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Expression of alpha-cardiac myosin heavy chain in normal and denervated rat muscle spindles

David L. McWhorter[a], Jon M. Walro[a,*], Steven A. Signs[b], Jun Wang[a]

[a]*Department of Anatomy, Northeastern Ohio Universities College of Medicine, P.O. Box 95, 4209 St. Rt. 44, Rootstown, OH 44272-0095, USA*
[b]*Department of Pharmacology, Northeastern Ohio Universities College of Medicine, Rootstown, OH 44272-0095, USA*

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Abstract

Whether any fibers in rat hindlimb muscles express α-cardiac myosin heavy chain (MHC) is uncertain. Expression of α-cardiac MHC mRNA and the polypeptide for which it codes were examined in control and denervated rat muscle spindles using in situ hybridization (ISH) and immunocytochemistry (ICC). Both nuclear bag_2 and bag_1 intrafusal fibers in the extensor digitorum longus (EDL) muscles expressed α-cardiac MHC and its precursor mRNA. Furthermore, denervation of the hindlimb down-regulated α-cardiac MHC mRNA expression in rat nuclear bag intrafusal fibers, even though they continued to display a strong affinity for anti-α-cardiac MHC monoclonal antibody. These data show that (1) intrafusal fibers express the α-cardiac MHC gene; (2) innervation regulates α-cardiac MHC gene expression at a pre-translational level; and (3) ISH is more sensitive than ICC to changes in α-cardiac MHC gene expression in adult rat muscle spindles.

Keywords: α-Cardiac myosin heavy chain mRNA; Rat; Intrafusal fibers; In situ hybridization; Denervation

Myosin heavy chain, a major polypeptide subunit of myosin, exists as several isoforms. At least two immunocytochemical (ICC) studies have reported that nuclear bag_1 and nuclear bag_2 intrafusal fibers of muscle spindles express an α-cardiac-like MHC [6,7]. However, electrophoresis of isolated human muscle spindles [8] and Northern blot analysis of rat skeletal muscle [5] failed to confirm the presence of α-cardiac MHC or its mRNA. Thus, whether nuclear bag fibers of muscle spindles express the α-cardiac MHC gene or a gene which codes for a similar MHC is unclear.

Factors regulating the expression of the α-cardiac-like MHC in muscle spindles are also poorly understood. Sensory innervation may be essential for the induction and maintenance of α-cardiac-like MHC, because this myosin isoform is only expressed in rat hindlimb skeletal muscle fibers receiving sensory innervation, i.e. intrafusal fibers [6]. Likewise, motor innervation may up-regulate nuclear bag fiber expression of the α-cardiac-like MHC. Three and 8 weeks after the motor innervation to rat hindlimb muscles is extirpated, α-cardiac-like MHC is no longer detectable in the nuclear bag_1 fiber, and its breadth of expression in the nuclear bag_2 fiber is reduced to the equatorial region of the fiber [6]. Delineation of how innervation regulates α-cardiac-like MHC expression is confounded by the observation that this polypeptide can still be detected in nuclear bag intrafusal fibers of adult rats 6 months after motor or sensory denervation (Wang and Walro, unpublished data). Because proteins have longer half-lives than mRNAs [10], post-translational analyses such as ICC may have limited value for studying factors that regulate MHC gene expression. Therefore, the objectives of this study were to determine whether nuclear bag intrafusal fibers express the α-cardiac MHC gene and to determine whether innervation regulates the expression of this gene in adult rat hindlimb muscle spindles.

Ten young adult Sprague–Dawley rats (175–225 g) were anesthetized with a chloral hydrate (0.1 ml/100 g body wt.)/ketamine (0.05 ml/100 g body wt.) cocktail. The right extensor digitorum longus (EDL) muscle of eight rats was denervated by removing a 5-mm segment from the right sciatic nerve. The right EDL muscles from two

* Corresponding author. Tel.: +1 216 3252511, extn. 350; fax: +1 216 3252524; e-mail: jmw@riker.neoucom.edu.

