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p75^{NTR} Antagonistic Cyclic Peptide Decreases the Size of β Amyloid-Induced Brain Inflammation

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Abstract

Amyloid beta ($A\beta$) was shown to bind the 75 kD neurotrophin receptor (p75^{NTR}) to induce neuronal death. We synthesized a p75^{NTR} antagonistic peptide (CATDIKGAEC) that contains the KGA motif that is present in the toxic part of $A\beta$ and closely resembles the binding site of NGF for p75^{NTR}. In vivo injections of $A\beta$ into the cerebral cortex of B57BL/6 mice together with the peptide produced significantly less inflammation than simultaneous injections of $A\beta$ and a control (CKETIADGAC, scrambled) peptide injected into the contralateral cortex. These data suggest that blocking the binding of $A\beta$ to p75^{NTR} may reduce neuronal loss in Alzheimer's disease.

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Conflict of Interest Statement Aspects of the work reported in this correspondence pertain to patents for which M.Y. and B.A.G. are co-inventors. These patents are assigned to the Trustees of Boston University (their employer) and licensed to SemaCo, Inc., a for-profit company in which M.Y. and B.A.G. hold equity, created to commercialize intellectual property arising out of their laboratory.

Keywords

p75 Neurotrophin receptor; Alzheimer's disease; Cyclic peptide; Brain; Inflammation

Introduction

Neuronal death in the presence of beta amyloid ($A\beta$) characterizes Alzheimer's disease (AD). We have demonstrated (Yaar et al. 1997, 2002) and others have confirmed (Kuner et al. 1998; Perini et al. 2002; Tsukamoto et al. 2003) that $A\beta$ induces apoptosis/necrosis of neurons by binding the 75 kD transmembrane neurotrophin receptor (p75^{NTR}), activating nuclear NF κ B (Kuner et al. 1998) as well as caspases 8, 9, and 3 (Perini et al. 2002). Our previous in vitro work has demonstrated that a p75^{NTR} antagonistic cyclic peptide (CATDIKGAEC) interferes with $A\beta$ signaling and rescues neurons from $A\beta$ -induced toxicity (Yaar et al. 2007).

p75^{NTR} interacts with receptors of the trk family, increasing receptor affinity for neurotrophins and leading to several beneficial effects on neurons including survival [reviewed in (Ibanez 2002)]. However, certain ligands like $A\beta$ bind and activate p75^{NTR} alone, without coordinate binding to receptors of the trk family, and induce cell death (Yaar et al. 1997, 2002).

Ibanez et al. (1992) showed that amino acids 29–35 (TDIKGKE) of NGF, that form one of its β hairpin loop structures, mediate p75^{NTR} binding, and if K (lysine) in position 34 is replaced by A (alanine), the resulting NGF mutant still binds p75^{NTR} but with 50% lower affinity (Ibanez et al. 1992). Interestingly, amino acid residues 28–30 of $A\beta$ are KGA, and computerized structure analysis of $A\beta$ suggests that these residues have a high probability of being in a β -loop configuration (White et al. 1994), constituting a receptor binding site. Indeed, a cyclic peptide containing the KGA motif that specifically binds p75^{NTR} but not p140^{trkA}, prevents $A\beta$ -induced apoptotic signaling and prevents $A\beta$ -induced neuronal death in vitro (Yaar et al. 2007).

We now report that in vivo injections of $A\beta$ together with this p75^{NTR} antagonistic cyclic peptide into the cerebral cortex of mice produced significantly less inflammation than simultaneous injections of $A\beta$ and a control peptide. Blocking $A\beta$ binding to p75^{NTR} may provide a novel approach to arresting the progression of AD.

Materials and Methods

Materials and Animals

The cyclic peptides were purchased from BioSource International. $A\beta$ (1–42) (Quality Controlled Biochemicals, Hopkinton, MA) was dissolved in sterile distilled water (0.2 mM) and incubated at 37°C for 1 day before use to allow peptide aggregation. About 14–16-week-old male C57BL/6 wild type (WT) or p75^{NTR}-null (p75^{NTR}^{-/-}) mice (Charles River Laboratories, Wilmington, MA) were used. Animal experiments were performed in accordance with the National Institutes of Health *Guide for the Care and Use of Laboratory Animals* and were approved by both the Veteran's Affairs and Boston University Animal Care committees.

Experimental Design

Two experiments were performed. In the first experiment mice received stereotactic injections of $A\beta$ (1–42, 0.2 mM) and diluent, or $A\beta$ (0.2 mM) plus antagonistic cyclic peptide (0.25 μ M, 1 μ M, or 10 μ M) into the cerebral cortexes as described (Kowall et al. 1992). Animals were sacrificed 7 days later and brains were processed for analysis. The entire brain was sectioned (each section 35 μ m thick) and at least 18 sections including a negative (lesion-free) section

at each end were collected and stained with cresyl violet (CV) to identify inflammatory cells (Kowall et al. 1992). In the second experiment, mice ($n = 15$) were injected with A β and p75^{NTR} antagonistic cyclic peptide (CATDIKGAEC) to one hemisphere as above, or A β and a scrambled peptide (CKETIADGAC) as control to the contralateral hemisphere. Brain sections were processed as above, the extent of inflammatory infiltrate was identified manually in each section and the total area occupied by inflammatory cells was determined [Macintosh scanner IPLab spectrum software (Signal Analytics, Vienna, VA)].

