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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 (βAPP) expression and abnormal, intracellular accumulation of full-length βAPP 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 βAPP and assessed the consequence of $A\beta_{1-42}$ 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 $A\beta_{1-42}$. Application of $A\beta_{1-42}$ to RyRs in bilayers resulted in an increased channel open probability and changes in gating kinetics, while addition of $A\beta_{1-42}$ to the rabbit SR vesicles resulted in RyR-mediated Ca^{2+} release. These data may relate altered βAPP metabolism in IBM to reductions in RyR-mediated Ca^{2+} release and muscle contractility.

Keywords: Inclusion body myositis; β-Amyloid; βAPP; 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 (Aβ), 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 (βAPP) 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),

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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-βAPP), which results in a muscle tissue-wide expression of βAPP (Sugarman et al., 2002), whereas, in the wild type holoAPP-expressing mouse, the transgene expression is driven by the myosin light chain promoter (MLC-βAPP), which results in targeted expression of βAPP 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 βAPP overexpressing transgenic mice.

2. Materials and methods

2.1. Muscle fiber preparation

MCK-βAPP 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