

Ca-ATPase isozyme expression in sarcoplasmic reticulum is altered by chronic stimulation of skeletal muscle

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Chronic stimulation of a predominantly fast skeletal muscle enhanced the expression of type I (slow muscle) Ca-ATPase and suppressed the expression of the type II (fast muscle) Ca-ATPase. Monoclonal antibodies IID8 and IIH11 against type I (slow) and type II (fast) isozymes respectively, were used to type the Ca-ATPases of the isolated SR (sarcoplasmic reticulum) by Western blots, and the Ca-ATPases of the muscle fibers by immunohistochemistry. Of the fibers from control muscles 80% stained for the type II isozyme and 20% for the type I isozyme. Following chronic stimulation all fibers stained for type I isozyme and none stained for type II isozyme. Ca-ATPase isozyme distribution in isolated SR confirmed this effect of chronic stimulation. The calcium uptake activities of homogenates of stimulated muscles were 22% of the control muscles. The Ca-ATPase and calcium-uptake activities of the isolated SR from stimulated muscles were, respectively, 32 and 45% of the control muscles.

Skeletal muscle transformation; Sarcoplasmic reticulum; Ca uptake; Ca-ATPase isozyme: (Ruthenium red)

1. INTRODUCTION

Chronically stimulated fast twitch (type II) mammalian skeletal muscle exhibits many of the physiological characteristics of slow twitch (type I) skeletal muscle. This functional transformation is now recognized to involve the expression of isozymes, of which the myofibrillar isozymes have been most extensively studied (for review, see [1]). The effect of chronic stimulation on the expression of the proteins involved in excitation-contraction coupling have not been extensively investigated in spite of the finding that type I and type II muscles express different isozymes of the Ca-ATPase of SR (sarcoplasmic reticulum) [2]. Leberer et al. [3] have reported that chronic stimulation of rabbit fast skeletal muscle does not lead to the expression of type I Ca-ATPase. They did find, as others before them, that stimulation depresses Ca-uptake and Ca-ATPase activity of muscle homogenates and isolated SR. Mabuchi et al. [4], on the other hand, reported at the 1989 Biophysical Society meeting, that indirect stimulation of the tibialis anterior of the rabbit increases the fraction of muscle fibers expressing the type I Ca-ATPase. In the following report we have examined the effect of chronic stimulation of the canine latissimus dorsi on the expression of type I and type II Ca-

ATPase isozymes and have functionally characterized the SR in homogenates and microsome fractions isolated from these muscles.

2. MATERIALS AND METHODS

In each of the 6 dogs studied the right latissimus dorsi was used as the control while the experimental muscle on the left side was stimulated, without a vascular delay period, at 2 Hz via the thoracodorsal nerve as described in [5]. In three of the dogs the experimental muscle was wrapped around a cardiac device [5], in the other three the muscle was stimulated in situ. No difference in any of the parameters studied could be discerned and the data from the 6 dogs were combined. Following the conditioning period (5.5–8) weeks, the animals underwent general anesthesia with pentobarbital. Latissimus dorsi from both sides were removed and rapidly placed in ice-cold saline. After trimming away the fat the muscles were weighed and homogenized in 2.7 vols of 0.25 M sucrose and 20 mM MOPS (3-(*N*-morpholino)propanesulfonic acid), pH 7.0. The SR enriched microsome fraction was isolated as in [6] and oxalate supported Ca-uptake was measured as in [7]. Ca-ATPase activity was measured in a coupled enzyme system [8] as the difference between the ATPase rate with 0.1 mM CaCl₂ or 10 mM EGTA (ethylene glycol-bis(β -aminoethyl ether)-*N,N,N',N'*-tetra-acetic acid). 5 mM sodium azide was added to both solutions to suppress the ATPase activity of mitochondrial fragments.

Immunofluorescence staining of transverse serial cryosections (8 μ m) from canine skeletal muscle was carried out as described by Jorgensen et al. [9], with either MAb (monoclonal antibody) IIH11 or MAb IID8 as first layer antibody at a concentration of 0.1 mg/ml, and with fluorescein-conjugated goat anti-mouse IgG (Boehringer Mannheim) at a 1:50 dilution as second layer antibody. Monoclonal antibodies IID8 and IIH11, which are specific for the type I (slow/cardiac muscle) Ca-ATPase and type II (fast muscle) Ca-

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ATPase, respectively, were prepared and purified as described by Jorgensen et al. [9].

