Structure and neurotoxicity of novel amyloids derived from the BRI gene

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Abstract

A number of human neurodegenerative diseases involve aggregated amyloid proteins in the brain, e.g. Alzheimer's disease (β-amyloid) and Parkinson's disease (α-synuclein). Other examples are rare familial dementias which involve the BRI gene. In a British family, mutation of the termination codon extends the reading frame of BRI to yield a furin-processed 34-residue peptide (Abri; British dementia peptide), 11 residues longer than the wild-type (WT). In a Danish family, a ten-base insertion also yields a 34-residue peptide (Adan; Danish dementia peptide). To explore the roles of Abri and Adan in neurodegeneration, we synthesized Abri and Adan in oxidized and reduced forms and generated transgenic mice colonies expressing the WT and mutated forms of BRI. We have generated transgenic mice colonies bearing the genes coding for WT-BRI, Adan and Abri under the control of the Thy1 promoter. Whereas WT-BRI transgenic mice express full-length WT-BRI protein in their brains, Adan protein is fully processed to small peptides.

FBD (familial British dementia) and FDD (familial Danish dementia) are rare autosomal dominant neurodegenerative disorders that share features of AD (Alzheimer's disease), including amyloid plaques surrounded by astrocytes and microglia, neurofibrillary tangles, neuronal loss and progressive dementia [1,2]. FBD is clinically characterized by the onset of dementia in the fourth decade, progressive spastic tetraparesis and cerebellar ataxia. FDD is similar, but also involves cataract formation and auditory loss. FBD and FDD are distinguished from AD and other dementing disorders by plaque deposition in the cerebellum and the accompanying cerebellar ataxia. Histological studies show that some of the amyloid deposits in FBD patients exhibit yellow-green birefringence under polarized light after staining with Congo Red, indicating the presence of amyloid-like fibrils with β-sheet structure [3], whereas some deposits do not stain with Congo Red. Immunohistochemical and biochemical analysis of plaques and vascular amyloid of FBD and FDD brains revealed that peptides of approx. 4 kDa named Abri (British dementia peptide) and Adan (Danish dementia peptide) are the main components of the insoluble amyloid deposits that are likely to be involved in pathogenesis [3]. The Abri and Adan peptides are fragments derived from a larger membrane-anchored precursor protein, termed BRI precursor protein, encoded by the BRI gene on chromosome 13 (Figure 1) [3]. FBD patients have a single nucleotide transition ($T \rightarrow A$) that converts the stop codon (TGA) into AGA, extending the length of the BRI precursor protein to 277 amino acids [3]. Furin-mediated processing of mutant BRI between Arg^{243} and Glu^{244} produces the 34-residue C-terminal peptide Abri [4]. A decamer duplication in the 3′ region of the BRI gene originates the peptide Adan that is associated with dementia in FDD. Cleavage by furin releases a peptide of 34 residues, which is identical with Abri and WT-BRI in its N-terminal 22 residues, but contains ten distinct C-terminal residues composed of mainly hydrophobic residues. Synthetic oxidized Abri and reduced Adan form soluble oligomers that are toxic to SH-SY5Y cells in culture (Figure 2) [5,6]. Oxidized Abri in solution initially forms toxic soluble oligomers, and, upon prolonged incubation, yields less toxic insoluble long interweaving fibrils [5]. These observations are in keeping with pathology showing that the brains of Abri patients contain both fibrillar and non-fibrillar deposits [7]. Similarly, small soluble β-amyloid oligomers, rather than fibrils, are the earliest species to compromise synaptic function in AD [8]. WT-BRI, which does not aggregate, is not toxic [5].

DNA constructs of WT-BRI, Abri and Adan, kindly donated by Dr E. McGowan and Dr J. Hardy (Mayo Clinic, Jacksonville, FL, U.S.A.) were inserted into vectors with the Thy1 promoter (a gift from Professor F. Van Leuven, Department of Human Genetics, Catholic University of Leuven, Leuven, Belgium), and fragments were injected at 1 ng/μl into eggs retrieved from superovulated B57 female mice, which were re-implanted into the oviducts of pseudopregnant females. Founders were separated after 3–4 weeks, and transgenics were identified by PCR of digested tail slices for the huBRI2 gene. Positive founders were mated with C57/BL6 mice. Western blots using antibody EN3 against a C-terminal region within the BRI proteins of brain homogenates of a second generation Adan PCR-positive male mouse (M1049) (lane 2; Figure 3) showed that the Adan protein had been processed to small fragments <4 kDa, whereas the brain of a second generation WT-BRI PCR-positive mouse contained the full-length WT-BRI protein of 25 kDa (lane 3). A PCR-negative mouse from the same litter as M1049 showed a faint immunostaining protein at 10 kDa

Key words: α-synuclein, β-amyloid, BRI gene, Danish dementia peptide (Adan), neurotoxicity, transgenic mice colony.

