EFFECTS OF LONG-TERM ELECTRICAL STIMULATION ON SARCOPLASMIC RETICULUM OF FAST RABBIT MUSCLE

B. U. RAMIREZ* and D. PETTE
Fachbereich Biologie der Universität Konstanz, Germany

Received 8 October 1974

1. Introduction

Long-term stimulation of the nerve innervating a fast muscle with the frequency pattern resembling that of a motoneuron to a slow muscle has been shown to produce marked changes in the activity pattern of enzymes of energy-supplying metabolism, the isozyme pattern of lactate dehydrogenase and some histochemical characteristics [1–3]. These changes reflect a transformation of the fast into a slower muscle. This transformation is emphasized by corresponding changes in the contractile parameters, e.g. enlargement in contraction time [4]. Recent studies [5] have indicated concomitant changes in the pattern of myosin light chains and the specific activities of Ca^{2+}-activated and K^+-EDTA-activated ATPase of myosin. However, these latter changes appeared only after a stimulation period of 3 weeks. On the other hand, the first changes in contractile parameters have been reported to occur as early as 4 days after the onset of stimulation [2]. We were interested therefore to investigate possible effects on the sarcoplasmic reticulum, since early effects upon this system have already been described after denervation [6]. As a matter of fact, the present study revealed that as soon as 1 day after the onset of stimulation a change occurs in the SDS-electrophoretic pattern of the sarcoplasmic reticulum proteins and it was found a concomitant decrease in the activity of the Ca^{2+}-stimulated ATPase.

* Present address: Departamento de Neurobiología, Universidad Católica de Chile, Casilla 114-D, Santiago/Chile

Abreviations: SDS: sodium dodecyl sulfate; EGTA: ethyleneglycol-bis-(β-aminoethyl ether)-*N,N'* tetraacetic acid; HEPES: *N*-2-hydroxy-ethylpiperazine-*N'*-2-ethanesulfonic acid.

2. Methods

The experiments were performed on male rabbits. Electrodes were implanted laterally to the peroneal nerve as previously described [2]. Stimulation was performed 8 hr a day with a frequency of 10 imp./sec and 0.15 msec duration. Animals were killed at various intervals and sarcoplasmic reticulum was prepared following mainly the procedure of [7] : a 20% homogenate of freshly excised muscle in 0.3 M sucrose buffered with 10 mM HEPES pH 7.5 was centrifuged at 9700 × *g* for 30 min. The supernatant was filtered through nylon gauze and centrifuged again at 9700 × *g* for 15 min. The supernatant was diluted with an equal volume of 1.2 M KCl in 10 mM HEPES pH 7.5 and centrifuged at 140 000 × *g* for 35 min. The sediment was resuspended in the sucrose medium to give a 1 mg protein per ml suspension. For sodium dodecyl sulfate-electrophoresis the sarcoplasmic reticulum fractions were heated for 2 min at 95°C in 5% SDS and were heated again for 1 min after addition of mercaptoethanol to a final concentration of 150 mM. 5 – 10 μg of protein were applied on slab gels prepared according to [8]. The gels were stained with 0.05% Coomassie Blue (R-250) and destained with 10% acetic acid. Gels were calibrated using cytochrome *c* (mol. wt 12 000), adenylate kinase (mol. wt 21 000), lactate dehydrogenase (mol. wt 35 000), aldolase (mol. wt 40 000) and phosphofructokinase (mol. wt 93 000).

ATPase activity at pH 7.0 was measured in a coupled optical test according to [9], a) in the presence of 0.2 mM $CaCl_2$ or, b) in the absence of Ca^{2+} and in the presence of 2 mM EGTA. According to Hughes and Yasin [10] activity measured in the

North-Holland Publishing Company – Amsterdam

presence of Ca^{2+} represents 'total' activity, that in the absence of Ca^{+}, the 'basal' activity. The difference between total and basal was designated as 'extra' activity.

3. Results and discussion

As may be seen from fig.1, changes in the SDS-electrophoretic pattern appeared already 1 day after onset of stimulation. Comparing the unstimulated fast tibialis anterior muscle with a typical slow muscle

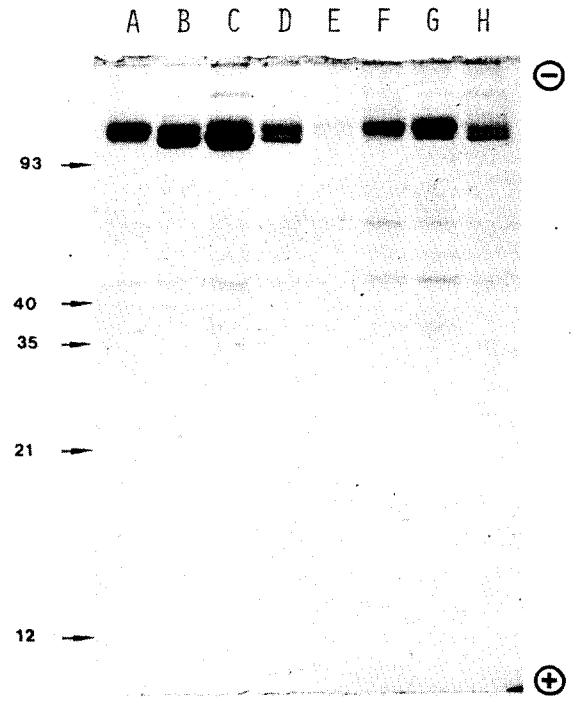

Fig. 1. SDS slab gel of sarcoplasmic reticulum from rabbit tibialis anterior and soleus muscles.
A) Unstimulated tibialis ant.contralateral to a stimulated one; B) tibialis ant. after 1 day stimulation; C) tibialis ant. after 3 days stimulation; D) tibialis ant. after 7 days stimulation; E) soleus muscle; F) tibialis ant. after 7 days denervation; G) tibialis ant. of an unoperated animal; H) soleus muscle. Calibration of the gel (mol. wt $\times 10^{-3}$) is indicated at the left margin.

