Myosin heavy chains in fibers of TTX-paralyzed rat soleus and medial gastrocnemius muscles

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1Département de Kinésiologie, Université de Montréal, Succursale Centre-ville, Montréal, Québec, Canada H3C 3J7; 2Institut National de la Santé et de la Recherche Médicale U 300, Montpellier 34060; and 3Unité de Neurobiologie Cellulaire, Centre National de la Recherche Scientifique, Marseille 13007, France

Cormery, Bruno, Françoise Pons, Jean-François Marini, and Phillip F. Gardiner. Myosin heavy chains in fibers of TTX-paralyzed rat soleus and medial gastrocnemius muscles. J. Appl. Physiol. 88: 66–76, 2000.—The expression of five myosin heavy chain (MHC) isoforms was analyzed in the rat soleus (Sol) and the deep and superficial medial gastrocnemius (dGM, sGM) muscle after 2 and 4 wk of TTX paralysis by using immunohistochemical techniques. In Sol, after 4 wk of paralysis, fibers containing type I MHC were either pure type I (14%) or also contained developmental (D; 76%), Ila (26%), or Ilx (18%) MHC. Values for corresponding fibers in dGM were 8.5, 65, 38, and 22%. Also, by 4 wk an increase was seen in the proportions of fibers expressing Ila MHC in Sol (from 16 to 38%) and dGM (from 24 to 74%). In a region of sGM in control muscles containing pure Ibx fibers, a major proportion (86%) remained pure after 4 wk of paralysis, with the remainder coexpressing Ibx and Ilx. The results indicate that TTX-induced muscle paralysis results in an increase in fibers containing multiple MHC isoforms and that the D isoform appears in a major proportion of these hybrid fibers.

fibers types; tetrodotoxin; compartmentalization; antibodies

There is ample evidence in the research literature that postural muscles such as the rat soleus (Sol) respond to reduced activity by upregulating the expression of myosin heavy chains (MHC) other than the predominant type I isoform. Changes in Sol MHC expression after spaceflight (1, 7, 8), spinal transection (39), spinal isolation (13), immobilization (18), hindlimb suspension (40), and TTX-induced paralysis (25) have indicated that this muscle responds, in general, by upregulating the expression of type II and, in some cases, embryonic and/or neonatal MHC, and by increasing the incidence of fibers containing more than one MHC species. Differences in the extent of the responses of Sol to these various interventions can be attributable to variations in the degree of inactivity remaining in the different models.

The response of muscles containing large proportions of type II muscle fibers is less clear. After 9 days of spaceflight, for example, in vastus intermedius and red vastus lateralis, which both contain high levels of type I plus Ila/x MHC, tendencies for decreased type I and type Ila and increased Ilx and Iib expression were noted. Such changes were not evident in the tibialis anterior and white vastus lateralis muscles, both of which contain very little type I MHC in control muscles (14). Jankala et al. (18) noted, in immobilized rat gastrocnemius and plantaris, decreased type Ila mRNA and increased type Ilx expression in the former, similar to the results of Haddad et al. (14). On the other hand, TTX-induced muscle paralysis resulted in decreased percentages of fibers expressing Iib, and no change in fibers expressing Ila and Ilx in extensor digitorum longus (25). In general, it would appear that the more the muscle's involvement in postural activities, and/or the higher the initial proportions of type I and Ila fibers in the muscle, the higher the impact of reduced activity on MHC expression.

Recently, evidence has been presented (6, 13, 25, 38, 39) that type I fibers in rat Sol are heterogeneous in their time course and extent of response to hyperthyroidism and reduced activity, individually and in combination, signifying several subpopulations of type I fibers may exist in this muscle. These reports have included combinations of MHCs (such as I/Ila and I/Iib) that were not consistent with the previously proposed sequential scheme of adaptation (I, Ila, Ilx, Iib) (37). TTX-induced disuse, which induces more complete muscle inactivity than does hindlimb suspension (24), appears to accentuate these adaptations in Sol (25). Whether similar responses occur in type I fibers in mixed muscles is not known. Examination of the synergist medial gastrocnemius would allow the comparison of type I fibers in this muscle with those in Sol, to determine whether these patterns of adaptive response are specific to Sol muscle, or constitute adaptive options present among type I fibers of muscles with a more mixed fiber composition.