Before immunoblotting, the proteins in the isolated SR from control and stimulated muscles were separated by SDS-PAGE (sodium dodecyl sulfate-polyacrylamide gel electrophoresis) [10], using 3% to 12% gradient slab gels and then electrophoretically transferred to nitrocellulose (0.45 μ m pore size) for 90 min at 1 A using 25 mM Tris (tris[hydroxymethyl]aminomethane), 195 mM glycine, 20% methanol (Towbin et al. [11]). Immunoblotting and color development of blots were performed as described by Jorgensen et al. [9]. Primary incubation of blots was carried out for 2 h with either MAb IIH11 or IID8 (5 μ g/ml in Blotto [Bovine Lactor Transfer technique Optimizer/PBS/5% nonfat dry milk], and secondary incubation with peroxidase-conjugated goat anti-mouse IgG (Boehringer Mannheim), at a 1:1000 dilution in BLOTTO, for 60 min with gentle agitation.

3. RESULTS AND DISCUSSION

The presence of type I and type II Ca-ATPases was investigated in control and chronically stimulated canine skeletal muscle using immunofluorescence and

immunoblotting with monoclonal antibodies IID8 and IIH11 previously shown to be specific for the type I and type II Ca-ATPase isozymes, respectively [9]. The control panels demonstrate the specificity of MAb's IIH11 and IID8 and lack of cross-reactivity. The immunofluorescence staining patterns in fig. 1a,c show that the control muscle is of mixed fiber type with approximately 20% of the fibers containing type I Ca-ATPase isozyme and 80% of the fibers containing type II Ca-ATPase isozyme. In contrast, all cells of the stimulated muscle were stained by the type I Ca-ATPase MAb and none of the cells were stained by the type II MAb. These results were reproducible in the 6 dogs studied. Mannon et al. [12], using a similar stimulation paradigm found by histochemical typing that 57% of the fibers in the control muscles stained for fast myosin and 46% stained for slow myosin isozymes. 100% of the fibers in the stimulated muscles were of the slow myosin type.

