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Platelet-activating factor antagonists protect amyloid-β damaged neurons from microglia-mediated death

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Abstract

Neurons treated with sub-lethal concentrations of amyloid-β_{1-42} developed phenotypic changes and selectively bound a CD14-IgG chimera; in co-cultures, microglia recognised and killed these amyloid-β_{1-42}-damaged neurons. Pre-treatment with the platelet-activating factor (PAF) antagonists (Hexa-PAF, CV6209 or ginkgolide B) reduced CD14-IgG binding to amyloid-β_{1-42}-damaged neurons, and the presence of PAF antagonists in co-cultures increased neuronal survival in a dose-dependant manner. PAF antagonists also protected neurons treated with HuPrP82–146, a peptide found in prion diseases. Second messenger studies demonstrated that the addition of PAF mimicked some of the effects of amyloid-β_{1-42} on neurons. PAF-damaged neurons bound CD14-IgG, and PAF-damaged neurons were killed by microglia in a CD14-dependent process. Neuronal death was inversely related to both the concentration of PAF, and the number of microglia added. The effects of PAF were reduced by an antagonist of the prostanoid D receptor (BWA868C) indicating that neuronal damage induced by PAF is partly mediated by prostaglandins. These observations are compatible with the hypothesis that sub-lethal concentrations of amyloid-β_{1-42} stimulate a cascade of second messengers including PAF and the prostaglandins. At nanomolar concentrations PAF induces a change in neuronal phenotype that activates microglia via the CD14 molecule, these activated microglia then kill the amyloid-β_{1-42} damaged neurons.

Keywords: Amyloid-β; CD14; Microglial cells; Neurotoxicity; Platelet-activating factor; Prion; Prostaglandins

1. Introduction

The senile plaques that are commonly found in the brains of Alzheimer's disease (AD) patients are formed following the deposition of fibrils containing amyloid-β peptides (Braak and Braak, 1997). The amyloid hypothesis of AD pathogenesis maintains that the primary event in AD is the accumulation and deposition of such fibrils, composed of peptides derived from abnormal proetolytic cleavage of the amyloid precursor protein (Esler and Wolfe, 2001; Hardy and Selkoe, 2002). It has been argued that the accumulation of large amounts of amyloid-β peptides leads to the subsequent disruption of neuronal processes, abnormal phosphorylation of tau and ultimately the dysfunction and death of neurons. Initially it was thought that fibril formation by amyloid-β peptides was required for neurotoxicity (Lorenzo and Yankner, 1994). More recently, smaller soluble oligomers of amyloid-β or amyloid-β-derived diffusible ligands (ADDLs) have been recognised as potent neurotoxins (Lambert et al., 1998; Klein et al., 2001). Several of the events that lead to neuronal loss in AD can be examined in vitro by incubating neurons with small hydrophobic peptides derived from the amyloid-β protein, such as amyloid-β_{1-42} (Yankner et al., 1989; Loo et al., 1993).

The addition of microglia to neurons treated with some amyloid-β-derived peptides results in increased neuronal loss (Meda et al., 1995), strengthening the hypothesis that amyloid-β peptides stimulate a microglia-mediated inflammatory response that contributes to the neuronal loss and cognitive decline that is characteristic of AD. There are some similarities between the neuropathology of AD and that of the

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transmissible spongiform encephalopathies (TSE)s, commonly known as prion diseases. In these diseases the key pathological event is the conversion of the cellular isoform of the prion protein (PrP^{C}) to a disease-associated isoform (PrP^{Sc}) (Prusiner, 1998). Once formed, PrP^{Sc} molecules self-aggregate to form fibrils which accumulate in association with infected neurons (Williams et al., 1997). A peptide derived from amino acid residues 82–146 of the human prion protein (HuPrP82–146) is found in the brains of patients with Gerstman-Sträussler-Scheinker (GSS) syndrome (a human prion disease), but not in normal brains, and is neurotoxic in vitro (Salmona et al., 2003).

Microglial cells are often found in close association with damaged neurons in both AD and prion diseases (Itagaki et al., 1989; Williams et al., 1997), and in tissue culture models, neurons damaged by prion-derived peptides or amyloid-β_{1-42} are killed by microglia (Brown et al., 1996; Meda et al., 1995). Treatment of neurons with either HuPrP82–146 or amyloid-β_{1-42} resulted in a changed phenotype that was recognised by microglia (Bate et al., 2004b; Fassbender et al., 2004). Currently, little is known about the process in which these peptides induce the change in neuronal phenotype. Our previous studies showed that the phenotypic changes induced in cortical neurons by HuPrP82–146 or amyloid-β_{1-42} correlated with the production of prostaglandins and that prostaglandins D_2 or E_2 alone were able to mimic some of the effects of amyloid-β_{1-42} on neurons (Bate et al., 2006). However, neurons treated with prostaglandins alone were not killed by microglia. Since prostaglandin production did not fully mimic all the effects of amyloid-β_{1-42} on neurons we examined the effects of other second messengers on neurons.

