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Muscle-specific overexpression of IGF-I improves E-C coupling in skeletal muscle fibers from dystrophic mdx mice

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Submitted 3 September 2007; accepted in final form 5 November 2007

Schertzer JD, van der Poel C, Shavlakadze T, Grounds MD, Lynch GS. Muscle-specific overexpression of IGF-I improves E-C coupling in skeletal muscle fibers from dystrophic mdx mice. Am J Physiol Cell Physiol 294: C161–C168, 2008. First published November 7, 2007; doi:10.1152/ajpcell.00399.2007.—Duchenne muscular dystrophy (DMD) is a lethal X-linked disease caused by the absence of functional dystrophin. Abnormal excitation-contraction (E-C) coupling has been reported in dystrophic muscle fibers from mdx mice, and alterations in E-C coupling components may occur as a direct result of dystrophin deficiency. We hypothesized that muscle-specific overexpression of insulin-growth factor-1 (IGF-I) would reduce E-C coupling failure in mdx muscle. Mechanically skinned extensor digitorum longus muscle fibers from mdx mice displayed a faster decline in depolarization-induced force responses (DIFR); however, there were no differences in sarcoplasmic reticulum (SR)-mediated Ca2+ resquestration or in the properties of the contractile apparatus when compared with nondystrophic controls. The rate of DIFR decline was restored to control levels in fibers from transgenic mdx mice that overexpressed IGF-I in skeletal muscle (mdx/IGF-I mice). Dystrophic muscles have a lower transcript level of a specific dihydropyridine receptor (DHPR) isoform, and IGF-I-mediated changes in E-C coupling were associated with increased transcript levels of specific DHPR isoforms involved in Ca2+ regulation. Importantly, IGF-I overexpression also increased the sensitivity of the contractile apparatus to Ca2+. The results demonstrate that IGF-I can ameliorate fundamental aspects of E-C coupling failure in dystrophic muscle fibers and that these effects are important for the improvements in cellular function induced by this growth factor.

The dystrophin-associated glycoprotein complex (DGC) is a multimeric array of membrane and cytoskeletal proteins that links the extracellular matrix with the cytoskeleton. In skeletal muscle, the DGC is composed of dystrophin, the syntrophins, the dystroglycans, and the sarcoglycans. The importance of the DGC is evident from the fact that a deficiency of almost any of its components constitutes a primary cause of one or more forms of muscular dystrophy. A number of members of the DGC are directly associated with components of excitation-contraction (E-C) coupling. A number of sarcoglycan subunits and dysferlin have been found to directly interact with skeletal muscle sarcoplasmic reticulum (SR) (16) and the dihydropyridine receptor (DHPR) (1), respectively. Considering that dystrophin associates with both sarcoglycans and dysferlin through the DGC, dystrophin is thought to be closely associated with both the sarcolemma and t-system. Abnormal E-C coupling has been reported in dystrophic muscle fibers from mdx mice (9, 33, 52), and these alterations in E-C coupling components may occur as a direct result of disruption and destabilization of the DGC in dystrophic muscle.

The mechanisms responsible for impairments in E-C coupling and contractile function in dystrophic muscle are poorly understood. It has been demonstrated that action potential-induced Ca2+ release from the SR is lower in intact single fibers isolated from flexor digitorum brevis (FDB) muscles of mdx mice when compared with controls (52). Interestingly, this effect was not associated with changes in the t-tubular voltage dependence of SR Ca2+ release, suggesting an intrinsic depression in ryanodine receptor (RyR1)-mediated SR Ca2+ release in dystrophic muscle (53). Similar results have been obtained in mechanically skinned fibers from the extensor digitorum longus (EDL) muscle of mdx mice (34). Direct activation of RyR-mediated SR Ca2+ release induced with submaximal caffeine concentrations (2–7 mM) is depressed in mechanically skinned EDL muscle fibers from mdx mice when compared with controls (34). These results support the hypothesis that intrinsic changes in RyR1-mediated SR Ca2+ release play a role in defective E-C coupling responses in dystrophic muscle. However, this effect may not be the only impairment in E-C coupling in dystrophic skeletal muscle.