Abbreviations used: Abri, British dementia peptide; AD, Alzheimer's disease; Adan, Danish dementia peptide; FBD, familial British dementia; FDD, familial Danish dementia.

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Figure 1 | Schematic showing the BRI precursor protein and peptides Abri and Adan released in the brains of patients with FBD and FDD

In FDD, there is a 10 base duplication occurring between codons 265 and 266 of the BRI gene, resulting in a frame shift in the gene, and a 277-residue precursor protein. In patients with FBD, there is a single base substitution at the stop codon, again generating a 277-residue protein. The mark indicates where furin cleavage releases the peptides Adan and Abri having 34 residues or the WT-peptide comprising 23 residues. Adapted from [9] with permission. ©2001 Portland Press Ltd.

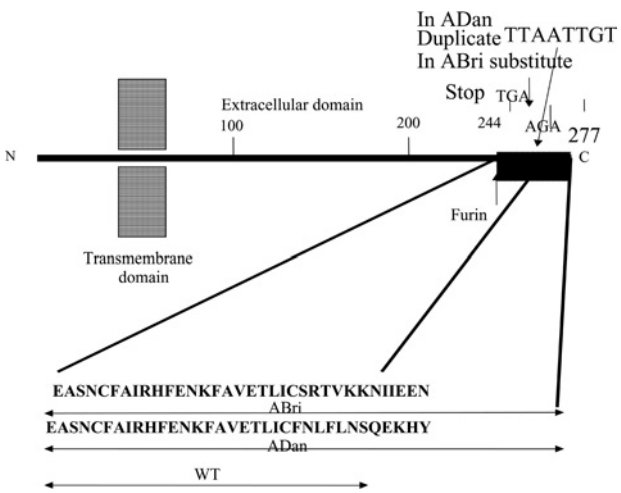


Figure 2 | Neurotoxicity of aged (20 days) and fresh solutions of BRI peptides at 200 μM, determined by LDH (lactate dehydrogenase) release (□) or MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2*H*-tetrazolium bromide] uptake (■) in SH-SY5Y cells in culture

Percentage toxicity is the LDH released compared with that released by 0.1% Triton, or the colour reduction compared with that obtained with 100 μM camptothecin. Results are averaged from six independent readings on separate batches of cells; S.D.s were <10% of averages recorded. Red, reduced; Ox/Oxid, oxidized.

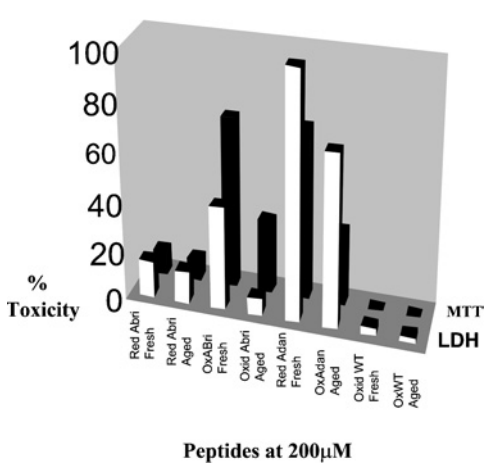
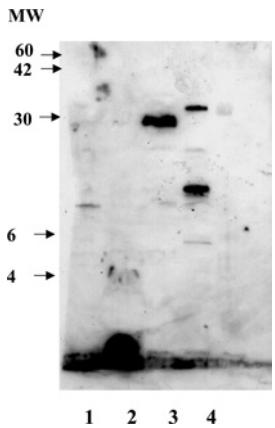


Figure 3 | Western blot of brain homogenates in RIPA buffer

Brains of mice were dissected, homogenized in 5.0 ml of RIPA buffer, and supernatants (20 μl) were separated by SDS/PAGE (10–20% gels). Western blot was performed with 1000-fold dilutions of EN3 antibody, kindly provided by Dr E. McGowan (raised to residues 229–241 of BRI). Molecular masses (MW) are given in kDa.



only (lane 1; Figure 3). Transgenic mice bearing the Abri gene have been generated but not yet examined for BRI expression. Human smooth muscle cells showed immunoreactive processed BRI proteins at approx. 20 and 32 kDa (lane 4; Figure 3). Adan mice at 8 months show mild deficits in object recognition, but are physically normal.

We conclude that mutation in familial dementia appears to increase the lability of the BRI protein to proteolytic processing [4].

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