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Isoform-specific proteolysis of apolipoprotein-E in the brain

David A. Elliott [a], Kayan Tsoi [a], Sandra Holinkova [a], Sharon L. Chan [a], Woojin S. Kim [a,b], Glenda M. Halliday [a,b], Kerry-Anne Rye [c], Brett Garner [a,b,*]

[a] *Prince of Wales Medical Research Institute, Randwick, NSW 2031, Australia*
[b] *School of Medical Sciences, Faculty of Medicine, University of New South Wales, Sydney, NSW 2052, Australia*
[c] *The Heart Research Institute, Sydney, NSW 2050, Australia*

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Abstract

Apolipoprotein-E (apoE) plays important roles in neurobiology and the apoE4 isoform increases risk for Alzheimer's disease (AD). ApoE peptides are biologically active and may be produced in the brain. It is unclear if apoE proteolysis is dependent on isoform or AD status and this was addressed here. Hippocampus, frontal cortex, occipital lobe and cerebellum samples were homogenized into fractions that were soluble in Tris-buffered saline (TBS), Triton X-100 or guanidine hydrochloride and analysed for apoE fragmentation by Western blotting. Approximately 20% of apoE3 was detected as fragments and this was predominantly as a 25 kDa peptide in TBS-soluble fractions. The concentration of TBS-soluble apoE fragments was two- to three-fold higher in apoE3 compared to apoE4 subjects. This difference was observed in all areas of the brain examined and was not related to AD status. Cathepsin-D treatment generated apoE fragments that were very similar to those detected in brain, however, no apoE isoform-specific differences in susceptibility to cathepsin-D proteolysis were detected. This indicates that proteolytic processing of apoE to form soluble fragments in the human brain is dependent on apoE isoform but not AD status.

Keywords: ApoE; ApoE-Fragmentation; Alzheimer's disease; Human-brain; Cathepsin-D

1. Introduction

Apolipoprotein-E (apoE) is a ∼34 kDa glycoprotein that plays a crucial role in lipid transport in the peripheral circulation and in the central nervous system (CNS) (Ladu et al., 2000; Mahley, 1988). In humans, apoE exists as three major isoforms apoE2, apoE3 and apoE4 which differ in their Cys/Arg composition at positions 112 and 158. ApoE2 contains Cys^{112}, Cys^{158}; apoE3 contains Cys^{112}, Arg^{158}; and apoE4 contains Arg^{112}, Arg^{158} (Rall et al., 1982). ApoE4 is a major genetic risk factor for late-onset Alzheimer's disease (AD) whereby possession of one or two copies of the ε4 allele confers a ∼5 or ∼10-fold increase, respectively, in AD risk (Corder et al., 1993; Strittmatter et al., 1993). In contrast, the ε2 allele is associated with decreased AD risk. ApoE isolated from cerebrospinal fluid (CSF) is present in the form of both spherical and discoidal lipoprotein complexes (Fagan et al., 1999; LaDu et al., 1998; Pitas et al., 1987). Astrocytes are thought to be the primary source of apoE in the brain, although microglia and neurons may also contribute to the CNS apoE pool under certain circumstances (Boschert et al., 1999; Elliott et al., 2007; LaDu et al., 1998; Mahley, 1988; Metzger et al., 1996; Xu et al., 1999, 2000).

ApoE has several proposed functions beyond lipid transport including roles in immunoregulation, oxidative stress, stabilization of neuronal microtubules, nerve regeneration

Abbreviations: Aβ, amyloid-β; Ab, antibody; AD, Alzheimer's disease; apoE, apolipoprotein-E; CatD, cathepsin-D; CNS, central nervous system; CSF, cerebrospinal fluid; CON, control; FC, frontal cortex; gHCl, guanidine hydrochloride; HI-Thr, heat-inactivated thrombin; Hippo, hippocampus; IDE, insulin degrading enzyme; NEP, neprilysin; NFT, neurofibrillary tangles; LBD, lipid-binding domain; NR, non-reduced; Occip, occipital lobe; PBS, phosphate buffered saline; POPC, 1-palmitoyl-2-oleylphosphatidyl choline; R, reduced; SDS, sodium dodecyl sulfate; PAGE, polyacrylamide gel electrophoresis; TBS, tris-buffered saline; TBS-X, TBS containing Triton X-100; Thr, thrombin.

* Corresponding author at: Prince of Wales Medical Research Institute, Sydney, NSW 2031, Australia. Tel.: +61 2 93991024; fax: +61 2 93991005.
E-mail address: brett.garner@unsw.edu.au (B. Garner).

