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Amyloid- β protein impairs Ca^{2+} release and contractility in skeletal muscle

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Abstract

Inclusion body myositis (IBM), the most common muscle disorder in the elderly, is partly characterized by dysregulation of β -amyloid precursor protein (β APP) expression and abnormal, intracellular accumulation of full-length β APP and β -amyloid epitopes. The present study examined the effects of β -amyloid accumulation on force generation and Ca^{2+} release in skeletal muscle from transgenic mice harboring human β APP and assessed the consequence of $\text{A}\beta_{1-42}$ modulation of the ryanodine receptor Ca^{2+} release channels (RyRs). β -Amyloid laden muscle produced less peak force and exhibited Ca^{2+} transients with smaller amplitude. To determine whether modification of RyRs by β -amyloid underlie the effects observed in muscle, *in vitro* Ca^{2+} release assays and RyR reconstituted in planar lipid bilayer experiments were conducted in the presence of $\text{A}\beta_{1-42}$. Application of $\text{A}\beta_{1-42}$ to RyRs in bilayers resulted in an increased channel open probability and changes in gating kinetics, while addition of $\text{A}\beta_{1-42}$ to the rabbit SR vesicles resulted in RyR-mediated Ca^{2+} release. These data may relate altered β APP metabolism in IBM to reductions in RyR-mediated Ca^{2+} release and muscle contractility.

Keywords

Inclusion body myositis; β -Amyloid; β APP; Ryanodine receptors; Excitation-contraction coupling

1. Introduction

Inclusion body myositis (IBM), the most common myopathy in the aging population, is characterized by progressive weakness of skeletal muscle (Askanas and Engel, 1995; Carpenter, 1996; Askanas et al., 1998). At the molecular level, IBM is characterized by an abnormal intracellular accumulation of numerous proteins, including β -amyloid proteins ($A\beta$), hyperphosphorylated tau, ubiquitin and superoxide dismutase 1. It is noteworthy that most of the proteins that accumulate within the muscle of IBM patients have also been linked to the pathogenesis of Alzheimer's disease (Selkoe, 2001). This makes it likely that both disorders share some common pathogenic steps. It is believed that increased levels of β -amyloid precursor protein (β APP) and its proteolytic products, such as β -amyloid peptides, and their subsequent aggregations into inclusions within the muscle cells contribute to IBM pathology (Sarkozi et al., 1993; Askanas and Engel, 1998a,b,c; McFerrin et al., 1998).

Although the etiology of IBM has been well characterized (Askanas and Engel, 2006; Engel and Askanas, 2006), the sequence of events that leads to the muscle pathology remains unclear. Since muscle weakness is a prominent feature of IBM, it is logical to predict that processes governing muscle force production could be severely affected in this disease. In skeletal muscle, force generation is regulated by the process known as excitation-contraction (E-C) coupling. In this process, a rapid cascade of events is initiated by an action potential that upon reaching the transverse tubules (TT) activates the L-type Ca^{2+} channels, the dihydropyridine receptors (DHPRs). Activated DHPRs rapidly trigger massive release of Ca^{2+} from the sarcoplasmic reticulum (SR) via Ca^{2+} release channels, the ryanodine receptors (RyRs) that reside in the junctional region of the SR immediately adjacent to the TT membrane (Melzer et al., 1995). The resulting release of Ca^{2+} produces a transient increase in intracellular $[Ca^{2+}]$, which activates the contractile apparatus of muscle fibers.

To date, there has been little attention given to the possibility that dysregulation of Ca^{2+} signaling during E-C coupling plays a significant role in IBM pathogenesis. However, there is growing evidence that alteration in intracellular Ca^{2+} handling is involved in the impairment of neuronal function in AD (Mattson et al., 2000; LaFerla, 2002). Recently, it was reported that in cortical neurons, accumulations of intracellular β -amyloid lead to a substantial increase in resting cytoplasmic $[Ca^{2+}]$ (Lopez et al., 2008). Previous investigations have also shown the involvement of endoplasmic reticular Ca^{2+} release channels, the RyRs (Kelliher et al., 1999; Chan et al., 2000; Smith et al., 2005; Stutzmann et al., 2006, 2007), in the neurotoxic cascade associated with AD. Similar to AD, Ca^{2+} homeostasis may also be disrupted in IBM muscle cells laden with abnormal amounts of β -amyloid. This has been supported by the observation that overexpression of $A\beta_{1-42}$ in skeletal myotubes results in a twofold elevation in resting myoplasmic $[Ca^{2+}]$ and an increased sensitivity of RyRs to activation by caffeine (Christensen et al., 2004). Further evidence of disrupted Ca^{2+} homeostasis in IBM has been provided by studies using transgenic mouse models of IBM in which either wild type holoAPP or APP harboring the Swedish double mutant transgene (APP_{SWE}) were selectively expressed in skeletal muscle (Moussa et al., 2006). Both of these models recapitulate some of the molecular and physiological features of IBM, including substantially augmented levels of $A\beta_{1-40}$ (Sugarman et al., 2002; Sugarman et al., 2006), $A\beta_{1-42}$ (Sugarman et al., 2002, 2006; Moussa et al., 2006), as well as oligomeric species of $A\beta_{1-42}$ (Moussa et al., 2006) in an age-dependent manner. However, the main difference between the two is that in the APP_{SWE} mouse, transgene expression is driven by the muscle creatine kinase promoter (MCK- β APP), which results in a muscle tissue-wide expression of β APP (Sugarman et al., 2002), whereas, in the wild type holoAPP-expressing mouse, the transgene expression is driven by the myosin light chain promoter (MLC- β APP), which results in targeted expression of β APP in fast-twitch muscle (Moussa et al., 2006). In addition to numerous structural and physiological changes exhibited by these IBM-transgenic mice, it has been demonstrated that chronic overproduction

