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Cultured muscle from myotonic muscular dystrophy patients: Altered membrane electrical properties

(tissue culture/electrophysiology)

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ABSTRACT **Myotonic muscular dystrophy (MyD) is an inherited human disease involving skeletal muscle as well as many other organ systems. We have approached the study of this disorder by growing normal and diseased human muscle in a primary tissue culture system and investigating some of the electrical properties of the resulting myotubes. The most distinctive abnormality noted in MyD myotubes was an increased tendency to fire repetitive action potentials. A decreased action potential afterhyperpolarization amplitude and the presence of depolarizing afterpotentials were also noted, as were a decreased resting membrane potential, decreased action potential amplitude and overshoot, and decreased outward-going rectification. Although the ionic basis of these abnormal properties *in vitro* is not clearly defined, changes in the slow outward-going potassium current offer the best explanation. Furthermore, MyD cell culture offers a valuable model for critical analysis of the molecular mechanisms underlying MyD deficits.**

Myotonic muscular dystrophy (MyD) is an inherited human disease with muscle wasting, weakness, and stiffness as prominent clinical manifestations. Other systems may also be involved, and patients may experience cardiovascular, ophthalmologic, endocrine, and gastrointestinal problems. The primary metabolic defect in MyD is not known.

One of the clinical hallmarks of this disorder is myotonia, which is manifested as stiffness and difficulty in relaxing a muscle after voluntary contraction (1). Electromyographically, myotonia is characterized by repetitive bursts of activity, which increase and decrease in frequency (2). Myotonia is seen not only in MyD but also in other syndromes such as human myotonia congenita (3, 4), which resembles myotonia in goats and appears to be related to a decrease in muscle membrane chloride conductance (5, 6). However, a decreased chloride conductance has not been demonstrated in MyD and the cause of myotonia in MyD remains undetermined.

Recent studies of erythrocytes (7, 8) and muscle (9) from patients with MyD suggest abnormalities in the surface membranes of different tissues. In biopsied MyD muscle fibers, these membrane abnormalities are expressed as a decreased resting membrane potential (4, 10–12) and an increased tendency to fire repetitive action potentials (11). The presence of denervation, degeneration, fat and connective tissue infiltration, and the decreased viability of biopsied tissue limit the possible electrophysiological analyses.

We have approached the study of human muscle disease by growing normal and diseased human muscle in a primary tissue culture system. Tissue culture has many advantages over the intact muscle preparation, but it has a major difficulty in that the abnormality underlying the disease may not be propagated in culture. Many human neuromuscular diseases (oculocraniosomatic neuromuscular syndrome with ragged red fibers, acid-maltase deficiency, and unusual myopathy with "cabbage" bodies) can be reproduced in culture (13). However, other investigators, as well as ourselves, have found no consistent morphological abnormalities in cultured dystrophic muscle (14, 15).

This paper demonstrates that MyD muscle grown in culture possess abnormalities in some fundamental electrical proper-

