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The effect of subacute denervation on the electrical anisotropy of skeletal muscle: Implications for clinical diagnostic testing ☆

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ABSTRACT

Objective: Applied electrical current flows preferentially along rather than across muscle fibers, a characteristic called anisotropy. In this study, we investigate the alteration in muscle anisotropy after denervation.

Methods: Eight adult male rats underwent sciatic nerve crush and the gastrocnemius was harvested from 1 to 2.5 weeks later. Muscle from 12 additional healthy rats was also obtained. Multifrequency electrical impedance measurements were made on the tissue and its conductivity and relative permittivity (i.e., its polarizability) calculated. Anisotropy of the tissue was determined by calculating conductivity and permittivity differences, subtracting transverse from longitudinal values. Muscle fiber and blood vessel quantification were also performed.

Results: The mean conductivity difference for sciatic crush animals was higher ($p < 0.05$) than for the healthy animals across the frequency spectrum, due to a greater increase in longitudinal conductivity than in transverse conductivity. For example, at 10 kHz, the conductivity difference was 0.15 S/m for healthy animals and 0.29 S/m for post-crush animals. Relative permittivity difference values, however, were similar between groups. There was a strong correlation of conductivity anisotropy to muscle fiber size but not to blood vessel area.

Conclusions: Anisotropy of muscle conductivity increases markedly after subacute denervation injury.

Significance: This alteration in anisotropy has direct relevance to the clinical application of electrical impedance myography. We also speculate that it may impact other forms of diagnostic testing, including needle electromyography and magnetic resonance imaging.

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1. Introduction

Skeletal muscle is highly anisotropic with applied electrical currents flowing more easily along muscle fibers than across them. This unique property of skeletal muscle was first appreciated in the early 1960s in studies assessing excised animal tissue (Burger and van Dongen, 1961; Rush, 1962; Rush et al., 1963; Fatt, 1964). In the 1990s, Shiffman and Aaron identified that muscle anisotropy could be assessed non-invasively through the application of surface impedance methods (Aaron et al., 1997). More recent work has suggested that the anisotropic characteristics of the tissue may be effective in distinguishing neurogenic from myopathic injury and could be used as an indicator of disease progression (Garmirian et al., 2009). However, the mechanisms underlying anisotropy and its alteration in diseased states remain poorly understood. In addition, how this property of muscle may impact standard electrophysiologic testing and magnetic resonance imaging (MRI) has not been explored.

As part of a larger research effort focused on the application of electrical impedance techniques to rat models of neuromuscular disease, we have been studying the effects of neurogenic injury on the electrical properties of skeletal muscle (Nie et al., 2006; Ahad and Rutkove, 2009). Although most of this work has focused on surface measurements, we can also study the electrical properties of the tissue directly, after animal sacrifice. We recently assessed the effect of acute denervation injury on the electrical properties of skeletal muscle, demonstrating consistent changes in its conductivity (Ahad et al., 2009) 1–2.5 weeks after sciatic nerve crush. In this supplemental analysis, we specifically assess the resulting alteration in the tissue's anisotropic characteristics and the possible mechanisms underlying this change. Moreover, since these characteristics impact the propagation of electrical signals through tissue and are indicative of changes in intra- and

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extracellular water content, we also discuss their potential relevance to more standard clinical diagnostic testing, including needle electromyography and MRI.

2. Methods

2.1. Rats

All animal studies were approved by the Institutional Animal Care and Use Committee of the Beth Israel Deaconess Medical Center, Boston, MA. A total of 20 rats were studied: 12 control rats and 8 rats after sciatic crush. All were adult male Wistar Rats, weighing between 420 and 480 g, obtained from Charles River Laboratories, Wilmington, MA and were acclimated for 48 h after arrival at our facility before any measurements were obtained. In the eight sciatic crush animals, an incision was made proximally in the thigh and the sciatic nerve exposed, being careful not to disturb surrounding tissues, with the animal anesthetized under isoflurane. The nerve was then crushed using a jeweler's forceps by applying pressure for approximately 30 s. The incision was then sutured closed and the animals allowed to recover until sacrifice, 1–2.5 weeks later (this time course, in part, a product of the complexity of the procedures, with one animal being studied per day).