of β -amyloid within the muscle of these animals leads to a substantial elevation in the resting, myoplasmic Ca^{2+} concentration (Moussa et al., 2006). Unlike their non-transgenic littermates, hemizygous APP-transgenic animals also developed muscle weakness and other hallmark pathologic features of IBM in an age-dependent manner, including structural and physiological alterations in muscle function.

Although there is a clear link between β -amyloid overproduction and Ca^{2+} dysregulation in skeletal muscle, little is known about the mechanisms involved. Therefore, the goal of the present study was to determine the effects of β -amyloid on various components of E-C coupling in β APP overexpressing transgenic mice.

2. Materials and methods

2.1. Muscle fiber preparation

MCK- β APP mice (Sugarman et al., 2002) were used in this study following a protocol approved by the Caritas St. Elizabeth's Medical Center Institutional Animal Care and Use Committee. Transgenic and age-matched non-Tg mice (20-24 months old) were euthanized by pentobarbital overdose. Flexor digitorum brevis (FDB) muscles were removed, placed in a Dulbecco's modified eagle medium (DMEM) (Invitrogen, Carlsbad, CA) solution containing 2 mg/ml collagenase A (Roche, Nutley, NJ) and incubated under gentle agitation for 3 h at 37 °C. Thereafter, muscles were removed from the enzyme-containing buffer and rinsed twice in DMEM. Muscles bundles were then transferred to DMEM supplemented with 10% bovine growth serum, 1% penicillin, 1% streptomycin and 1% glutamine and gently triturated with a polished glass pipette until a significant portion was dissociated to single cells. Myofibers were plated onto ECM-coated (Sigma, St. Louis, MO) glass-bottom dishes (MatTek, Ashland, MA) and allowed to settle to the bottom of the dish over night in an incubator at 5% CO_2 and 37 °C.

2.2. Fluorescence recording

All reagents, unless otherwise indicated, were purchased from Sigma-Aldrich (St. Louis, MO). Cells were bathed in a normal Ringer solution containing in (mM): [125]NaCl, [5]KCl, [1.2] MgSO_4 , [6]glucose, [25]HEPES, [2] CaCl_2 , pH 7.4. Myofibers were loaded at room temperature for 30 min in Ringer solution supplemented with Ca^{2+} indicator dye (magFluo-4AM, 5 μM (Molecular Probes, Eugene OR)). Cells were later washed several times with Ringer to terminate further loading and placed in a 37 °C incubator for de-esterification of the dye. To eliminate the motion artifacts due to muscle contraction, *N*-benzyl-*p*-toluene sulphonamide (BTS, 50 μM), an inhibitor of the myosin II ATPase, was added to the bathing solution. Whole cell fluorescence changes were detected using an IonOptix fluorescence system (IonOptix, Milton, MA) interfaced with an inverted Zeiss Axiovert 200 microscope equipped with a Neofluar 40 $\times$ oil-immersion objective. Changes in fluorescence were detected by a photo multiplier tube (PMT).

Ca^{2+} transients were elicited by applying supra-threshold rectangular pulses (1 ms duration) through two platinum electrodes placed on opposite sides of the experimental chamber. Changes in intracellular $[\text{Ca}^{2+}]$ were characterized as changes in Fluo-4 fluorescence intensity. All experiments were conducted at room temperature (22 °C). Detected changes in fluorescence within each cell were analyzed using IonOptix software (IonOptix, Milton, MA). The resulting fluorescence changes were corrected for the background fluorescence within individual cells by dividing the magnitude of change in fluorescence (ΔF) by the mean baseline fluorescence intensity (F_0) to give the $\Delta F/F$ values.

