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NF-κB activated by ER calcium release inhibits Aβ-mediated expression of CHOP protein: Enhancement by AD-linked mutant presenilin 1

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Abstract

Mutations in presenilin which result in early-onset Alzheimer disease (AD) cause both increased calcium release from intracellular stores, primarily endoplasmic reticulum (ER), and changes in NF-κB activation. Some studies have also reported that neurons containing AD-linked mutant presenilins (mPS1) show increased vulnerability to various stresses, while others report no differences in neuronal death. The majority of these reports center on potential changes in ER stress, because of the enhanced ER calcium release seen in mPS1 neurons. One of the primary death effectors of ER stress is CHOP, also termed GADD153, which acts to transcriptionally inhibit protective cellular molecules such as Bcl-2 and glutathione. Because both CHOP and NF-κB are activated by increased intracellular calcium and stress, yet have diametrically opposite effects on neuronal vulnerability, we sought to examine this interaction in greater detail.

We observed that IP3-mediated calcium release from ER, stimulated by Aβ exposure, mediated both CHOP expression and NF-κB DNA binding activity. Further, specific inhibition of NF-κB resulted in greater expression of CHOP, while activation of NF-κB inhibited CHOP expression. The enhanced release of calcium from IP3-mediated ER stores in mPS1 neurons stimulated increased NF-κB compared to normal neurons, which inhibited CHOP expression. Upon blockage of NF-κB, exposure to Aβ caused significantly greater Aβ-mediated CHOP expression and death in mPS1 neurons compared to normal neurons. Thus, AD-linked PS1 mutations disrupt the balance between stress-induced NF-κB and CHOP, resulting in greater dependence on stress-induced NF-κB activation in mPS1 neurons.

Keywords: Presenilin; NF-κB; Amyloid; Neuronal death; Alzheimer disease; Endoplasmic reticulum; Calcium regulation; GADD153; CHOP; Cell culture; EMSA

Neurons exposed to amyloid beta peptide 1–42 ($A\beta_{1-42}$) exhibit disrupted calcium regulation resulting in increased cytoplasmic calcium, which is a hallmark of neurodegenerative pathways (LaFerla, 2002; Mattson and Chan, 2003; Canevari et al., 2004; Katayama et al., 2004). A portion of this calcium originates from intracellular stores, primarily endoplasmic reticulum (ER) (Mattson et al., 2001; Mattson and Chan, 2003; Verkhratsky and Toescu, 2003). Disruption of ER calcium, such as that resulting from $A\beta_{1-42}$ exposure, can lead to a phenomenon termed ER stress, which may in turn modulate responses which can either stimulate or inhibit cellular death pathways (Berridge, 2002; Lehotsky et al., 2003; Verkhratsky and Toescu, 2003; Katayama et al., 2004). Among the pro-death responses is expression of the death effector C/EBP homologous protein (CHOP; Oyadomari and Mori, 2004) also called GADD153, a transcription factor which acts to inhibit transcription of protective proteins such as Bcl-2 and to decrease glutathione (DeGracia et al., 2002). Inhibition of calcium release from ER protects neurons from Aβ-mediated death (McCullough et al., 2001; Suen et al., 2003; Ferreiro et al., 2004), as does inhibition of CHOP expression (Mattson et al., 2000), thus both ER-mediated calcium release and CHOP expression play critical roles in Aβ-mediated neuronal death.

Somewhat paradoxically, excess calcium release and ER stress also activate the anti-apoptotic transcription factor NF-κB

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(Pahl and Baeuerle, 1995, 1996, 1997). Mobilization of calcium from internal (Pahl and Baeuerle, 1996; Quinlan et al., 1999; Suen et al., 2003) or extracellular (Kanno and Siebenlist, 1996) sources activates NF-κB, which in turn protects against excitotoxicity. Conversely, buffering calcium (Shatrov et al., 1997) or preventing calcium influx (Kanno and Siebenlist, 1996; Lazaar et al., 1998) decreases NF-κB activity. NF-κB-mediated transcription can protect neurons from a variety of insults relevant to neurodegenerative disorders, including $A\beta_{1-42}$, the excitotoxin NMDA, and a number of oxidative insults (Barger et al., 1995; Mattson et al., 1997; Taglialatela et al., 1997; Glazner et al., 2000; Glazner and Mattson, 2000; Cardoso and Oliveira, 2003). NF-κB protects cells by inducing the expression of genes that promote cell survival such as those encoding for anti-oxidant (manganese superoxide dismutase (MnSOD); (Mattson et al., 1997) calcium stabilizing (calbindin D28K) (Cheng et al., 1994) and anti-apoptotic proteins (Bcl-2, Bcl-XL) (Mattson et al., 1997; Tamatani et al., 1999, 2000).

