INTRODUCTION

Spasticity is a disabling condition that occurs secondary to upper motor neuron lesions such as stroke, spinal cord injury, and cerebral palsy (CP). Individuals with spasticity have increased muscle tone, stiffness, exaggerated reflexes, weakness and joint contractures, all of which lead to considerable functional impairment. Although the etiology of spasticity is neural, there is increasing evidence that skeletal muscle is altered by this condition (1). Since the giant cytoskeletal protein, titin, bears the majority of passive force in mammalian skeletal muscle (2), the purpose of this study was to measure the passive mechanical stiffness of spastic and non-spastic single muscle cells and, after selective titin degradation, to determine whether titin could account for the differences in stiffness measured.

METHODS

This study was performed with the full approval of the Human Research Protection Program at the University of California, San Diego and Children’s Hospital, San Diego. Following informed consent, muscle biopsies were obtained from children with spasticity (all had CP, n=18) and non-spastic children (n=15) who were undergoing other, unrelated orthopaedic procedures.

Biopsies were immediately pinned to cork board and placed in a relaxing solution for 60 minutes containing [mM]: imidazole [59.4], KCH3PO4[86.0], Ca(KCH3O2)2[0.13], Mg(KCH3O2)2[10.8], K-EGTA [5.5], KH2PO4[1.0], Na2ATP [5.1], and 50.0 µM leupeptin to prevent hyper-contraction and proteolytic degradation. Biopsies were stored at -20 °C in storage solution containing KCl, EGTA, MgCl2, imidazole, Na2ATP, NaN3, glutathione, glicerol and leupeptin until analyzed (no more than 2 weeks). Single fibers (5-6 mm in length) were dissected in chilled relaxing solution and then cut in half. One half was immediately placed in buffer solution for analysis of titin size and titin and myosin heavy chain content by quantitative gel electrophoresis. The other half was secured, via 9-0 silk suture loops, to a micromanipulator and a force transducer. Resting fiber length and diameter were measured. Fibers were then elongated in 250 µm increments, during which sarcomere length and tension were measured. Two minutes after stress relaxation, fibers were again stretched until reaching approximately 60% of ultimate strain. Stress-strain curves were generated from these data.

In some cases (n=8 spastic, n=5 normal) fibers, while still tied to the apparatus, were incubated in a very dilute (0.037%) trypsin solution (Invitrogen Corp., San Diego, CA) at room temperature for 10 minutes. These same fibers were again elongated and tangent modulus recalcualted. Fibers were then stored in buffer solution for subsequent gel electrophoresis. Electrophoresis was performed on each fiber (before and after trypsin digestion) to verify the selective degradation of titin and to quantitify titin size in each fiber. Results were analyzed by linear regression, compared between normal and spastic subjects by ANOVA. Significance level (α) was set to p<0.05.

RESULTS

Quantitative analysis of protein gels demonstrated that the trypsin protocol selectively degraded most of the cell’s titin. This was based on an approximate 80% reduction loss in the titin N2A band (19±14.5% of pre-digestion values) with no measurable change in other bands such as the myosin heavy chain band which showed no significant change (107±11.4% of pre-digestion values). Across all samples, there was a strong, negative correlation between tangent modulus and titin size (p<0.01, r2=0.61). The larger the titin, the lower the modulus, consistent with titin-mediated stiffness in these cells (Fig. 1). However, since samples were obtained across a variety of muscles, a subset of fibers from the same muscle (vastus lateralis, VL) were analyzed pre- and post-titin degradation. Tangent modulus was significantly higher for spastic VL cells (58.6±10.3 kPa) compared to normal VL muscle cells (44.9±12.5 kPa; p<0.03; Fig. 2, open bars). However, after selective titin degradation, there was no significant difference between spastic and normal VL cells (18.8±0.9 kPa) VL cells (p>0.7; Fig. 2 filled bars).

REFERENCES


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