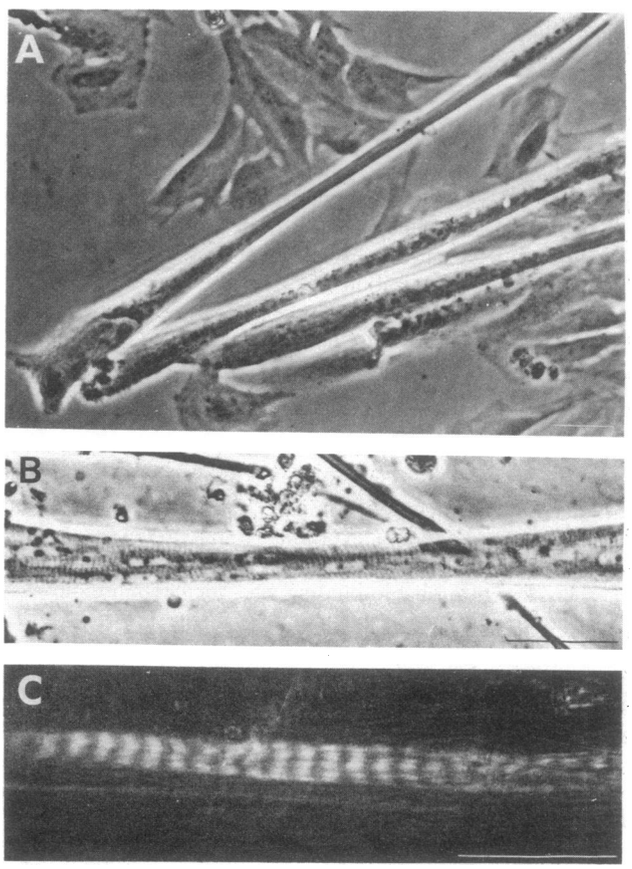

FIG. 1. Photomicrographs of control human myotubes 3–4 weeks in culture, showing variations in morphology. Myotubes had a diameter of 10–20 μm and variable length (50 μm to over 1000 μm) and often underwent extensive bifurcation. No differences in morphology or rate of cell fusion have been detected in cultured myotubes. (*A*) Low-power phase-contrast photomicrograph showing variations in morphology of myotubes. (*B*) High-power phase-contrast photomicrograph of portion of myotube showing striations and peripheral nuclei (with nucleoli). (*C*) High-power polarized-light photomicrograph of portion of myotube showing birefringence. All calibration bars are 50 μm.

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Abbreviations: MyD, myotonic muscular dystrophy; RP, resting potential; AP, action potential; DAP, depolarizing afterpotential.

ties. These abnormalities are remarkably similar to those observed in muscle fibers from biopsied specimens. A preliminary report of this work has appeared in abstract form (16).

METHODS

Muscle biopsies were obtained from the vastus lateralis muscle with the consent of control patients or patients with electromyographic evidence of myotonia and clinical features of MyD. These studies were carried out between February and November 1979. The cultures were prepared from biopsy specimens by trypsin dissociation using a modification of a technique described by Yasin (17). Well-developed myotubes were grown from both control and MyD biopsy specimens after 3–5 weeks in culture (Fig. 1) We have observed no morphological differences in the rate of development, maturity (e.g., appearance of striations), or size of control and MyD myotubes at the light microscope level.

Individual cultures were studied 3–5 weeks after plating, using standard electrophysiological techniques. The Ringer's solution contained 148 mM NaCl, 4.5 mM KCl, 2.0 mM $CaCl_2$, 1.0 mM $MgCl_2$, 10.0 mM glucose, 0.44 mM NaH_2PO_4, and 12.0 mM $NaHCO_3$ and was bubbled with 5% CO_2 to maintain a pH of 7.2. All experiments were performed at room temperature, 20 ± 1°C, to ensure constant temperature of cultures and perfusing solutions and to prolong the life of the cultures.

Microelectrodes had a tip diameter of 1 μm or less and were filled with 3 M KCl, producing a dc resistance of 30–50 MΩ. Myotubes were impaled with single microelectrodes and demonstrated stable resting potentials and stable high input resistance (usually greater than 50 MΩ), suggesting that the myotubes were not damaged by penetration. Due to their small size, myotubes could be penetrated routinely by only a single microelectrode. The membrane potential was simultaneously monitored during current injection by using a chopped current injection method (18). Measurements of properties that required membrane polarization, such as steady-state current voltage plots and effects of membrane polarization on action potential characteristics, were performed on "short" myotubes. Short myotubes have been defined by linear cable analysis, which has demonstrated that myotubes shorter than approximately 200 μm can be considered to be isopotential and uniformly polarized during current injection (unpublished observations).

RESULTS

Resting Potential (RP) and Action Potential (AP) Abnormalities. One of the most conspicuous abnormalities noted in MyD myotubes was an approximately 10-mV-decreased RP (Table 1). A previous study (10 controls and 6 MyD biopsies, not shown) also demonstrated an approximate 10-mV-decreased RP for MyD cultures (RP difference = 9.7 mV, $P < 0.0005$).

