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Pro-oxidant diet enhances β/γ secretase-mediated APP processing in APP/PS1 transgenic mice

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Abstract

The etiology of Alzheimer's disease (AD) is complex with oxidative stress being a possible contributory factor to pathogenesis and disease progression. TASTPM transgenic mice expressing familial AD-associated amyloid precursor protein (APPswe) and presenilin transgenes (PS1M146V) show increased brain amyloid beta (Aβ) levels and Aβ plaques from 3 months. We tested if enhancing oxidative stress through diet would accelerate Aβ-related pathology. TASTPM were fed a pro-oxidant diet for 3 months resulting in increased brain levels of protein carbonyls, increased Nrf2, and elevated concentrations of glutathione (GSH). The diet increased both amyloid precursor protein (APP) and Aβ in the cortex of TASTPM but did not alter Aβ plaque load, presenilin 1, or β-secretase (BACE1) expression. TASTPM cortical neurons were cultured under similar pro-oxidant conditions resulting in increased levels of APP and Aβ likely as a result of enhanced β/γ secretase processing of APP. Thus, pro-oxidant conditions increase APP levels and enhance BACE1-mediated APP processing and in doing so might contribute to pathogenesis in AD.

Keywords: Oxidative stress; APP; Alzheimer's disease; TASTPM mice; BACE; presenilin

1. Introduction

The majority of cases of Alzheimer's disease (AD) are sporadic and lack hereditary associations (Selkoe, 2001) although numerous risk factors have been proposed. In postmortem AD tissue, markers of oxidative stress have been found associated with all classes of biomacromolecules (Markesbery and Lovell, 2007; Moreira et al., 2008; Reddy et al., 2009; Sultana et al., 2009) and are apparent at the onset of disease, preceding increases in amyloid beta (Aβ), Aβ plaques, and neurofibrillary tangles (NFTs) (Nunomura et al., 2000). In Down syndrome patients, 8-hydroxynonenol (Nunomura et al., 1999) and nitrotyrosine levels are elevated in neurons before the deposition of Aβ plaques (Nunomura et al., 2000), and markers of lipid peroxidation are found in Tg2576 mice before Aβ deposition (Pratico et al., 2001). There are, however, also many reports suggesting that Aβ can induce oxidative stress (Behl, 1999) and Aβ and iron have a synergistic role in inducing oxidative stress (Pike et al., 1993).

Epidemiological studies have suggested that a diet rich in polyphenolic antioxidants confers protection against neurodegenerative diseases (Dai et al., 2006; Kuriyama et al., 2006), whereas dietary intake of iron, which is associated with oxidative stress, increases the risk of neurodegeneration (Powers et al., 2003). It may be possible therefore to use dietary manipulation as a potential modulator of the onset and progression of AD.

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Superoxide dismutase 2 (SOD2) is enriched around amyloid plaques (Furuta et al., 1995) and inactivation of superoxide dismutase 2 in an amyloid precursor protein (APP) transgenic mouse model accelerated the onset of behavioral deficits and exacerbated cerebrovascular amyloidosis, suggestive of a link between oxidative load and amyloid pathology (Esposito et al., 2006). Given the evidence supporting a role for oxidative stress in the pathogenesis of AD we hypothesized that the administration of a pro-oxidant diet to mice carrying AD-causing mutations would lead to enhanced Aβ pathology. In this report, we describe how a pro-oxidant diet increased the levels of APP and soluble Aβ in TASTPM transgenic mice bearing human mutant APP and presenilin 1 (PS1) transgenes (APPswe, PS1M146V) likely as a result of enhanced β/γ secretase-mediated processing rather than increased β-secretase (BACE1) expression.

2. Methods

2.1. Materials

Bench chemicals from Sigma-Aldrich (Poole, UK), Fisher (Loughborough, UK), or VWR (Lutterworth, UK). Cell culture reagents from Invitrogen (Paisley, UK). Protein carbonyl assay kit from Cayman Chemicals (Boldon, UK). Glutathione assay kit from Sigma (Poole, UK). Aβ enzyme-linked immunosorbent assay (ELISA) kits from IBL (Hamburg, Germany), and Wako (Neuss, Germany). BioVeris immunoassays for Aβ utilized the following monoclonal antibodies: biotinylated 6E10 (Senetek PLC, Maryland Heights, MO, USA), G210 (for detection of $A\beta_{1\text{-}40}$; the Genetics Company, Zurich, Switzerland) and 5G5 ($A\beta_{1\text{-}42}$; GlaxoSmithKline, Harlow, UK). Luciferase plasmids from Stratagene (Amsterdam, The Netherlands) except for APP-Gal4 and PFr-luciferase plasmids which were kindly donated by Dr. Michael Perkinton.