2.3. Muscle contractile experiments

These experiments were performed according to previously described methodology (Spangenburg et al., 1998). In brief, single EDL muscles from 12-month-old non-Tg and MLC- β APP mice were surgically excised with ligatures at each tendon (5-0 silk suture) and mounted in an *in vitro* bath between a fixed post and force transducer (Aurora 300B-LR) operated in isometric mode. The muscle was maintained in physiological saline solution (PSS; pH 7.6) containing in (mM) [119]NaCl, [5]KCl, [1]MgSO₄, [5]NaHCO₃, [1.25]CaCl₂, [1]KH₂PO₄, [10]HEPES, [10]dextrose, and maintained at 30 °C under aeration with 95% O₂, 5% CO₂ throughout the experiment. Resting tension, muscle length and stimulation current were iteratively adjusted for each muscle to obtain optimal twitch force. During a 5 min equilibration, single twitches were elicited at every 30 s with electrical pulses (0.5 ms) via platinum electrodes running parallel to the muscle. Optimal resting tension was determined and isometric tension was evaluated by 250 ms trains of pulses delivered at 1, 10, 20, 40, 60, 80, 100, 150, 300 Hz. Following a 15 min rest period, the muscle was stimulated to fatigue by delivering tetanic trains (100 Hz for 100 ms) every 2 s for 5 min. After the experimental protocol, the muscle rested for 5 min at which time muscle length was determined with a digital micrometer, muscle was trimmed proximal to the suture connections, blotted and weighed. The cross-sectional area for each muscle was determined by dividing the mass of the muscle (g) by the product of its length (L_0 , mm) and the density of muscle (1.06 g/cm³, Mendez and Keys, 1960) and was expressed as square millimeters. Muscle output was then expressed as isometric tension (g/mm²) determined by dividing the tension (g) by the muscle cross-sectional area. The rate of fatigue was determined by linear least squares regression (Origin, 7.5) of the initial fast phase of the fatigue decrement. The magnitude of fatigue was determined by the mean of the final three force measurements during the fatigue bout in each genotype.

2.4. Preparation of the peptides

Lyophilized A β ₁₋₄₂ peptide was purchased from Biosource Corp (Carlsbad, CA). Peptide was rehydrated in a buffer containing 5% DMSO and 2.5% 1 M TRIS (pH 7.4). Rehydrated peptide was rapidly frozen and stored at -80 °C until further use.

2.5. In vitro Ca²⁺ ATPase and Ca²⁺ release assay

Membrane isolation was done as described previously (Ikemoto et al., 1988). Briefly, rabbit SR membrane vesicles were prepared from back and hind leg skeletal muscles (Pel-Freez Biologicals, Rogers AR) by differential centrifugation of muscle homogenates. Vesicles (0.2 mg/ml of protein) were added to the cuvette containing 500 μ l of buffer solution (150 mM KCl-MOPS (pH 7.2) and 2 μ M Fluo-3 (pentammonium salt)). Changes in Fluo-3 fluorescence were monitored with a luminescence spectrometer Perkin-Elmer LS55 (Perkin-Elmer, Waltham, MA). Solutions in the cuvette were constantly stirred by a magnetic stirrer. Resting fluorescence was recorded in the absence of MgATP. Ca²⁺ uptake was stimulated by addition of 2.1 μ l MgATP (0.5 mM) to the cuvette. In the β -amyloid-induced release assay, synthetic peptide was added after Ca²⁺ uptake was initiated and a steady base line was attained.

2.6. Single channel recording

SR vesicles containing RyR1 were prepared from the back and leg muscles of New Zealand rabbits and SR vesicles containing RyR2 were prepared from sheep heart and were reconstituted into artificial lipid bilayers as previously described (Laver et al., 1995). Lipid bilayers were formed from phosphatidylethanolamine and phosphatidylcholine (8:2, w/w) in *n*-decane (50 mg/ml). During experiments the *cis* (cytoplasmic) and *trans* (luminal) solutions contained 250 mM Cs⁺ (230 mM CsCH₃O₃S, 20 mM CsCl) and various concentrations of CaCl₂. The composition of the *cis* and *trans* solutions were altered either by aliquot addition of stock solutions containing 10 mM peptide, 5% DMSO and 2.5% 1 M Tris (pH 7.4). In

addition, we could alter the composition of the *cis* solution by local perfusion which allowed solution exchange within ~ 1 s (O'Neill et al., 2003). Solutions were pH buffered with 10 mM TES and solutions were titrated to pH 7.4 using CsOH. Free $[Ca^{2+}]$ of 100 nM (1 mM $CaCl_2$, 4.5 mM BAPTA) estimated using published association constants (Marks and Maxfield, 1991) and the program 'Bound and Determined' (Brooks and Storey, 1992). Bilayer apparatus and data recording methods are described elsewhere (Laver et al., 2004). Electrical potentials are expressed using standard physiological convention (i.e., cytoplasmic side relative to the luminal side at virtual ground). Measurements were carried out at 23 ± 2 °C. The current signal was digitally filtered at 1 kHz with a Gaussian filter and sampled at 5 kHz.

2.7. Statistics

Statistical analysis was performed using Student's *t*-test for two independent populations. Differences were considered to be statistically significant at $p < 0.05$. All data are presented as mean $\pm$ S.E.M.

