in most patients with symptoms of neuromuscular disease the involvement of intercostal muscle, even if it is suspected, may be difficult to demonstrate, and where this muscle is more obviously affected during the advanced stages of some diseases the diagnostic value of muscle biopsy is doubtful. However, the motor-point muscle biopsy is already established as a diagnostic aid and the present study has demonstrated the viability of such biopsies *in vitro*. There can be little doubt, therefore, that further electrophysiological investigations of the motor-point muscle biopsy could add considerably to our knowledge of neuromuscular disease without adding to the discomfort of the patient.

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