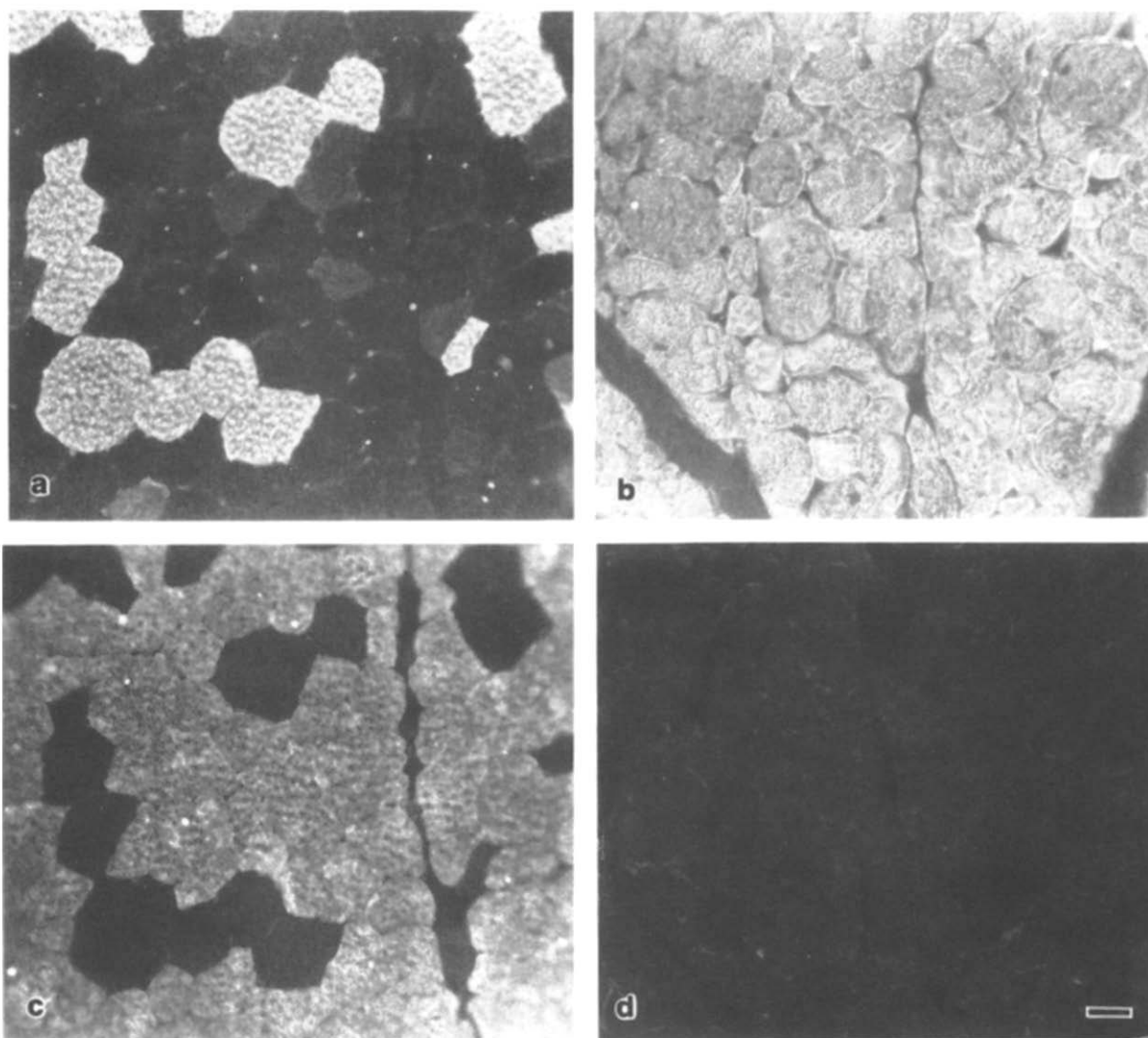


Fig. 1. Immunofluorescence staining of control and conditioned canine muscle using anti-type I and anti-type II Ca-ATPase antibodies. Transverse serial sections of control (a,c) and conditioned (b,d) canine skeletal muscle were stained by indirect immunofluorescence technique with MAb IID8 against type I Ca-ATPase (a,b) and with MAb IIH11 against type II Ca-ATPase (c,d) as described in section 2.

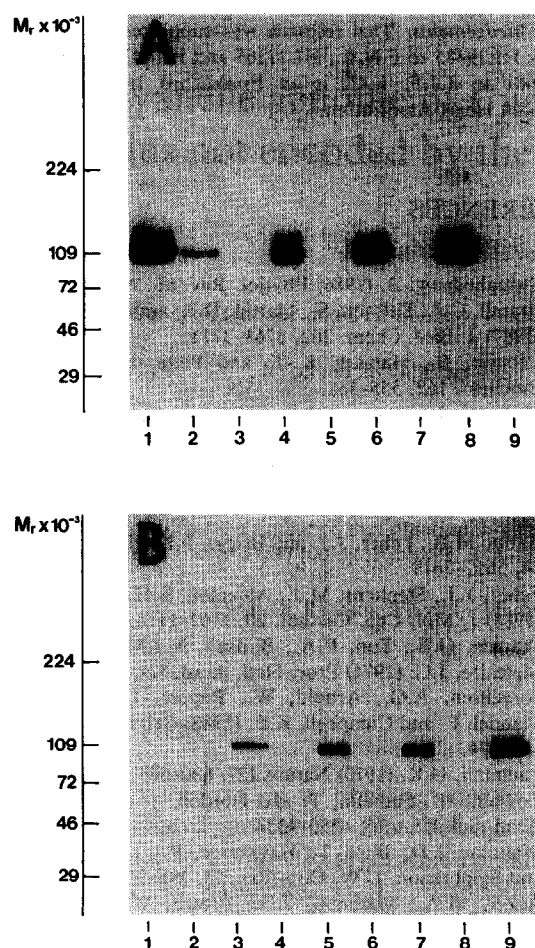


Fig.2. Immunoblot staining of sarcoplasmic reticulum from control and conditioned muscle. Sarcoplasmic reticulum was stained with MAB IID8 (type I) and MAB IIH11 (type II) as described in section 2. Lane (1), microsomal membranes from rabbit skeletal muscle; (2) canine skeletal muscle; (3), canine cardiac muscle; 4, 6, and 8 sarcoplasmic reticulum membranes from control muscles; 5, 7, and 9, sarcoplasmic reticulum from conditioned muscles. A, staining with MAB IID8 (type I), B, staining with MAB IIH11 (type II). 20 μ g protein in each lane. SDS-PAGE was performed under non-reducing conditions.

Table 1

Functional characterization of SR from conditioned type II skeletal muscle

	Nonconditioned	Conditioned
Calcium uptake		
Homogenate ^a		
– ruthenium red	9.3 \pm 2.2	2.6 \pm 1.3
+ ruthenium red	19.6 \pm 4.5	4.4 \pm 2.0
Sarcoplasmic reticulum ^b		
– ruthenium red	3.0 \pm 0.4	1.5 \pm 0.6
+ ruthenium red	5.9 \pm 0.8	1.9 \pm 0.8
Calcium ATPase		
Sarcoplasmic reticulum ^c	4.6 \pm 0.5	2.1 \pm 0.5

Ruthenium red was added to a concentration of 50 μ M to the homogenate and 2 μ M to the SR. Values are means \pm SE of observations on material from 4 dogs.