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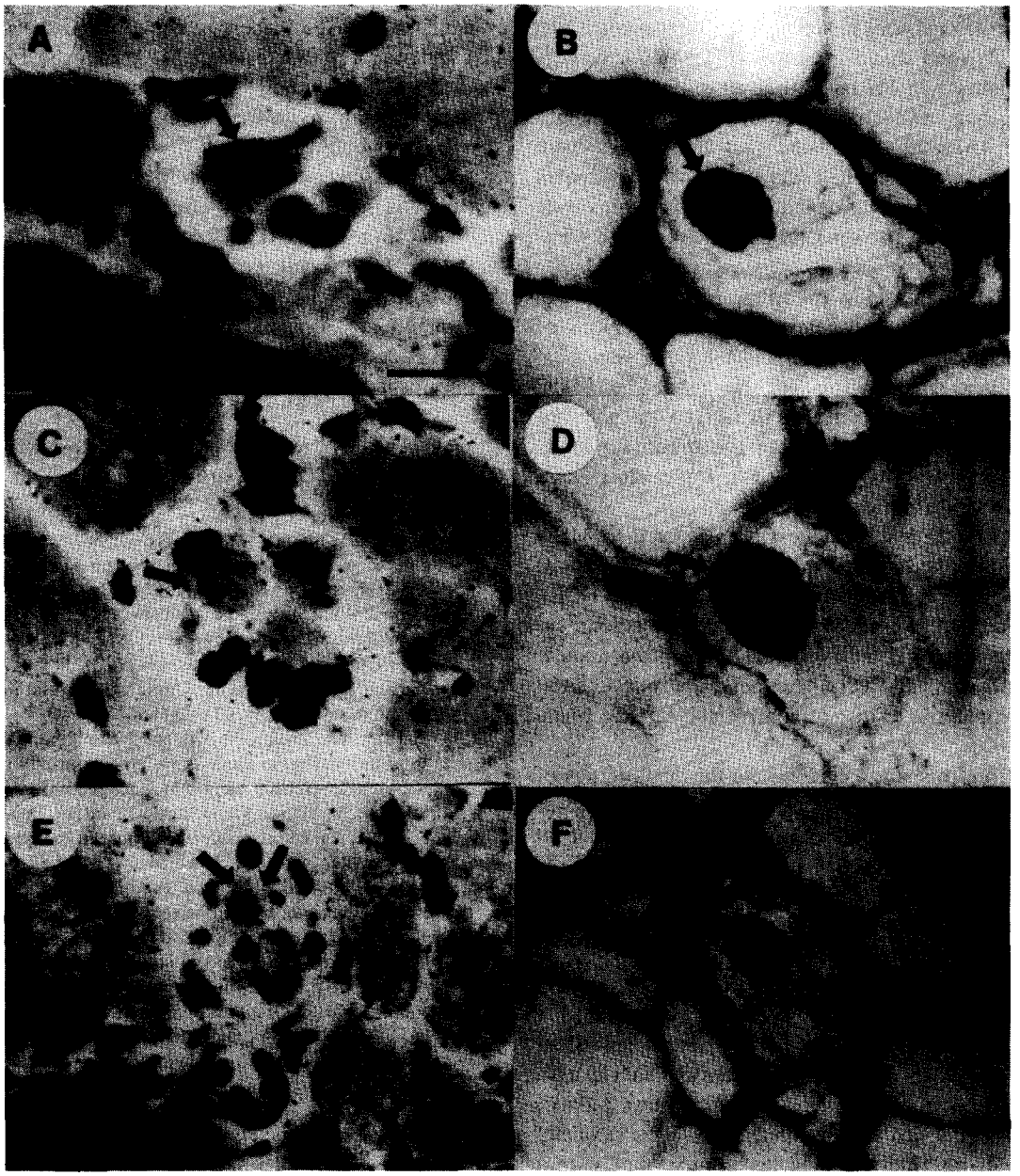

Fig. 1. Three muscle spindles from control (A,B), 3-day denervated (C,D), and 21-day denervated (E,F) rat EDL muscles reacted with an α-cardiac MHC mRNA probe and counterstained with toluidine blue (A,C,E) or reacted with an α-cardiac MHC MAb (B,D,F). Note that the bag_2 fiber (arrows) in the control spindle expresses both the α-cardiac mRNA (A) and α-cardiac MHC (B). However, the mRNA is no longer detectable in bag_2 fibers (arrows) in the 3- and 21-day denervated spindles (C,E), yet the same bag_2 fibers (arrows) retain their affinity for the α-cardiac MHC antibody (D,F). Scale bar = 20 μm in (A–F).

