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Myosin heavy chain isoform mRNA and protein levels after long-term paralysis

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Abstract

To assess the long-term influence of paralysis on muscle phenotypic mRNA and protein expression, the effects of spinal cord transection (ST) on myosin heavy chain (MyHC) isoform mRNA and protein levels in the soleus and medial gastrocnemius (MG) muscles of rats were analyzed. Control soleus contained predominantly MyHC-I with low amounts of MyHC-IIa and IIx mRNAs. After ST, MyHC-I mRNA decreased to ~15%, MyHC-IIa was increased by 75–200%, and MyHC-IIx was elevated by 8–10×. Low level expression of MyHC-IIb was observed post-ST, suggesting that reduced activity is not a primary stimulus for MyHC-IIb expression. Adaptations in mRNA preceded protein adaptations in the soleus. Although MyHC-I protein in the MG was reduced post-ST, no other consistent changes occurred. The relative lack of adaptation to ST by the MG suggests that the reduced activity and load bearing encountered by the MG were insufficient to induce a change in muscle phenotype.

Keywords: Muscle; Myosin heavy chain; Paralysis; Reverse transcriptase-polymerase chain reaction; Spinal cord injury

Spinal cord injury (SCI) results in debilitating decrements in sensory and motor function of the affected limbs. Following paralysis induced by SCI, rodent slow skeletal muscles, such as the soleus, acquire fast muscle characteristics, including an increased content of fast (type II) MyHC isoforms at the expense of slow (type I) MyHC isoforms [1–5]. In normally slow muscles, the transitions in MyHC isoform content following paralysis impact the functional properties of the muscle [1]. Thus, it is important to identify the intracellular mechanisms that induce the transitions in MyHC isoform expression after SCI.

In mammals, each MyHC isoform is encoded by a single gene. The adult slow MyHC is encoded by a gene (myh7) on chromosome 14 in the mouse and human and on chromosome 15 in the rat, and is also known as the β-cardiac MyHC expressed in cardiac muscle [6]. The slow β-cardiac MyHC gene sits in tandem with the α-cardiac MyHC gene (myh6) which together display coordinated expression patterns in cardiac muscle [7]. However, the α-cardiac MyHC isoform is not typically expressed at appreciable levels in the extrafusal fibers of adult skeletal muscles. The adult fast MyHC isoforms (IIa, IIx, and IIb) are encoded by individual genes (myh2, myh1, and myh4, respectively) that are clustered together on chromosomes 17, 11, and 10 in the human, mouse, and rat, respectively [6,8–10]. Two skeletal muscle MyHC isoforms that are expressed primarily during development are encoded by genes that are in tandem with IIa, IIx, and IIb. The embryonic MyHC isoform is situated upstream and the neonatal MyHC is situated downstream of the fast MyHC cluster [6,8–10]. Neither the embryonic nor the neonatal MyHC isoform proteins are observed in adult control or paralyzed muscles [3].

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To date, the cellular and molecular mechanisms that lead to the increased expression of fast and decreased expression of slow MyHC isoforms following a SCI are unknown. Knowledge of the time-course of the adaptations in the expression of the MyHC isoform mRNAs would be useful in designing the most efficacious rehabilitative strategies to counter the deleterious influence of paralysis and to identify the cellular signaling mechanisms responsible for the altered expression of the MyHC genes.

To identify the cellular and molecular mechanisms that contribute to muscle adaptations after a complete spinal cord transection (ST), it is important to determine how the products of the various MyHC isoform genes are modulated after ST. Therefore, in the present study, the MyHC mRNA isoform content of the soleus (a slow antigravity muscle) and the medial gastrocnemius (MG, a fast antigravity muscle) were quantified in control rats and at 15, 30, 90, 180, and 360 days post-ST. Specifically, the MyHC protein isoform proportions of the soleus and MG of control and ST rats were quantified by high-resolution SDS–PAGE [11] and the MyHC isoform mRNAs were quantified by reverse transcriptase-polymerase chain reaction (RT-PCR) [12]. We hypothesized that ST would result in: (1) a time-dependent increase in fast (IIa and IIx) and decrease in slow (I) MyHC mRNA and protein levels in the soleus; (2) a time-dependent increase in MyHC-IIb and decrease in MyHC-I mRNA and protein in the MG; and (3) more rapid adaptations in MyHC mRNA levels than protein levels.

