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Neurobiology of Aging 33 (2012) 1001.e1–1001.e6

NEUROBIOLOGY OF AGING

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Altered ryanodine receptor expression in mild cognitive impairment and Alzheimer's disease

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Received 17 December 2010; received in revised form 3 March 2011; accepted 15 March 2011

Abstract

Intracellular Ca^{2+} dysregulation is an underlying component of Alzheimer's disease (AD) pathophysiology, and recent evidence implicates the ryanodine receptor (RyR) in the disease pathway. Three genes code for different RyR isoforms and each gene transcript gives rise to several alternatively spliced messenger RNAs (mRNAs). These variants confer distinct functionality to the RyR channel, such as altering Ca^{2+} release properties or subcellular localization. Changes in RyR isoform expression and alternative splicing have not been examined for potential roles in AD pathogenesis. Here, we compare mRNA levels of the RyR2 and RyR3 isoforms as well as specific alternatively spliced variants across vulnerable brain regions from postmortem samples of individuals with no cognitive impairment (NCI), mild cognitive impairment (MCI), and AD. We find an increase in RyR2 transcripts in MCI brains compared with no cognitive impairment. In addition, there is a reduction in a RyR2 splice variant, associated with an antiapoptotic function, in MCI and AD brains. These alterations in RyR expression at early disease stages may reflect the onset of pathologic mechanisms leading to later neurodegeneration.

Keywords: Alternative splicing; Alzheimer's disease; Apoptosis; Calcium; Endoplasmic reticulum; Mild cognitive impairment; Pre-mRNA splicing; Ryanodine receptor

1. Introduction

Alzheimer's disease (AD) is characterized histologically by amyloid and tau deposits, and cognitively by impaired memory function. Although the etiology of AD is unknown, the contribution of aberrant endoplasmic reticulum (ER) Ca^{2+} release to AD progression is recognized as a pathogenic factor (Bezprozvanny and Mattson, 2008; LaFerla, 2002; Stutzmann, 2007). In particular, the ryanodine receptor (RyR), an ER-resident Ca^{2+} channel, is implicated in AD-linked Ca^{2+} dyshomeostasis (Bezprozvanny and Mattson, 2008; Goussakov et al., 2010; Smith et al., 2005; Stutzmann et al., 2006).

RyR is coded for by 3 genes: RyR1, 2, and 3. RyR2 and RyR3 are expressed in the brain at moderate to high levels while RyR1 is expressed at lower levels in restricted regions (Hertle and Yeckel, 2007). Tightly regulated RyR-mediated Ca^{2+} release is necessary for neuronal viability as well as higher cognitive functions, such as learning and memory, as well as the underlying synaptic plasticity via RyR2 and RyR3 isoforms (Baker et al., 2010; Fitzjohn and Collingridge, 2002; Galeotti et al., 2008; Zhao et al., 2000). By contrast, sustained Ca^{2+} dysregulation likely contributes to neurodegeneration and cognitive impairment (Khachaturian, 1994; Stutzmann, 2007). Consistent with this, upregulated RyR levels are found in human AD brains (Kelliher et al., 1999), while in animal models of AD, RyR2, and RyR3

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doi:10.1016/j.neurobiolaging.2011.03.011

isoforms are upregulated at early and late stages, respectively (Chakroborty et al., 2009; Supnet et al., 2006, 2010; Zhang et al., 2010).

Prior to AD diagnosis, patients often present with a less severe condition termed mild cognitive impairment (MCI) suggesting this may be a risk factor or precursor to AD (Boyle et al., 2006; Petersen et al., 2001a, 2001b). Little is known about Ca^{2+} dysregulation and RyR expression patterns in MCI. In healthy brains, RyR ribonucleic acid (RNA) transcripts are alternatively spliced, giving rise to messenger RNA (mRNA) variants which code for RyR isoforms with altered function, localization, and/or ligand affinity (George et al., 2007; Jiang et al., 2003; Leeb and Brenig, 1998). However, the pattern of alternative RyR splice variants in AD and MCI brains has not been investigated. To address this, we examined changes in RyR isoforms and alternatively spliced variants in samples from persons with no cognitive impairment (NCI), MCI, and AD to determine if there are alterations in RyR patterns within vulnerable brain regions specific to dementia stage. We document a significant increase in RyR2 mRNA expression in human midtemporal cortices of MCI brains, and differential expression of a RyR2 splice variant in MCI and AD brains that may increase vulnerability to apoptosis. Additionally, we reveal novel findings of differential alternative splicing of RyR3 across several brain regions.