In this study, we examined changes in MHC contents of muscle fibers of two ankle extensors, Sol and medial gastrocnemius (GM), after 2 and 4 wk of complete inactivity produced by silencing of the sciatic nerve via chronic TTX application. The examination of the GM allowed us to determine the response of two fairly distinct compartments, the deep (dGM) and superficial...
MHC RESPONSE TO DISUSE IN FAST AND SLOW MUSCLES

Table 1. MAb specificity

<table>
<thead>
<tr>
<th>MAbs</th>
<th>IIa</th>
<th>IIX</th>
<th>IIb</th>
<th>D</th>
<th>Ref. Nos.</th>
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<tbody>
<tr>
<td>Sc-71</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
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<tr>
<td>8H8</td>
<td>-</td>
<td>+</td>
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<td>14E8</td>
<td>-</td>
<td>-</td>
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<td>16G73</td>
<td>-</td>
<td>-</td>
<td>+</td>
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<td>-</td>
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</table>

MAb, monoclonal antibody; MHC, myosin heavy chain; D, developmental.

(sGM) regions of the same muscle, which differ in nerve supply, fiber composition, and postural involvement (9, 15, 28, 41). Using an immunohistochemical approach, we were able to analyze and compare the appearance of hybrid fibers containing two or more MHCs in two neighboring muscles (Sol and dGM) and two compartments of the same muscle (sGM and dGM).

MATERIALS AND METHODS

Experimental animals and treatments. During the experiments in this study, animals were treated according to the guidelines of the Canadian Council of Animal Care, and all procedures were approved by the University of Montreal Animal Ethics Committee. Female Sprague-Dawley rats initially weighing 200 to 225 g were randomly allocated to either control or TTX groups. The procedures and effectiveness with regard to the TTX-induced disuse model have been discussed in prior publications (11, 12, 34, 36). In the TTX group inactivity of the left triceps surae was produced by continuous superfusion of the sciatic nerve with TTX during 2 (TTX2) or 4 (TTX4) wk. TTX was delivered to the nerve by using a system consisting of a miniosmotic pump (Alzet, model 2002 or 2004) and connecting Silastic tubing and cuff (Dow Corning), with a technique described in detail elsewhere (34). The concentration of TTX in the pump was 500 µg/ml in the Alzet 2002 pump (14 days) and 1 mg/ml in the Alzet 2004 pump (28 days). Solutions also contained penicillin and streptomycin (200 IU/ml and 200 µg/ml, respectively, in pump 2002, and double those in pump 2004).

The pumps were placed either subcutaneously on the animal’s back or intraperitoneally while the rat was under anesthesia (ketamine-xylazine, 80 mg/kg, 10 mg/kg body wt). After recovery from anesthesia in a warm cage, each rat was housed in a plastic cage until the terminal experiment. A coating of saturated picric acid solution was applied to the paralyzed hindlimb to prevent automutilation. Paralysis occurred between 24 and 72 h after pump implantation. Postoperative care included verification of paralysis (absence of toe-spreading reflex, no response to toe pinching, and no extensor response during passive dorsflexion or during locomotion) and the presence of automutilation. Two groups of sham-operated rats, Sham 14 days and Sham 28 days, in which the TTX solution was replaced with sterile 0.9% NaCl, as well as a group of unmanipulated controls, were included. For the TTX2 group, 14 days after the implant procedure each rat was anesthetized as above and weighed, the cuff was removed, and the connecting tubing was ligated. Twenty-four to 36 h later, animals were anesthetized with pentobarbital sodium, the left Sol and GM were excised, trimmed, and weighed, and the animals were killed with an overdose of pentobarbital sodium.

