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Prostaglandin D_2 mediates neuronal damage by amyloid-β or prions which activates microglial cells

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Abstract

Microglial cells killed neurons damaged following incubation with sub-lethal concentrations of peptides derived from either the human prion protein (HuPrP82-146) or amyloid-β_{1-42} (a peptide found in Alzheimer's disease). HuPrP82-146 or amyloid-β_{1-42} induced phenotypic changes in neurons that caused them to bind a CD14-IgG chimera. In co-cultures microglial cells produced interleukin (IL)-6 in response to HuPrP82-146 or amyloid-β_{1-42} damaged neurons. The binding of the CD14-IgG chimera to HuPrP82-146 or amyloid-β_{1-42} damaged neurons was reduced by pre-treatment with cyclo-oxygenase (COX)-1 inhibitors and in co-cultures, COX-1 inhibitors significantly increased neuronal survival. Studies with individual prostaglandins demonstrated that the addition of prostaglandin D_2, or prostaglandin E_2, but not other prostaglandins ($F_2\alpha$, H_2, I_2 or 15-dJ_2), mimicked the effects of amyloid-β_{1-42} on neurons. Thus, prostaglandin D_2 or E_2 damaged neurons bound the CD14-IgG chimera, and in co-cultures prostaglandin D_2 damaged neurons activated microglial cells. These effects were mediated via the DP prostanoid receptor; DP receptor agonists BW245C or SQ27986 induced neuronal damage, while the DP receptor antagonist BWA868C was neuroprotective in co-cultures. These results indicate that prostaglandin D_2, produced following activation of COX-1 by sub-lethal concentrations of HuPrP82-146 or amyloid-β_{1-42}, causes phenotypic changes in neurons that activates microglial cells and leads to neuronal loss.

Keywords: Amyloid-β; Prion; Cyclo-oxygenase; Prostaglandins; CD14; Microglial cells

1. Introduction

The aggregation and deposition of misfolded proteins within the brain is a common feature of neurodegenerative diseases including Alzheimer's disease (AD) and the transmissible spongiform encephalopathies (TSEs), commonly known as prion diseases. In AD, the progressive deposition of amyloidgenic fibrils contributes to the formation of senile plaques (Braak and Braak, 1997). These fibrils are composed of amyloid-β peptides derived from proteolytic cleavage of the amyloid precursor protein (Kang et al., 1987). The key pathological event of the TSEs, is the conversion of the cellular isoform of the prion protein (PrP^C) to a disease-associated isoform (PrP^{Sc}) (Prusiner, 1998). PrP^{Sc} molecules self-aggregate to form fibrils which accumulate in association with infected neurons. In both diseases the build up of insoluble fibrillar protein complexes are thought to initiate neuronal damage.

The dysfunction and subsequent loss of neurons, that is central to the clinical symptoms of AD or TSEs, can be modelled in vitro by culturing neurons with peptides derived from the amyloid-β protein (Yankner et al., 1989), or from the prion protein (Forloni et al., 1993). In those studies microglial cells were shown to kill neurons incubated with peptides derived from amyloid-β (Meda et al., 1995) or PrP (Brown et al., 1996). In addition, some fibrillar forms of amyloid-β or PrP peptides stimulated microglial cells to produce inflammatory mediators (Del Bo et al., 1995). Such observations underlie the hypothesis that deposition of amyloid-β or PrP^{Sc} stimulates a microglial cell-mediated inflammatory response that contributes to the neuronal loss and cognitive decline that is characteristic of AD or TSEs. This hypothesis is strengthened by in vivo observations that amyloid plaques are associated

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with microglial cells and astrocytes (Itagaki et al., 1989;Williams et al., 1997a).

In the current study the interactions between microglial cells and neurons incubated with sub-lethal (nanomolar) concentrations of PrP or amyloid-β peptides were studied in an attempt to model the early stages of neuronal dysfunction/neurodegeneration. Raised levels of arachidonic acid metabolites are associated with the neurodegenerative processes observed in both TSEs (Williams et al., 1997b) and AD (Montine et al., 1999). Epidemiological studies have demonstrated that prophylactic use of non-steroidal anti-inflammatory drugs (NSAIDs), drugs which inhibit the cyclo-oxygenases (COX) and reduce prostaglandin production, are beneficial in AD (McGeer et al., 1996). However, the mechanisms by which these drugs have their beneficial effect remain controversial. In this study we investigated the effects of pharmacological manipulation of the arachidonic acid cascade on prion or amyloid-β damaged neurons. The binding of a CD14-IgG chimera was used to determine changes in neuronal phenotype following previous observations that microglial cells respond to amyloid-β damaged neurons by a process that is dependent on the CD14 protein (Bate et al., 2004). These studies are complemented by co-culture studies in which microglial responses to damaged neurons were measured via the production of interleukin (IL)-6, and the survival of damaged neurons.