Materials and methods

Animals. Young adult (~150 g) female Sprague–Dawley rats were assigned randomly to either control or ST groups. Rats in the ST groups were anesthetized with ketamine (75 mg/kg body mass) and xylazine (10 mg/kg body mass) and under aseptic conditions, subjected to a complete ST at a mid-thoracic level according to Talmadge et al. [3,4] and as described in detail by Roy et al. [13]. The ST and age-matched control rats were sacrificed at 15, 30, 90, 180, and 360 days post-ST via a lethal dose of pentobarbital ($n = 5$–6 rats per group at each time point). The soleus and MG muscles were rapidly dissected and cleaned of excess connective tissues, weighed, frozen in isopentane chilled by liquid nitrogen, and stored at −70 °C until later analysis. The maintenance and care procedures for ST animals have been described in detail previously [13]. This study was approved by the University of California, Los Angeles, Animal Care and Use Committee, and followed the American Physiological Society Animal Care Guidelines.

Myosin heavy chain isoform analyses. Frozen soleus and MG muscles were cut transversely into halves and one-half was homogenized and subjected to high-resolution gel electrophoresis for the assessment of MyHC isoform content as described in detail by Talmadge and Roy [11]. The gels were dried and scanned with an Alpha Innotech ChemiImager 5500 video densitometric system and the MyHC isoform data are presented as a percentage of a given isoform relative to the total MyHC content. The MyHC protein isoform data for the soleus muscle have been reported previously [3], however they are presented here to facilitate comparisons between the adaptations in MyHC isoform protein and mRNA levels. Sample gels showing the positions of MyHCs-I, IIa, IIx, and IIb for the soleus and MG muscles in control and ST rats are presented in Fig. 1.

The other half of each soleus and MG muscle was used for RT-PCR quantification of adult MyHC isoform mRNAs. Total RNA was isolated from each muscle half using the Trizol technique and cDNA was synthesized using Superscript II (Invitrogen) according to the manufacturer's instructions. An aliquot of synthesized cDNA (~200 ng of reverse transcribed RNA) was subjected to PCR with MyHC isoform specific primers (one primer set per MyHC isoform) and an internal calibration standard in a DNA Engine (MJ Research) thermocycler as described in detail by Wright et al. [12] and modified by di Maso et al. [14]. The PCR products, including the products generated from the internal calibration standard (control fragments) and the products generated from the cDNA (specific fragments), were electrophoresed on agarose gels, stained with SYBR Green I (Molecular Probes), and quantified (specific fragment intensity/control fragment intensity) using an Alpha Innotech ChemiImager 5500 video densitometric system (Fig. 1A).

Statistical procedures. All data are reported as mean values (proportion of each isoform relative to the total of all isoforms) ± standard error of the mean. Statistical procedures included a two-way (group × time) analysis of variance followed by the Bonferroni test for multiple comparisons with the α-level set at $p \leqslant 0.05$. Because there were no significant time-dependent changes in MyHC isoform protein or mRNA in control rat soleus and MG (data not shown), the data for the control rats from all time points were condensed into a single group for ease of presentation and are presented as time point zero in Figs. 2–4.

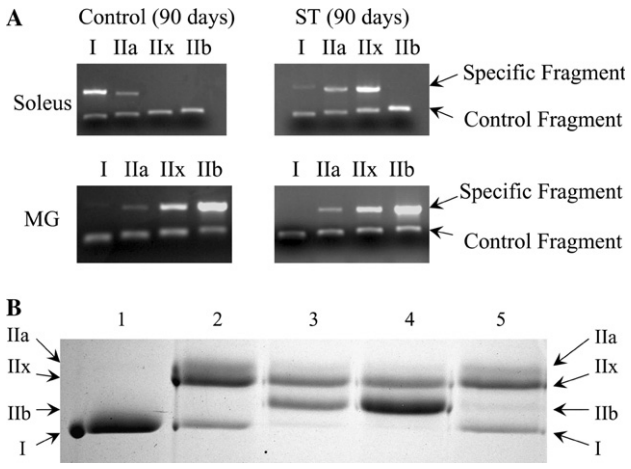

Fig. 1. Representative MyHC isoform RT-PCR (A) and SDS–PAGE (B) gels of soleus and medial gastrocnemius (MG) muscles from control (90 day time point) and spinal cord transected (ST, 90 day time point) rats. (A) Agarose gels of RT-PCR products showing the positions of the specific and control fragments for each of the MyHC isoforms. The control rat soleus contains predominantly MyHC-I and some IIa, whereas 90 days after ST the soleus contains predominantly MyHC-IIx and IIa mRNA isoforms. The control and ST rat MG both contain predominantly MyHC-IIb and IIx and small amounts of MyHC-IIa mRNA isoforms. (B) MyHC region of Coomassie-blue stained SDS–PAGE gel of control rat soleus (lane 1), ST rat soleus (lane 2), control rat MG (lane 3), and ST rat MG (lane 4). Rat diaphragm is shown in lane 5 as a control to show the positions of all four adult MyHC isoform bands.