Both control and MyD myotubes generated all-or-none overshooting APs followed by an afterhyperpolarization when stimulated by a short depolarizing current pulse or by anode break excitation (unpublished data; cf. refs. 19 and 20). AP characteristics were examined at the myotube's normal RP by anode break stimulation with a hyperpolarizing pulse 50 ms in duration with an amplitude sufficient to evoke an all-or-none AP. It was not possible to study the AP characteristics at a standard membrane potential because the majority of myotubes were too long

Table 1. Average RP and AP characteristics of cultured myotubes

Biopsy specimen	*n*	RP, mV	Afterpotential, mV	*n*	AP amplitude, mV	Overshoot, mV	Half-amplitude duration, ms
Controls							
1	14	−46.9 ± 1.7	−2.9 ± 0.8	13	77.2 ± 2.2	30.9 ± 2.3	10.0 ± 0.6
2	22	−49.1 ± 1.4	−6.7 ± 2.0				
3	14	−58.3 ± 2.9	−4.4 ± 0.7	5	94.0 ± 3.0	33.2 ± 5.1	7.0
4	9	−54.8 ± 1.2	−6.1 ± 0.4				
5	34	−58.2 ± 1.8	−7.8 ± 0.9	22	97.1 ± 2.4	36.5 ± 1.3	8.8 ± 0.4
6	4	−54.5 ± 2.6	−7.4 ± 0.4	4	96.3 ± 4.9	43.7 ± 7.8	8.3 ± 0.5
7	12	−48.9 ± 1.3	−3.7 ± 0.6	12	86.2 ± 1.9	37.3 ± 1.7	11.8 ± 0.4
8	11	−51.6 ± 2.0	−6.8 ± 0.5	10	81.8 ± 2.8	31.3 ± 2.8	12.9 ± 0.4
Mean ± SEM and totals	120	−52.8 ± 1.5	−5.7 ± 0.6	66	88.3 ± 3.4	35.5 ± 2.0	9.8 ± 0.9
MyD							
1	16	−39.8 ± 2.8	−4.3 ± 0.8	19	64.7 ± 3.9	26.3 ± 2.3	7.6 ± 0.4
2	9	−35.0 ± 4.3	−4.0 ± 1.0	12	65.0 ± 3.5	26.8 ± 2.2	7.4 ± 0.5
3	15	−39.5 ± 1.7	−0.3 ± 1.2	16	76.3 ± 3.5	35.7 ± 3.0	8.3 ± 0.7
4	10	−46.9 ± 2.5	−3.7 ± 0.7	11	71.8 ± 4.2	24.8 ± 3.7	7.5 ± 0.4
5	10	−45.2 ± 1.8	−6.1 ± 1.1	24	74.0 ± 2.2	31.5 ± 2.0	7.1 ± 0.2
6	37	−43.4 ± 1.1	−1.0 ± 0.8	35	66.0 ± 2.2	22.9 ± 1.6	10.7 ± 0.7
7	47	−45.7 ± 1.1	−2.0 ± 0.8	43	83.1 ± 2.2	39.1 ± 1.4	9.7 ± 0.5
8	16	−34.2 ± 2.6	+2.0 ± 2.0				
9	17	−50.6 ± 2.7	+0.6 ± 2.1	4	86.3 ± 2.4	21.7 ± 7.8	8.5 ± 0.5
Mean ± SEM and totals	177	−42.3 ± 1.8	−2.1 ± 0.9	164	73.4 ± 2.9	28.6 ± 2.2	8.4 ± 0.4

AP amplitude, peak amplitude of AP measured with respect to the RP; afterpotential, maximal amplitude of AP afterpotential (hyper- or depolarizing) measured with respect to the RP; overshoot, AP amplitude expressed with respect to 0 mV; and half-amplitude duration, duration of AP measured at half AP amplitude. Measurements were made from 2–10 cultures prepared from each biopsy specimen and averaged, giving equal weight to each measurement value; ± indicates SEM. *n* is the total number of fibers sampled from each biopsy (*n* on the left refers to RP and afterpotential measurements and *n* on the right refers to the remaining AP measurements). APs were evoked from the myotube's normal RP with 50-ms hyperpolarizing pulses sufficiently large to evoke an all-or-none AP.

