Human and Rat Skeletal Muscle Adaptations to Spinal Cord Injury

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Abstract/Résumé
Results of studies of rodent skeletal muscle plasticity are often extrapolated to humans. However, responses to “disuse” may be species specific, in part because of different inherent properties of anatomically similar muscles. Thus, this study quantified human and rat m. vastus lateralis (VL) fiber adaptations to 11 weeks of spinal cord injury (SCI). The m. VL was taken from 8 young (54 d) male Charles River rats after T-9 laminectomy (n = 4) or sham surgery (n = 4). In addition, the m. VL was biopsied in 7 able-bodied and in 7 SCI humans (31.3 ± 4.7 years, mean ± SE). Samples were sectioned and fibers were analyzed for type (I, IIa, IIb/x), cross-sectional area (CSA), succinate dehydrogenase (SDH), α-glycerol-phosphate dehydrogenase (GPDH), and actomyosin adenosine triphosphatase (αTAPase) activities. Rat fibers had 1.5- to 2-fold greater SDH and GPDH activities while their fibers were 60% the size of those in humans. The most striking differences, however, were the absence of slow fibers in the rat and its four-fold greater proportion of IIb/x fibers (80% vs. 16% of the CSA) compared to humans. SCI decreased SDH activity more in rats whereas atrophy and IIa to IIb/x fiber shift occurred to a greater extent in humans. It is suggested that the rat is a reasonable model for studying the predominant response to SCI, atrophy. However, its high proportion of IIb/x fibers limits evaluation of the mechanical consequences of shifting to “faster” contractile machinery after SCI.

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On transpose fréquemment chez les humains les résultats des études sur la plasticité des muscles squelettiques des rongeurs. Notons cependant que l’adaptation au non-usage peut être spécifique à l’espèce et due en partie aux différentes propriétés intrinsèques des muscles apparentés sur le plan anatomique. Cette étude s’intéresse à la caractérisation des adaptations des fibres du muscle vastus lateralis (VL) d’humains et de rats, 11 semaines après une lésion médullaire (SCI). Le muscle VL est prélevé chez 8 jeunes rats Charles River (54 jours) après une laminectomie en T-9 (n = 4) ou une chirurgie simulée (n = 4). De plus, des biopsies du muscle VL ont été pratiquées chez 7 humains valides et 7 traumatisés médullaires (âge moyen: 31,3 ans; erreur type: 4,7 ans). Les échantillons musculaires sont analysés pour évaluer la proportion de fibres du type I, IIa, IIb/x, la surface de coupe (CSA), l’activité de la succinate déshydrogénase (SDH), de l’α-phosphoglycérate déshydrogénase (GPDH), et de l’adénosine triphosphatase de l’actomyosine (qATPase). L’activité de la SDH et de la GPDH est de 1,5 à deux fois plus élevée chez les rats que chez les humains bien que la dimension des fibres musculaires chez le rat représente 60% de celle des humains. La différence la plus notable est l’absence de fibres musculaires lentes chez le rat et la présence de quatre fois plus de fibres IIb/x que chez l’humain (CSA de 80% comparativement à 16%). Chez les rats, on observe une plus grande diminution de l’activité de la SDH alors que l’atrophie et la proportion de fibres transitionnelles IIa – IIb/x sont plus marquées. En conclusion, le rat se révèle un modèle acceptable pour étudier l’atrophie qui constitue la principale adaptation à une lésion médullaire. Toutefois, sa grande proportion de fibres IIb/x constitue une limite à l’évaluation des propriétés mécaniques d’un complexe contractile devenu plus rapide à la suite de la lésion médullaire.

Introduction

Studies of skeletal muscle plasticity have often used rodents as models (Baldwin, 2000; Holloszy, 1983). The stringent control, relative inexpense, and apparent ease of using invasive procedures make studies of rodents quite attractive. Consequently, these important studies have provided the bulk of information concerning skeletal muscle responses to acute and chronic alterations in loading history, to several “pathological” conditions, and to select therapies (Baldwin, 2000). Most of these studies are conducted in an effort to increase our understanding of a basic biological process. It is hoped that the results of such work will result in the development of interventions that are successful in lower mammals and, consequently, can be evaluated for treating humans. Therefore an integrated approach to demonstrate the important role that skeletal muscle plays in human health and disease is warranted (Baldwin, 2000).

