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Glimepiride protects neurons against amyloid-β-induced synapse damage

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ABSTRACT

Alzheimer's disease is associated with the accumulation within the brain of amyloid-β (Aβ) peptides that damage synapses and affect memory acquisition. This process can be modelled by observing the effects of Aβ on synapses in cultured neurons. The addition of picomolar concentrations of soluble Aβ derived from brain extracts triggered the loss of synaptic proteins including synaptophysin, synapsin-1 and cysteine string protein from cultured neurons. Glimepiride, a sulphonylurea used for the treatment of diabetes, protected neurons against synapse damage induced by Aβ. The protective effects of glimepiride were multi-faceted. Glimepiride treatment was associated with altered synaptic membranes including the loss of specific glycosylphosphatidylinositol (GPI)-anchored proteins including the cellular prion protein (PrP^C) that acts as a receptor for $Aβ_{42}$, increased synaptic gangliosides and altered cell signalling. More specifically, glimepiride reduced the Aβ-induced increase in cholesterol and the Aβ-induced activation of cytoplasmic phospholipase A_2 ($cPLA_2$) in synapses that occurred within cholesterol-dense membrane rafts. $Aβ_{42}$ binding to glimepiride-treated neurons was not targeted to membrane rafts and less $Aβ_{42}$ accumulated within synapses. These studies indicate that glimepiride modified the membrane micro-environments in which Aβ-induced signalling leads to synapse damage. In addition, soluble PrP^C, released from neurons by glimepiride, neutralised Aβ-induced synapse damage. Such observations raise the possibility that glimepiride may reduce synapse damage and hence delay the progression of cognitive decline in Alzheimer's disease.

1. Introduction

Alzheimer's disease (AD), a genetically heterogeneous disease that is the most common form of dementia, is a complex neurological disorder characterized by a progressive dementia (Selkoe, 2002; Tanzi, 2005). The amyloid hypothesis of AD pathogenesis maintains that the main event leading to AD is the production of toxic amyloid-β (Aβ) peptides derived from proteolytic cleavage of the amyloid precursor protein by β and γ secretases (De Strooper et al., 2010; Hardy and Selkoe, 2002). The cognitive decline in AD patients coincides with increasing concentrations of Aβ in the brain (Naslund et al., 2000) which leads to the subsequent disruption of neuronal processes, abnormal phosphorylation of tau and synapse damage. Some of the events that lead to neurodegeneration in AD can be examined by incubating cultured neurons with Aβ peptides.

In this study we measured concentrations of synaptic proteins in cultured primary cortical neurons incubated with Aβ preparations to study the molecular mechanisms involved in synapse degeneration. Since soluble Aβ oligomers that can diffuse throughout the brain are regarded as highly potent neurotoxins (Haass and Selkoe, 2007; Lambert et al., 1998) soluble forms of "natural Aβ" were isolated from brain extracts. The addition of soluble Aβ oligomers reduced the amounts of synaptophysin, synapsin-1, cysteine string protein (CSP) and vesicle-associated membrane protein (VAMP)-1 in cultured neurons indicative of synapse damage (Bate et al., 2010) thus providing a reliable tissue culture model of the synapse

Abbreviations: AD, Alzheimer's disease; Aβ, amyloid-β; PrP^C, cellular prion protein; p-CMPS, chloromercuriphenylsulphonate; $cPLA_2$, cytoplasmic phospholipase A_2; CSP, cysteine-string protein; DRMs, detergent-resistant membranes; DMSO, di-methyl sulphoxide; GPI, glycosylphosphatidylinositol; HPTLC, high performance thin-layer chromatography; PI, phosphatidylinositol; PLAP, phospholipase A_2-Activating Peptide; PLC, phospholipase C; PAGE, polyacrylamide gel electrophoresis; PG, prostaglandin; VAMP-1, vesicle-associated membrane protein.

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damage that is observed in AD. The biological effects of these Aβ oligomers occur at picomolar concentrations, similar to those in extracts from human brain or cerebrospinal fluid (Mc Donald et al., 2010; McLean et al., 1999; Wang et al., 1999).

Numerous biochemical, epidemiological, pharmacological and genetic studies demonstrated that cholesterol is a risk factor for the development of AD (Jick et al., 2000; Li et al., 2007; Puglielli et al., 2003). The requirement of cholesterol for the formation of specific membrane micro-domains called lipid rafts (Rajendran and Simons, 2005) is thought to be is a critical factor affecting AD pathogenesis. The observations that $A\beta_{42}$ accumulates within rafts (Kawarabayashi et al., 2004; Oshima et al., 2001) and that rafts are essential for the formation of signalling platforms (Mayor and Rao, 2004) suggests that $A\beta_{42}$ triggers the events that lead to neurotoxicity from within rafts. This hypothesis is supported by the observation that cholesterol depletion protected neurons against Aβ-induced neurodegeneration (Bate and Williams, 2007; Wang et al., 2001). However, cholesterol synthesis inhibitors are regarded as crude pharmacological tools as cholesterol depletion also affects many other neuronal processes including the sensitivity of neurotransmitter receptors (Allen et al., 2007). The observation that rafts exist as multiple heterogeneous subsets containing different proteins and with different functions (Pike, 2004) raised the possibility that compounds that alter the function of specific rafts involved in Aβ-induced neurodegeneration could be discovered. The factors that affect the formation and function of rafts are inadequately understood. Rafts contain many proteins attached to cell membranes via glycosylphosphatidylinositol (GPI) anchors (Legler et al., 2005; Mayor and Riezman, 2004). As GPI anchors promote the formation of rafts (Brown and London, 2000), drugs that affect GPI anchors may consequently affect the composition and function of rafts.