The muscles, with their internal surfaces face down, were then placed onto a flat piece of aluminum (0.5-mm thick) covered with a thin layer of embedding compound (Tissue-Tek, Miles, Elkhart, IN). Muscles were slightly stretched and allowed to recoil passively to a length at which no visible slack was evident and were then covered with a layer of embedding compound and flash-frozen in melting isopentane. Muscles were subsequently stored at –80°C until immunohistochemical processing. For the TTX4 group the same procedures were followed, except that in three rats the muscles were excised after in situ recording of the muscle, muscle unit, and motoneuron properties. No differences in immunohistochemical responses were observed between these six muscles (3 GM and 3 Sol) and eight others that were harvested immediately after anesthetization. Samples were allowed to warm to cryostat temperature (~20°C) and were mounted on metal chucks with paraffin-based embedding compound (Tissue-Tek) for cutting of a series of 10-µm cross sections for immunohistochemical processing.

Immunohistochemical procedures. The indirect immunofluorescence technique was performed on serial cryostat sections (10 µm) of skeletal muscles with each anti-MHC antibody [monoclonal antibody (MAb)] at various dilutions. Five MAb's were selected on the basis of their reactivity toward adult MHC and that of one MAb toward developmental (D) MHC (Table 1). The specificities of the MHC antibodies 4C10 (antidevelopmental), 8H8 (anti-I), SC-71 (anti-IIa), and 15F4 (anti-IIa+IIX) have been described elsewhere (2, 21, 22).

Fig. 1. Anti-fast myosin heavy chain (MHC) 16G7 and MHC monoclonal antibody (MAb) 14E8 were prepared with psoas myosin as antigen. Protein contents of whole tissue extracts obtained from adult rat muscles soleus (s) and diaphragm (d) were separated by performing SDS-PAGE (0.75 mm thick, 4% stacking gel, 7% separating gel). Lanes 1 and 2, silver-stained gel; lanes 3–4 and 5–6, similar gels that were electrophoresed onto nitrocellulose sheets and treated with MAb's 16G7 (IIX) or 14E8 (IIb), respectively. MAb's were revealed by using rabbit antimouse IgG coupled to alkaline phosphatase. MAb 14E8 detected single slow-migrating band in diaphragm, corresponding to IIb MHC and nothing in slow soleus. Anti-fast 16G7 MHC MAb recognized migrating bands in diaphragm, corresponding to IIX MHC.
Fig. 2. Micrographs of serial sections from control (A–F) and TTX4 (G–L) soleus muscle, the sciatic nerve of which was continuously superfused with TTX for 4-wk period. Serial cross sections with MAbs against MHC type I (A and G), IIa (B and H), IIa+IIx (C and I), IIx (D and J), IIb (E and K), and developmental (D; F and L) are shown. Fiber-type areas in A–F: 1, IIa; 2, I. Fiber-type areas in G–L: 1, I+IIa+IIx+D; 2, IIx+D; 3, IIa+IIx+D; 4, I. Scale bar in F = 100 µm for control muscle and in L = 50 µm for TTX4 muscle.

Table 2. Body and muscle weights

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>Body Wt, g</th>
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<th>GM Wt</th>
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</thead>
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<tr>
<td></td>
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<td>mg/g</td>
<td>mg</td>
</tr>
<tr>
<td>2 Wk</td>
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</tr>
<tr>
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<td>6</td>
<td>256 ± 7</td>
<td>137 ± 6</td>
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<td>TTX</td>
<td>8</td>
<td>241 ± 11</td>
<td>80.1 ± 8*</td>
<td>0.332 ± 0.037*</td>
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<tr>
<td>4 Wk</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Control</td>
<td>6</td>
<td>259 ± 15</td>
<td>141 ± 4</td>
<td>0.544 ± 0.02</td>
</tr>
<tr>
<td>TTX</td>
<td>8</td>
<td>261 ± 17</td>
<td>67.2 ± 7*</td>
<td>0.256 ± 0.048*</td>
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Values are means ± SD. n, No. of muscles; Sol, soleus; GM, medial gastrocnemius. *Significantly different from control at P < 0.05.
SC-71 was kindly provided by S. Schiaffino, University of Padua, Italy (30). The anti-fast MHCs 16G7 and 14E8 were prepared with psoas myosin as antigen and characterized by Western blotting and immunofluorescence and are specific to the IIx and IIb MHCs, respectively (from laboratory of F. Pons, Fig. 1).

