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ApoE Promotes the Proteolytic Degradation of Aβ

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SUMMARY

Apolipoprotein E is associated with age-related risk for Alzheimer's disease and plays critical roles in Aβ homeostasis. We report that ApoE plays a role in facilitating the proteolytic clearance of soluble Aβ from the brain. The endolytic degradation of Aβ peptides within microglia by neprilysin and related enzymes is dramatically enhanced by ApoE. Similarly, Aβ degradation extracellularly by insulin-degrading enzyme is facilitated by ApoE. The capacity of ApoE to promote Aβ degradation is dependent upon the ApoE isoform and its lipidation status. The enhanced expression of lipidated ApoE, through the activation of liver X receptors, stimulates Aβ degradation. Indeed, aged Tg2576 mice treated with the LXR agonist GW3965 exhibited a dramatic reduction in brain Aβ load. GW3965 treatment also reversed contextual memory deficits. These data demonstrate a mechanism through which ApoE facilitates the clearance of Aβ from the brain and suggest that LXR agonists may represent a novel therapy for AD.

INTRODUCTION

Alzheimer's disease (AD) is characterized by the accumulation and deposition of Aβ peptides within the brain, leading to the perturbation of synaptic function and neuronal loss that typifies the disease (Tanzi and Bertram, 2005). Genetic analysis of familial forms of AD has established the centrality of APP processing and Aβ production to disease pathogenesis. Aβ peptides are normally produced by neurons in the brain and cleared through efflux into the peripheral circulation (Zlokovic et al., 2005) and through their degradation by proteinases within the brain (Hardy and Selkoe, 2002).

An isoform of apolipoprotein E, ApoE4, has been shown to confer dramatically increased risk for late-onset AD (LOAD) (Roses et al., 1995); however, the basis for this remains one of the major unanswered questions of disease pathogenesis. ApoE plays critical roles in regulating brain Aβ peptide levels, as well as their deposition and clearance (Holtzman, 2001; Zlokovic et al., 2005). Thus, processes that regulate ApoE expression and functional state could affect its ability to influence brain Aβ homeostasis. ApoE is the predominant apolipoprotein in the brain and is synthesized and secreted mainly by astrocytes (but also by microglia; Xu et al., 2006, 2000) within unilamellar HDL-like particles (Fagan et al., 1999). ApoE is lipidated principally through the action of the ATP-binding cassette transporter ABCA1 (and related transporters), which acts in a variety of cell types to transfer both phospholipids and cholesterol to ApoE (Hirsch-Reinshagen et al., 2004; Wahrle et al., 2004), and, in this way, ApoE acts to traffic lipids throughout the brain. The lipidation status of ApoE is an important functional parameter, governing its conformation (Fisher and Ryan, 1999), intrinsic stability (Hirsch-Reinshagen et al., 2004; Wahrle et al., 2004), and interactions with membrane receptors (Dergunov et al., 2000; Ladu et al., 2006). Importantly, ApoE binds to Aβ, and this, too, is influenced by its lipidation status (Tokuda et al., 2000). Studies with *APP* transgenic mice have demonstrated ApoE isoform-specific effects on the propensity of Aβ to be deposited in the brain (E4 > E3 > E2), the nature of the deposits, and a gene-dosage-related influence on the magnitude of these effects (Holtzman, 2004). ApoE lipidation status is also a significant determinant of whether its interaction with Aβ leads to efflux of the peptides from the brain or, alternatively, to the formation of fibrils and their deposition into plaques (Bell et al., 2007; LaDu et al., 1995; Morikawa et al., 2005; Tokuda et al., 2000). Recently, three independent studies have reported that inactivation of the *Abca1* gene in *APP*-expressing transgenic mice resulted in reduced levels of ApoE. Remarkably, these mice exhibited a seemingly paradoxical elevation of brain Aβ peptide levels and a doubling of Aβ plaque burden without a significant effect on Aβ generation (Hirsch-Reinshagen et al., 2005; Koldamova et al., 2005a; Wahrle et al., 2005). The outcomes of these studies strongly suggested that lipidated forms of ApoE act to enhance the clearance of Aβ peptides from the brain. The aim of the present study was

to establish the mechanism by which ApoE and its lipidation affect Aβ homeostasis.

Liver X receptors (LXRs) are ligand-activated transcription factors that induce the expression of *Apoe*, *Abca1*, and other genes of lipid metabolism (Beaven and Tontonoz, 2006). LXRs act physiologically as cellular cholesterol sensors and are activated by oxysterols. There are two LXR isoforms, LXRα and LXRβ (encoded by *Nrlh3* and *Nrlh2*, respectively), both of which are expressed in the brain (Wang et al., 2002), and their activation results in the rapid and robust increase in the levels of lipidated forms of ApoE (Cao et al., 2007; Jiang et al., 2003; Liang et al., 2004). Thus, regulation of LXR transcriptional activity provides a mechanism to regulate brain ApoE levels and its lipidation status.