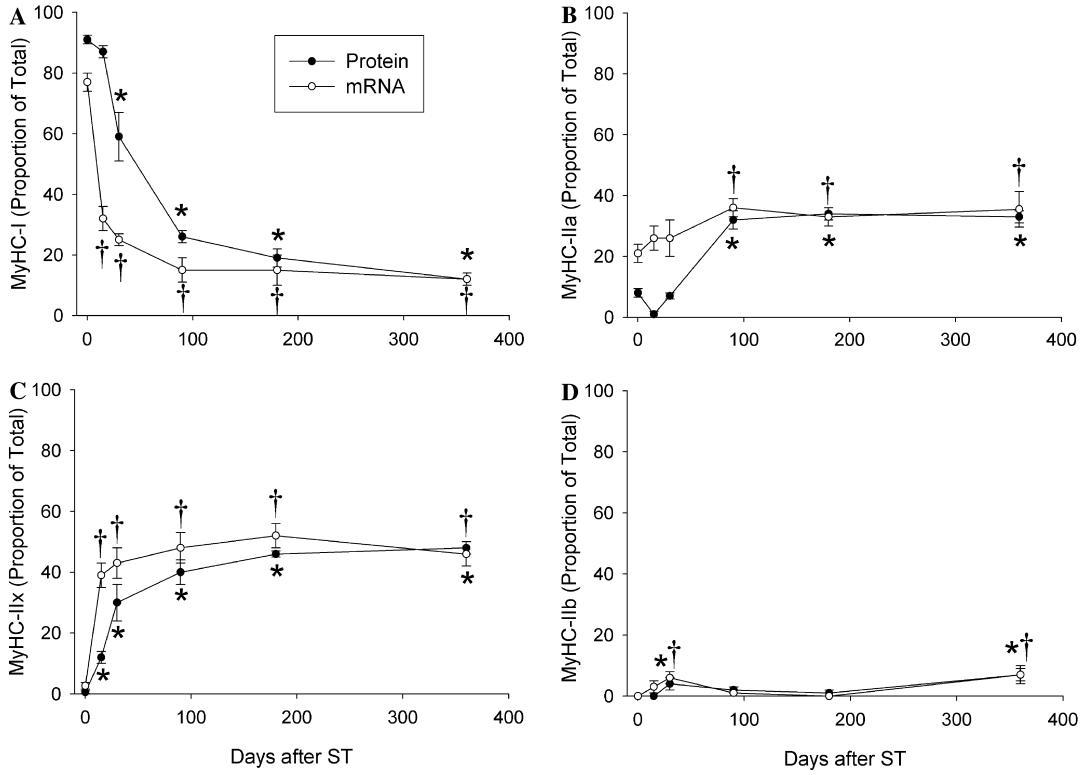

Fig. 2. The percentage composition of adult MyHC isoform mRNAs (open symbols) and protein (closed symbols) from the soleus muscle of ST rats. Control data for all time points were combined and plotted as time point zero (see Materials and methods) for ease of presentation because no significant time-dependent differences were found in control samples. (A) MyHC-I, (B) MyHC-IIa, (C) MyHC-IIx, and (D) MyHC-IIb. Values are plotted as means ± standard error of the mean (SEM). Abbreviations, same as in Fig. 1. * and † denote that the protein and mRNA levels at that time point are significantly ($p \leqslant 0.05$) different from control (time zero), respectively.

Results and discussion

The levels of the MyHC isoform mRNAs in soleus muscles of ST rats adapted in a similar manner as the proteins that they encode. As expected, the adaptations in the mRNAs preceded the changes in protein, as evidenced by the leftward shift of the mRNA curves relative to the protein curves (Fig. 2). The rate of decrease in MyHC-I mRNA was greatest during the first 15 days after ST, whereas the rate of MyHC-I protein loss was greatest between 15 and 30 days after ST (Fig. 3A). The rate of increase in both MyHC-IIa and -IIx mRNA was greatest during the first 15 days, and net MyHC-IIa and -IIx protein accretion did not begin until 15-30 days after ST (Figs. 3B and C). Thus, the net changes in mRNA preceded the net changes in protein. These data are coherent with the idea that a modulation of the MyHC protein isoform content occurs as a consequence of altered mRNA content. This relationship is consistent with the wealth of data showing that MyHC isoform protein levels are primarily regulated at the level of gene transcription [15–18].