Several observations suggest there is opportunity, in fact a frank need, to increase our understanding of human skeletal muscle plasticity in health and disease. For example, the standard of health care in the modern world has resulted in many individuals living a long time with a debilitating disease such as multiple sclerosis, living long after a spinal cord injury or heart attack, and/or simply having a longer life span (Baldwin, 2000). In all, deterioration of skeletal muscle probably compromises functional capacity and may contribute to poor health (Bauman et al., 1999; Kent-Braun et al., 1997; Massie et al., 1996). However, it seems prudent to caution that in the use of animal models at the expense of human studies, an integrated approach needs to be taken.
The inherent characteristics of skeletal muscle have a marked influence on the nature and magnitude of its adaptive response to altered loading (Pette and Staron, 2000; Roy et al., 1991). At the same time, they can differ markedly between anatomically similar skeletal muscles in humans and, for example, rats. As a result, adaptive responses to an intervention may not necessarily be comparable between species. Accordingly, this study tested the hypothesis that m. vastus lateralis (VL) of humans and rats would show divergent responses to 11 weeks of SCI.

Methods

The m. VL was taken from 8 young (54 d littermates) male Charles River rats 11 weeks after SCI (T-9 laminectomy, \( n = 4 \)) or sham surgery (\( n = 4 \)). Additionally, percutaneous needle biopsy samples were taken 11 weeks post injury from m. VL of 7 patients with complete SCI as well as 7 recreationally active, able-bodied controls (31.3 ± 4.7 years, mean ± SE), matched for age, gender, body mass, and previous activity level. Excised muscle tissue was mounted in an embedding medium and subsequently frozen in 2-methyl butane cooled to its freezing point in liquid nitrogen. Attempts to control for variations in fiber CSA were made by freezing animal muscles at resting length and standardizing freezing intervals between species. Samples were stored at –70 °C until analyzed. Subjects were informed of the protocol and signed an informed consent prior to participation in the study.

HISTOCHEMICAL ANALYSES

Samples were removed from the freezer and placed in a cryostat microtome at –20 °C to warm. Serial sections (6, 10, or 14 µm) were cut and placed onto cover slips for immediate assay by both qualitative and quantitative histochemical techniques. Images were acquired using a Sony xc 77 CCD camera attached to an Olympus bh-2 microscope linked to a Macintosh Quadra 800 computer. Images were saved and analyzed using NIH image software (written by Wayne Rasband at the US National Institutes of Health, zippy.nimh.nih.gov), as done previously (Castro et al., 1999; 2000; Gregory et al., 2001; Kent-Braun et al., 1997).

Sections (10 µm) were assayed qualitatively for myofibrillar adenosine triphosphatase activity (hATPase) using the techniques of Brooke and Kaiser (1970) and of Guth and Samaha (1969). Single fibers were analyzed for type by microdensitometric determination of optical density. The myosin ATPase composition, represented by the optical density of the fiber and based on its pH lability, is correlated to the myosin heavy chain (MHC) composition of the given fiber (Staron and Pette, 1986). In an attempt to simplify comparisons between species, fibers were classified as either type I, IIa, or IIb/x, as done previously (Castro et al., 1999; 2000; Gregory et al., 2001).

Quantitative histochemical determination of succinic dehydrogenase activity (SDH) and alpha-glycerol phosphate dehydrogenase (GPDH) activity were determined by the microdensitometric technique described by Blanco et al. (1988) and Martin et al. (1985), respectively. Images were captured using the same tools as for hATPase with the exception that a narrow pass interference filter with peak emission of 570 nm was used so as to assess maximal absorption of Nitro-blue
tetrazolium-diformazan (NBT-dfz), the in-vitro reaction end-product. Enzyme activities were obtained from the difference in optical density between samples incubated in the presence and absence of substrate and are expressed as μmol fumarate/L tissue/min and μmol glycerol-3-phosphate/L tissue/min for SDH and GPDH activities, respectively.

The method of Blanco and Sieck (1992) was modified and used for quantitative determination of actomyosin adenosine triphosphatase (qATPase) activity in single fibers. Briefly, 6 μm serial sections were incubated in one of 6 solutions of different ATP concentrations (0–3 mM). The use of incubations of different concentrations is necessary because, in contrast to the assays for SDH and GPDH, the qATPase reaction is non-substrate limited. The Michaelis-Menten model of enzyme kinetics was used to analyze the data, and the reciprocal of the y-intercept of the Lineweaver-Burk plot was taken as the maximum velocity (Vmax) of the qATPase in OD/min. Enzyme activity, expressed as mmol Pi per liter of tissue per minute, was determined using the Lambert-Beer equation with a molar extinction coefficient for lead-sulfide of 1450 M⁻¹ cm⁻¹.