2.2. Impedance measurement system

Impedance measurements were made using a lock in amplifier, Signal Recovery Model 7280, Advanced Measurement Technology Inc., Oak Ridge, TN coupled with a very low capacitance active probe (Model 1103 of Tektronix, Beaverton, OR) as previously described (Esper et al., 2006).

2.3. Sacrifice and measurements of the electrical constant

After obtaining surface impedance measurements (reported elsewhere), the entire gastrocnemius muscle was immediately excised at its proximal extent just below the knee and distally by cutting the gastrocnemius tendon at its insertion into the calcaneus. The animal was then immediately killed with anesthetic overdose. From this muscle, an approximately 1 cm × 1 cm square of about 0.4 cm height was further excised with a scalpel.

This tissue was immediately placed into a 1 cm × 1 cm × 2 cm plastic measuring cell between two stainless steel current electrodes with the fiber orientation perpendicular to the metal electrodes (providing longitudinal measurements), similar to the approach of Baumann et al. (1997), as previously described (Ahad et al., 2009). After longitudinal measurements were performed, the muscle was rotated 90° such that the muscle fibers were parallel to the stainless steel plates and measurements repeated (transverse measurements). To ensure consistent temperature, the entire cell was maintained at 37 °C through the use of a heating pad surrounding the cell.

2.4. Quantitative histology

Measurements were performed on eight normal animals and all of the crush animals. After impedance measurements were completed as above, the muscle tissue was immediately frozen in isopentane cooled in liquid nitrogen for histological analysis (see below) and stored at −80 °C until ready for use. The frozen tissue was cut into10 μm thick sections and stained with Hematoxylin and Eosin. Standard non-biased, blinded stereological measurements (Mayhew et al., 1997) were made on a Zeiss Axiophot microscope with a LUDL motorized stage interfaced with a Dell Optiflex 380 computer running Stereo Investigator software (MBF Biosciences, Inc, Williston, VT) as previously described (Ahad et al., 2009). For each slide, a histogram of cell size (in cross-sectional area and diameter) is obtained.

These sections were also used to quantify vascular lumen area. Images were captured at 40× from one section of gastrocnemius muscle per animal and all vessels were used for quantification using the public domain NIH image program (http://rsb.info.nih.gov/nih-image/), measuring the entire area inside the endothelial cell layer.

2.5. Data analysis

Calculation of the electrical constants, the conductivity and relative permittivity, was performed as previously described (Ahad et al., 2009). The conductivity is a measure of the inherent ability of electrical current to flow freely through the muscle. The relative permittivity is a measure of the inherent polarizability of the tissue. Anisotropy for these two parameters at each frequency was assessed by defining a conductivity difference (longitudinal–transverse conductivity) and a relative permittivity difference (longitudinal–transverse relative permittivity). Standard two-group methods (Mann–Whitney and unpaired *t*-tests) were used for two-group comparisons. Spearman rank correlation was used to assess the relationship between anisotropy values and muscle fiber and blood vessel area. All analyses were two-tailed, with $\alpha = 0.05$ and were performing using SPSS Statistical Software, Version 17.0 (SPSS, Inc, Chicago, IL).

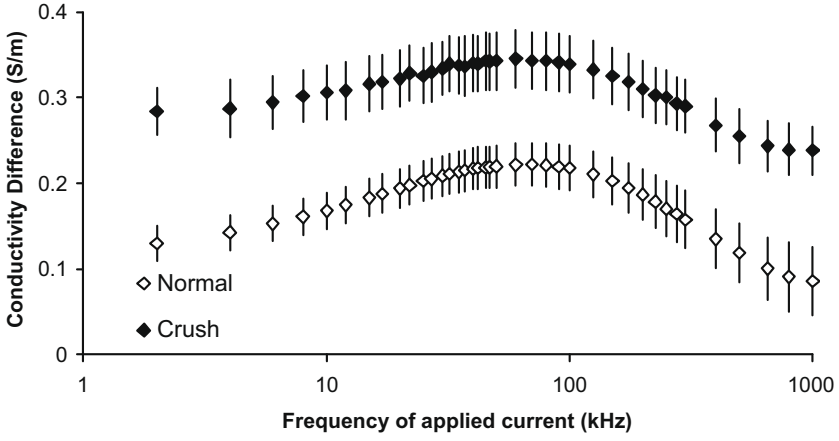

Fig. 1. Conductivity differences in normal and sciatic crush animals from 2 kHz to 1 MHz, mean ± standard error.

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