Table 1
ATPase activity of the sarcoplasmic reticulum obtained from m.tibialis anterior after different periods of stimulation and from soleus muscle of the rabbit.

Series	Control	Stimulated	Stimulated / Control	N
A) m.tibialis anterior				
Unoperated animal	0.36 ±0.23	–	–	3
1 day stimulation	0.37 ±0.12	0.31 ±0.09	0.84	3
2 days stimulation	0.28	0.22	0.79	1
3 days stimulation	0.31 ±0.13	0.23 ±0.08	0.74	5
1 week stimulation	0.23 ±0.02	0.16 ±0.04	0.70	2
3 weeks stimulation	0.25 ±0.10	0.21 ±0.10	0.84	8
B) m.soleus	0.05 ±0.06	–	–	12

The ATPase activity represents the difference between the 'total' ATPase activity measured in the presence of 0.2 mM $CaCl_2$, 20 mM $MgCl_2$ and 200 mM KCl, and the 'basal' ATPase activity measured in the absence of Ca^{2+} and in the presence of 2 mM EGTA, 20 mM $MgCl_2$ and 200 mM KCl, using a coupled optical test for detecting the ADP produced after splitting from ATP at pH 7.0 [9]. The muscle contralateral to the stimulated one was always used as control. Results are given as mean values of the number of experiments indicated under N ± standard deviation.

(soleus), it can be seen that two bands with mol. wt about 123 000 and 110 000 are present in the pattern of the slow muscle (E,H), whereas the pattern of the unstimulated fast muscle (A,G) shows one strong band with a mol. wt of about 120 000. However, a second but faintly stained band of about 100 000 can also be seen in the pattern of this muscle (A,G).

Stimulation of the fast muscle leads to pronounced increase of this lighter component (B,C,D). In order to investigate whether this band represents a degradation product of the 120 000 component the corresponding pattern of a denervated muscle was also analysed (F). However, no increase in the 100 000 mol. wt component was seen in this case. Therefore it may be suggested that the observed increase in the lighter component of the pattern of the stimulated muscle results from synthesis. The relatively short half-life of endoplasmic reticulum proteins [11] would be in line with this suggestion.

Although no direct evidence of the nature of these components is provided in the present study, their molecular weight is in the range of that described by Meissner and Fleischer [12] for the phosphoprotein intermediate of the Ca^{2+} pump.

The stimulation induced also a decrease in the Ca^{2+} stimulated 'extra' ATPase activity (see Methods) of the sarcoplasmic reticulum. Table 1 summarizes data measured at various periods of stimulation. It may be seen that even after 1 day of stimulation there was a slight decrease in activity which persisted after long-lasting stimulation. Although the decrease was only about 20–30% it has been found in a great number of animals. An interesting finding concerns a slight and progressive decrease of the enzyme activity also in the contralateral unstimulated muscle which was taken in each case as the respective control value. This may result from reflex activity provoked by the contralateral stimulation.

The present findings demonstrate early changes in the sarcoplasmic reticulum induced by indirect muscle stimulation. It is tempting to assume that these are also related to the early changes in contractile characteristics observed under the same conditions of stimulation [2].

Acknowledgements

This study was supported by Deutsche Forschungsgemeinschaft, Sonderforschungsbereich 138 'Biologische Grenzflächen und Spezifität' und Schwerpunktprogramm 'Enzymdiagnostik'. B.U.R. is grateful to Deutscher Akademischer Austauschdienst for a stipend. The authors wish to thank Drs D. Brdiczka and E. Weidekamm for helpful suggestions and discussions.

References

[1] Pette, D., Staudte, H. W., Vrbová, G. (1972) Die Naturwissenschaften 59, 469–470.
[2] Pette, D., Smith, M. E., Staudte, H. W., Vrbová, G. (1973) Pflügers Arch. 338, 257–272.
[3] Cotter, M., Hudlicka, O., Pette, D., Staudte, H. W., Vrbová, G. (1973) J. Physiol 230, 34P.
[4] Salmons, S., Vrbová, G. (1969) J. Physiol. (Lond.) 201, 535–549.
[5] Sréter, F. A., Gergely, J., Salmons, S., Romanul, F. (1973) Nature (New.Biol.) 241, 17–19.
[6] Margreth, A., Salviati, G., DiMauro, S., Turati, G. (1972) Biochem. J. 126, 1099–1110.
[7] Yasin, R., Hughes, B. P., Parker, J. A. (1973) Lab. Invest. 29, 207–215.
[8] Laemmli, U. K. (1970) Nature 227, 680–685.
[9] Weidekamm, E., Brdiczka, D., submitted to Biochim. Biophys. Acta.
[10] Hughes, B. P., Yasin, R. (1973) Biochem. J. 136, 1129–1132.
[11] Arias, I. M., Doyle, D. and Schimke, R. T. (1969) J. Biol. Chem. 244, 3303.
[12] Meissner, G., Fleischer, S. (1971) Biochim. Biophys. Acta 241, 356–378.