Glimepiride, a sulphonylurea used to treat diabetes, activates an endogenous GPI-phospholipase C (GPI-PLC) (Movahedi and Hooper, 1997) leading to the release of GPI-anchored proteins including the cellular prion protein (PrP^C) that has been identified as a receptor that mediates Aβ-induced synapse damage (Lauren et al., 2009). Consequently glimepiride was reported to reduce membrane cholesterol (Bate et al., 2009) and affect the distribution of raft-resident proteins (Müller et al., 2005). Here we report that glimepiride protected neurons against Aβ-induced synapse damage. It did not affect the incorporation of $A\beta_{42}$ into neurons, rather it reduced the Aβ-induced changes in cell membranes and the activation of cytoplasmic phospholipase A_2 ($cPLA_2$) in synapses.

2. Materials and methods

2.1. Primary neuronal cultures

Cortical neurons were prepared as described (Lesuisse and Martin, 2002). Neurons were plated (2×10^5 cells/well) in 48 well plates pre-coated with poly-L-lysine in Ham's F12 containing 5% foetal calf serum for 2 h. Cultures were shaken (600 r.p.m for 5 min) and non-adherent cells removed by 2 washes in PBS. Neurons were grown in neurobasal medium containing B27 components supplemented with nerve growth factor (5 nM) for 10 days. Immunohistochemistry showed that the cells were greater than 90% neurofilament positive. Neurons were subsequently pre-treated with test compounds including glimepiride, glipizide, p-chloromercuriphenylsulphonate (p-CMPS) or 0.2 units/ml phosphatidylinositol (PI)-PLC derived from *Bacillus cereus* (all from Sigma) for 1 h before the addition of test samples including Aβ or Phospholipase A_2-Activating Peptide (PLAP) (Bachem) for 24 h. All experiments were performed in accordance with European regulations (European Community Council Directive, 1986, 56/609/EEC).

2.2. Isolation of synaptosomes

Synaptosomes were prepared from cultured neurons on a discontinuous Percoll gradient as described (Thais et al., 2006; Westmark et al., 2011). Neurons were homogenized at 4 °C in 1 ml of SED solution (0.32 M sucrose, 50 mM Tris–HCl, pH 7.2, 1 mM EDTA, and 1 mM dithiothreitol) and centrifuged at 1000×*g* for 10 min. The supernatant was transferred to a 4-step gradient of 3, 7, 15, and 23% Percoll in SED solution and centrifuged at 16,000×*g* for 30 min at 4 °C. Synaptosomes were collected from the interface of the 15% and 23% Percoll steps and washed in PBS before use. Isolated synaptosomes were pre-treated for 1 h and incubated with peptides for 1 h.

2.3. Cell/synaptosome extracts

Treated neurons/synaptosomes were washed 3 times with PBS and homogenised in a buffer containing 150 mM NaCl, 10 mM Tris–HCl, pH 7.4, 10 mM EDTA, 0.2% SDS, mixed protease inhibitors (4-(2-aminoethyl) benzenesulfonyl fluoride, Aprotinin, Leupeptin, Bestain, Pepstatin A and E-46) and a phosphatase inhibitor cocktail (PP1, PP2A, microcystin LR, cantharidin and p-bromotetramisole) (Sigma) at 10^6 cells/ml. Nuclei and cell debris were removed by centrifugation (300×*g* for 5 min).

2.4. Isolation of detergent-resistant membranes (DRMs)/membrane rafts

Membrane rafts were isolated by their insolubility in non-ionic detergents. Briefly, samples were homogenised in an ice-cold buffer containing 1% Triton X-100, 10 mM Tris–HCl, pH 7.2, 150 mM NaCl, 10 mM EDTA and mixed protease inhibitors and nuclei and large fragments were removed by centrifugation (300×*g* for 5 min at 4 °C). The post nuclear supernatant was incubated on ice (4 °C) for 1 h and centrifuged (16,000×*g* for 30 min at 4 °C). The supernatant was reserved as the detergent soluble membrane (DSM) while the insoluble pellet was homogenised in an extraction buffer containing 10 mM Tris–HCL, pH 7.4, 150 mM NaCl, 10 mM EDTA, 0.5% Nonidet P-40, 0.5% sodium deoxycholate, 0.2% SDS and mixed protease inhibitors at 10^6 cells/ml, centrifuged (10 min at 16,000×*g*) and the soluble material was reserved as the DRM fraction.

2.5. Western blotting

Samples were mixed with Laemmli buffer, heated to 95 °C for 5 min and proteins were separated by electrophoresis on 12% polyacrylamide gels (PAGE) under standard denaturing conditions. Proteins were transferred onto a Hybond-P PVDF membrane by semi-dry blotting. Membranes were blocked using 10% milk powder; PrP was detected by incubation with mAb ICSM18, synaptophysin with MAB368 (Abcam), CSP with rabbit polyclonal anti-CSP ((sc-33154) Santa Cruz), VAMP-1 with mAb 4H302 (Abcam), synapsin-1 with a rabbit polyclonal antibody (515200, Invitrogen), caveolin with rabbit polyclonal antibodies (Upstate) and Thy-1 with a rat mAb (Abcam). Bound antibodies were visualised using a combination of biotinylated anti-mouse/rat/rabbit IgG (Sigma), extravidin-peroxidase and enhanced chemiluminescence.

2.6. Isolation of PrP^C and Thy-1

PrP^C and Thy-1 were prepared from neurons as described (Bate and Williams, 2012). Briefly, cells were homogenised in a buffer containing 10 mM Tris–HCl pH 7.4, 100 mM NaCl, 10 mM EDTA, 0.5% Nonidet P-40, 0.5% sodium deoxycholate and mixed protease inhibitors (as above). Cell debris was removed by centrifugation