Force production during repeated t-tubular Na+ depolarizations decreases at a faster rate in mechanically skinned EDL muscle fibers from mdx mice when compared with control mice (34). This effect, coupled with a reduced repriming rate of depolarization-induced contractile responses (DIFRs) and a

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DUCHENNE MUSCULAR DYSTROPHY (DMD) is caused by mutations in the dystrophin gene and results in a progressive decline in skeletal, cardiac, and smooth muscle function (3, 22). Several impairments in function have been characterized in the skeletal muscles of mdx mice, a widely used animal model that harbors a mutation in the dystrophin gene and lacks functional levels of functional dystrophin (42). Skeletal muscles from mdx mice undergo cycles of degeneration and regeneration and have lower maximal forces when corrected for overall muscle cross-sectional area (i.e., normalized or specific force) (28). A deeper understanding of the intracellular mechanisms responsible for these pathophysiological effects in dystrophic muscle will help provide the basis for developing novel therapeutic interventions for DMD, beyond correction of the primary genetic defect.
IGF-I attenuates E-C coupling failure in mdx muscle fibers

In dystrophic muscle, the contractile performance is impaired by a combination of intrinsic defects in the excitation-contraction (E-C) coupling system and extrinsic muscle damage (34). The E-C coupling impairment includes reduced SR Ca2+ release, decrease in intracellular Ca2+ transients, and impaired Ca2+ cycling due to structural degradation of the SR membrane and t-tubular system necessary for excitation of Ca2+ release (34). The long-term efficacy of treatments for DMD is limited by the progressive nature of muscle damage and the necessity for lifelong treatments (34). Therefore, there is a great interest in identifying factors that could improve the contractile performance of dystrophic muscle fibers, which could have therapeutic relevance to DMD.

In this study, our aim was to investigate the effects of IGF-I on various aspects of E-C coupling in single muscle fibers from dystrophic mice. We assessed E-C coupling in mechanically skinned single muscle fiber segments from wild-type nondystrophic control C57BL/10ScSn mice (hereafter referred to as BL/10), littermate transgene null dystrophic mice (simply referred to as dystrophic control C57BL/10ScSn mice (hereafter referred to as BL/10)), and transgenic dystrophic mdx mice that overexpress the IGF-I Ea isoform in skeletal muscle (mdx/IGF-I) (40). The transgenic mice were generated by interbreeding of mdx mice with transgenic mice that overexpress the IGF-I Ea isoform in skeletal muscle driven by the myosin light chain promoter were used (mdx/IGF-I). The transgenic mice were generated by interbreeding of mdx mice with transgenic mice that overexpress the IGF-I Ea isoform in skeletal muscle driven by the myosin light chain promoter were used (mdx/IGF-I). The transgenic mice were generated by interbreeding of mdx mice with transgenic mice that overexpress the IGF-I Ea isoform in skeletal muscle driven by the myosin light chain promoter were used (mdx/IGF-I). The transgenic mice were generated by interbreeding of mdx mice with transgenic mice that overexpress the IGF-I Ea isoform in skeletal muscle driven by the myosin light chain promoter were used (mdx/IGF-I).

In this study, our aim was to investigate the effects of IGF-I overexpression on various aspects of E-C coupling in dystrophic muscle fibers. Acutely, IGF-I overexpression improves the contractile performance of dystrophic muscle fibers by stimulating a Ca2+-dependent signaling cascade, resulting in binding of the cAMP-response element binding protein (CREB) to the promoter region of DHPR (55, 56). The gene-specific primers used for PCR genotyping were 5'-CTAGGC-CACAGAATTTGGAAGATT-3' and 5'-GACATTGCTTCTGTA-3' for the transgene and 5'-GAGCTGACTTTGTAGGCTTCA-3' for the dystrophic control. The transgenic overexpression of IGF-I in the skeletal muscle fibers acutely regulates the selective Ca2+ influx without altering the voltage-sensing properties (8).

