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BRAIN RESEARCH

Research Report

AP-2β regulates amyloid beta-protein stimulation of apolipoprotein E transcription in astrocytes

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ARTICLE INFO

Article history:
Accepted 8 January 2012
Available online 18 January 2012

Keywords:
Alzheimer's disease
Amyloid beta-protein
Apolipoprotein E
Activator protein-2
Beta-adrenergic receptor
Neuroprotection

ABSTRACT

Two key players involved in Alzheimer's disease (AD) are amyloid beta protein (Aβ) and apolipoprotein E (apoE). Aβ increases apoE protein levels in astrocytes which is associated with cholesterol trafficking, neuroinflammatory responses and Aβ clearance. The mechanism for the increase in apoE protein abundance is not understood. Based on different lines of evidence, we propose that the beta-adrenergic receptor (βAR), cAMP and the transcription factor activator protein-2 (AP-2) are contributors to the Aβ-induced increase in apoE abundance. This hypothesis was tested in mouse primary astrocytes and in cells transfected with an apoE promoter fragment with binding sites for AP-2. Aβ(42) induced a time-dependent increase in apoE mRNA and protein levels which were significantly inhibited by βAR antagonists. A novel finding was that Aβ incubation significantly reduced AP-2α levels and significantly increased AP-2β levels in the nuclear fraction. The impact of Aβ-induced translocation of AP-2 into the nucleus was demonstrated in cells expressing AP-2 and incubated with Aβ(42). AP-2 expressing cells had enhanced activation of the *apoE* promoter region containing AP-2 binding sites in contrast to AP-2 deficient cells. The transcriptional upregulation of apoE expression by Aβ(42) may be a neuroprotective response to Aβ-induced cytotoxicity, consistent with apoE's role in cytoprotection.

1. Introduction

ApoE is involved in neurodegeneration and regeneration and its gene expression and protein levels are elevated after neuronal injury and in Alzheimer's disease (AD) (Cedazo-Mínguez, 2007; Haasdijk et al., 2002; Ignatius et al., 1986; Li et al., 2010a; Seitz et al., 2003; Yamada et al., 1995; Zarow and Victoroff, 1998). Metabolism and clearance of the amyloid beta protein (Aβ) is associated with apoE (Deane et al., 2008; Fan et al., 2009). Aβ increases cellular apoE levels, but the mechanism for this increase has not been established (Hu et al., 1998; Igbavboa et al., 2003, 2006; LaDu et al., 2000, 2001). Different lines of evidence have lead us to propose that Aβ may be stimulating apoE transcription involving the beta-adrenergic receptor (βAR), cAMP, and the transcription factor, activator protein-2 (AP-2). Brain tissue of patients with AD showed increased apoE mRNA levels as compared with control individuals (Yamada et al., 1995; Yamagata et al., 2001;

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doi:10.1016/j.brainres.2012.01.017

Zarow and Victoroff, 1998) although there have been reports that apoE mRNA levels were lower in AD patients or unchanged (Oyama et al., 1995; Poirier et al., 1991). Cyclic AMP (cAMP), a second messenger that is also upregulated in AD and after astrocyte activation, increased apoE expression and protein secretion (Cedazo-Mínguez et al., 2001; LaDu et al., 2001; Martinez et al., 2001a,b; Prapong et al., 2001). Aβ directly binds to the βAR and alters its internalization and degradation (Wang et al., 2010, 2011). Aβ(42)-induced stimulation of apoE protein levels in mouse primary astrocytes by activation of βAR and a cAMP-dependent pathway (Igbavboa et al., 2006). The transcription factor AP-2 regulates apoE gene expression in astrocytoma cells which is mediated by cAMP (García et al., 1996). AP-2 is an inducible cell type-specific transcription factor family consisting of five closely related proteins (α, β, γ, δ and ε) that regulate the expression of specific target genes (Damberg, 2005; Eckert et al., 2005). AP-2α and β are the most abundant isoforms in the brain. The proximal region of the *apoE* has AP-2 consensus sequences (Du et al., 2005; Lahiri, 2004; Maloney et al., 2007).

The present study determined whether the Aβ-induced increase of apoE protein abundance in astrocytes is due to stimulation of apoE gene expression mediated by binding of the transcription factor AP-2 to the *apoE* promoter region. We tested this hypothesis in mouse primary astrocytes and in cells transfected with a luciferase reporter gene under the control of an *apoE* promoter fragment containing AP-2 binding sites. Based on our previous studies, we used soluble untreated Aβ(42) because this form, but not aggregated or oligomeric Aβ, increased apoE protein levels and altered cholesterol distribution in the Golgi complex and plasma membrane of mouse primary astrocytes (Igbavboa et al., 2003, 2006, 2009). In the present study, we demonstrate that Aβ(42) increased apoE mRNA levels which were inhibited by βAR antagonists. Aβ(42) significantly increased AP-2β levels but significantly reduced AP-2α levels in the nuclear extract. Cells expressing AP-2 showed Aβ(42)-induced activation of a co-expressed luciferase reporter gene construct under the control of an *apoE* promoter fragment containing AP-2 binding sites in contrast to cells not expressing AP-2. These novel findings demonstrate for the first time that Aβ(42) stimulates apoE gene expression by specifically inducing activation of the transcription factor AP-2β.