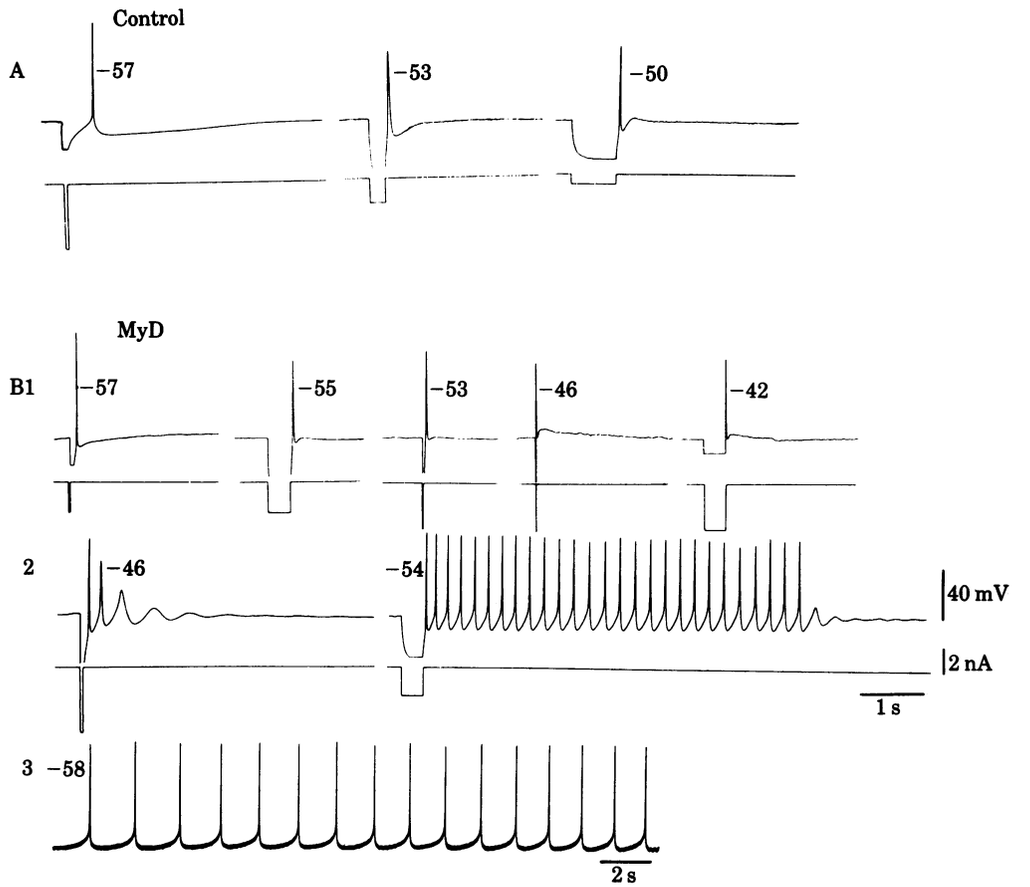

FIG. 2. Comparison of control (*A*) and MyD (*B*) activity in response to single anode break stimulus. Numbers associated with each trace refer to the RP of the cell under conditions of no current injection. The duration of the anode break stimulus was not found to affect the resulting firing activity. Control myotubes typically fired a single AP followed by an afterhyperpolarization of variable magnitude and duration. The activity resulting from stimulating MyD myotubes was much more variable than observed in control myotubes. Many MyD myotubes had depolarizing afterpotentials (DAPs, Fig. 2 *B1* and *B2*). Sometimes, MyD myotubes were seen to repetitively fire APs continuously (Fig. 2*B3*) with no stimulus. No correlation was seen between RP and repetitive firing. As can be seen, control and MyD myotubes with similar RPs often had very different repetitive firing and afterpotential characteristics.

to uniformly polarize with artificial current injection.

In MyD myotubes the AP amplitude was reduced by 14.9 mV ($P < 0.005$) and the AP overshoot was reduced by 6.9 mV ($P < 0.05$). No significant differences were detected in AP half-amplitude duration. The AP afterhyperpolarization amplitude of MyD myotubes was significantly less than the afterhyperpolarization amplitude of control myotubes (-5.7 ± 0.6 for control and -2.1 ± 0.9 mV for MyD, $P < 0.005$). Cells from two MyD biopsies possessed average positive afterpotentials (MyD patients 8 and 9). It is clear that measurements of both the amplitude of the AP and its afterhyperpolarization could be influenced by the decreased RP.