Sections were incubated with one of the primary antibodies (30 min at 37°C), followed by incubation with fluorescein-conjugated rabbit anti-mouse IgG (Nordic Immunological Laboratories). Incubation with the MAb was in solution 1 [(in mM) 130 NaCl, 2 KCl, 10 Na2HPO4, and 1 KH2PO4, as well as 2% BSA, pH 7.4], and all washes were in solution 1 without BSA (solution 2) (2). A final wash was done with solution 2 containing 2% paraformaldehyde. Stained sections were mounted in Permount and keep at 4°C to diminish fading. Stained cross sections were photographed by using an Olympus fluorescence microscope with a Nikon automatic camera attachment. For Sol, photographs were taken from two portions of the muscle area. For GM, photos were taken from each of the dGM (that region containing the highest proportion of type I fibers) and sGM (furthest from the deep region) muscle regions. At least 275 fibers were analyzed per muscle section.

Fig. 3. Deep region of medial gastrocnemius. A–F: serial sections from a control muscle. G–L: serial sections from a TTX4 muscle. Serial cross sections with MAb against MHC type I (A and G), IIa (B and H), IIa + IIx (C and I), IIx (D and J), IIb (E and K), and developmental (F and L). In control (A–F), labeled fibers are fiber 1, (I), fiber 2 (IIa), fiber 3 (IIx), fiber 4 (IIb). In TTX4 (G–L), labeled fibers are fiber 1, (I), fiber 2 (IIa + IIx), fiber 3 (IIa + IIx + IIb), fiber 4 (IIa + IIx + IIb + D). Scale bar in F = 100 µm, in L = 50 µm.

Statistical analysis. A one-way ANOVA was conducted to identify direct inactivity effects. When differences were detected, a Neuman-Keuls post hoc test was used. All statistical
analyses were performed by using Statistica software. Statistical significance was set at $P < 0.05$.

RESULTS

Body and muscle weights. All TTX groups gained weight during the TTX treatment, from 226 ± 8 g at the time of insertion of the pump to 250 and 260 g after 2 and 4 wk, respectively (see Table 2). As expected, TTX inactivation produced significant reductions in muscle weight (expressed as mg or as mg/g body wt) of the Sol and GM muscles compared with their respective control muscles. After 2 and 4 wk the Sol muscle was 42 and 53% smaller than time-matched control muscles. Corresponding values for GM were 40 and 48%, respectively.

Immunohistochemical responses to inactivity. Immunohistochemical reactions using the panel of antibodies are shown in Figs. 2-4 for each of the muscles examined. In control Sol, the majority of fibers contained type I MHC (84%), and the remainder were IIa (14%) and hybrid fibers containing mixtures of I+IIa, I+IIx, or IIa+IIx (~2%). In dGM, fibers were positive for anti-I (30%), anti-IIa (24%), anti-IIx (43%), or anti-IIb (5%), with very few fibers (5%) showing the presence of more than one MHC. In sGM, 94% of fibers were type

![Figure 4. Superficial region of medial gastrocnemius. A–F: serial sections from a control muscle. G–L: serial sections from a TTX4 muscle. Serial cross sections with MAbs against MHC type I (A and G), IIa (B and H), IIa+IIx (C and I), IIx (D and J), IIb (E and K), and developmental (F and L). In micrographs from control muscle, labeled fibers are fiber 1 (I), fiber 2 (IIa), fiber 3 (IIx), fiber 4 (IIb). In TTX4 micrographs, labeled fibers are fiber 1 (I+IIa+IIx+D), fiber 2 (IIb+IIx), fiber 3 (IIa+IIx+IIb+D), fiber 4 (IIa+IIx+D). Scale bar in F = 100 µm, in L = 50 µm.](image-url)
IIb and 6% were IIx, with no evidence of fibers containing more than one heavy chain species (Figs. 5–7).