Mechanically skinned single muscle fibers. Mice were anesthetized with pentobarbital sodium (60 mg/kg ip, Nembutal, Rhone Merieux, Pinkenba, QLD, Australia), and the right EDL muscle was surgically excised for single fiber analysis. The muscle was blotted on filter paper and placed in a Petri dish containing paraffin oil at room temperature. Muscles were pinned at resting length to the base of a dish that was layered with Sylgard gel (Dow Corning, Midland, MI). Single muscle fibers were isolated from as close to the surface of the muscle as possible, and the sarcolemma was peeled away from the t-tubular membrane and contractile apparatus under a dissecting microscope using fine forceps, as described previously (43). The mechanically skinned fiber was then attached to one end of a piezoresistive force transducer (AES01, SensoNor, Horten, Norway) using braided silicone Deknatel, size 10; 0.2 mm, and the other end of the fiber was clamped between a pair of forceps fixed to a micromanipulator (17). Average sarcomere length of each fiber was adjusted to a length slightly longer than optimal to reliably measure depolarization-induced force responses (DIFRs), as described previously (34). All experiments were conducted at room temperature (23 ± 2°C).

The compositions of solutions and experimental procedures have been described thoroughly elsewhere (19, 25, 35, 37, 43). All solutions had pH 7.10 ± 0.01, and free Mg2+ concentration ([Mg2+]) was 1 mM, unless specified otherwise. Free [Ca2+] in Ca2+ was verified using a Ca2+-sensitive electrode (Orion Research, MA). Refer to the online supplemental Table 1 for the composition of specific solutions.

Depolarization-induced force responses. Mechanically skinned muscle fibers were polarized by incubating the fiber in a potassium hexamethylenediamine-tetraacetic acid solution (K-HDTA) for 2 min. The t-tubular membrane system was then depolarized by rapidly transferring the fiber into a Na-HDTA solution, causing a transient depolarization-induced force response (DIFR). The muscle fiber was depolarized in the K-HDTA solution for 30 s before eliciting another depolarization in the Na-HDTA solution, as described previously (34). This protocol was repeated to produce DIFRs until the peak amplitude of the DIFR reached <50% of the initial value (n ≥ 10 fibers per group).

To test the acute effects of IGF-I exposure on DIFRs, EDL muscles from mdx mice were surgically excised and incubated in saline (control) or saline containing 10 μg/ml Long-R-IGF-I (Gropex, Adelaide, SA, Australia) for 15 min. Muscles were blotted on filter paper, skinned fiber segments were isolated under paraffin oil, and DIFRs were assessed as described previously (n = 11 fibers per group).

Caffeine-induced force responses and SR Ca2+ accumulation. Initially, mechanically skinned muscle fibers were equilibrated in a wash solution for 30 s followed by thorough depletion of SR Ca2+ stores, achieved by transferring the fiber preparation into a release solution containing 30 mM caffeine and 0.02 mM free [Mg2+] (19). The presence of 0.5 mM EGTA in the release solution ensured that the level of Ca2+ during caffeine-induced release did not maximally activate the contractile apparatus, which is necessary to allow quantitative evaluation of the amount of Ca2+ released. Ca2+ release from the SR was estimated from the relative areas under the caffeine-induced force response (15, 26, 47). The fiber was left in the release solution for 2 min to ensure complete SR Ca2+ depletion, before being washed for 30 s. Thereafter, the fiber was reloaded with Ca2+ in load solution (0.2 μM Ca2+, pH 6.7, where pCa = −log10[Ca2+]) for various lengths of time (10, 20, 30, and, 60 s), before being equilibrated for 30 s in wash solution, and subsequently SR Ca2+ was released in release solution. Overexpression of IGF-I resulted in an

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increase in the maximum Ca\(^{2+}\)-activated force response. Therefore, relative areas under the caffeine-induced force responses of each fiber were normalized to the corresponding maximum Ca\(^{2+}\)-activating force allowing comparison of results between fibers from different animals. Data were fitted with a standard exponential association equation giving the rate at which the SR accumulated Ca\(^{2+}\) (s\(^{-1}\)) but not the amount of SR Ca\(^{2+}\) accumulated (Table 1).

**SR Ca\(^{2+}\) leak.** The percentage of Ca\(^{2+}\) lost from the SR due to the passive leak was assessed, as described previously (29, 47). The fiber was loaded for 20 s in loading solution. The fiber preparation was then placed in wash solution for 30 s followed by SR Ca\(^{2+}\) content released in release solution (Ca\(^{2+}\) leak in 30 s). The fiber preparation was then placed in wash solution before reloading for 20 s in load solution and transferred to wash solution for 90 s, and the remaining SR Ca\(^{2+}\) was released in release solution (Ca\(^{2+}\) leak in 90 s). The 30-s Ca\(^{2+}\) leak was then repeated, and the area (corrected for proportionality between area and SR Ca\(^{2+}\) content) under the test run was divided by the area of the test run. The total Ca\(^{2+}\) leak in 60 s was reported as a percentage of the total Ca\(^{2+}\) leak in 60 s.

