Calcium uptake and bioelectrical activity of denervated and myotonic muscle

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SUMMARY Calcium uptake on muscle microsomal fraction has been investigated in connection with bioelectrical activity in some muscle diseases. The findings showed a significant increase of calcium uptake in denervated muscle, which exhibited spontaneous bioelectrical activity (fibrillations). In myotonias, a low calcium uptake was peculiar to Steinert’s disease but not to myotonia congenita. In other muscle diseases, such as progressive muscular dystrophy (Duchenne’s type) or Charcot-Marie-Tooth’s disease, the ability of muscle microsomal fraction to bind calcium was not changed. Starting with the key role of calcium in excitation-contraction coupling, the implications of calcium uptake disturbances in muscle electrogenesis are discussed.

The role of calcium ions in muscle function is now an irrefutable fact, but few studies have been made of its role in muscle diseases (Kuhn and Stein, 1966; Samaha, Schroeder, Rebeiz, and Adams, 1967; Radu, Pendefunda, Blücher, Radu, Darko, and Gödri, 1969).

In the present study we explored the relations between spontaneous and myotonic activity in the electromyogram and uptake of calcium, as a metabolic parameter, and some quantitative histo-enzymological aspects.

MATERIAL AND METHODS

Table 1 summarizes the diagnoses in the case material. All the biopsies were obtained from deltoid and tibialis anterior muscles.

<table>
<thead>
<tr>
<th>TABLE 1</th>
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<tr>
<td>CALCIUM-UPTAKE AND T1/T2 RATIO (MEAN VALUES)</td>
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</table>

<table>
<thead>
<tr>
<th>Group</th>
<th>Cases (no.)</th>
<th>Ca uptake (μM/mg hr -37°C)</th>
<th>T1</th>
<th>T2</th>
<th>T1/T2 ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Fibrillations</td>
<td></td>
<td></td>
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<td></td>
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</tr>
<tr>
<td>Kugelberg-Welander</td>
<td>6</td>
<td>5.14 ± 0.44* Not estimated</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Amyotrophic lateral</td>
<td>3</td>
<td></td>
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<td></td>
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<tr>
<td>2. Normal</td>
<td></td>
<td></td>
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<tr>
<td>Duchenne’s muscular</td>
<td>7</td>
<td>3.84 ± 0.41 0.94 ± 0.11</td>
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<td></td>
</tr>
<tr>
<td>dystrophy</td>
<td>10</td>
<td>4.39 ± 0.52 Not estimated</td>
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<tr>
<td>Charcot-Marie-Tooth</td>
<td>5</td>
<td>3.92 ± 0.21 Not estimated</td>
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<tr>
<td>Myositis ossificans</td>
<td>1</td>
<td>3.61 Not estimated</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3. Myotonias</td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Steinert</td>
<td>7</td>
<td>2.73 ± 0.31† 2.09 ± 0.28†</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Thomsen</td>
<td>5</td>
<td>4.11 ± 0.49 0.38 ± 0.08†</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Debré-Semelaigne</td>
<td>1</td>
<td>3.52 0.88</td>
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* = P < 0.05; † = P < 0.01 calculated against normal.

The calcium uptake in the microsomal fraction of muscle homogenate was investigated by the method of Brody (1966). In brief, the muscle sample was homogenized in four volumes of Tris-maleate (5 mM) buffered solution of sucrose (250 mM), pH 7.4, at 4°C. The mitochondria and the myofibrils were sedimented by centrifugation at 9,000 g for 30 minutes. The supernatant was subsequently centrifuged at 30,000 g for 90 minutes and the microsomal fraction was resuspended in the sucrose solution. Immediately a 0.03 ml microsomal suspension was incubated with a solution containing potassium oxalate (2 mM), potassium chloride (150 mM), ATP (2 mM), and calcium chloride (50CaCl2, 0.1 mM), with a final volume of 4 ml, for 60 minutes at 37°C. After incubation the mixture was filtered through a very fine filter. The filter was rinsed in four successive stages with 10 ml buffered sucrose and the persistent radioactivity was measured in a scintillation counter. The results (μM) was referred to 1 mg birette protein.

From the same muscle samples the ratio between type I(T1) and type II(T2) muscle fibres was investigated, using succinic dehydrogenase as ‘marker’ for T1 and phosphorylase as sarcoplasmic enzyme (T2).