Specifically, the decreased RP of MyD myotubes could be expected to increase the amplitude of the afterhyperpolarization and decrease the amplitude of the AP. To study these effects, we depolarized the membrane potential of control "short" myotubes that were considered to be uniformly polarized during current injection. Depolarization from -55 mV to -35 mV increased the afterhyperpolarization amplitude by approximately 5 mV, decreased the AP amplitude by approximately 20 mV, but did not significantly affect the AP overshoot. Therefore, the reduction in afterpolarization amplitude of approximately 3.5 mV in MyD myotubes is probably an underestimate, and although the AP overshoot appears independent of RP, at least part of the decrease in AP of MyD myotubes may be due to the decreased RP.

Increased Repetitive Firing and Decreased Outward-Going Rectification Abnormalities. Another conspicuous difference between control and MyD myotubes in culture was the tendency of MyD myotubes to fire multiple APs upon stimulation (Fig. 2 *B2* and *B3*). Of the 360 control and 370 MyD myotubes examined, approximately 20% of the MyD myotubes and 2% of the control myotubes fired more than one AP when stimulated by anode break excitation. The increased repetitive firing behavior of MyD myotubes is not believed to be due primarily to their decreased RP because: (*i*) no correlation was seen between repetitive firing behavior and RP (compare frame 2 of Fig. 2*A* and frames 2 and 3 of Fig. 2*B1* with frame 2 of Fig. 2*B2*); and (*ii*) steady-state depolarization of the membrane potential of control or MyD myotubes with large RPs did not increase the likelihood of repetitive firing upon stimulation.

The AP afterpotential of control myotubes was typically hyperpolarizing (Fig. 2*A*), in contrast to the MyD afterpotential, which exhibited much more variability (Fig. 2*B*). Approximately 50% of the MyD myotubes exhibited a normal or reduced AP afterpotential that was hyperpolarizing. The remaining 50% of the MyD myotubes exhibited either a depolarizing afterpotential (DAP; e.g., Fig. 2*B1*, frames 4 and 5) or a small, short, afterhyperpolarization followed by a delayed DAP (e.g., Fig. 2*B1*, frames 2 and 3). Myotubes that fired multiple APs when stimulated (Fig. 2 *B2* and *B3*) had conspicuous DAPs.