The ST-induced down-regulation of MyHC-I mRNA in the soleus occurs very rapidly after ST (Fig. 2A). The mRNA levels drop to ~40% of control within the first 15 days and approach a new steady state within the first 30 days after ST, whereas the protein levels are maintained at ~95% of control 15 days post-ST and do not approach steady state until ~90 days post-ST. The delayed reduction in MyHC-I protein content relative to mRNA is likely caused by the relatively slower process of remodeling of the myofibrillar apparatus versus mRNA degradation. For instance, the estimated half-life of MyHC protein is ~54 days [19], whereas that of MyHC mRNA is ~1 day or less [20,21]. Our data on the ST-induced reduction in MyHC-I protein content of the soleus are consistent with a virtually complete inhibition of MyHC-I synthesis after ST and the ~ 54 day half-life of MyHC protein. As shown in Fig. 2A, the proportion of MyHC-I protein is decreased to approximately one-half of control at some point between 30 and 90 days post-ST.

The up-regulation of MyHC-IIx mRNA was nearly complete by 15 days post-ST, whereas the protein levels did not reach their final steady state until ~90 days post-ST (Fig. 2C). This delayed response in the protein adaptations is also likely a consequence of the remodeling process that includes the time required for efficient translation and incorporation of the newly synthesized protein into the myofibrillar apparatus. The primary

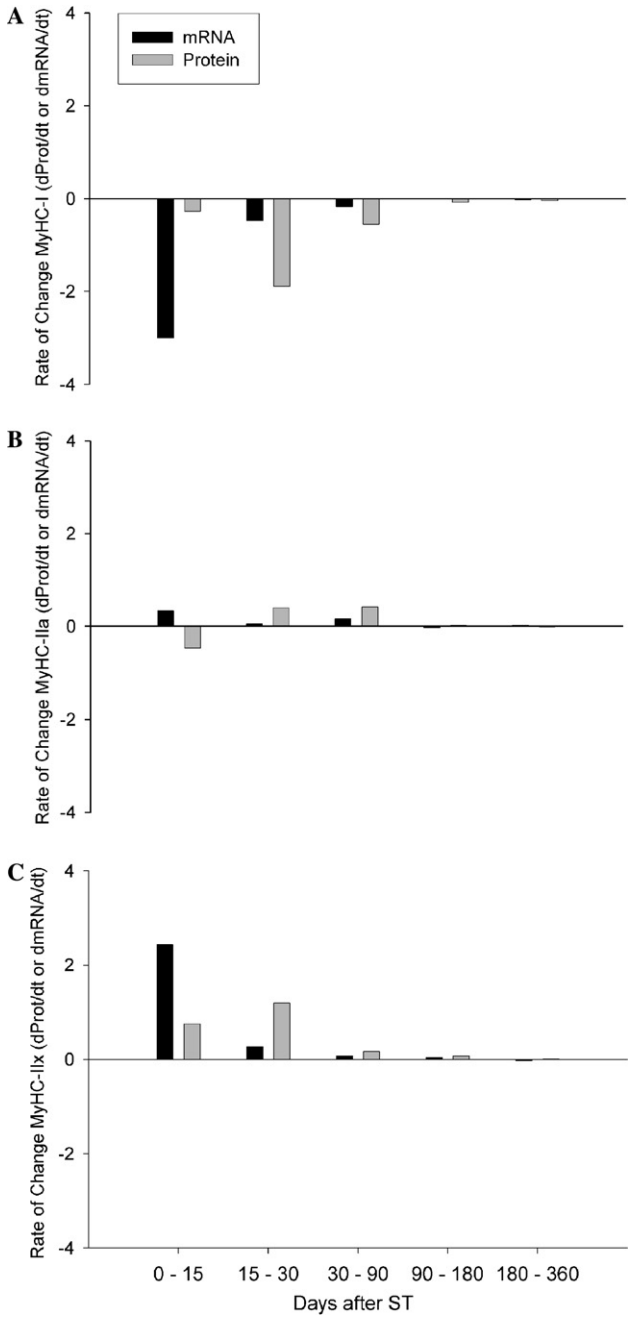

Fig. 3. Rates of change of MyHC-I (A), MyHC-IIa (B), and MyHC-IIx (C) in the soleus muscle after ST. The plotted values are calculated as the change in mean protein isoform proportion divided by the time interval (dProt/dt) or the change in mean mRNA isoform proportion divided by the time interval (dmRNA/dt). Negative values represent a decrease in the amount of that isoform and positive values an increase in that isoform during the time interval. The units of the plotted values are change in % MyHC/day.