3. Results

3.1. Effects of β -amyloid on force production and fatigue development

The effect of intracellular β -amyloid, produced by proteolytic processing of β APP, on muscle contractility *in vitro* was assayed in single EDL muscles from age matched non-Tg and MLC- β APP mice. The force vs. stimulation frequency relationship was determined and peak force at each stimulation frequency was normalized to muscle cross-sectional area (see Section 2). There was no difference in the EDL muscle mass between the non-Tg and transgenic animals (9.2 ± 1.1 mg non-Tg vs. 8.2 ± 1.6 mg MLC- β APP). At stimulation frequencies greater than 60 Hz, EDL muscles from MLC- β APP animals ($n = 4$) produced less muscle force than EDL muscles from non-Tg animals ($n = 4$) (Fig. 1A). A detailed characterization of the contractile kinetics of the twitch (i.e., 1 Hz) and peak tetanic (i.e., 300 Hz) revealed a prolonged time to peak force at 300 Hz in the transgenic muscle (0.22 ± 0.04 s for MLC- β APP vs. 0.15 ± 0.03 s for non-Tg, $p < 0.05$) (Fig. 1B). No significant differences were observed in relaxation time between genotypes (Fig. 1C) ($p > 0.05$).

Muscle fatigability was assessed with tetanic trains delivered every 2 s during a period of 5 min (see Section 2). The rate of fatigue development (0.56 ± 0.14 s $^{-1}$ for non-Tg vs. 0.45 ± 0.022 s $^{-1}$ for MLC- β APP) was less rapid and the magnitude of fatigue ($29.4 \pm 0.82\%$ of initial for non-Tg vs. $34.3 \pm 1.46\%$ of initial for MLC- β APP) was smaller in the EDL muscles from transgenic animals compared to the EDL muscles from non-Tg controls ($p < 0.05$) (Fig. 1D).

3.2. Effects of β -amyloid on Ca^{2+} release

To assess the effect of the β APP transgene and intracellular β -amyloid accumulation on SR Ca^{2+} release, individual, enzymatically dissociated FDB muscle fibers prepared from the MCK- β APP ($n_{\text{animals}} = 4$) and non-Tg ($n_{\text{animals}} = 4$) animals were field stimulated with five successive single action potential pulses delivered at 0.05 Hz. Muscle fibers readily exhibited Ca^{2+} release activity with similar kinetic properties in response to each stimulus. Fig. 2 presents the averaged Ca^{2+} transients from non-Tg (Fig. 2A) and MCK- β APP muscle fibers (Fig. 2B). As demonstrated in Fig. 2C Ca^{2+} transients from the MCK- β APP muscle fibers exhibited smaller peak amplitudes (0.19 ± 0.01 $\Delta F/F$, $n = 23$) than those from the non-Tg fibers (0.27 ± 0.02 $\Delta F/F$, $n = 21$, $p < 0.05$).

The time course of decay in FDB muscle fibers from both animals could be well described by a single exponential function. Fig. 2D presents the comparison of decay time constants obtained from MCK- β APP and non-Tg Ca^{2+} transients. In non-Tg fibers decay time constant was 12.9 ± 0.72 ms ($n = 21$), where as in MCK- β APP fibers it was 18.4 ± 1.6 ms ($n = 23$). Despite the

finding there was no correlation between the peak amplitude and decay rate of Ca^{2+} transients in the non-Tg myofibers ($R = -0.007$), there was a weak correlation between these parameters in the MCK- β APP cells ($R = 0.27$). This is consistent with the possibility that a reduced magnitude of Ca^{2+} release in the MCK- β APP cells contributed to the differences in Ca^{2+} clearance reported here. The full duration at half-maximal amplitude (FDHM) of Ca^{2+} transients from non-Tg (7.3 ± 0.6 ms) and MCK- β APP transgenic fibers (10.7 ± 0.9 ms) is presented in Fig. 2E. The increase in duration of Ca^{2+} transients in MCK- β APP cells suggests that overproduction of β -amyloid resulted in slower Ca^{2+} clearance after stimulation. Despite the significant prolongation of the decay phase of the fluorescent transient in MCK- β APP myofibers a full return to the pre-stimulation baseline (i.e., resting $[\text{Ca}^{2+}]$) was readily achieved after each stimulation.