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and apoptosis (Arai et al., 1999; Elliott et al., 2007; Mahley et al., 1996; Mahley and Rall, 2000; Miyata and Smith, 1996; Strittmatter et al., 1994). ApoE is also associated with amyloid plaques that are a characteristic of AD and substantial evidence indicates apoE plays a role in amyloid-beta (Aβ) peptide clearance (Beffert et al., 1999; Bell et al., 2007; Jordan et al., 1998). Recent studies demonstrate that lipidated apoE promotes the extracellular degradation of Aβ by insulin degrading enzyme (IDE) and also confirm that apoE targets Aβ for intracellular degradation in microglia (Jiang et al., 2008). Despite intense research into the biological function of apoE, the precise mechanism by which the apoE4 isoform increases AD risk remains to be fully elucidated. Many differences between apoE3 and apoE4 structure and function have been reported that are potentially relevant to AD and these include the findings that: domain interaction mediated by a salt bridge between Arg^{61} and Glu^{255} in apoE4 reduces lipid-binding capacity (Xu et al., 2004), lipidated apoE4 has a lower affinity for Aβ (LaDu et al., 1994; Tokuda et al., 2000), apoE4 is less efficient at stabilizing microtubules (Strittmatter et al., 1994), apoE4 exhibits weaker antioxidant activity (Miyata and Smith, 1996), and apoE4 is structurally less stable (Clement-Collin et al., 2006; Morrow et al., 2000) when compared to apoE3.

It is also recognised that apoE undergoes proteolytic cleavage in the brain to form truncated fragments; some of which preferentially associate with neurofibrillary tangles (NFT) and amyloid plaques (Aizawa et al., 1997; Cho et al., 2001; Harris et al., 2003; Huang et al., 2001; Marques et al., 1996). The generation of apoE fragments in the brain is very likely to be physiologically significant as several studies have demonstrated that truncated apoE (and apoE-mimetic peptides) exert potent bioactive properties that regulate neuronal signalling (Gay et al., 2007; Hoe et al., 2005) and (depending on the fragments analysed) may promote neurodegeneration (Chang et al., 2005; Clay et al., 1995; Crutcher et al., 1994; Huang et al., 2001; Marques et al., 1996; Tolar et al., 1999, 1997; Wellnitz et al., 2005) or stimulate neuroprotective and anti-inflammatory pathways (Aono et al., 2003; Laskowitz et al., 2001; Li et al., 2006; Lynch et al., 2003, 2005; Singh et al., 2008).

Studies by Huang et al. suggested apoE3 and apoE4 are both cleaved in the brain to form a carboxy terminal truncated ∼29–30 kDa fragment and that the relative amount of this fragment is increased in AD (Huang et al., 2001). A quantitatively minor group of fragments in the range of ∼14–20 kDa was also detected but only in AD subjects expressing the apoE4 isoform (Huang et al., 2001). The apoE fragments detected in the AD brains were highly enriched in an insoluble fraction of the brain homogenates which required a cocktail of strong detergents (4% SDS/1% Nonidet P-40/1% sodium deoxycholate) to be solubilized and thus detected (Huang et al., 2001). This group proposed that the formation of the ∼29 kDa apoE fragment may be due to a chymotrypsin-like protease and detailed studies in transgenic mice indicated that neuronal expression of human apoE3 was accompanied predominantly by formation of the ∼29 kDa fragment in the brain, whereas expression of apoE4 was accompanied by the lower molecular weight fragments (Brecht et al., 2004). Importantly, transgenic expression of these forms of apoE in astrocytes (the principal source of apoE in the human brain) did not result in detectable apoE fragmentation in mouse brain (Brecht et al., 2004). Similarly, transgenic expression of human apoE3 or apoE4 under the control of the endogenous mouse apoE promoter did not result in detectable apoE fragmentation in the brains of young (12–20 weeks old) mice (Riddell et al., 2008).

Earlier studies by Crutcher et al. also indicated that apoE fragmentation occurs in the human brain (Marques et al., 1996). In these seminal studies, the major apoE fragments were referred to as 22 kDa fragments that were thought to be equivalent to the 22 kDa thrombin cleavage product (Marques et al., 1996). Further studies showed that the 22 kDa thrombin cleavage product of apoE was toxic to primary neurons in culture and that the apoE4 22 kDa fragment was more toxic than the apoE3 fragment (Tolar et al., 1997, 1999). Subsequent work utilised an ELISA technique to show that the concentration of apoE 22 kDa fragment was increased in brains from AD cases compared to controls and that within the AD cases, apoE4 genotype was associated with higher levels of apoE fragmentation. A more recent study from this group redefined the major apoE fragment in the human brain as a 24 kDa fragment and shows that similar patterns of apoE fragmentation can be induced by treating recombinant human apoE with cathepsin-D (CatD) (Zhou et al., 2006). These findings agree with data published by Rebeck et al., who reported that AD brain homogenate contained an apoE N-terminal fragment at 23–25 kDa and a C-terminal fragment of 10 kDa (Cho et al., 2001).

Although apoE fragments have been detected in the human brain, it is not currently clear if their formation is influenced mostly by apoE isoform or the presence of AD. The extent to which apoE fragments exist in a soluble (and therefore potentially bioactive) form as well as the relative distribution of different sized fragments in soluble and insoluble compartments in the human brain is also not clear. In this investigation we conducted a comprehensive analysis of apoE fragmentation in multiple regions of the human brain and examined the influence of apoE3/apoE4 isoform and AD disease status on apoE proteolysis.

2. Materials and methods

2.1. Human brain tissue

Brain tissue samples were obtained through the Australian Brain Donor Program with ethics approval from the University of New South Wales Human Research Ethics Committee (approval No. HREC03322). Cortical neuritic plaques and neurofibrillary tangles were assessed according to current