rats at 3, 7, 14, and 21 days after denervation, and from two control rats were excised and frozen. Sets of semi-serial, 10 μm-thick transverse sections were cut in a cryostat, thaw-mounted on glass slides and processed for in situ hybridization (ISH) and ICC according to the procedures outlined by Siegel [12], and Kucera and Walro [2], respectively. A 40-mer oligonucleotide complementary to bases 216–256 from the 3′ end terminus of the α-cardiac

MHC cDNA [4] was synthesized and tailed with [^{35}S]dATP for use as a probe. This sequence was selected because of its low homology with other MHC isoform sequences. After processing for ISH, sections were counterstained with 1% toluidine blue. Other sections in each set were reacted with BA-G5, ALD 19, or MF-30 monoclonal antibodies (MAbs) raised against the α-cardiac [9], slow tonic [1], and avian neonatal [11] MHCs, respectively. The latter two MAbs were used to distinguish between bag_1, bag_2 and chain intrafusal fibers [3]. Eight control and 16 adult denervated muscle spindles were examined. α-Cardiac MHC expression was evaluated qualitatively by comparing densities of silver grains over intrafusal fibers with background densities overlying extrafusal fibers.

In control EDL muscles, α-cardiac MHC mRNA was present in both the juxtaequatorial and polar regions of the nuclear bag_2 fiber (Fig. 1A), and the polar region of the bag_1 fiber. Distribution of the α-cardiac MHC polypeptide was similar to that of its mRNA for both nuclear bag fibers (Fig. 1B). Neither the α-cardiac MHC mRNA nor its protein were detected in any nuclear chain intrafusal or extrafusal fibers.

As soon as 3 days after denervation, α-cardiac MHC mRNA could not be detected in either the nuclear bag_1 or nuclear bag_2 fibers of any muscle spindles (Fig. 1C,E). However, at all time points up to 21 days after denervation, the intensity of BA-G5 binding to both the nuclear bag_1 and nuclear bag_2 fibers of denervated muscle spindles was comparable to that of control nuclear bag fibers (Fig. 1D,F).

Results from this study corroborate the specificities of both the BA-G5 [9] and F88.12F8 [6] MAbs for α-cardiac MHC, and demonstrate that both nuclear bag fibers of rat skeletal muscle express the α-cardiac MHC gene. These data also show that α-cardiac MHC mRNA is approximately located in the same regions of intrafusal fibers as the polypeptide for which it codes.

The absence of α-cardiac MHC mRNA in denervated nuclear bag intrafusal fibers supports the hypothesis that expression of α-cardiac MHC fibers is neural-dependent [6] and shows that motor and/or sensory innervation regulate expression of this gene at the pre-translational level. Pedrosa et al. [6] reported that 7 days after neonatal denervation of rat hindlimb muscles, approximately one-third of muscle spindles examined had only one fiber weakly to moderately reactive to anti-α-cardiac MHC MAb. Furthermore, 3 weeks after neonatal deefferentation of rat hindlimb muscles, the reactivity of the nuclear bag_2 fibers to the anti-α-cardiac MHC MAb was decreased and limited to a shorter region of the fiber, and nuclear bag fibers were completely unreactive to the anti-α-cardiac MHC MAb. In the present investigation, the intensities of reactivity of both nuclear bag fibers to α-cardiac MAb were comparable to that of control adult nuclear bag fibers 3 weeks after denervation of adult rat hindlimb muscles. Differences in the sensitivities of the two anti-α-cardiac MHC MAbs used in these two investigations could account for the observed differences in reactivity, but slower MHC turnover in adult compared to neonatal intrafusal fibers seems more plausible. In denervated muscles, the BA-G5 MAb may be binding to α-cardiac MHC either still incorporated in the thick filaments and/or to a free cytoplasmic pool of α-cardiac MHC monomers.

The absence of α-cardiac MHC mRNA in nuclear bag fibers as soon as 3 days after denervation indicates that denervation down-regulates α-cardiac MHC gene expression, although no decrease in binding of anti-α-cardiac MHC MAb may be noted for several weeks. Consequently, ISH may be a better tool than ICC for studying factors that regulate gene expression in adult nuclear bag intrafusal fibers.

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