3.3. Effects of $\text{A}\beta_{1-42}$ peptide on Ca^{2+} uptake by SERCA

To determine whether $\text{A}\beta_{1-42}$ peptide can directly modulate SERCA function, we performed an *in vitro* Ca^{2+} uptake assay. In these experiments SR vesicles prepared from rabbit skeletal muscle were induced to take up Ca^{2+} by stimulating SERCA with MgATP (see Section 2). Vesicles were pretreated on ice with 10 μM of synthetic form of $\text{A}\beta_{1-42}$ peptide for 10 min. Representative time courses of Ca^{2+} uptake in the absence and in the presence of $\text{A}\beta_{1-42}$ are shown in Fig. 3. The initial portion of each trace was collected prior to stimulation of Ca^{2+} uptake by SERCA. Addition of MgATP resulted in an immediate decrease of Fluo-3 fluorescence due to Ca^{2+} uptake by the vesicles. Neither the half-time (8.9 ± 0.85 s control; 8.1 ± 0.44 s $\text{A}\beta_{1-42}$, $n = 7$) nor the magnitude of Ca^{2+} uptake (3.6 ± 0.06 F/F_{max} control; 3.7 ± 0.08 F/F_{max} $\text{A}\beta_{1-42}$, $n = 7$), were significantly affected by $\text{A}\beta_{1-42}$. Together these results demonstrate that an acute application of $\text{A}\beta_{1-42}$ does not affect SERCA function.

3.4. Recovery from inactivation

We tested the possibility that β -amyloid could alter the recovery of Ca^{2+} release from inactivation (Caputo et al., 2004; Capote et al., 2005). This series of experiments employed a double stimulation protocol where fluorescence transients were elicited by two action potentials separated by varying intervals between the first and the second stimulus (Fig. 4A). The development of inactivation during the first Ca^{2+} release was evaluated by measuring the change in the peak amplitude of a second Ca^{2+} transient (F_2) with respect to the amplitude of the first (F_1). The time course of recovery from inactivation can be determined by plotting of the fractional peak amplitude F_2/F_1 vs. the time between the stimuli. Fig. 4B shows the mean fractional peak values obtained in FDB fibers from non-Tg ($n = 13$) and MCK- β APP ($n = 17$) transgenic animals. The time constants for recovery from inactivation (τ) for both groups of cells were determined by fitting the experimental data points by a single exponential function. As evident from the graph MCK- β APP fibers exhibited a slower time course of recovery ($\tau = 39.6 \pm 0.04$ ms) than the non-Tg fibers ($\tau = 30 \pm 0.03$ ms).

3.5. $\text{A}\beta_{1-42}$ -induced Ca^{2+} release

Fig. 5 demonstrates the ability of $\text{A}\beta_{1-42}$ peptide to elicit Ca^{2+} release from the SR vesicle. In these experiments rabbit SR vesicles were actively loaded with Ca^{2+} , allowed to achieve a steady state condition and subsequently challenged by application of synthetic $\text{A}\beta_{1-42}$ peptide (10 μM). Addition of the vehicle solution in which β -amyloid peptide was rehydrated (Fig. 5A), alone, did not have an effect on the vesicles ($n = 5$), whereas addition of $\text{A}\beta_{1-42}$ peptide almost immediately resulted in a modest, but sustained elevation in the fluorescence of the indicator dye (Fig. 5B, $n = 10$), signifying that the presence of $\text{A}\beta$ peptide resulted in generation of a Ca^{2+} release. The amount of Ca^{2+} released from the vesicles was substantially smaller than that released by application of submaximal dosages of RyR-specific agonist, 4-Chloro-*m*-Cresol (4-CmC, 100 μM , not shown). To confirm that Ca^{2+} release observed after addition

of A β peptide was mediated by RyRs, vesicles were pretreated with 5 μ M ruthenium red, a RyR antagonist, for 10 min prior to loading of the vesicles with Ca $^{2+}$. As shown in Fig. 5C, pretreatment with ruthenium red prevented the appearance of A β ₁₋₄₂-induced Ca $^{2+}$ release ($n = 5$).

3.6. A β ₁₋₄₂ modulation of RyRs in bilayers

To determine whether A β ₁₋₄₂ modulates the function of RyRs, single channel recordings were performed on RyR1 reconstituted in planar lipid bilayers. As demonstrated in Fig. 6A, at a cytoplasmic (*cis*) Ca $^{2+}$ concentration of 100 nM, which is in the physiological range of the resting myoplasmic Ca $^{2+}$ concentration, RyRs exhibited a low channel open probability ($P_0 = 0.013 \pm 0.005$, $n = 5$). In all five experiments the addition of 2 μ M of A β ₁₋₄₂ peptide to the *cis* side of the bilayer caused a marked increase in RyR activity, resulting in a 10-fold increase in channel open probability (Fig. 6B), without changes in channel conductance. This increase was associated with a twofold increase in mean open time (Fig. 6D) and a fivefold decrease in mean closed time (Fig. 6E). The activating effect of the peptide was observed within 10 s of application to the channel and lasted for the duration of the experiment (>150 s). In one experiment, five RyR Ca $^{2+}$ channels in a single bilayer were subjected to three applications of 2 μ M of A β ₁₋₄₂ peptide separated by two periods of continuous washout. Each time, the peptide-induced activation was completely reversed within 20 s of perfusing away the peptide.

