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 1

CALCIUM-UPTAKE AND $\frac{T_1}{T_2}$ RATIO (MEAN VALUES)			
Group	Cases (no.)	Ca uptake ($\mu\text{M}/\text{mg}/\text{hr } 37^\circ\text{C}$)	$\frac{T_1}{T_2}$ ratio
1. Fibrillations			
Kugelberg-Welander	6	5.14 $\pm$ 0.44*	Not estimated
Amyotrophic lateral sclerosis	3		
2. Normal	7	3.84 $\pm$ 0.41	0.94 $\pm$ 0.11
Duchenne's muscular dystrophy	10	4.39 $\pm$ 0.52	Not estimated
Charcot-Marie-Tooth	5	3.92 $\pm$ 0.21	Not estimated
Myositis ossificans	1	3.61	Not estimated
3. Myotonias			
Steinert	7	2.73 $\pm$ 0.31†	2.09 $\pm$ 0.28†
Thomsen	5	4.11 $\pm$ 0.49	0.38 $\pm$ 0.08†
Debré-Semelaigne	1	3.52	0.88

* = $P < 0.05$; † = $P < 0.01$ 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 ($^{45}\text{CaCl}_2$, 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 1.0 mg burette 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);

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.

FIG. 4. The distribution of the individual calcium uptake values.

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 in 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

(a)

(b)

FIG. 5. Histoenzymology: (a) phosphorylase, (b) succinic dehydrogenase. (Amyotrophic lateral sclerosis.) (a) and (b) are complementary. The reciprocal activity is manifest in the large fibres only, the small fibres exhibiting weak activity for both enzymes.

FIG. 6. Histochemistry: (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.

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. 7. Histochemistry: (a) succinic dehydrogenase, preponderance of type II muscle fibres, (b) phosphorylase activity. (Thomsen's disease.)

structure. Nevertheless, the hypothetical hypersensitivity of this sarcoplasmic reticulum packed with calcium may be correlated with the electrophysiological data of Lenman (1965) and McComas and Mrózek (1967) concerning the significant reduction of the resting potential in denervated muscle, and also to the findings of Nastuk and Liu (1966) relating to changes in chemosensitivity of muscle postjunctional membrane produced by calcium. In addition Wechsler (1966) showed that the sarcoplasmic reticulum in human denervated muscle remains relatively preserved morphologically.

A disturbance in the function of relaxing factor could be *a priori* incriminated in myotonia. The findings of Kuhn and Stein (1966), Samaha *et al.* (1967), and Radu *et al.* (1969) support this hypothesis; particularly for Steinert's disease. The last group of authors think that the ATP-ase/Ca-uptake ratio, illustrating two features of relaxing factor, are likely to be the best criteria of the myotonic state, the more so as this ratio is directly proportional to the relaxation time of myotonic muscle (Radu, unpublished data).

It is interesting that the decreased calcium uptake more frequently corresponds to 'myotonic bursts', while the normal values found in Thomsen's disease are associated only with an atypical repetitive activity (called by us 'myotonic equivalence') in the EMG. This intriguing observation must be related to the findings with microelectrodes showing a decrease of the resting potential in Steinert's disease (McComas and Mrózek, 1969), but not in Thomsen's disease (Riecker, Dobbelsstein, Röhl, and Bolte, 1964; McComas and Mrózek, 1969), to the preferential atrophy of type I fibres peculiar also to myotonic dystrophy (Engel and Brooke, 1966; Brooke and Engel, 1969; Radu *et al.*, 1969) and to the lesions of the sarcoplasmic reticulum recognized by electron microscopy in the same disease by Monticone and Gabella (1967). The increased percentage of type I muscle fibres in dystrophia myotonica (Engel and Brooke, 1966; Radu *et al.*, 1969) must also be taken into account and the relative reduction of relaxing activity of the microsomal fraction originating in this fibre type as compared with the same fraction from type II (Takauji, Yamamoto, and Nagai, 1967). The consequences of this preferential lesion on the relaxing phase are not difficult to predict.

Possibly some of the peculiar morphometabolic 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.

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