**Relative SR Ca\(^{2+}\) sensitivity.** To determine the effect of IGF-I overexpression on the RyR, a caffeine dose-response curve was determined from the forces produced by the contractile apparatus after SR Ca\(^{2+}\) release induced by low caffeine concentrations (4, 34). Each fiber was then removed and replaced by the contractile apparatus after SR Ca\(^{2+}\) release induced by low caffeine concentrations (4, 34). Each fiber was then removed and placed back in the relaxing solution for a further 2 min. Force responses were generated at each caffeine concentration by loading the fiber preparation with caffeine and then placed back in the relaxing solution at each pCa. The peak of each caffeine contraction was expressed as a percentage of the peak caffeine-activated force (46). Data points were fitted with a Hill equation producing two parameters: the pCa\(^{50}\) (i.e., the Hill coefficient, indicative of the steepness of the force-pCa relationship) and the Hill sensitivity, which is the slope of the linear fit of caffeine sensitivity data.

**RESULTS**

Overexpression of IGF-I attenuates E-C coupling failure in dystrophic muscle fibers. Transient DIFRs were evoked by rapid substitution of K-HDTA for Na-HDTA in mechanically skinned muscle fibers. During a protocol of repeated depolarizing and depolarizing events, fibers from EDL muscles of mdx mice demonstrated a more rapid decline in DIFRs compared with controls, as reported previously (34). Rundown in DIFRs to 50% of peak amplitude occurred in 4.1 ± 0.5 and 12.5 ± 0.6 min in skinned fibers from mdx and BL/10 mice, respectively (P < 0.05; Fig. 1, A and B; n = 12 fibers per group). Transgenic overexpression of IGF-I in the skeletal muscles of mdx mice (mdx/IGF-I) prevents the rapid rundown in DIFRs, such that each muscle fiber was reduced significantly in EDL muscles of mdx mice compared with controls (P < 0.05; Fig. 1, A and B; n = 10 fibers). Importantly, when EDL muscles from mdx mice were bathed in IGF-I, the rundown in DIFRs was not different from saline controls (5.9 ± 0.7 min in skinned fibers from mdx and BL/10 mice, respectively; P = 0.14). These results indicated that long-term overexpression is the important underlying mechanism for the IGF-I-mediated changes in E-C coupling rather than an acute effect of IGF-I on DIFR.

**IGF-I overexpression increases dihydrolipidine receptor isoforms critical for Ca\(^{2+}\) regulation.** Semiquantitative RT-PCR analysis showed that transcript levels of the majority of E-C coupling components linking the t-tubular system to SR Ca\(^{2+}\) release were unchanged in EDL muscles of mdx and BL/10 mice with one notable exception (Fig. 2). Transcript levels of DHRpα282 were reduced significantly in EDL muscles of mdx mice when compared with controls (P < 0.05; Fig. 2). Transcription levels of SERCA2a and sarcolipin were higher in EDL muscles of mdx mice compared with controls (P < 0.05; Fig. 2). Transgenic overexpression of IGF-I in a dystrophic skeletal muscle did not alter transcript levels of the majority of the E-C coupling components, except for specific DHRp subunits. Transcript levels of DHRpα1, DHRpβ1, and DHRpα282 were higher

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**Table 1. Properties of mechanically skinned single fibers from EDL muscles of BL/10, mdx, and mdx/IGF-I mice**