We showed that PAF was able to reproduce many of the effects of amyloid-β_{1-42} on primary cortical neurons. Thus, cortical neurons treated with otherwise sub-lethal concentrations of platelet-activating factor (PAF) were killed by microglial cells. In addition, we demonstrated that PAF antagonists were neuroprotective in co-cultures containing microglia and amyloid-β_{1-42}-damaged neurons. The killing of PAF damaged neurons by microglial cells was dependent on the CD14 protein (expressed by microglia) and neurons treated with PAF selectively bound a CD14-IgG chimera. Finally, we showed that the neurotoxicity of PAF was dependent on the activation of the prostanoid D receptor. These studies indicate that PAF may be involved in the neuronal changes that occur early during AD or prion disease and which lead to the activation of microglial cells.

2. Methods

2.1. Primary neuronal cultures

Primary cortical neurons were prepared from brains removed from embryonic mice as previously described (Bate et al., 2004b). Neuronal progenitors were seeded at 500,000 cells per well in 48 well plates in plating medium (RPMI containing 5% foetal calf serum (FCS)). After 24 h, medium was changed to neurobasal medium (NBM) containing B27 components (Invitrogen, Paisley, UK), 100 U/ml penicillin, 100 μg/ml streptomycin and 2 mM glutamine; cultures were used 5 days later. For toxicity studies, neurons were treated with varying concentrations of drug or peptide as indicated. After 3 h, cultures were washed 3 times to remove unbound peptide/drug and microglia were added. For pre-treatment studies, neurons were treated with drugs for 3 h before the addition of peptides. After a further 3 h, cultures were washed 3 times to remove unbound drug/peptide, and microglia were added. Neuronal viability was determined 4 days after the addition of peptides, and after the removal of the loosely adherent microglia by shaking (240 r.p.m for 15 min), by a colourimetric assay using the tetrazolium salt WST-1 (Roche Diagnostics Ltd, Lewes, UK). The number of microglia (cells staining positive for F4/80 (Serotech, Kidlington UK) left in co-cultures after shaking was typically less than 2%.

2.2. Microglia

Were prepared by dissociating cerebral cortices of newborn mice as previously described (Bate et al., 2004b). Microglia were isolated from C57Bl/B6 wildtype ($CD14^{+/+}$) mice, or from CD14 knockout ($CD14^{-/-}$) mice (Moore et al., 2000). For co-culture studies, isolated microglia were suspended in NBM containing B27 components and 2 mM glutamine and added to neurons. In some experiments, microglia were first incubated with polyclonal antibodies to either CD14 or ICAM-1 (R & D systems) for 2 h prior to their addition to neurons. In other studies microglia were added to a transwell system (Falcon companion multiwell™ plates and inserts); a compartment that separated microglia from the treated neurons by a distance of 0.8 mm. This allowed soluble factors to diffuse into the neuronal compartment but prevented the migration of cells. The insert compartment containing microglia was then removed prior to determining neuronal survival.

2.3. CD14-IgG/ICAM-1-IgG chimera binding assays

Neurons were treated with drugs and/or peptides for 3 h prior to the addition of NBM supplemented with 10 ng/ml of a CD14-IgG chimera (containing amino acids 1–345 of murine CD14 fused to the Fc region of human IgG1 via a polypeptide linker, R&D Systems) and 10 ng/ml of an ICAM-1-IgG chimera (R&D Systems) for 1 h. Neurons were washed 3 times with PBS to remove unbound chimera. Cells were collected and membranes were prepared by extraction in a buffer containing 10 mM Tris–HCl, pH 7.8, 100 mM NaCl, 10 mM EDTA, 0.5% Nonidet P-40, 0.5% sodium deoxycholate and 5 mM phenylmethylsulphonylflouride (PMSF) at 1×10^6 cells per ml. The amounts of neuron-bound CD14-IgG or ICAM-1-IgG were determined in a sandwich enzyme-linked immunoassay (ELISA). Membrane preparations were applied to immunoplates pre-coated with either anti-CD14 or anti ICAM-1 polyclonal antibodies (both R&D systems). Bound chimeras were detected using a biotinylated anti-human IgG antibody and amplified using extravidin-alkalinephosphatase and *p*-nitrophenyl phosphate (all Sigma). Absorbance was read at 450 nM and the amount of neuron-bound chimera was calculated by reference to standard concentrations of the chimeras.

2.4. Peptides

A synthetic peptide containing amino acid residues 1 to 42 of the amyloid-β protein (amyloid-β_{1-42}) and a control consisting of the same amino acids in reverse order (amyloid-β_{42-1}) were obtained from Bachem (St Helens, UK). Peptides containing amino acid residues 82 to 146 of the human PrP protein (HuPrP82–146) (Salmona et al., 2003), a control peptide (HuPrP82–146scrambled) were also used (a gift from Professor Mario Salmona, Milan, Italy). Stock solutions of peptides were stored at 1 mM in distilled water and thawed on the day of use. Samples were sonicated before dilution in culture medium and addition to cells.

2.5. Drugs

PAF (1-*O*-alkyl-2-acetyl-*sn*-glycerol-3-phosphocholine), Hexa-PAF (1-*O*-alkyl-2-acetyl-*sn*-glycerol-3-phospho-(*N*,*N*,*N*-trimethyl), hexanolamine), prostaglandins E_2 and D_2 were obtained from Novabiochem (Nottingham, UK). Lyso-PAF (1-*O*-alkyl-*sn*-glycerol-3-phosphocholine), AH13205, SQ27986,