Rapid reduction in parvalbumin concentration during chronic stimulation of rabbit fast twitch muscle

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Chronic indirect stimulation of fast twitch rabbit muscle induced a rapid reduction in parvalbumin concentration. When compared to the unstimulated contralateral muscle, parvalbumins were reduced to 55% following 6 days of stimulation. Prolonged stimulation further reduced parvalbumins so that they were undetectable after 28 days. The time course of these changes appears to be related with the previously observed changes in the sarcoplasmic reticulum.

Fast and slow twitch muscle *Chronic stimulation* *Parvalbumin* Ca^{2+}

1. INTRODUCTION

Parvalbumins are low M_r Ca^{2+} binding proteins present in high concentrations in mammalian skeletal muscle [1,2]. Although the precise function is unknown, it has been suggested that they may serve as an intracellular Ca^{2+}–Mg^{2+}-buffering system [3], a regulator of various Ca^{2+}-dependent cellular processes [4] or a soluble relaxing factor [5]. These functions are probably limited for fast twitch muscle, however, as parvalbumins are either undetectable [6] or present in very low concentrations [2] in slow twitch muscle.

Over the past several years we have expanded upon a model which utilizes chronic indirect electrical stimulation of fast twitch skeletal muscle to induce a controlled transformation of its characteristics to those of slow twitch muscle. This type of stimulation results in significant alterations in contractile characteristics [7], sarcoplasmic reticulum [8], enzyme patterns [9] and contractile proteins [10]. In light of the fact that parvalbumins appear functionally significant only in fast twitch muscle, we have examined the effect of chronic stimulation upon the concentration of parvalbumins in extensor digitorum longus (EDL) muscle of the rabbit. The purpose of this investigation was to determine if stimulation produces a reduction in parvalbumins which would be consistent with a fast to slow transformation. In addition, the time course of such changes could help to identify a cause and effect relationship between alterations in parvalbumins and those seen in the Ca^{2+} sequestering membranes of the sarcoplasmic reticulum. As a matter of fact, parvalbumin decreases in response to the stimulation protocol and this change is one of the earliest observed so far.

2. METHODS

Electrodes were implanted laterally to the left lateral popliteal nerve of male rabbits as in [9]. Stimulation was performed 12 h/day (1 h stimulation followed by 1 h rest) at 10 Hz with impulses of 0.15 ms at 2–8 V using a telestimulation system. Animals were sacrificed at various time intervals, and the EDL muscles were frozen in liquid nitrogen and stored at −80°C until assayed for parvalbumin concentration and enzyme activity.

Parvalbumins were purified as in [1] which employs ion-exchange chromatography on a DE-52 cellulose column as the initial step followed by gel filtration on Sephadex G-75. The extent of purity of the parvalbumin preparation was determined by

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2-dimensional electrophoresis, the ultraviolet spectrum and $^{45}Ca^{2+}$ binding. Protein concentration of parvalbumins was measured by dry wt and the relationship $E_{258}-E_{268} = 102 \times N$ where N is the number of phenylalanine residues i.e., 5.5 for rabbit [11]. The concentration of parvalbumins in the EDL muscle samples was determined by a modification of the method in [2].

About 100 mg of tissue was pulverized by percussion under liquid nitrogen and diluted 1:10 with quartz distilled water. The extracts were homogenized with a modified dentist drill and centrifuged at 40000 × g for 30 min. To five 50-μl samples of the supernatant fraction, 0.5–4.0 μg of purified parvalbumins were added, and the samples electrophoresed on 10% polyacrylamide slab gels in the absence of SDS with 25 mM Tris/glycine (pH 8.3) as the running buffer (fig.1). A standard curve for use in determining the amount of parvalbumins present was obtained by simultaneously electrophoresing purified parvalbumins (4–12 μg) with the muscle extracts. The gels were stained overnight with Coomassie blue, destained for 8 h and scanned at 550 nm. Integration of the peaks yielded a series of points from which a straight line could be constructed and the amount of parvalbumin determined by extrapolation to the value, which corresponded to zero added parvalbumin. For determination of the activity of 3-oxoacid CoA transferase about 10 mg of tissue from the same fiber bundle used for determination of parvalbumins was extracted [12] and activity measured as in [13].