<table>
<thead>
<tr>
<th></th>
<th>BL/10 (n = 11)</th>
<th>mdx (n = 12)</th>
<th>mdx/IGF-I (n = 10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Specific force, kN/m(^2)</td>
<td>180.7 ± 13</td>
<td>219.8 ± 23</td>
<td>304.5 ± 27*</td>
</tr>
<tr>
<td>Ca(^{2+}) sensitivity, pCa(_{50})</td>
<td>5.86 ± 0.01</td>
<td>5.88 ± 0.02</td>
<td>6.06 ± 0.02*</td>
</tr>
<tr>
<td>n(_H)</td>
<td>3.73 ± 0.22</td>
<td>4.57 ± 0.04</td>
<td>4.18 ± 0.77</td>
</tr>
<tr>
<td>SR Ca(^{2+}) leak, % in 60 s</td>
<td>16.7 ± 5</td>
<td>19.0 ± 4</td>
<td>7.2 ± 3*</td>
</tr>
<tr>
<td>SR Ca(^{2+}) accumulation, s(^{-1})</td>
<td>0.05 ± 0.01</td>
<td>0.03 ± 0.01</td>
<td>0.08 ± 0.03</td>
</tr>
<tr>
<td>Slope of linear fit of caffeine sensitivity data</td>
<td>9.21 ± 1.01</td>
<td>9.39 ± 0.73</td>
<td></td>
</tr>
</tbody>
</table>

Values are means ± SE. pCa\(_{50}\), [Ca\(^{2+}\)] required to elicit 50% maximum force; n\(_H\), Hill coefficient indicative of the steepness of the force-pCa and force-caffeine dose-response relationship; SR, sarcolemmal reticulum; *Significantly different (P < 0.05) from all other conditions.
IGF-I overexpression increases force production, increases Ca\textsuperscript{2+} sensitivity, and decreases SR Ca\textsuperscript{2+} leak. The maximum Ca\textsuperscript{2+}-activated force, Ca\textsuperscript{2+} sensitivity, and cooperative binding of Ca\textsuperscript{2+} (Hill coefficient as determined from the steepness of the force-pCa relationship) were not different in fibers from mdx and nondystrophic BL/10 mice (Table 1; Fig. 4). However, fibers from mdx/IGF-I mice generated significantly greater maximum Ca\textsuperscript{2+}-activated force and exhibited an increased sensitivity to Ca\textsuperscript{2+} as evidenced by a leftward shift of the force-pCa relationship without alterations in the Hill coefficient compared with fibers from both mdx and control BL/10 mice (P < 0.05; Table 1; Fig. 4).

There was no difference in the ability of the SR to accumulate Ca\textsuperscript{2+} in EDL muscle fibers from mdx mice and BL/10 controls (P > 0.05; Table 1). Transgenic overexpression of IGF-I in the skeletal muscles of mdx mice increased SR Ca\textsuperscript{2+} accumulation in single EDL muscle fibers compared with nondystrophic (BL/10) and mdx mice (P < 0.05; Table 1), as indicated by a greater area under the caffeine-induced force response. The apparent increase in the amount of Ca\textsuperscript{2+} released by mdx/IGF-I muscle fibers compared with BL/10 and mdx fibers could be due to either the increase in Ca\textsuperscript{2+} sensitivity of the mdx/IGF-I fibers (Table 1; Fig. 4) and/or an increase in the amount of releasable SR Ca\textsuperscript{2+}. To determine whether the increase in Ca\textsuperscript{2+} sensitivity influenced the interpretation of the SR Ca\textsuperscript{2+} accumulation rates, the caffeine-induced force responses of mdx/IGF-I fibers were normalized to the shift of the force-pCa curve (0.02 pCa units; Fig. 4; Table 1). The relative force produced by the caffeine-induced force responses (normalized to maximum Ca\textsuperscript{2+}-activated force) of mdx/IGF-I fibers were converted into the corresponding relative forces produced by those in the BL/10 control fibers using the typical force-pCa curves for mdx/IGF-I and BL/10 control fibers. Taking into consideration the left shift in pCa\textsubscript{50} of 0.2 pCa units, there was statistically no difference between mdx/IGF-I and BL/10 fibers (P < 0.05).

Passive SR Ca\textsuperscript{2+} leak was not different in EDL muscle fibers from mdx and control mice (Table 1). However, EDL muscle fibers from mdx/IGF-I mice demonstrated a 57% and
pathology in mdx mice such as muscle degeneration and contraction-induced injury (2, 39, 40). The present study highlights the potential role of IGF-I-induced changes in E-C coupling as an underlying mechanism for the cellular benefits of increased IGF-I in dystrophic muscle.