The physiologic concentration of cytoplasmic ATP is a strong stimulator of RyR activity (Laver et al., 2001). In the presence of 2 mM ATP (near maximal ATP effect) and 100 nM Ca $^{2+}$, RyRs had a $P_0 = 0.085 \pm 0.025$ ($n = 5$). Addition of 2 μ M of A β ₁₋₄₂ peptide to the *cis* side of the bilayer caused a twofold increase in P_0 (Fig. 5C).

Specificity of A β ₁₋₄₂ effects on RyR1 was determined either by addition of peptide to the bilayers that did not contain the RyRs, by addition to the luminal (*trans*) side of the bilayer or by testing its effects on the cardiac isoform of the channel (RyR2), which shares over 60% amino acid sequence identity with RyR1. There was no detectable effect of the peptide when it was applied to naïve bilayers. In three experiments addition of 2 μ M of A β ₁₋₄₂ peptide to the luminal side decreased P_0 of RyR1 by $7 \pm 12\%$. Furthermore, there was no detectable effect of the peptide when it was applied to bilayers that contained RyR2 (not shown). Together, these results confirm that A β ₁₋₄₂ can modulate the cytoplasmic domain of RyR1 and have an acute effect on the function of the channel.

4. Discussion

The objectives of this work were to determine the changes in specific components of the excitation-contraction coupling machinery and to investigate putative mechanisms of muscle weakness associated with β -amyloid accumulation in IBM. We used two mouse models of IBM, MCK- β APP and MLC- β APP, in which substantial alterations in resting [Ca $^{2+}$]_i have been previously reported (Moussa et al., 2006). Here, we report that two primary elements involved in the E-C coupling process, specifically, the action potential-induced Ca $^{2+}$ release by the RyRs and the force generation, are altered in fast twitch skeletal muscle that is chronically exposed to intracellular β -amyloid. In addition we demonstrate that an interaction of A β ₁₋₄₂ peptide with the RyRs leads to the modification of channel function, which could account for the previously reported changes in resting [Ca $^{2+}$]_i.

The present results shed new light on the putative mechanism of β -amyloid-induced muscle weakness. In both mouse models used in this study, β APP transgene expression is targeted specifically to skeletal muscle. The MLC- β APP model could be considered to be a more stringent approximation of the disease since β APP overexpression is limited only to the fast twitch muscle fibers (Moussa et al., 2006). In MCK- β APP mice the transgene is expressed

ubiquitously in skeletal muscle tissue (Sugarman et al., 2002, 2006) with a generally higher expression in the fast-twitch muscle (Sugarman et al., 2006). Since the cross-section of a muscle is a mosaic of fast and slow-twitch muscle fibers (Pette and Staron, 2001), we did not utilize the MLC- β APP mouse to monitor Ca^{2+} transients due to the inability to distinguish between the β -amyloid-affected and unaffected fibers. Because of these characteristics we chose to use intact muscle preparations from the MLC- β APP mice to assess the changes in force production and a dissociated muscle fiber preparation from the MCK- β APP mouse to investigate the changes in action potential-evoked Ca^{2+} transients. Based on previous findings (Sugarman et al., 2002; Christensen et al., 2004; Moussa et al., 2006) we believe that the effects observed here were likely due to age-dependent intramuscular accumulation of metabolic derivatives of APP (Sugarman et al., 2002; Moussa et al., 2006). However, we can not rule out the possibility that full length APP, independent of β -amyloid deposits, also contributed to abnormalities in Ca^{2+} release and contractility.

The effects of β -amyloid proteins on Ca^{2+} homeostasis and Ca^{2+} signaling have been investigated in great detail in neurons and more recently in skeletal muscle. There are several aspects of Ca^{2+} handling with respect to the current data that can be considered. These include the changes in resting $[\text{Ca}^{2+}]_i$ and alterations in stimulated Ca^{2+} release. With regard to the former, we have previously established that accumulation of intracellular β -amyloid results in a substantial elevation in myoplasmic Ca^{2+} (Christensen et al., 2004; Moussa et al., 2006). Although the sources of elevated Ca^{2+} remain to be established, since RyR is the major intracellular Ca^{2+} release channel, it has to be considered to be a primary candidate. In this work we demonstrate that RyR function is modulated by a 42 amino acid species of β -amyloid ($\text{A}\beta_{1-42}$). To our knowledge this is the first report of a direct modulation of RyR function by β -amyloid. It is important to note that $\text{A}\beta_{1-42}$ did not have an effect on the luminal domain of these channels and did not change the membrane properties when added to naïve bilayers, thus excluding the possibility that the observed changes in RyR properties were due to anything other than the interaction between $\text{A}\beta$ peptide and the cytoplasmic domain of the channel. It is possible that a large portion of the increased cytoplasmic resting Ca^{2+} in transgenic muscle cells is attributed to an increased open probability of RyR channels (i.e., Ca^{2+} leak), while the cells are at rest. However, since it has been reported that these peptides are capable of modifying the properties of the L-type (Weiss et al., 1994; Ueda et al., 1997; Pierrot et al., 2004; Lopez et al., 2008) and capacitative Ca^{2+} entry channels (Lopez et al., 2008), it is likely that an abnormal contribution of extracellular Ca^{2+} could play a role as well.