adaptations in the expression of MyHC mRNA isoforms, i.e., the increase in MyHC-IIx and the decrease in MyHC-I mRNAs, appear to occur within the first 2 weeks post-ST. Thus, the major changes in cellular signaling events that influence MyHC expression also likely occur within the initial 2 weeks. Furthermore, these observations suggest that the expression of MyHC-I and -IIx is highly sensitive to chronic changes in the level of muscle activation and/or load bearing.

In contrast to the rapid changes in MyHC-I and IIx mRNAs, the MyHC-IIa mRNA levels increase at a slower rate and become significantly higher than control at 90 days post-ST (Fig. 2B). The differential response across the MyHC isoform mRNAs suggests that although the primary regulation of MyHC expression is accomplished within the first 15 days post-ST, the signals that drive the ultimate increase in IIa expression are either delayed or slower acting. These results support the idea that separate cellular and molecular signals are involved in the increased expression of MyHC-IIa and MyHC-IIx mRNAs. Despite following the adaptations for periods up to 1-year post-ST, no consistent adaptations in MyHC-IIb protein or mRNA were observed in the soleus muscle (Fig. 2D). This observation is in agreement with previous shorter duration studies that show only minor increases in MyHC-IIb in the rat soleus after chronic reductions in activity and/or load bearing [22–24].

As shown by Fig. 4, the only consistent adaptation in MyHC isoform expression in the MG was a decrease in MyHC-I protein levels beginning 90 days post-ST. Otherwise, the MG showed no consistent adaptations in the expression of MyHC isoform mRNAs or proteins. These results emphasize the muscle-specific nature of muscle plasticity. The fact that the MG did not transition towards a faster phenotype (i.e., neither IIx nor IIb was increased consistently after ST) was unexpected, since previous data from our laboratory demonstrated that spinal cord isolation (SI), a model of complete inactivity, induces an increase in MyHC-IIb in the MG at the expense of MyHCs-I and IIa [25]. This suggests that the reductions in activity associated with ST were insufficient to induce an adaptation in MyHC isoform expression in the MG. The greater level of adaptation observed in the soleus relative to the MG may be related to the larger absolute decrease in electrical activation, as measured by electromyographic activity, observed after ST for slow compared to fast muscles. For instance, in cats, the soleus displayed a greater absolute decrease in electromyographic activity than the lateral gastrocnemius (a fast muscle) [26]. It is also possible that the MG is somewhat resistant to reduced activity induced adaptation simply because it starts as a fast muscle, whereas the soleus is normally a slow muscle.

The observation that MyHC-IIb was not substantially elevated in either the MG or the soleus up to 1-year (~1/3rd of the animal's life-span) after ST suggests that high level expression of this isoform is not simply associated with low levels of daily activation or load bearing. Although, complete inactivity induces an increase in MyHC-IIb in the MG [25]. Alternative or addi-