The shifts in single-fiber MHC species with complete inactivity are summarized in Table 3. Despite the initial different distributions of MHC isoforms at the single-fiber level, both Sol and dGM showed significant increases in the proportions of fibers containing IIa, IIx, and D MHC by 4 wk of paralysis. The magnitude of these changes was clearly different in these two muscles; the proportions of fibers containing IIx and IIa MHC were higher in dGM at 4 wk. Interestingly, the increase in proportions of fibers containing D MHC as well as the proportions of fibers containing more than one MHC, were similar in Sol and dGM at 2 and 4 wk of paralysis, as they were in control muscles (Table 3). The sGM responded to muscle paralysis with an increase in the proportion of fibers expressing IIx MHC and an increase in fibers containing more than one MHC type, but to a lesser extent than was the case in Sol or dGM.

Fibers containing type I MHC. In both Sol and dGM, the proportion of “pure type I” fibers decreased with paralysis, such that, by 4 wk, it had decreased from 84 to 13% in Sol, whereas in dGM it had decreased from 30 to 8%. Thus a higher proportion of the original pure type I fibers remained pure in dGM (8 of 30), compared with Sol (13 of 84), after 4 wk of paralysis. Virtually all hybrid fibers containing type I MHC also contained D MHC isoform, in both Sol and dGM. In addition, many of these hybrid fibers also contained, in order of predominance, types IIa and/or IIx MHC, in proportions similar in both muscles (Fig. 8).

At 2 wk, the most prominent hybrids in fibers containing type I MHC in Sol were I+D, I+IIa+IIx+D, I+IIa, and I+IIx+D. By 4 wk, I+D became the predominant hybrid (32% of all Sol fibers), the proportions of these other hybrid combinations were larger, and significant proportions of I+IIa+D became evident (Figs. 5 and 6). This pattern was similar in dGM. Thus it appeared that, at least for previous type-I-containing fibers that began expressing other MHC species when inactivated, the expression of D MHC preceded the expression of IIx and IIa MHCs, with a few exceptions.

Fibers containing IIb MHC. In the sGM the majority of fibers contained exclusively type IIb MHC, both in control and in paralyzed muscles. After 4 wk of paralysis, only 16% of fibers containing IIb MHC contained an additional MHC, which was always IIx. No MHC other than IIb and IIx appeared in the sGM as a result of paralysis. An interesting observation was that MHC-IIb-containing fibers in dGM responded quite differently from those in sGM. In the former, hybrid fibers containing IIb MHC also contained, at 2 and 4 wk of paralysis, IIx, IIa, and/or D isoform (Figs. 6 and 7).

Responses of type IIa and IIx fibers. In Fig. 9, we have presented a summary of the analysis of those fibers not containing type IIb.
treated in previous figures, i.e., fibers in paralyzed muscles that did not contain type I and IIb MHC. In Sol, where the proportion of MHC-IIa-containing fibers changed very little, almost all IIa-containing fibers became hybrid fibers by 2 wk and remained so at 4 wk. Most of these fibers contained both IIx and D MHC, in addition to the original IIa. After 2 wk of inactivation the hybrids included, in order of predominance, IIa+IIx+D, IIa+IIx, and IIa+D. By 4 wk, the incidence of the IIa+IIx+D hybrid increased (to ∼13% of all fibers). Thus it appeared that original type IIa fibers in the Sol expressed IIx and D MHC, whereas IIx fibers expressed, with a slower time course, IIa and D MHC.

DISCUSSION

In this study, we have demonstrated that, similar to the case in Sol, a decrease in the activity of one of its synergists of mixed fiber type results in an increased incidence of fibers containing more than one, and up to four, MHC isoforms. Examination of the variety of MHC mixtures that are produced from originally pure fiber types reveals some degree of similarity in proportions between these two different muscles (Figs. 5 and 6), suggesting a certain degree of commonality related to fiber type, and not only to muscle. Finally, TTX paralysis results in the expression of D MHC isoforms in the majority (75–76%) of the muscle fibers in Sol and dGM regions.