There are many reports of $\text{A}\beta$ peptide producing pores in lipid bilayers (Arispe et al., 1993a,b; Arispe, 2004; Ambroggio et al., 2005). These pores have been shown to pass Ca^{2+} (Arispe et al., 1993b) and this has been proposed to underlie the cytotoxic action of $\text{A}\beta$ peptide. It is interesting that we do not detect $\text{A}\beta$ pore formation in bilayers. The discrepancy might lie in the experimental differences between studies. There are several experimental differences but the most significant one is likely to be the method of peptide application. In other studies, $\text{A}\beta$ pores were formed by inserting liposomes containing $\text{A}\beta$ peptide into lipid bilayers, whereas in our study $\text{A}\beta$ peptide was applied to lipid bilayer via perfusion of the aqueous solutions containing the peptide. In previous studies, the liposomes were combined with $\text{A}\beta$ peptide using dehydration and sonication lipid/peptide mixtures, a process that requires several hours. This is a much longer period of peptide exposure than was used in this study, which were only several minutes.

The ability of $\text{A}\beta_{1-42}$ peptide to induce Ca^{2+} release through the RyRs was also confirmed by an *in vitro* Ca^{2+} release assay, where it was shown that its application to RyR-enriched SR vesicles resulted in a sustained Ca^{2+} release that was sensitive to inhibition by a RyR-specific antagonist, ruthenium red. Although the exact implication of an SR Ca^{2+} leak in IBM remains

to be investigated, the possibility exists that this aberration could lead to decrease in force production and more pervasive Ca^{2+} -dependent metabolic defects.

Although overproduction of β -amyloid has been implicated in the clinical progression of IBM (Askanas et al., 1996; Askanas and Engel, 2006) and in the development of deficits in motor performance in β APP transgenic animals (Sugarman et al., 2002; Moussa et al., 2006), specific effects of β -amyloid on muscle force development have not been investigated. The results presented here demonstrate that chronic overexpression of β APP, which leads to an accumulation of β -amyloid products (Sugarman et al., 2002, 2006; Moussa et al., 2006), results in a reduced contractility of skeletal muscle. A decline in contractility could be explained, in part, by a reduction in Ca^{2+} release during muscle stimulation. Such an effect was observed in individual muscle fibers from MCK- β APP transgenic animals, in which action potential evoked Ca^{2+} transients were substantially smaller than Ca^{2+} transients in non-Tg cells. Since we have established that $\text{A}\beta_{1-42}$ peptide modulates RyR function, this interaction itself could lead to changes in SR Ca^{2+} release. One of the possibilities is that a population of β -amyloid-modified RyR1 channels is less responsive to activation by DHPRs and therefore the net RyR-dependent Ca^{2+} flux would be reduced during myofiber activation. In this regard, a suppression of RyR-dependent Ca^{2+} release could explain the apparent resistance to fatigue seen in the MLC- β APP EDL muscles. It is well acknowledged that a reduction in the releasable pool of SR Ca^{2+} release under-pins the depression in SR Ca^{2+} release during the fatigue process (Allen et al., 2002, 2008; Westerblad and Allen, 2003). It is possible that due to a reduction in an action potential-induced Ca^{2+} release, the releasable SR content is spared longer in the β -amyloid affected muscle, thus making it relatively fatigue resistant compared to the non-Tg muscle. This apparent benefit is, however, outweighed by the overall reduction in force generating capacity of the muscle; its primary function. In addition to a reduced magnitude of Ca^{2+} release and a reduction in contractility, Ca^{2+} transients from MCK- β APP as well as force transients from the MLC- β APP muscle fibers also exhibited a modestly reduced rate of recovery to baseline. The reduced rate of recovery was likely due to a slower rate of SERCA-mediated Ca^{2+} reuptake by SR. As β -amyloid does not appear to modify the capacity of the SERCA pumps directly (Fig. 3) we speculate that mitochondrial abnormalities, reduced ATP availability or more wide-spread metabolic deficiencies linked to β -amyloid-mediated pathologies (Grant et al., 1999; Casley et al., 2002) could reduce the efficiency of SERCA.

In summary, the present data demonstrate considerable alterations in SR Ca^{2+} release and contractility in skeletal muscle of IBM mice potentially arising from β -amyloid modulation of RyR function. Since Ca^{2+} handling is a major determinant of force generation in skeletal muscle, we suggest that better understanding of the mechanisms for β -amyloid-mediated changes in Ca^{2+} homeostasis may lead to improved therapeutic strategies for IBM.

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Fig. 1.