2. Methods

2.1. Human brain samples

Postmortem samples were obtained through the Rush Religious Orders Study conducted by the Rush Alzheimer's Disease Center, Chicago, IL, USA, taken from 13 persons with NCI (age: 74–90 years; mean age, 85.8, male [M]:female [F] 3:10), 11 with MCI (age, 75–92 years; mean age, 86.7, M:F 3:8), and 11 with AD (age, 80–95 years; mean age, 86.4, M:F 4:7). The postmortem intervals (PMIs) ranged from 1 hour to 27 hours. Average postmortem intervals for the NCI, MCI, and AD groups were 8.1, 7.0, and 7.6 hours, respectively. Clinical diagnoses of NCI, MCI, and AD have been previously reported in detail (Bennett et al., 2002).

2.2. RNA extraction and reverse transcription

Tissue samples were stored at −80 °C and all samples were kept on dry ice during RNA extraction. For RNA isolation, 100 mg of tissue was homogenized in Trizol-Reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's protocol. Following DNase treatment of 2 μg of total RNA (DNA Free Kit; Ambion), complementary DNA (cDNA) synthesis with random primers was performed using the High Capacity Reverse Transcription kit (Applied Biosystems, Carlsbad, CA, USA).

2.3. Quantitative real-time PCR

Target gene expression was analyzed by real-time polymerase chain reaction (PCR) using the 7500 RT-PCR System and SYBR Green detection (Applied Biosystems). Cycling parameters were as follows: 2 minutes at 50 °C, 10 minutes at 95 °C, and then 36 cycles at 95 °C for 15 seconds, followed by 60 °C for 60 seconds. A dissociation phase was added to the end of each cycle to determine product purity. Beta 2 microglobulin (β2M) was used as a reference control gene (Coulson et al., 2008). Primer sequences for RyR isoforms are as follows:

RyR2 5′ TGCTGGCTTGGGGCTGGAGA3′ and 5′ ACCATGGGCAGCGTCCACAG 3′; RyR3 5′ GACATGCGAGTCGGCTGGGC 3′ and 5′ GATGCCAACGCTGGCCCCTG 3′.

Primer specificities and product purity was supported by the presence of a single peak present on the dissociation curve for each primer set. Specificities were further validated by the presence of a single amplicon for each primer set at the predicted sizes after agarose gel electrophoresis of products with ethidium bromide staining. Each sample was evaluated in triplicate, and interrun calibrators were employed as an indicator of systematic variation. Amplification data were analyzed using the comparative cycle threshold method after normalization to β2M. Primer efficiencies were validated prior to analysis using ΔCt, cDNA dilution assays.

2.4. Semiquantitative PCR

PCR cycling parameters for RyR cDNA were 10 minutes at 95 °C, 40 cycles of 95 °C for 30 seconds, 60 °C for 60 seconds, and 72 °C for 60 seconds. Reactions were allowed to run to completion for 5 minutes at 72 °C, followed by a 4 °C hold. Products were separated on agarose gels and stained with ethidium bromide. Data were recorded using a Gelprint 2000i CCD imaging system (Bio Image Systems, Inc., Jackson, MI, USA) and quantitated with Metamorph Imaging 7.0 software (Molecular Devices, Sunnyvale, CA, USA). Primers were designed to flank the alternative splice sites. Primer sequences and product sizes: RyR2 30 base pair (bp) 5′ CCATCAGTATGACACAGGC 3′ and 5′ CATTATTGTTGCGTCCTTGC 3′, with product sizes of 191 (+30) and 161 (−30) bp. For alternative splicing of the RyR3 isoform the primers are:

RyR3 87 bp 5′ GAAGGTTGAGAAGCCGGAAG 3′ and 5′ CAGGGTTGGTGCCATATACC 3′ with product sizes of 295 (+87) bp and 208 (-87) bp. RyR3 82 bp 5′ CATTGATGAATCTGGACAGCAC 3′ and 5′ CCAGTGTGTCAACCATCTGC 3′ with product sizes of 320 (+82) bp and 258 (-82) bp.

To confirm splice variant identity, amplification products from the semiquantitative PCR were extracted from gels, purified and sequenced at the Genomics Core Facility, Northwestern University, Chicago, IL, USA.