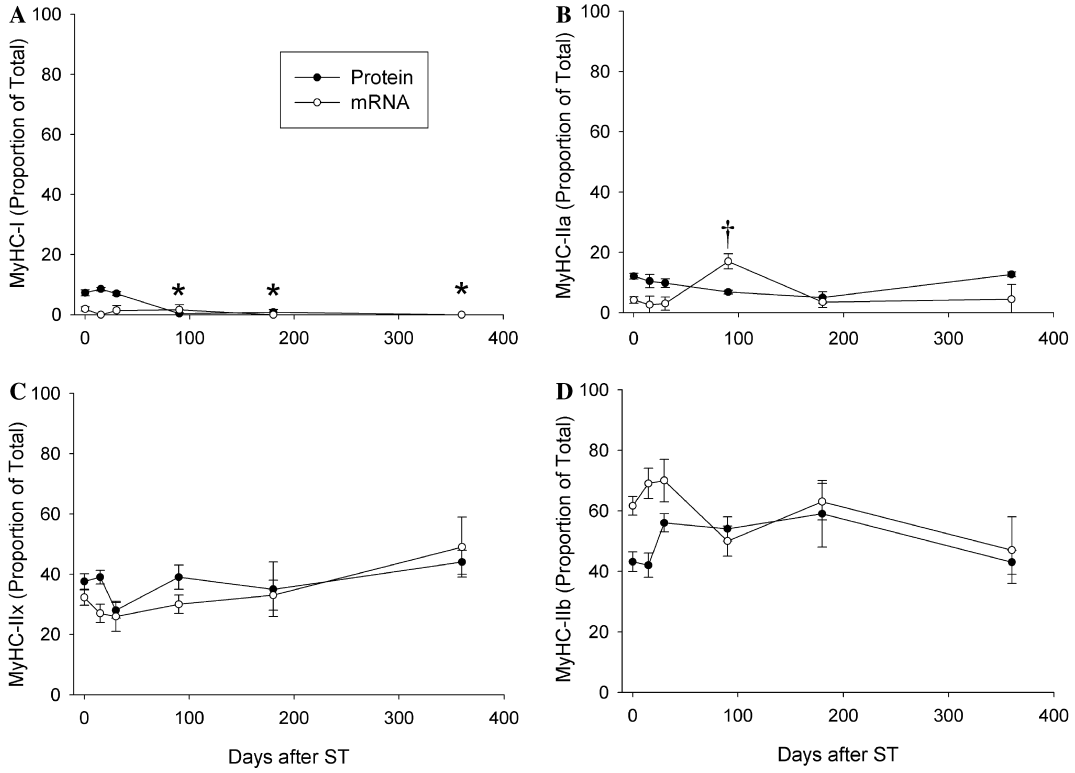

Fig. 4. The percentage composition of adult MyHC isoform mRNAs (open symbols) and protein (closed symbols) from the MG muscle of ST rats. Control data for all time points were combined and plotted as time point zero (see Materials and methods) for ease of presentation because no significant time-dependent differences were found in control samples. (A) MyHC-I, (B) MyHC-IIa, (C) MyHC-IIx, and (D) MyHC-IIb. Values are plotted as means ± standard error of the mean (SEM). Abbreviations, same as in Fig. 1. * and † denote that the protein and mRNA levels at that time point are significantly ($p \leqslant 0.05$) different from control (time zero), respectively.

tional signaling factors may need to be involved to induce MyHC-IIb expression in rat slow muscle. For instance, decreased load bearing and thyroid hormone treatment both cause a transformation of slow muscle towards fast, but neither one alone invokes appreciable MyHC-IIb expression [15]. However, in combination reduced load bearing with thyroid hormone treatment induces large increases in MyHC-IIb expression in the rat soleus [27].

Comparison to other models of reduced activity and load bearing distinguishes ST as a unique model for MyHC adaptations. For instance, studies using hindlimb suspension or spaceflight as models of reduced load bearing do not show nearly the same level of slow muscle transformation towards a fast phenotype as ST [22]; however, the majority of spaceflight and hindlimb suspension studies have been performed over a period of only a few weeks, compared to the present 1-year long study [26,28–32]. Thus, the duration of unloading or reduced activity may be responsible for the observed differences. In addition, SI, a model of complete electrical inactivity, does not result in up-regulation of MyHC-IIa in the rat soleus to the extent caused by ST [23,24]. For instance, Grossman et al. [23] demonstrated that at 60 days after SI the rat soleus contains ~20% MyHC-IIa protein, compared to the present study which found over 30% after 90 days of ST. In addition, Huey et al. [24], found no significant increases in MyHC-IIa mRNA up to 90 days after SI. As mentioned previously, SI also results in elevated expression of MyHC-IIb in the MG [25], which was not observed with ST. The SI model does result in a similar adaptive response in the expression of MyHCs-I and IIx in the soleus. Collectively, these data suggest that the low level of remaining electromechanical activity in ST compared to SI may be important in inducing MyHC-IIa expression in the soleus and preventing MyHC-IIb expression in the MG.

In summary, the soleus muscle responds to paralysis by up-regulating the mRNAs for MyHCs-IIx and -IIa, and down-regulating MyHC-I in a time-dependent fashion. The fast MG muscle showed less of a response to paralysis in MyHC isoform expression than the slow soleus muscle. Thus, there is a muscle-specific response to chronic reductions in neuromuscular activity. Finally, MyHC-IIb expression was not substantially influenced by ST in either fast (MG) or slow (soleus) rat ankle extensor muscles, suggesting that ST alone has little influence on the expression of this isoform.

Acknowledgments

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