2. Methods

2.1. Primary cortical neurons

Primary cortical neurons were prepared from embryonic mice as previously described (Bate et al., 2001) and seeded at 5×10^4 cells/well into 48-well plates. After 24 h plating medium was changed to neurobasal medium (NBM) containing B27 components (Invitrogen, Paisley, UK), 100 U/ml penicillin, 100 μg/ml streptomycin, 2 mM glutamine, with 5 μM cytosine arabinoside to prevent the proliferation of astroglial cells; cultures were used 5 days later.

2.2. Microglial cells

Primary microglial cell cultures were prepared by dissociating cerebral cortices of newborn mice as previously described (Bate et al., 2001). Isolated microglial cells were suspended in NBM containing B27 components and added to treated neurons to give neuron:microglial cell ratios as described. In some experiments isolated microglial cells were treated for 3 h with drugs prior to being washed 3 times and added to treated neurons.

2.3. Cell viability assays

Neurons were treated with varying concentrations of peptides or prostaglandins for 3 h, cultures were washed 3 times to remove unbound drug/peptide prior to the addition of microglial cells. For inhibition 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 microglial cells were added. After 4 days cultures were shaken to remove the loosely adherent microglial cells (15 min at 200 rpm) and neuronal survival was determined by addition of 500 μg/ml of the tetrazolium salt WST-1 (Roche Diagnostics Ltd, Lewes, UK). The tetrazolium salts are cleaved to formazan by mitochondrial dehydrogenases and the amount of dye formed directly correlates to the number of metabolically active cells. Optical density was read on a spectrophotometer at 450 nm and percentage survival was calculated by reference to untreated cells incubated with WST-1 (100%).

2.4. CD14-IgG binding assay

Neurons were treated with peptides/prostaglandins for 24 h, prior to the addition of NBM containing B27 components and 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, Abingdon, UK) for 1 h. Neurons were then washed 3 times to remove unbound chimera, collected, counted, 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 phenylmethylsulphonylfluoride (PMSF). The amount of neuron-bound CD14-IgG chimera was determined in an enzyme-linked immunoassay (ELISA) by applying membrane preparations to Nunc maxisorb immunoplates pre-coated with goat anti-CD14 polyclonal antibodies (R&D systems). Bound secondary biotinylated anti-human IgG antibody (Sigma, Poole, UK) was amplified using extravidin-alkaline phosphatase (Sigma) and detected using the *p*-nitorophenol indicator. Absorbance was read at 450 nM and the amount of neuron-bound CD14-IgG chimera was calculated by reference to standard concentrations of the CD14-IgG chimera. Neurons were incubated with an ICAM-1-IgG chimera (R&D Systems, Abingdon, UK) as a control.

2.5. Peptides

Synthetic peptides containing amino acids corresponding to residues 82–146 of the human prion protein (PrP82-146), a peptide found in the brains of patients with Gerstman-Straussler-Scheinker (GSS) syndrome (Tagliavini et al., 1991) and a control peptide containing amino acid residues 82–146 in a scrambled sequence (PrP82-146sc) were synthesized by solid-phase chemistry and purified by reverse-phase HPLC. The neurotoxic peptide corresponding to amino acid residues 1–42 of the amyloid-β protein (amyloid-β_{1-42}) and a control peptide (amyloid-β_{42-1}) were obtained from Bachem (St Helens, UK). Stock solutions were thawed on the day of the experiment and were mixed thoroughly and sonicated before addition to cells.

2.6. IL-6 assays

The levels of IL-6 in cultures were determined using a sandwich enzyme-linked immunoassay (ELISA) (R&D Systems) as a measure of microglial cell activation.

2.7. Drugs

Polymyxin B, acetyl salicylic acid, ibuprofen, SC-236, SC-560, valeryl salicylate, DuP-697, FR122047, prostaglandin G_2, misoprostol, butaprost AH13205, AH23848, SQ27986, BWA868C and BW245C were obtained from Sigma. LM-1685, diclofenac, baicalein, Prostaglandins 15d-J_2, E_2, D_2, $F_2\alpha$, H_2 and I_2 were obtained from Novabiochem, (Nottingham, UK). Stock solutions were dissolved in ethanol or di-methyl sulphoxide (DMSO) and diluted appropriately. Vehicle controls consisted of equal dilutions of ethanol or DMSO.

2.8. Statistical analysis

Comparison of treatment effects was carried out using one- and two-way analysis of variance techniques. Post hoc comparisons of means were performed as necessary. For all statistical tests significance was set at the 1% level.

3. Results

3.1. Microglial cells kill amyloid-β_{1-42} or HuPrP82-146-damaged neurons

In the current study the survival of primary cortical neurons incubated with sub-lethal concentrations of amyloid-β_{1-42} or