Images saved for SDH, GPDH, and qATPase assays were matched to those saved for hATPase, thus allowing enzyme activities obtained for individual fibers in serial sections to be matched and expressed relative to fiber type and cross-sectional area. This provides a qualitative indicator of fiber properties via hATPase, quantitative estimates of both glycolytic and oxidative energy supply via GPDH and SDH activities, respectively, and a quantitative estimate of energy demand via qATPase activity. The averages of a minimum of 100 fibers of each type were used for calculation of fiber cross-sectional area and muscle enzyme activities.

STATISTICS

Statistical analyses were done using SPSS 10.0 software. Comparisons made between species in control muscles were done using a planned comparison from a one-within/one-between analysis of variance (ANOVA). Comparisons made between species for adaptations over time were made using the interaction term from a one-within/one-between ANOVA. Inter-species changes in fiber type percentage were tested via t-tests. Differences were considered significant at \( p < 0.05 \).

Results

COMPARISONS BETWEEN RAT AND HUMAN CONTROL VL

Average fiber size was 63% greater in humans than in rats \( (p \leq 0.0001, \text{Table 1}) \). The magnitude of this difference was most dramatic in type IIa fibers, with human fibers being 131% larger (Table 1). Rats had no type I fibers and a greater relative proportion of type IIb/x fibers (Table 1). This differed greatly from the mosaic of human phenotypes (Figure 1). As a result, the relative cross-sectional area (CSA) occupied by fast and by IIb/x fibers was greater in the rat \( (p \leq 0.0001, \text{Table 1}) \). A twofold and a threefold greater average fiber SDH and GPDH activity, respectively, was also found in the rat \( (p \leq 0.0001, \text{Table 1}) \). As with fiber CSA, species differences in enzyme activities were greatest in type IIa fibers. Average fiber qATPase activity was 30% lower in rats than in humans \( (p \leq 0.0001, \text{Table 1}) \).
Cross-sectional comparisons in both species showed that average fiber CSA and SDH activity were lower and that there was an apparent shift in the proportion of fast fiber subtypes, IIa → IIb/x after SCI. However, the decrease in fiber CSA was greater in humans ($p \leq 0.0001$) while the opposite was the case for SDH activity ($p = 0.026$, Table 1). The 77% increase in the proportion of type IIb/x fibers in humans was five times the 14% increase noted for rats ($p \leq 0.0001$, Figure 1). No changes in GPDH and qATPase activities or fast fiber percentages were noted with injury (Table 1).

**Table 1 Fiber Type for Succinate Dehydrogenase Activity (SDH), Glycerol Phosphate Dehydrogenase Activity (GPDH), Actomyosin Adenosine Triphosphatase Activity (qATPase), and Percent Cross-sectional Area (%CSA)**