In the symmetrical muscle of the other side electromyography (EMG) was recorded for 40 minutes on magnetic tape. The fibrillation and myotonic discharges were selected and photographed with a three channel oscillograph. To improve relaxation a dose of Flaxedil 0.5 mg/kg was given occasionally in some patients.

RESULTS

For electromyographic reasons the subjects were subdivided into three groups:

Group 1: cases presenting fibrillation potentials (Fig. 1);
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**FIG. 1.** Electromyography: fibrillations (Kugelberg-Welander's disease).

**FIG. 2.** Electromyography: repetitive activity of 'myotonic equivalence' type (Thomsen's disease).

**FIG. 3.** Electromyography: 'myotonic bursts' (Steinert's disease); $\int$: integrated EMG.
Group 2: cases without spontaneous bioelectrical activity or myotonia, including also the normal subjects.

Group 3: cases with myotonia (Figs. 2 and 3).

In the first group the mean value of the calcium uptake was significantly increased ($P < 0.05$). In all cases, with one exception, the amount of calcium binding was higher than the normal mean value and in 66% of cases it was greater than the upper limit of normal (Fig. 4, Table 1).

The cases classified in the third group showed two different tendencies: in dystrophia myotonica the uptake of calcium was generally decreased while this parameter remained near the normal limits in the patients with Thomsen's disease (myotonia congenita).

In other cases of muscle disorders in which spontaneous bioelectrical activity or myotonia was not found (group 2), the values of calcium uptake were normal. However, three interesting exceptions must be stressed:

1. Case (a), an association of progressive muscular dystrophy with poliomyelitis with fibrillation and high uptake of calcium.
2. Case (b), a patient presenting progressive muscular dystrophy with very large pseudohypertrophy of calf muscles and short 'myotonic bursts' in the EMG had a subnormal calcium uptake.
3. Case (c), myositis ossificans: in this case the uptake of calcium was not changed in the microsomal fraction but was much increased in the myofibrillar fraction.

The histoenzymological findings did not indicate preferential atrophy of a particular fibre type and denervated muscle. If the large fibres seem to be of type II, the small fibres exhibit a general weak reactivity for both enzymes studied, with some variations from one specimen to another (Fig. 5a, b). An estimate of the ratio $T1/T2$ would have been uncertain.

Type I atrophy proved to be characteristic of
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myotonic dystrophy (Figs. 6a, b) but not of Thomsen's disease. The ratio between type I and type II muscle fibres was greater than unity in Steinert's disease but less than unity in myotonia congenita (Figs. 6a, b, 7).

DISCUSSION

The results of the present investigation indicate firstly that the denervated muscle which exhibits spontaneous bioelectrical activity also exhibits a significant increase in the amount of calcium binding in the microsomal fraction. Because the increased uptake of calcium was not found in other muscles chronically deprived of their nerve supply but without fibrillations on EMG, it may be suggested that the genesis of fibrillation must be related to a hyperactive metabolic mechanism for coupling the bioelectrical changes at membrane level with the contraction-relaxation induced myofibrillar activity.

Brody (1966), on whose work we have based our experiments, stressed the correlation between fibrillation activity and uptake of calcium, concluding that the sarcoplasmic reticulum in denervated muscle had enhanced release and binding of calcium and that this provides an explanation for fibrillation. It remains to be proved whether the basic disturbance is the persistent sequestration of the cation in the sarcoplasmic reticulum, as speculated by Howell, Fairhurst, and Jenden (1966), or the facilitation of the calcium carried to and from this

FIG. 6. Histoenzymology: (a) phosphorylase, (b) succinic dehydrogenase. (Steinert's disease.) (a) and (b) are complementary. The preferential atrophy of type I muscle fibres and their percentual preponderance are clear.

FIG. 7. Histoenzymology: (a) succinic dehydrogenase, preponderance of type II muscle fibres, (b) phosphorylase activity. (Thomsen's disease.)
features of myotonic dystrophy—namely, the preferential atrophy of type I fibres, which are much more sensitive to dystrophy than type II (Bajusz, 1965; Dubowitz, 1968)—could result from the associated dystrophy.

REFERENCES