MyD myotubes that did not fire multiple APs in response to

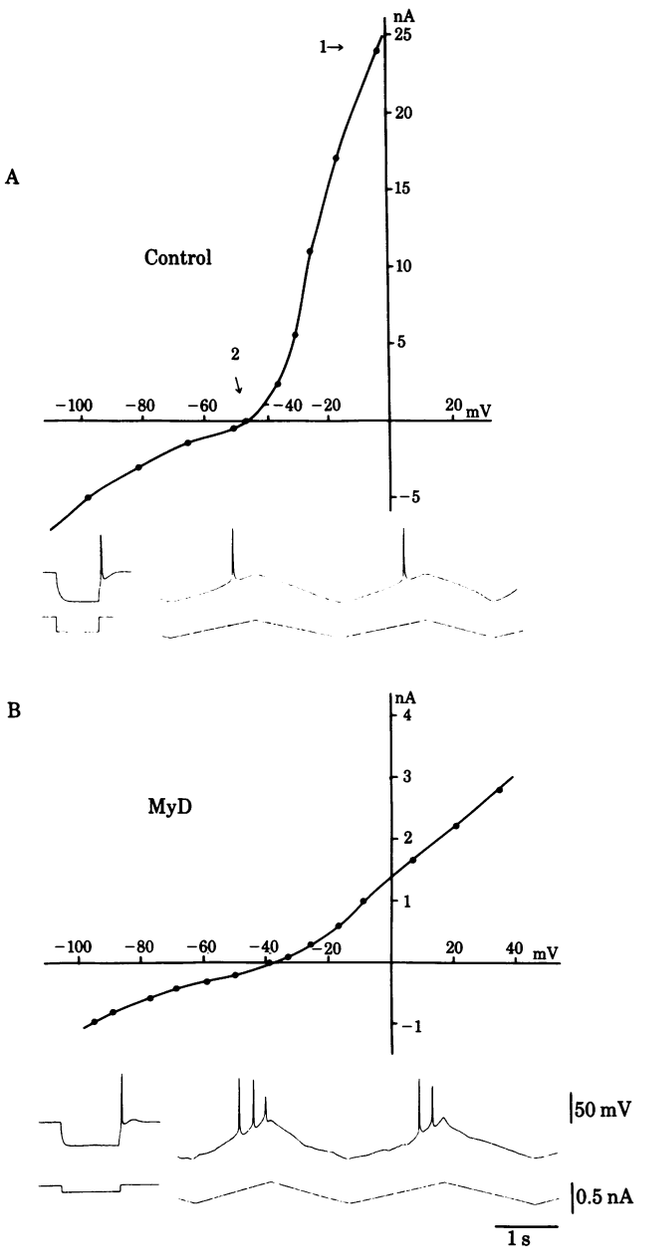

FIG. 3. Comparison of steady-state current–voltage (*I–V*) plots and repetitive firing behavior of control and MyD myotubes. Both myotubes were short (approximately 200 μm) with similar diameters (approximately 25 μm). (*A*) Upper, representative steady-state *I–V* plot from control myotube. The steady-state voltage response (abscissa) is plotted versus the magnitude of a long (approximately 1 s) rectangular current pulse (ordinate) injected into the myotube. Inward-going rectification is seen in the membrane potential region near RP (arrow 2) and well-developed outward-going rectification (arrow 1) is seen at depolarized membrane potentials (RP = −45 mV). Lower, a single AP is elicited by anode break stimulation (left) as well as by the depolarizing phase of a slowly varying current ramp (right). The ramp was adjusted so that its most positive phase reached the myotube's normal RP. Upper traces, membrane potential; lower traces, injected current. (*B*) Upper, representative steady-state *I–V* plot of MyD myotube. The MyD *I–V* plot shows a region of inward-going rectification compared to control myotube *I–V* plots (RP = −39 mV). Lower, anode break stimulation of a MyD myotube elicits a single AP (left) but a slowly varying current ramp injected into the same myotube elicits multiple APs (right).

anode break stimulation could often be made to fire repetitively in response to a slowly depolarizing current ramp (Fig. 3*B*). A ramp frequency of 0.3 Hz was most effective in producing repetitive firing activity. Approximately 30% of the MyD myotubes and 10% of the control myotubes that fired a single AP with anode break stimulation could be made to fire two or more APs with the ramp test. The ramp test was found to be particularly effective in producing multiple AP firing in MyD myotubes that demonstrated a reduced afterhyperpolarization or a DAP with anode break stimulation.

The relationship between afterpotential amplitude and repetitive firing behavior was further examined in MyD myotubes by correlating the number of APs evoked during the ramp test and the peak amplitude of the hyper- or depolarizing afterpotential after a single AP (evoked by anode break stimulation). This analysis demonstrated a highly statistically significant correlation between the number of APs and the afterpotential amplitude (correlation coefficient = 0.60, $P < 0.01$ and $n = 40$ myotubes). It is expected that this correlation coefficient is an underestimate because myotubes with afterhyperpolarizations greater than −2 mV usually fired only a single AP with the ramp test, which would have the effect of decreasing this correlation.

Steady-state current–voltage (*I–V*) plots of control and MyD fibers were compared in order to examine the slow current processes that are expected to underlie the repetitive firing behavior seen in many MyD myotubes. Control steady-state *I–V* plots typically exhibited a region of inward-going rectification at membrane potentials slightly depolarized from rest and well-developed outward-going rectification at more depolarized membrane potentials (Fig. 3*A*). Control steady-state *I–V* plots were similar to one another, whereas MyD *I–V* plots exhibited considerable variability. Approximately one-fourth of the MyD *I–V* plots were indistinguishable from control plots; however, the majority of MyD *I–V* plots exhibited significantly decreased outward-going rectification (Fig. 3*B*). The outward-going rectification properties of individual control and MyD myotube *I–V* plots were estimated by measuring the increase in slope conductance between the two standard membrane potentials of −50 and 0 mV. The slope conductance ratio, therefore, provides a quantitative measure of the degree of outward-going rectification of an *I–V* plot (measured at −50 mV). This procedure to quantify the outward-going rectification in muscle fiber steady-state *I–V* plots has also been used by other investigators (21). The slope conductance ratio of MyD *I–V* plots (2.3 ± 0.3, $n = 13$, *I–V* plots) was found to be significantly ($P < 0.001$) less than the slope conductance ratio of control *I–V* plots (5.0 ± 0.7, $n = 11$, *I–V* plots). Thus, *I–V* plots obtained with MyD myotubes have approximately half the slope conductance ratio of *I–V* plots obtained with control myotubes.