2.2. Animals

Heterozygote transgenic mice overexpressing both the hAPP695swe mutation (TAS10) and the presenilin-1 M146V mutation (TPM) were generated by standard techniques as previously described (Howlett et al., 2004) and supplied from the GSK colony maintained by Charles River. Wild type animals were of the C57BL/6 line. Full details including background strains and expression are described in Howlett et al. (2004). Feeding studies were conducted at King's College London under appropriate Personal and Project Home Office Licenses.

2.3. Dietary regimen

TASTPM and wild type animals were fed either a control diet or pro-oxidant modified diet (see below) for 3 months starting at 3 months of age. Each animal was housed individually and had ad libitum access to water and food. The pro-oxidant diet was replaced every 2 days. Animals were weighed weekly in order to monitor health and palatability of diet.

2.4. Dietary composition

The control diet (Harlan, Bicester, UK) was standard mouse chow 2018 containing both vitamin and mineral premixes. The modified pro-oxidant diet (Harlan TD.06186) contained the same basic food groups; the vitamin premix had been removed although lower essential levels of vitamin A, vitamin B12 and vitamin E were present. The mineral premix lacked selenium. In addition an absorbable form of iron, ferric citrate, was added to assist the induction of oxidative stress.

2.5. Tissue preparation

Animals were sacrificed and the brains removed and hemisected. One hemisect was immersion-fixed in 4% paraformaldehyde in 0.1-M phosphate buffer saline (PBS, pH 7.4) for 48 hours for immunohistochemistry (Howlett et al., 2004). The cortex, hippocampus, cerebellum, and striatum from the remaining hemisect were frozen on dry ice for immunoblotting and ELISA. Brain regions were homogenized in 50 mM Tris HCl, 5 mM ethylene glycol tetraacetic acid EGTA, 10 mM ethylenediaminetetraacetic acid EDTA, pH 7.4, containing 2 μg/mL aprotinin, 2 μg/mL leupeptin, 2 μg/mL pepstatin A, 20 μg/mL phenylmethanesulfonylfluoride PMSF. Lysates were centrifuged at 1000*g* for 5 minutes at 4 °C and supernatants retained as a total homogenate. Homogenates were centrifuged at 20,000*g* for 20 minutes at 4 °C and the supernatant retained as a crude cytosolic fraction.

2.6. Measurement of protein carbonyls and glutathione

Total protein carbonyl and glutathione levels from brain homogenates were measured using commercial kits according to the manufacturer's instructions (see Materials). Briefly, protein carbonyl concentration (nmol/mL) was calculated by incubating samples with 2,4 dinitropheylhydrazine (DNPH) and the levels of protein hydrazones formed were analyzed spectrophotometrically at 370 nm (Turner Biosystems Inc, Sunnyvale, CA, USA). Glutathione levels were calculated by measuring the reduction of 5,5′-dithiobis(2-nitrobenzoic acid) (DTNB) to thiobis-2-nitro benzoic acid (TNB) which was measured spectrophotometrically at 412 nm. The concentration was estimated from a glutathione standard curve (0–50 μM).

2.7. Western blotting

Protein samples (20 μg) diluted in loading buffer (final concentration: 62.5 mM Tris, 2% sodium dodecyl sulfate SDS), 10% glycerol, 5% mercaptoethanol, 0.0025% bromophenol blue) were separated by electrophoresis alongside SeeBlue (Invitrogen) markers (7–250 kDa range). Gels were transferred onto either nitrocellulose membrane (1 hour) or polyvinylidene fluoride (PVDF) (1.5 hour) (GE Healthcare, Chalfont St Giles, UK). Blots were probed with an antiAPP antibody (polyclonal antibody Cat no. 9452, Cell Signaling Technology, Danvers, MA) against the C-terminus of full