<table>
<thead>
<tr>
<th>FT</th>
<th>CSA</th>
<th>SDH</th>
<th>GPDH</th>
<th>qATPase</th>
<th>%CSA</th>
</tr>
</thead>
<tbody>
<tr>
<td>CON Rat</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>I</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>IIa</td>
<td>2599</td>
<td>1944</td>
<td>181.1</td>
<td>26.1</td>
<td>20.7</td>
</tr>
<tr>
<td>± 26</td>
<td>± 32⁺</td>
<td>± 4.7⁺</td>
<td>± 2.1</td>
<td>± 1.9</td>
<td></td>
</tr>
<tr>
<td>IIb/x</td>
<td>3446</td>
<td>1567</td>
<td>200.6</td>
<td>31.3</td>
<td>79.3</td>
</tr>
<tr>
<td>± 43</td>
<td>± 22⁺</td>
<td>± 2.7⁺</td>
<td>± 1.7</td>
<td>± 3.0⁺</td>
<td></td>
</tr>
<tr>
<td>CON Human</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>I</td>
<td>4765</td>
<td>859</td>
<td>37.3</td>
<td>20.6</td>
<td>34.6</td>
</tr>
<tr>
<td>± 323</td>
<td>± 83</td>
<td>± 3.1</td>
<td>± 2.9</td>
<td>± 2.6</td>
<td></td>
</tr>
<tr>
<td>IIa</td>
<td>6012</td>
<td>720</td>
<td>54.2</td>
<td>35.8</td>
<td>50.2</td>
</tr>
<tr>
<td>± 512⁺</td>
<td>± 75</td>
<td>± 3.4</td>
<td>± 3.2⁺</td>
<td>± 3.9⁺</td>
<td></td>
</tr>
<tr>
<td>IIb/x</td>
<td>5111</td>
<td>618</td>
<td>62.9</td>
<td>40.1</td>
<td>15.2</td>
</tr>
<tr>
<td>± 421⁺</td>
<td>± 60</td>
<td>± 3.1</td>
<td>± 3.8⁺</td>
<td>± 0.5</td>
<td></td>
</tr>
<tr>
<td>SCI Rat</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>I</td>
<td>–</td>
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<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>IIa</td>
<td>1254</td>
<td>1608</td>
<td>197.4</td>
<td>27.9</td>
<td>9.5</td>
</tr>
<tr>
<td>± 30</td>
<td>± 47⁺⁺</td>
<td>± 3.9⁺⁺</td>
<td>± 2.0</td>
<td>± 0.6</td>
<td></td>
</tr>
<tr>
<td>IIb/x</td>
<td>1833</td>
<td>1141</td>
<td>213.3</td>
<td>33.4</td>
<td>90.5</td>
</tr>
<tr>
<td>± 42</td>
<td>± 26⁺⁺</td>
<td>± 5.4⁺⁺</td>
<td>± 2.3</td>
<td>± 1.1⁺⁺</td>
<td></td>
</tr>
<tr>
<td>SCI Human</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>I</td>
<td>2545</td>
<td>688</td>
<td>45.9</td>
<td>21.7</td>
<td>39.7</td>
</tr>
<tr>
<td>± 202</td>
<td>± 67</td>
<td>± 3.3</td>
<td>± 3.1</td>
<td>± 4.6</td>
<td></td>
</tr>
<tr>
<td>IIa</td>
<td>2633</td>
<td>525</td>
<td>60.2</td>
<td>36.2</td>
<td>33.4</td>
</tr>
<tr>
<td>± 354⁺⁺</td>
<td>± 42</td>
<td>± 3.7</td>
<td>± 3.3⁺⁺</td>
<td>± 6.0⁺⁺</td>
<td></td>
</tr>
<tr>
<td>IIb/x</td>
<td>2569</td>
<td>428</td>
<td>63.9</td>
<td>39.7</td>
<td>26.9</td>
</tr>
<tr>
<td>± 347⁺⁺</td>
<td>± 45</td>
<td>± 2.9</td>
<td>± 3.2⁺⁺</td>
<td>± 5.1</td>
<td></td>
</tr>
</tbody>
</table>

*Note:* Data are presented as mean ± SE. *Value > control rat; ’Value > control human; **Value > SCI rat; ’’Value > SCI human ($p < 0.05$).
Figure 1. hATPase images captured of human m. VL in control (upper left) and SCI (upper right) subjects, and rat VL in control (lower left) and SCI (lower right) animals.

Discussion

The unique aspect of this study was in the comparisons of adaptations of fibers of the same major locomotor muscle, m. VL, to complete SCI in humans and in the rat. Of the variables assessed, the results, although preliminary in nature, indicate that fiber atrophy is the major adaptation of m. VL to 11 weeks of SCI. The loss of fiber size occurred to a greater extent in humans while the opposite was the case for the decline in SDH activity. Neither response appeared to be particularly fiber-type dependent, thus the high proportion of IIb/x fibers in the rat would not be overly problematic. However, the predominance of fibers of this type would seemingly limit conversion to faster muscle, as appeared to be the case. Before addressing these issues, a few comments concerning inherent characteristics of m. VL are warranted.
The small size and high SDH activity of rat compared to human fibers of *m. VL* in combination with the slightly lower qATPase activity in this rodent are characteristics of muscle with a substantial resistance to fatigue. The smaller fibers provide a shorter diffusion distance, while the activities of SDH and qATPase, which are used as estimates of energy supply and energy demand, respectively, are determinants of several mechanical characteristics of skeletal muscle. In addition, resistance to fatigue is strongly related to their ratio (SDH:qATPase) (Tesch et al., 1985; Van Der Laarse et al., 1991). The rat *m. VL* has no slow fibers, unlike the human *m. VL* which is about one-third slow contractile machinery. This would suggest a high energy demand of contraction, noting that qATPase activity of fast fibers is 1.5 to 3 times that of slow fibers (Barany, 1967). However, the high proportion of fast fibers in the rat *m. VL*, and in fact in its hind limb, is countered by the unique hierarchy of its fibers with respect to markers of aerobic-oxidative metabolism. This hierarchy in the rat followed IIa > I > IIb/x (Rivero et al., 1998) vs. that of humans being I > IIa > IIb/x (Gregory et al., 2001). Thus the impact of the high, inherent proportion of fast fibers in the rat is seemingly lessened.