DISCUSSION

These studies have demonstrated abnormalities in some of the fundamental electrical properties of cultured muscle from MyD patients. The abnormalities in electrical properties include: (*i*) decreased average RP (*ii*) decreased AP amplitude and overshoot; (*iii*) decreased AP afterhyperpolarization amplitude; (*iv*) decreased outward-going rectification; and (*v*) increased tendency to fire repetitive APs. These abnormalities in cultured muscle are remarkably similar to abnormalities observed in intact muscle studied immediately after biopsy. Several investigators have noted a decreased RP in intact MyD muscle (4, 10–12), and Gruener (11) reported that intact MyD muscle fibers have a decreased overshoot and fire trains of APs in response to the injection of a depolarizing current ramp unlike control muscle fibers. Thus, although the expression of the MyD defect inculture may be influenced by the culturing process itself, the physiological abnormalities demonstrated in cultured MyD myotubes suggests similarities of the intact fiber and tissue culture systems.

Of the five changes noted above, the tendency to fire repetitive action potentials would appear to be the most significant, because myotonia is the hallmark of skeletal muscle in this disorder *in vivo*. Of the other abnormalities in electrical properties, the decreased AP afterhyperpolarization amplitude may well be of primary importance, and the decreased RP may be of secondary importance. The secondary importance of the RP is pointed out by its lack of correlation with repetitive firing and the observation that depolarizing MyD or control myotubes does not increase their tendency to fire repetitive APs.

The increased tendency of MyD myotubes to fire repetitive APs is a reflection of the hyperexcitability characteristic of adult MyD muscle and appears to be closely associated with the properties of the AP afterpotential. Many MyD myotubes exhibited DAPs, which were particularly pronounced in those myotubes that repetitively fired, in response to either a single stimulus or a slowly depolarizing ramp. Such DAPs are indicative of nerve and muscle preparations that fire repetitive APs and have been observed in systems such as spinal motoneurons (22, 23), hippocampal neurons (24), molluskan neurons (25), and lobster stretch receptors (26).

The AP afterhyperpolarization typical of control myotubes (and some MyD myotubes) would be expected to repolarize the membrane potential below the RP, preventing a new AP from being evoked. However, a DAP after an AP would prevent the membrane potential from hyperpolarizing to the RP and would provide a sufficiently large depolarizing current to evoke a new AP, producing repetitive firing.

Either an increase in a slow time course inward current or a decrease in a slow time course outward current can be expected to underlie the depolarizing afterpotential. Of the two possibilities, we postulate that a defect in slow outward K^+ current is involved, because the defective MyD membrane properties such as AP afterhyperpolarization and outward-going rectification are most likely dependent on slow K^+ currents. This decrease in slow K^+ outward current could be due either to a K^+ conductance abnormality or to a decrease in K^+ equilibrium potential. The decreased RP could also be explained by a defective K^+ conductance, defective K^+ equilibrium potential, or both, especially because the resting permeability of the control myotubes is primarily dependent upon K^+ (Na^+ permeability to K^+ permeability ratio = 0.14 ± 0.06, unpublished observations).

Although a defect in K^+ permeability and associated currents could cause all of the observed MyD abnormalities, alterations in other membrane currents could also explain the defects. For example, a defect causing delayed Na^+ current inactivation is known to be capable of reducing AP afterhyperpolarization amplitude and producing repetitive firing (27, 28). In order to distinguish between alterations in the K^+ and Na^+ currents it will be necessary to understand the defects in terms of the amplitude of the currents as well as their kinetics, especially their inactivation kinetics.