Muscle atrophic responses. After 2 wk of paralysis the atrophy of the ankle extensors GM and Sol was of similar magnitude (58–60% of control muscle wt) and did not change very much by 4 wk (48–52% of control, Table 1). These results are consistent with previous reports for the TTX model (4, 23, 33–35) and are similar to the effects of denervation (17, 27, 31). In the model of spinal isolation, however, the slow Sol demonstrates a more pronounced atrophy of muscle mass and fiber area (64–75% lower than control after 60 days) than that seen with TTX-induced inactivity (11, 35), and this atrophy is more pronounced than that seen in fast muscles by 4 wk (13). The more pronounced Sol atrophy with spinal cord isolation may be attributable,
as suggested by Grossman et al. (13), to the differences in the presence of functional afferences onto motoneurons innervating the silenced muscles in the TTX-paralysis condition. It is presently unknown to what extent motoneurons, the axons of which are silenced by TTX blockade, continue to generate action potentials or subliminal decreases in membrane potential when the rats are locomoting during the period of TTX paralysis. The lesser extent of atrophy of the Sol during TTX-induced paralysis, compared with spinal isolation, may suggest that periodic motoneuron depolarizations exert an atrophy-attenuating influence on innervated muscles, via a myotrophic mechanism. In motoneurons innervating the lobster claw muscle, depolarization of motoneuron cell bodies of insufficient magnitude to result in action potential generation can evoke changes in efficacy of neuromuscular junctions that are similar to those seen after chronic electrical stimulation of axons (20). Such a proposal would also suggest that type I fibers, and muscles containing a preponderance of this fiber type, are more prone to this influence than are type II fibers.

Response of type I fibers. Previous studies have demonstrated that inactivity induced by TTX, spinal transection, denervation, or more recently in spinal isolation results in a shift in MHC expression from type I to type II in muscles containing a predominance of type I fibers (13, 26, 32, 39). A recent report of the effects of TTX paralysis indicated an increase in the proportion of fibers containing type I MHC in the Sol after only 2 wk of inactivity (25). In our experiment, we observed a transient and nonsignificant increase in the number of Sol fibers containing type I MHC at 2 wk, which subsided and became significantly decreased by 4 wk. A similar trend was evident in dGM. In more detail, we compared the propensity of fibers that express the type I MHC to express or reexpress other MHCs in both Sol and dGM. A specific question raised

![Fig. 8. Changes in composition of fibers containing type I MHC during 2 and 4 wk of paralysis.](http://jap.physiology.org/) Downloaded from 10.220.33.4 on April 5, 2017 http://jap.physiology.org/ Downloaded from
was whether TTX paralysis would result in combinations not consistent with the I-Ilx-IIb transition scheme, as has been shown in other models that include the component of reduced neuromuscular activity. Our results showed the I/IIx combination in a small proportion of Sol fibers, as has been previously shown after hindlimb suspension (38), spaceflight (38), spinal cord transection and isolation (13; 39), and TTX paralysis (25), further suggesting that short periods of reduced activity promote the expression of IIx MHC, without the intermediate expression of type IIa. This combination was also evident in dGM at 2 wk, thus attesting to the propensity of a proportion of type I fibers in muscles other than Sol to this adaptation scheme.

After 1 mo of complete inactivity 13 and 8% of the Sol and dGM, respectively, expressed a pure type I fiber with the rest of the fibers coexpressing two to four other MHCs. This may indicate a heterogeneous population of type I fibers in the rat Sol and GM. The same phenomenon has been observed previously, but only in rat Sol, after short periods of reduced muscle activity (6, 25, 38, 39) The α-cardiac MHC, which reacts similarly to the β-MHC with our antibodies, has been demonstrated in rabbit and human muscles, but not yet in rats, except after TTX paralysis (25). Thus a subpopulation of type I fibers may indeed respond to inactivity by expression of the α-cardiac heavy chain, whereas other subpopulations express type II and D forms.