Data showing that rat muscles are made up of predominately fast muscle fiber types are not new (Armstrong and Phelps, 1984). Additionally, previous work on single fibers has described functional and adaptive differences to various interventions by slow and by fast fiber subtypes (Roy et al., 1991). Thus, the fact that human muscles, unlike the rat, typically contain a mosaic of phenotypic expression would lead us to expect that these species might adapt differently to disuse. Nonetheless, the overall decrease in fiber size and SDH activity, combined with a shift between fast fiber subtypes (IIa → IIb/x), was common to both species. However, the magnitude of change in these variables was different. Humans showed the greatest reduction in size and percentage of type IIa fibers, given their approximate 63% larger fibers and 1.5 times greater proportion of this fast subtype in able-bodied individuals. Rat fibers showed a greater reduction in SDH activity, given their approximate 150% higher inherent activity for this enzyme. Thus the original hypothesis of divergent responses is accepted.

Given the described changes resulting from SCI, the response with the greatest impact is most likely fiber atrophy. The atrophic response that typically accompanies an injury of this type has been linked to many of the secondary pathologies associated with SCI and has been shown to be the limiting factor in exercise capacity in such patients (Bauman et al., 1999; Hopman et al., 1998). Therefore the greater decrease in size, both absolute and relative, in the human suggests a greater overall insult from SCI compared to the rat.

The most obvious inherent difference between these two species was in the percentage of fast fibers. Conventional wisdom suggests slow to fast fiber conversion after SCI (Pette and Staron, 2000; Roy et al., 1991). However, this could have little impact in the rat, given that nearly all of its hind limb inherently consists of fast fibers. The one exception is the *m. soleus*, which occupies approximately 5% of the hind limb and contains a high percentage of slow fibers (Armstrong and Phelps, 1984). Interestingly, this muscle converted to a predominantly fast muscle after the 11 weeks of SCI in these same animals (Gregory et al., 2003). In contrast, we did not find a decrease in the percentage of slow fibers 11 weeks after SCI in human *m. VL* in this study, nor in these same subjects 24 weeks after injury (Castro et al., 1999; Talmadge et al., 2002). Evaluation of affected muscles that inherently
contain slow fibers in long-term SCI patients, nonetheless, suggests that this slow → fast shift does eventually occur in humans (Martin et al., 1992). Clearly, when this occurs, it would be expected to have a much greater impact on human than on rat muscle, i.e., an increase in contraction speed (Gerrits et al., 1999; Shields et al., 1997).

The IIa → IIb/x shift was the major phenotypic adaptation that occurred in m. VL in humans and rats. The potential for change in the rat, however, was minimal because of its high inherent proportion of type IIb/x fibers. The fact that only ~15% of the CSA of m. VL in able-bodied humans is occupied by fibers of this type leaves room for greater phenotypic adaptations. This may partly account for the extreme fatiguability evident in human m. VL reported in long-term SCI individuals. On the other hand, the minimal increase in type IIb/x fibers that can occur in rats could be viewed as countering its greater decline in SDH activity with regard to resistance to fatigue. The potential influence of conversion to a faster muscle upon contraction mechanics and resistance to fatigue is based in part on a lack of plasticity in qATPase activity after SCI. This does appear to be the case after short-term injury in humans (Castro et al., 2000), while persons with long-term injury may actually show a decline (Martin et al., 1992). However, it is not so clear that loss of force during contraction reflects fatigue in the classical sense, given that SDH activity has been reported to explain little of the performance decrement during electrical stimulation in long-term SCI patients (Martin et al., 1992; Rochester et al., 1995).

The results of this study suggest that the major adaptation of m. VL to 11 weeks of SCI is fiber atrophy in both humans and rats. Neither this response nor the decline in SDH activity was shown to be fiber-type dependent. Accordingly, it is suggested that the rat is a reasonable model for the evaluation of mechanistic aspects of, and probable therapies for, these responses to short-term SCI. Such studies should not be conducted at the expense of human work, however, realizing that human experiments are the ultimate testing ground. On the other hand, it is suggested that studies of phenotypic expression in rats be conducted with caution. Most important, the inherent slow phenotype of human skeletal muscle is extremely resistant to change. Even when altered by such an extreme incident as chronic SCI, human skeletal muscle takes on the inherent characteristics of the rat hind limb. Accordingly, the importance of figuring out how the rat functions with such a high proportion of fast fibers in its hind limb could be valuable, given that it has been difficult to increase the proportion of slow fibers in paralyzed human muscle (Anderson et al., 1996).

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