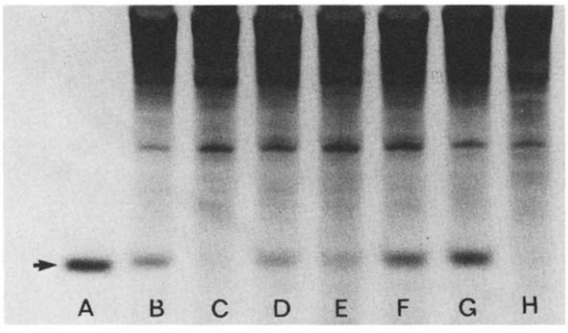

Fig.1. Polyacrylamide gel electrophoresis of purified parvalbumins and of aqueous extracts of EDL muscles stimulated for different periods of time, and of normal soleus muscle. (A) 10 μg purified parvalbumins; (B) unstimulated EDL; (C) 28-d; (D) 18-d; (E) 12-d; (F) 6-d; and (G) 2-d stimulated EDL; (H) soleus.

3. RESULTS AND DISCUSSION

As illustrated in fig.1, parvalbumins migrate separately as a single band in polyacrylamide gel electrophoresis and can, therefore, be easily quantitated by scanning without any interference from other proteins. Parvalbumins were undetectable in normal slow twitch soleus muscle. The concentration of parvalbumins in the unstimulated contralateral EDL ranged from 0.6–1.0 mg/g muscle wet wt. For the purpose of assessing the effect of stimulation, this wide animal to animal variation is negated by the ability to compare the concentration in the stimulated muscle with that of the unstimulated contralateral muscle. Stimulation for 2 days had no effect on parvalbumin concentration (table 1). However, following 6 days of stimulation the concentration in the stimulated muscle decreased to as much as 55% of that of the control muscle. Since soluble acidic proteins are very susceptible to degradation [14], a reduced rate of synthesis would be sufficient to explain this rapid decrease. Reduced synthesis might result from a decrease in

Table 1

The effects of varying periods of time of stimulation upon parvalbumin concentration and activity of 3-oxoacid CoA transferase in extensor digitorum longus muscle of the rabbit

Animal No.	Days stimulated	Parv-albumins	3-oxoacid CoA transferase
1	2	1.0	0.85
2	2	1.0	0.76
3	6	0.867	1.72
4	6	0.554	1.43
5	12	0.876	1.49
6	12	0.320	2.02
7	18	0.322	2.35
8	18	0.394	2.14
9	28	0	6.01
10	28	0	7.24
11	28	0.579	2.23

Results are given as quotients of contents and activities in the stimulated as compared to the contralateral unstimulated muscle

transcription due to a switch from fast to slow type gene expression. Altered transcriptional activity has recently been shown to occur for myosin light chains during stimulation-induced muscle transformation [15].

In [8] similar reductions in the ATPase activity and Ca^{2+} uptake capacity of sarcoplasmic reticulum were reported after just 2 days of stimulation. However, in those experiments stimulation was for 24 h/day, i.e., twice that used in the present experiments. It was suggested that the early contractile changes resulting from chronic stimulation could be related to changes in intracellular Ca^{2+} flux. This hypothesis is supported by the data from the present experiments as the observed decrease in parvalbumins could represent the loss of Ca^{2+} buffering capacity, and hence increase the level of cytoplasmic free Ca^{2+} during contraction.

When stimulation was prolonged, the level of parvalbumins continued to decrease with time until, after 28 days, the concentration was below that which could be detected (table 1). It appears, therefore, that with respect to parvalbumins long term stimulation results in a fast to slow transformation similar to that observed with other cellular systems.

Table 1 shows a considerable difference in the values between individual muscles measured at 6, 12 and 28 days. In order to determine if these differences might be related to the quality of stimulation, the activity of 3-oxoacid transferase was measured. This enzyme has previously been found to be most sensitive to increases in muscle activity [9]. At 28 and 12 days the enzyme activity was considerably higher in those muscles which also had lower parvalbumin concentration. Therefore, it is conceivable that these muscles were stimulated at an intensity greater than those displaying higher levels of parvalbumins.

The critical question with regard to the data from the present experiments is how the time course of the reduction in parvalbumins compares with that of the alterations previously observed for sarcoplasmic reticulum [8,16,17]. If the reduction in parvalbumins precedes that of the Ca^{2+} sequestering system, an increased level of free Ca^{2+} in the cytoplasm might be related to a decrease in the synthesis of the Ca^{2+} pumping ATPase. This causal relationship has been proposed to be essential in the regulation of the development of the sarcoplasmic reticulum in embryonic muscle [18]. This question is presently under investigation.

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