The results confirmed that the force responses elicited from repeated t-tubular depolarizations decreased at a faster rate in skeletal muscles fibers from dystrophic mdx mice compared with controls (34). It should be noted that any differences, particularly in DIFR rundown, between this study and Plant and Lynch (34) can be explained by the different ages of animals used in each study (34). In Plant and Lynch (34) the mice were 9 wk of age, whereas in the present study the mice were 24 wk of age. It has been proposed that there is a progressive degeneration in the Ca\(^{2+}\)-handling properties of skeletal muscles of mdx mice (10), which may account for any differences observed between the results reported in Plant and Lynch (34) and those of the present study. This is an interesting finding that warrants further investigation since it highlights a loss of E-C coupling as a contributing mechanism in the pathophysiology of skeletal muscles in mdx dystrophic mice.

Regardless of differences between studies, IGF-I overexpression in mdx muscle prevented a faster rundown in DIFR. These changes occurred despite similar transcript levels for mdx and nondystrophic muscle for the majority of E-C coupling components responsible for t-tubular signal propagation and coupling to SR-mediated Ca\(^{2+}\) release. These findings are consistent with reports that both protein levels of most DHPR isoforms and net charge movement are preserved in mdx muscle (23, 12). In fact, protein levels of DHPR\(_{\alpha 1}\), DHPR\(_{\alpha 2}\) (presumably DHPR\(_{26}\)), RyR1, SERCA1, Na\(^{+}\)-K\(^{+}\)-ATPase, and calsequestrin have been reported not to be different between muscles of mdx and control mice (6). Furthermore, it has been shown that the impaired Ca\(^{2+}\) release in response to single action potentials is independent of changes in t-tubular propagation or voltage characteristics in dystrophic muscle fibers (53).

Overexpression of IGF-I did not alter the ability of the SR to accumulate Ca\(^{2+}\) or the caffeine threshold of mdx muscle fibers.
indicating that although overexpression of IGF-I did improve E-C coupling in \textit{mdx} muscle, these changes were not at the level of the RyR. This study reports the novel finding that transcript levels of DHPRe282 are lower in EDL muscles of \textit{mdx} mice. Given the lack of knowledge about this DHPR subunit in skeletal muscle, the significance of this result is unclear. Several changes in transcript levels indicate alterations in regulators of Ca^{2+} resequestration at the level of the SR, including increases in SERCA2a and sarcoplasmic transcripts in EDL muscles of \textit{mdx} mice compared with nondystrophic mice. SERCA2a has previously been shown to be increased in EDL muscles of \textit{mdx} mice compared with controls, and sarcoplasmic transcripts are increased in other myopathies, such as dysferlinopathies (5, 11). Sarcoplasmic has been reported to inhibit SERCA-mediated Ca^{2+} resequestration, slow the time course of contraction, and reduce force production in skeletal muscle, possibly by diminishing SR Ca^{2+} stores (30, 44). Given that the SR in dystrophic muscle has a reduced Ca^{2+} binding capacity, potentially mediated by reduced levels of luminal SR casequestrin-like proteins such as sarcalumenin (6, 14), further investigation of SR Ca^{2+} regulation in dystrophic skeletal muscle is warranted.

IGF-I overexpression in the skeletal muscles of dystrophic \textit{mdx/IGF-I} mice induced several changes that attenuated impairments in E-C coupling. The restoration in the time course of repeated DIFRs with IGF-I overexpression in dystrophic muscle was associated with increased transcript levels of DHPRe1, DHPRe0, and DHPRe282. These data are consistent with previous reports that increased IGF-I levels can prevent age-related E-C coupling failure by reversing reductions in DHPR levels (21, 38, 49).

The DHPRe1 subunit is considered the major subunit of the DHPR complex as it is the pore-forming subunit and necessary for interactions with RyRs (18), whereas the DHPRe0 subunit has been demonstrated to be a potent regulator of the open probability of L-type calcium channels (20). Very little is known about the action of the DHPRe282 subunit, and while it is not strictly necessary for E-C coupling (32), the probable role of the a282 subunit is in the lifetime of the channel complex in the plasma membrane, either by enhancing trafficking to the plasma membrane or by reducing turnover of channels (13). Interestingly, transcript levels of DHPRe282 were decreased in EDL muscles of \textit{mdx} mice and increased in \textit{mdx/IGF-I} mice, highlighting an attractive candidate for investigation in DMD and other myopathies.