2. Results

2.1. Aβ(42) increases apoE mRNA expression levels which is inhibited by βAR antagonists in mouse primary astrocytes

Data in Fig. 1A show that Aβ(42) increased apoE mRNA levels in a time-dependent manner. Significant stimulation occurred after the 30 min Aβ treatment with the peak level reached after 60 min. Data were normalized to β-actin mRNA whose expression level was unaffected by Aβ(42). We also found that Aβ(42) treatment significantly increased apoE protein levels (Fig. 1B) which is in agreement with earlier reports (Igbavboa et al., 2003, 2006). This increase in apoE protein levels is consistent with Aβ increasing apoE mRNA levels seen in Fig. 1A.

It has been reported that Aβ(42) stimulation of apoE protein abundance was inhibited by βAR antagonists (Igbavboa et al., 2006). Therefore, it was determined whether the stimulatory effect of Aβ(42) on apoE mRNA seen in Fig. 1A could be inhibited by βAR antagonists. The non-selective antagonist propranolol and the selective beta-adrenergic receptor antagonists, betaxolol for the β_1 receptor and ICI 118551 for the β_2 receptor were used. Incubation conditions and drug concentrations were the same as previously reported for effects of Aβ(42) on apoE protein levels (Igbavboa et al., 2006). Data in Fig. 1A showed that Aβ(42) significantly increased apoE mRNA levels as determined by endpoint RT-PCR. Those results were confirmed as seen in Fig. 1C using qRT-PCR. Moreover, the Aβ-induced stimulation of apoE mRNA levels was significantly inhibited by the non-selective antagonist propranolol ($p \leq 0.01$) and the β_2AR antagonist ICI 118551 ($p \leq 0.005$), with ICI having a greater inhibitory effect as shown in Fig. 1C. The β_1AR antagonist betaxolol did not significantly inhibit effects of Aβ(42) on apoE mRNA expression levels (Fig. 1C). This absence of a significant effect of the β_1AR antagonist on apoE mRNA levels is similar to what we observed on apoE protein levels (Igbavboa et al., 2006). We now demonstrate in mouse primary astrocytes that both apoE mRNA and protein levels are increased by Aβ(42) and that these stimulatory effects of Aβ(42) are inhibited by β_2AR antagonists.

2.2. Aβ(42) effects on AP-2α and β protein levels in the nuclear fraction and cell lysate in mouse primary astrocytes

We propose that Aβ(42) increases apoE protein abundance through a pathway that involves βAR activation and binding of the transcription factor AP-2 to the apoE promoter region. Reports indicate that of the five AP-2 isoforms, AP-2α and AP-2β are the most abundant isoforms in the brain; AP-2γ is co-expressed with AP-2α and AP-2β in several brain regions, but its expression level is the lowest among the three (Coelho et al., 2005; Damberg, 2005; Moser et al., 1995; Oulad-Abdelghani et al., 1996; Shimada et al., 1999). Effects of Aβ(42) on protein levels of AP-2α and AP-2β were determined in the nuclear extract of primary astrocytes. Aβ incubation significantly reduced AP-2α levels (Fig. 2A) and significantly increased AP-2β levels (Fig. 2B) in the nuclear extract. Total protein levels of AP-2α and AP-2β in the cell lysates of Aβ treated and control cells did not differ (Figs. 2C and D) suggesting that the Aβ-induced increase of AP-2β and the reduction of AP-2α in the nuclear fraction were due to a redistribution of the proteins and not synthesis or inhibition of degradation.

2.3. Aβ(42) stimulates apoE promoter activity

Data in Fig. 2B showed that Aβ(42) significantly increased AP-2β protein levels in the nuclear fraction of astrocytes. There is evidence that the proximal *apoE* promoter activity in astrocytes is regulated by binding of the transcription factor AP-2 to two sites located on the *apoE* proximal promoter region (García et al., 1996). We determined whether Aβ(42) would activate the *apoE* promoter region containing AP-2 binding sites. DITNC1 (immortalized rat astrocytes) and AP-2 deficient HepG2 cells were transfected with a luciferase reporter gene under the control of an apoE promoter fragment that included the AP-2 binding sites. Cells were incubated with Aβ(42). Fig. 3 clearly shows