^a μ mole Ca/min \times g wet weight of muscle

^b μ mole Ca/min \times mg protein

^c μ mole ADP/min \times mg protein

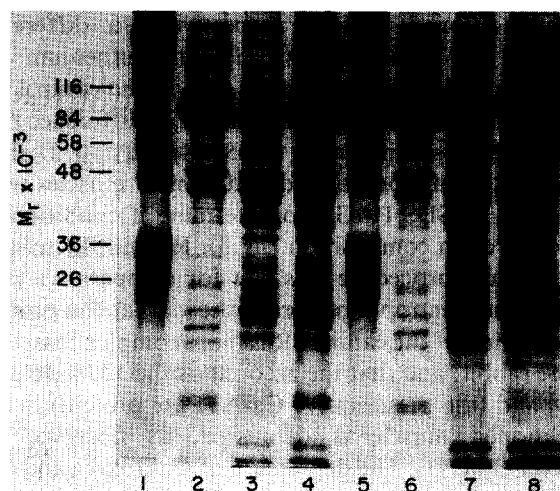


Fig.3. SDS-PAGE electrophoretogram of SR from control and conditioned muscles. SR was prepared from control and conditioned muscle as described in section 2. Fifty μ g protein was applied to each lane, subjected to SDS-PAGE [10] on a 10% gel, and stained with Coomassie Blue. 1, Prestained molecular weight standards. Their molecular weights are indicated on the left of the figure. 2 and 6, rabbit skeletal SR. 3 and 4, SR from control muscles 2 and 3. 7 and 8, SR from conditioned muscles 2 and 3.

Western immunoblot analysis of isolated SR membranes from control and stimulated muscles confirmed the findings observed in the muscle fibers. SDS-PAGE was carried out under non-reducing and reducing conditions (fig.2). Control muscles showed a strong reaction with the MAb IIH11 against the type II isozyme, while conditioned muscles reacted only with MAb IID8 against the type I isozyme. Thus both immunofluorescence and immunoblotting analysis show that chronic stimulation of latissimus dorsi enhances expression of type I isozyme and suppresses expression of type II isozyme. The failure by Leberer et al. [3] to obtain suppression of the type II Ca-ATPase isozyme and enhancement of the type I isozyme may have been caused by the discontinuous nature of their stimulus, which was 12 h on and 12 h off.

Calcium uptake and calcium ATPase activities were measured in homogenates and SR fractions prepared from control and conditioned muscles. The calcium uptake and Ca-ATPase activities of the SR were depressed 68 and 54% respectively (table 1), which is similar to that observed by Leberer et al. [3] in rabbit muscle. Calcium uptake rates of the corresponding muscle homogenates were depressed 78%. The discrepancy between the effect of conditioning on calcium-uptake in the homogenates and the isolated SR may be due to increases in connective tissue in the stimulated muscle [13]. Calcium uptake rates in both homogenates and isolated SR were stimulated by Ruthenium red (table 1), a compound which closes calcium channels in the SR and thereby stimulates calcium uptake in vesicles with open channels [14]. Ruthenium red increased homogenate calcium uptake 2.18 and 1.84 fold in the control

and conditioned muscles respectively, a difference which is not statistically significant. Ruthenium red stimulated calcium uptake 1.79 fold in the control SR and 1.38 fold in the experimental SR, a difference that is statistically significant at the 5% level.

The SR from conditioned and control muscles were subjected to SDS-PAGE to determine if conditioning affects protein composition (fig.3). Rabbit SR isolated by the same procedure was run for comparative purposes. The electrophoretogram shows that the protein composition of the canine SR is more complex than that of the rabbit, and that the amount of the 109 kDa protein band (the presumptive Ca-ATPase protein) in the conditioned muscle is depressed, as observed by Leberer et al. [3]. The depression of Ca-uptake and Ca-ATPase activities are probably related to this decrease in enzyme density and may also be related to the switch in Ca-ATPase isozyme.

The experiments reported here suggest that chronic stimulation of a motor nerve stimulates the expression of the isogene for type I Ca-ATPase and suppresses the expression of the type II isozyme. The studies by Brandl et al. [2], which show that these isozymes of the Ca-ATPase arise from distinct isogenes, make it likely that expression of these isozymes is regulated by expression of their respective isogenes. The observation that chronic stimulation can change the isozyme of the Ca-ATPase expressed in SR raises the possibility that slow muscle isoforms of other proteins involved in excitation-contraction coupling are expressed in response to chronic muscle stimulation.

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