Fig. 9. Changes in fibers other than those containing type I and IIb MHC in Sol (left) and dGM (right). Values are means ± SD. *Significantly different from control. **Significant difference between TTX2 and TTX4 groups. P < 0.05.
alyzed muscles contained also at least one other MHC isoform. However, the response of previously pure type IIb fibers differed significantly in dGM and sGM compartments; in the latter, copresence of more than one heavy chain was restricted to a minor proportion of these fibers and consisted of only the IIb-IIx combination. In dGM, appearance of myosin isoforms seemed to occur in a sequential manner (IIb > IIx > Ia +D). This difference in the response of type IIb fibers is consistent with previous findings of differences in type IIb myofibrillar ATPase (3), fast-fatigable motor unit properties (10), and type II fiber atrophic responses (11) between deep and superficial regions of this muscle. Whether type IIb fibers in the superficial region would have eventually shown a higher degree of coexistence of other MHC with a longer period of paralysis is unknown.

It must be recognized, however, that comparison of the response of the small proportion (5%) of IIb fibers in dGM with all of the fibers of sGM is tenuous. Indeed, perhaps these fibers within the two different muscle regions contain slightly different isoforms not distinguishable by using our antibodies. In addition, it is possible that the more robust plasticity of the IIb fibers in the dGM signifies basal levels of expression of other isoforms in control muscles that were not detectable by using our qualitative techniques.

The expression of some type IIb MHC was not observed in the Sol of rats after 30 days of TTX blockade (Figs. 5 and 8). The same results have been observed in previous studies with TTX blockade (25, 33), denervation (26), and spinal isolation (13). The capacity of Sol muscle fibers to express the IIb gene under normal conditions has been demonstrated (16, 18), and type IIb expression appears to be upregulated in rat Sol under conditions of spaceflight (38), spinal transection (39), and hindlimb suspension (38), especially when the latter is combined with a hyperthyroid state (6). Thus type IIb MHCs may be expressed in type I fibers under conditions of abnormal or unloaded contractile activity that remain in these latter models.

Responses of type IIA and IIX fibers. In general, our results suggest that type IIA fibers are quicker to respond to complete inactivity than are type IIX fibers. This conclusion is based on the analysis of fibers other than those containing type I and type IIb MHC, which were therefore assessed as originally containing either type IIA, IIX, or a combination of these isoforms (Fig. 9). At 2 wk, in Sol and dGM, virtually all type IIA-containing fibers (the proportions of which had not changed) were hybrid, whereas, at the same time, pure IIX fibers still were present. Thus, as one might expect, the rapidity of adaptation to inactivity in the alteration of expression of MHCs among fast MHC-containing fibers appears to be proportional to the original level of activity of the muscle fibers: IIA > IIX > IIb (dGM) > IIb (sGM). However type I fibers are evidently slower to respond than are the IIA fibers, which supports the proposal of Talmadge et al. (39) from their analysis of the effects of spinal cord transection on the rat Sol.

Reexpression of the D MHC isoform. The antibody used in this study recognized both the embryonic and neonatal forms of MHC (2, 21, 22). Normally, these isoforms are not expressed in rat muscle fibers at 3 and 8 wk, respectively, after birth (5, 19). Our findings of the reexpression of these isoforms in inactive muscles are consistent with the report of the appearance of embryonic MHC in TTX-paralyzed Sol (25) and of both embryonic and neonatal MHC in TTX-paralyzed Sol and tibialis anterior (29). In addition, 19% of Sol fibers reexpress the D isoform (neonatal and/or embryonic) in combination with the four adult isoforms (I, IIA, IIX, IIb) after 2 wk of spaceflight (1). On the other hand, few or no embryonic isoform-containing fibers were found after 1 mo of spinal transection (39) or 2 mo of spinal isolation (13). It thus appears that the neonatal isoform may be the primary D isoform reexpressed during muscle inactivity. The importance of reactivation of the D isoform (probably neonatal) program is supported by the significant proportion of fibers containing D MHC in the muscles examined in this study (76% of Sol fibers, 75% of dGM fibers). Interestingly, the propensity for fibers to express D isoforms was similar to their propensity to express other than original MHC isoforms (see above), leading to the suggestion that reexpression of the D isoform accompanies the conversion of muscle fiber nuclei to a state in which MHCs other than the original are synthesized. An exception, once again, appears to be the type IIb fibers in sGM, which, when they adapted, expressed IIX, but not D, MHC.

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