Muscle contractility and performance. (A) Muscle contractility was evaluated by assaying the force vs. stimulation frequency relationship in EDL muscles *in vitro*. Square wave pulses (500 μ s) were delivered at 1, 10, 20, 40, 60, 80, 100, 150, and 300 Hz. Muscle specific force from non-Tg (open circles) and MLC- β APP (filled circles) (normalized to cross-sectional area) is shown at each stimulation frequency. (B-C) The time to peak force (B) and half-time to relaxation (C) was calculated at twitch (1 Hz) and at peak tetanus (300 Hz). (D) Muscle fatigue was assayed with repetitive trains of tetanic pulses (100 Hz for 100 ms) delivered at 0.5 Hz over 5 min. Asterisks indicate statistical significance ($p < 0.05$).

Fig. 2.

Reduced Ca^{2+} release in MCK- β APP muscle fibers. A-B. Mean MagFluo-4AM fluorescence transient from non-Tg (A) ($n = 21$) and MCK- β APP (B) ($n = 23$) FDB muscle fibers, measured in response to 1 ms action potential. (C-E) Peak amplitude ($\Delta F/F$), decay time constant (τ , ms) and full duration at half-maximal amplitude (FDHM, ms) of Ca^{2+} transients from non-Tg and MCK- β APP muscle fibers from the same cells presented in panels A and B, respectively. Asterisks indicate statistical significance ($p < 0.05$).

Fig. 3.

Effect of $A\beta_{1-42}$ on Ca^{2+} uptake by SERCA. Representative Fluo-3 fluorescence time courses of Ca^{2+} uptake into SR vesicles in the absence of treatment (light grey, control), after 10 min pretreatment with synthetic $A\beta_{1-42}$ peptide (10 μ M) (black), or after 5 min pretreatment with 30 μ M cyclopiazonic acid (grey, CPA). The initial portion of each trace was collected prior to stimulation of Ca^{2+} uptake by SERCA. Addition of MgATP (arrow) resulted in an immediate decrease of Fluo-3 fluorescence due to Ca^{2+} uptake by the vesicles.

Fig. 4.

Recovery from inactivation in non-Tg and MCK-βAPP mouse fibers. Double-pulse experiments were conducted in FDB muscle fibers isolated from non-Tg and MCK-βAPP transgenic mice. Fibers were field stimulated with 2 pulses separated by 10, 30, 50, 70, 90, 120, 150 and 200 ms. The fibers were allowed to rest for 60 s between records. (A) Representative records of fluorescence transients elicited by two action potentials separated by 10, 30 or 120 ms stimulus intervals. Pulse protocol is shown below each record. (B) The mean values of the fractional amplitude of the second transient with respect to the first (F_2/F_1) were plotted as a function of the time interval between the stimuli. Experimental points were fitted to a single exponential function, which represents the time course of recovery from Ca^{2+} -dependent inactivation. The time constants of recovery from inactivation for non-Tg and MCK-βAPP fibers were 30 ± 0.03 ms ($n = 13$) and 39.6 ± 0.04 ms ($n = 17$), respectively.

Fig. 5.

Aβ₁₋₄₂-induced Ca²⁺ release. A. Representative time course of Fluo-3 fluorescence in the absence of Aβ₁₋₄₂ treatment. Experiments were conducted in the same manner as described in Fig. 3. Vehicle application phase (black bar) was initiated following the completion of an active Ca²⁺ uptake phase stimulated by addition MgATP ($n = 5$). B. Representative time course of Ca²⁺ release from SR vesicles in the presence of Aβ₁₋₄₂ (10 μM). Peptide application phase (black bar) was initiated following the completion of an active Ca²⁺ uptake phase stimulated by addition MgATP ($n = 10$). C. The same experiment as described for B, except that vesicles were pretreated with ruthenium red (5 μM, $n = 5$) for 10 min prior to initiation of active Ca²⁺ uptake. 4-chloro-*m*-cresol (4-CmC, 100 μM), a RyR1 specific agonist was applied to confirm the inhibition of RyRs by ruthenium red.

Fig. 6.

Effect of Aβ₁₋₄₂ on RyRs in bilayers. A. Single channel recording of a RyR1 in a planar lipid bilayer. The current baseline and open states are labeled 'C' and 'O', respectively. At the beginning of the recording the channel was activated by 100 nM Ca²⁺ in the cytoplasmic (*cis*) bath. Rapid perfusion of the bath with *cis* solution plus 2 μM of synthetic Aβ₁₋₄₂ peptide commenced at the time indicated by the arrow. Complete solutions exchange took ~1 s (see Section 2). (B-E) Combined data from five experiments showing the effects of 2 μM of Aβ₁₋₄₂ peptide on channel open probability (P_{Open}) (B and C), mean open time (τ_{Open}) (D) and mean closed time (τ_{Closed}) (E). Panels B and C show the effect of 2 μM of Aβ₁₋₄₂ peptide from paired measurements on P_0 before and after addition of peptide in its absence of ATP (B), or in the presence of 2 mM ATP (C). Asterisks indicate statistical significance ($p < 0.05$).