Though a minority of Alzheimer disease (AD) cases, familial AD (FAD) represents a very early onset, rapidly progressing form of the disease. The mutations causing FAD are 100% penetrant, and all persons carrying one of the mutant genes will develop the disease. FAD is most commonly caused by mutations in presenilin (PS)1 and PS2 (Levy-Lahad et al., 1995; Sherrington et al., 1996), which are integral membrane proteins found primarily in the plasma membrane and ER. Mutations in PS1 lead to increased production of $A\beta_{1-42}$ and enhanced plaque accretion (Annaert et al., 2000; Fraser et al., 2000). In addition, cultured cells containing human AD-linked presenilin mutations (mPS1) exhibit increased calcium release from intracellular stores in response to stress (Leissring et al., 1999a,b, 2001; Schneider et al., 2001). Presenilin mutants in neurons cause increased calcium release from both ER-resident IP3 receptors (Stutzmann et al., 2004; 2007 and ryanodine receptors (Smith et al., 2005; Lee et al., 2006; Stutzmann et al., 2007). In normal and mPS1 neurons, specific inhibition of ER calcium release protects neurons from death caused by $A\beta_{1-42}$ (Mattson et al., 2000). The presence of mutant presenilin in a cell line shows aberrant NF-κB DNA binding activity following exposure to $A\beta_{1-42}$ (Guo et al., 1998), including an enhanced early NF-κB activation which may also result from the greater flux of calcium from ER in these cells. Of particular interest is a recent report that NF-κB activity directly suppresses CHOP expression in a cancer cell line (Nozaki et al., 2000), though this has not been demonstrated in neurons.

Though calcium disruption by mPS1 is well accepted, there are conflicting reports regarding increased vulnerability of mPS1 neurons to stress. Cells containing mPS1 have been reported to be more easily killed by exposure to Aβ42 (Guo et al., 1998), while other studies show no increased vulnerability (Siman et al., 2000). The observation that NF-κB can suppress CHOP in tumors (Nozaki et al., 2001) raises the possibility of a similar mechanism at work in neurons, and may shed light on the role of mPS1 in neuronal vulnerability. Here we examined in greater detail the link between NF-κB and CHOP in mPS1 neurons exposed to $A\beta_{1-42}$.

Methods and materials

Antibodies against CHOP, as well as antisense oligonucleotides for CHOP (5′GAC TCA GCG CCA TGA C3′), were purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA). IκB antisense oligonucleotides (5′GCG CTC GGC CGC CTG GAA CAT GGC3′) and Neurobasal culture media was purchased from Invitrogen/Gibco BRL (Grand Island, NY, USA). Anti-mouse IgG and anti-goat IgG antibodies conjugated with peroxidase were purchased from Biocan (Mississauga, ON, Canada). DC Protein assay kit was provided by Bio-Rad (Mississauga, ON, Canada). Chemi-Glow Western blot detection kits were ordered from Canberra Packard. Fura-2 was purchased from Molecular Probes (Eugene, OR, USA).

Cell culture

Brains were removed from embryonic 16-day-old C57B mice or mice containing the M146V mutation in PS1 (Guo et al., 1999), and cerebral cortices and hippocampal regions dissected and placed in separate dishes containing HBSS Ca^{+2}/Mg^{+2}-free media. Tissues were mechanically dissociated by trituration and seeded in 6-well culture plates (treated with poly-D-lysine) in Neurobasal containing 10% fetal bovine serum (FBS) and incubated overnight, and media was changed to Neurobasal without FBS the next day. Experiments were performed on day 7–8 after plating. Cortical cells were used for Western blot experiments, while hippocampal cultures were used in survival experiments.

Quantification of neuronal survival

Neuronal survival was quantified by established methods (Mattson et al., 1995). In brief, viable neurons in pre-marked fields (20× objective) were counted before experimental treatment and at time points following treatment. Neurons that died in the intervals between examination points were usually absent, and the viability of the remaining neurons was assessed by morphological criteria. Neurons with membranes and soma with a smooth round appearance were considered viable, whereas neurons with fragmented or distended membranes and vacuolated soma were considered nonviable. Neurons were counted in four random fields per culture, and the percentage of surviving neurons per culture was calculated.

Western blotting

Immunoblotting was performed using methods previously described (Glazner et al., 2001). Briefly, after experimental treatment, cultures were washed once with PBS and scraped with Laemmli buffer (0.125 M Tris–HCl pH 6.8, 4% SDS). Samples were boiled and pushed through a 27 gauge needle to fully denature proteins and remove biological activity. A Bradford assay was used to determine protein concentration. Proteins (10–50 μg/lane) were separated in a 12% acrylamide gel by electrophoresis and transferred to a nitrocellulose membrane. Membranes were blotted with 1:1000 dilution of antibody and