Regardless which specific current represents the primary defect, our demonstration of altered electrical properties provides cogent evidence that functional abnormalities characteristic of MyD can be expressed in isolated MyD myotubes grown *in vitro*. Additionally, these studies support the usefulness of cell culture as a valuable model for critical analyses of the molecular mechanisms underlying MyD deficits.

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1. Appel, S. H. & Roses, A. D. (1978) in *The Metabolic Basis of Inherited Disease*, eds. Stanbury, J. B., Wyngaarden, J. B. & Frederickson, D. S. (McGraw-Hill, New York), pp. 1260–1281.
2. Bucthal, F. (1976) in *Pathogenesis of Human Muscular Dystrophies*, ed. Rowland, L. P. (Excerpta Medica, Amsterdam), pp. 203–218.
3. Lipicky, R. J. (1977) in *Pathogenesis of Human Muscular Dystrophies*, ed. Rowland, L. P. (Excerpta Medica, Amsterdam), pp. 747–758.
4. McComas, A. & Mrozek, K. (1968) *J. Neurol. Neurosurg. Psychiatry* **31**, 441–447.
5. Lipicky, R. J. & Bryant, S. H. (1966) *J. Gen. Physiol.* **50**, 89–111.
6. Lipicky, R. J., Bryant, S. H. & Salmon, J. H. (1971) *J. Clin. Invest.* **50**, 2091–2103.
7. Miller, S. E., Roses, A. D. & Appel, S. H. (1976) *Arch. Neurol.* **33**, 172–174.
8. Plishker, G., Gitelman, G. & Appel, S. H. (1978) *Science* **200**, 323–324.
9. Schotland, P. L., Bonnila, E. & Wakayama, T. (1980) *Muscle Nerve* **3**, 21–27.
10. Gruener, R. (1977) in *Pathogenesis of Human Muscular Dystrophies*, ed. Rowland, L. P. (Excerpta Medica, Amsterdam), pp. 242–258.
11. Gruener, R. (1979) *Muscle Nerve* **2**, 165–172.
12. Hoffman, W., Alston, W. & Rowe, F. (1966) *Electroencephalogr. Clin. Neurophysiol.* **21**, 521–537.
13. Askansas, V. & Engel, W. K. (1976) in *Pathogenesis of Human Muscular Dystrophies*, ed. Rowland, L. P. (Excerpta Medica, Amsterdam), pp. 856–871.
14. Geiger, R. S. & Garwin, J. R. (1975) *J. Neuropathol. Exp. Neurol.* **16**, 532–543.
15. Witkowski, J. A., Durbridge, M. & Dubowitz, V. (1976) *In Vitro* **12**, 98–106.
16. Merickel, M., Gray, R., Chauvin, P. & Appel, S. H. (1979) *Soc. Neurosci. Abstr.* **5**, 378 (abstr.).
17. Yasin, R., Van Bars, G., Nurse, K., Al-Ami, S., Landon, D. & Thompson, E. (1977) *J. Neurol. Sci.* **32**, 347–360.
18. Merickel, M. (1980) *J. Neurosci. Methods* **2**, 87–96.
19. Fishbach, G. D., Nameroff, M. & Nelson, P. G. (1970) *J. Cell Physiol.* **78**, 289–300.
20. Merickel, M. & Gray, R. (1979) *Biophys. J.* **25(2)**, 200a.
21. Lorkovic, H. (1979) *Pflügers Arch.* **379**, 89–93.
22. Calvin, W. H. (1973) in *Control of Posture and Locomotion*, eds. Stein, R. B., Pearson, K. E., Smith, R. S. & Redford, J. B. (Plenum, New York), pp. 173–178.
23. Nelson, P. G. & Burke, R. E. (1967) *Exp. Neurol.* **17**, 16–26.
24. Kandel, E. R. & Spencer, W. A. (1961) *J. Neurophysiol.* **24**, 243–259.
25. Thompson, S. (1976) *J. Neurophysiol.* **39**, 253–161.
26. Grampp, W. (1966) *Acta Physiol. Scand.* **67**, 116–126.
27. Tasaki, I. & Takenaka, T. (1964) *Proc. Natl. Acad. Sci. USA* **52**, 804–810.
28. Armstrong, C. M., Bezanilla, F. & Rojas, E. (1973) *J. Gen. Physiol.* **62**, 375–391.