IGF-I overexpression also increased the sensitivity of the contractile apparatus to Ca^{2+}, as evidenced by the leftward shift of the force-pCa relationship. This effect could effectively compensate for impairments in E-C coupling as it would result in increased force production at a lower [Ca^{2+}]. As such, even if DHPR-coupled RyR1-mediated Ca^{2+} release was impaired to some extent in dystrophic muscle (52), an increase in Ca^{2+} sensitivity may compensate and allow some degree of force production. Muscle fibers from EDL muscles of \textit{mdx/IGF-I} mice also displayed 39% and 69% higher specific forces (sPo) compared with fibers from \textit{mdx} and control mice, respectively (P < 0.05; Table 1). This finding suggests that at the cellular level, IGF-I-mediated changes in dystrophic muscle E-C coupling may include additional alterations in the contractile apparatus, such as changes in troponin C or in myosin heavy chain isoforms (4). Whereas these factors are important for understanding IGF-I influences on the contractile apparatus in \textit{mdx} muscle fibers, their investigation is beyond the scope of the present study.

IGF-I is known to acutely increase charge movement through DHPRs and increase peak twitch Ca^{2+} in aged skeletal muscle (8). We hypothesized that the same would happen in dystrophic skeletal muscle as evidenced by an increase in DIFR. As such, we tested the effect of acute exposure to exogenous IGF-I on mechanically skinned muscle fibers from \textit{mdx} mice and showed no alterations to E-C coupling as measured by repeated DIFRs. This contrasts with the Class I IGF-I Ea-mediated improvements of E-C coupling in dystrophic \textit{mdx/IGF-I} muscle fibers. Whether these differences reflect the mode of delivery (acute exogenous vs. long-term endogenous) or the isoform of IGF-I is not clear, but these are important issues to resolve for future potential therapeutic administration of IGF-I. The beneficial effects of IGF-I in \textit{mdx/IGF-I} muscles occurred in the absence of changes in transcript levels of the RyR and other associated SR Ca^{2+} release proteins and with no alteration in the functional properties of SR Ca^{2+} release when assessed by direct activation with caffeine.

Although somewhat contentious, most evidence to date supports the notion that SR Ca^{2+} resequestration is compromised in dystrophic skeletal muscle (11, 24, 27). Our results show that at the cellular level, the rate of SR Ca^{2+} loading was not impaired in single muscle fibers from \textit{mdx} mice and that IGF-I overexpression had no influence on SR Ca^{2+} accumulation. Interestingly, single fibers from \textit{mdx} mice showed an increase in transcript levels of sarcolipin, but IGF-I overexpression did not alter sarcolipin, SERCA1, or SERCA2a transcript levels. Transgenic overexpression of IGF-I dramatically reduced SR Ca^{2+} leak in muscle fibers of \textit{mdx/IGF-I} mice. A reduction in SR Ca^{2+} leak could enhance net SR Ca^{2+} resequestration and indicates an increase in the SR Ca^{2+} holding capacity. Furthermore, this result is consistent with the hypothesis that IGF-I promotes resealing of various membranes in dystrophic skeletal muscle fibers (40).

E-C coupling failure has been proposed as a contributing mechanism to the force deficit in skeletal muscle immediately following contraction-induced injury (36, 51, 54). We have shown previously that systemic administration of IGF-I at a relatively low dose reduces contraction-induced injury in tibialis anterior muscles of \textit{mdx} mice (39). The present study supports the hypothesis that IGF-I reduces muscle damage by attenuating E-C coupling failure. Identifying therapies that can reduce contraction-induced injury in dystrophic muscle and understanding their mechanisms of action is of paramount importance since dystrophic muscle fibers are highly susceptible to this type of trauma that aggravates the dystrophic pathology. E-C coupling failure in dystrophic muscle and the role of IGF-I and other factors to enhance E-C coupling should be investigated because correcting defective Ca^{2+} signaling during various stressors, such as repeated contractions or contraction-induced injury, may ameliorate the dystrophic pathology. The findings provide mechanistic insight into the benefits of muscle-specific IGF-I expression in dystrophin-deficient muscle and further highlight the therapeutic potential of IGF-I for muscular dystrophy.
ACKNOWLEDGMENTS

We thank Professors D. G. Stephenson and G. D. Lamb for helpful discussions regarding this manuscript.

GRANTS

This work was supported by research grants from the Muscular Dystrophy Association (USA) and the National Health and Medical Research Council of Australia (Project number: 350439).

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