The brain possesses robust intrinsic Aβ clearance mechanisms (Tanzi et al., 2004). Aβ peptides are proteolytically degraded within the brain principally by neprilysin (NEP) (Iwata et al., 2000) and insulin-degrading enzyme (IDE, insulysin) (Kurochkin and Goto, 1994). Genetic inactivation of these genes (Farris et al., 2003; Iwata et al., 2001) or administration of inhibitors of these proteinases into the brain results in substantial elevation of Aβ levels in the brain and induction of plaque deposition (Dolev and Michaelson, 2004). Conversely, overexpression of IDE or neprilysin lowered brain Aβ levels and reduced plaque formation (Hemming et al., 2007; Leissring et al., 2003). It has been argued that the predominant mode of Aβ42 clearance from the brain is through its proteolytic degradation because this peptide is not efficiently exported through the vasculature (Deane et al., 2004). Microglia, the brain's resident macrophages, play an essential role in Aβ clearance through their ability to take up and degrade soluble and fibrillar forms of Aβ (Rogers et al., 2002). Moreover, both microglia and astrocytes secrete proteinases, including IDE, that mediate the degradation of Aβ peptides in the extracellular milieu (Qiu et al., 1998).

Despite considerable effort, the cellular mechanisms through which ApoE influences Aβ clearance remain unresolved. We report that ApoE acts to facilitate the proteolytic degradation of Aβ, a previously unappreciated action of this apolipoprotein. Moreover, the lipidation status of ApoE is a critical determinant of its ability to stimulate Aβ degradation, and this finding provides a mechanistic explanation of the increased Aβ levels and deposition observed in APP-expressing mice lacking the *Abca1* gene. Importantly, we demonstrate that elevation of lipidated forms of ApoE, through activation of LXRs, results in reduced Aβ peptide and plaque levels in an animal model of AD and is associated with improved contextual memory. Therapeutic agents that increase the abundance of highly lipidated forms of ApoE, including LXR agonists, may attenuate disease pathogenesis and represent a promising strategy for the treatment of AD.

RESULTS

Microglia Efficiently Take Up and Degrade Soluble Aβ

We first investigated the contribution of microglia to the clearance of soluble Aβ. We found that these cells rapidly take up soluble forms of Aβ from the extracellular milieu (Figure 1A) through a nonsaturable (Figure 1B) macropinocytotic uptake mechanism (S.M. et al., unpublished data). The internalized Aβ is rapidly trafficked to a late endosomal/lysosomal compartment (Figure 1C). Indeed, incubation of BV-2 microglia with soluble Aβ42 resulted in the loss of Aβ from the medium, with complete clearance of the peptide within 24 hr (Figure 1D) that is reflective of both of its uptake into microglia as well as degradation by proteinases secreted by these cells into the extracellular milieu. Microglia efficiently degrade internalized soluble Aβ, and we observed little or no resecretion of Aβ into the medium (Figure 1E). The intracellular degradation of Aβ by microglia is carried out principally by neprilysin and related proteases whose activity can be inhibited by phosphoramidon and thiorphan (Iwata et al., 2000; Tanzi et al., 2004), because in the presence of these protease inhibitors the degradation of internalized soluble Aβ was dramatically inhibited (Figure 1F).

HDL Apolipoproteins Enhance the Cellular Degradation of Soluble Aβ by Microglia

ApoE has been postulated to facilitate the clearance of Aβ peptides from the brain, and we tested whether ApoE and related apolipoproteins influenced the ability of microglia to degrade soluble Aβ. ApoA-I is another major HDL-associated apolipoprotein both in the periphery and in the brain (Koch et al., 2001). Importantly, it functions and is trafficked in a manner similar to ApoE (Smith et al., 2004; Wang et al., 2001). ApoA-I is also lipidated by ABCA1 (Denis et al., 2004) and avidly binds Aβ peptides (Harr et al., 1996; Koldamova et al., 2001), thus providing an independent measure of HDL apolipoprotein function. BV-2 microglia (Figure 2A) or primary microglia (Figure 2B) were incubated with soluble Aβ42 in the presence of exogenously supplied ApoE or ApoA-I. Coincubation with purified human ApoE or ApoA-I resulted in a dramatic stimulation of Aβ clearance from the microglia. ApoE and ApoA-I elicited a similar reduction in intracellular Aβ levels, demonstrating that the observed effects are reflective of the common actions of these apolipoproteins. The effect of ApoE on microglial Aβ clearance is dose dependent (Figure 2C). We measured the efficiency with which ApoE stimulated the clearance of Aβ from microglia by monitoring, in parallel, both the cumulative uptake of Aβ using fluorescently labeled Aβ42 and the intracellular levels of the intact Aβ42 peptide by ELISA (Figures 2D–2F). Fluorescently labeled Aβ is taken up by microglia and degraded; however, the fluorophore is retained within the cells, and the total cellular fluorescence is an independent measurement of total Aβ uptake (S.M. et al., unpublished data). We found that the ApoE-stimulated reduction in intracellular Aβ levels was a consequence of enhanced degradation and was observed at both 3 and 9 hr of incubation. These data demonstrate that the degradation of soluble Aβ within microglia is significantly enhanced by HDL apolipoproteins.

Activation of LXR increased the expression of ApoE and ABCA1 (Burns et al., 2006; Koldamova et al., 2003; Sun et al., 2003), resulting in elevated levels of lipidated ApoE in astrocytes (see Figures S1A and S1B available online). Microglia express both LXR isoforms and respond to LXR agonists (Zelcer et al., 2007). These cells also express and secrete lipidated forms of ApoE (Xu et al., 2000). We tested whether elevation of lipidated ApoE levels would result in enhanced Aβ degradation by microglia by treating these cells with an agonist of LXRs. BV-2 microglia (Figure 3A) or primary microglia (Figure 3B) were pretreated