Results

Visual inspection of CV-stained sections showed that the inflammatory infiltrate surrounding brain areas injected with A β alone was consistently larger than that produced in contralateral sites injected with A β and the cyclic peptide (Fig. 1). Brain lesions in hemispheres injected with the antagonistic cyclic peptide in addition to A β were significantly smaller than those produced by injections of A β and the scrambled peptide ($P < 0.025$, paired t -test).

To further confirm the role of p75^{NTR} in A β -induced inflammation, wild type (WT, $n = 18$) or p75^{NTR}-null (p75^{NTR}^{-/-}, $n = 22$) C57BL/6 mice were injected with A β (0.2 mM) to one cortical hemisphere and diluent to the contralateral hemisphere, and total lesion area was determined as above. In WT mice brain lesions in A β injected hemispheres were $75 \pm 24\%$ (mean $\pm$ SEM) larger than in diluent injected hemispheres ($P < 0.02$, paired t -test). However, in p75^{NTR}^{-/-} mice, brain lesions in A β injected hemispheres were only $27 \pm 21\%$ (mean $\pm$ SEM) larger than in diluent injected hemispheres ($P = 0.56$, paired t -test). Thus, in mice lacking p75^{NTR} there was no significant difference in inflammation between A β and diluent alone.

Discussion

Neuroinflammation in Alzheimer's disease contributes to the brain's response to A β deposits and to the ensuing neuronal apoptosis and necrosis (reviewed in (Stege and Bosman 1999; Tan et al. 1998; Yaar et al. 1997)). Activated microglia and astrocytes are recruited to the area and assemble within and around A β plaques (El Khoury et al. 1996). Together with T lymphocytes, these cells are activated to produce inflammatory mediators including cytokines IL-1 β , IL-6, TNF- α , and monocyte chemoattractant protein 1 (Stege and Bosman 1999). Additionally, these inflammatory cells produce reactive oxygen species (ROS) (Tan et al. 1998) and nitric oxide (Goodwin et al. 1995), ultimately increasing neuronal injury and inflammation. Interestingly, inflammatory cell cytokines like IL-1 enhance A β synthesis (Goldgaber et al. 1989) and are then activated by newly synthesized A β to produce more inflammatory mediators, generating a positive feedback cycle that further aggravates neuronal injury (Klegeris et al. 1994). Although innate immune responses to injurious stimuli is generally beneficial, a prolonged response such as that present in AD is detrimental, adversely affecting vulnerable neurons and leading to further neuronal degeneration (Craft et al. 2005).

Injections of A β into murine cortex was reported to produce areas positive for glial fibrillary acidic protein immunoreactivity, increased astrocyte density, and increased level of markers for oxidative damage (Klein et al. 1999). In aged primates, A β intracerebral injections produced lesions containing argyrophilic, thioflavine S fluorescent, Alz 50, and ubiquitin immunoreactive neurons (McKee et al. 1998), demonstrating A β neurotoxicity, and possibly identifying the initial damage caused by A β deposition in the brain. Here we report that a p75^{NTR} antagonistic cyclic peptide, known to block A β -induced neuronal death in vitro (Yaar et al. 2007), decreases inflammation in cerebral cortices of mice injected with A β together with the antagonistic cyclic peptide as compared to those injected with A β and a control scrambled peptide.

Senile plaques in AD are characterized by extra-cellular accumulation of A β containing primarily A β 42 (Knauer et al. 1992). Although in humans these plaques evolve over many years, when A β 42 is injected into murine cortex it rapidly leads to AD-like changes, primarily recruitment of activated glial cells and increased level of ROS (determined by immunostaining for 8-hydroxydeoxyguanosine) (Klein et al. 1999; Kowall et al. 1991), leading to neuronal degeneration (Kowall et al. 1991). Using this murine model, we show a beneficial anti-inflammatory effect of a p75^{NTR} antagonistic cyclic peptide, not observed with an irrelevant scrambled cyclic peptide or in mice lacking p75^{NTR}. The studies, however, do not provide information on the cyclic peptide ability to prevent neuronal death in vivo or on its long-term effects in preventing the reported pathological, biochemical, and behavioral decrements associated with Alzheimer's disease. However, in combination with the prior in vitro observation that the cyclic peptide prevents A β -mediated neuronal apoptosis/necrosis (Yaar et al. 2007) the data suggest that the recruitment of microglia and other inflammatory cells that would have otherwise been activated in response to neuronal injury (Stege and Bosman 1999; Tan et al. 1998; Yaar et al. 1997). Indeed, degenerating neurons are known to release pro-inflammatory mediators such as heat shock proteins and interleukins (IL-1 and -8) to recruit and activate inflammatory cells (Gupta et al. 2006). Alternately, or in addition, microglia and astrocytes are known to express p75^{NTR} (Heese et al. 1998; Perini et al. 2002) and to be activated by A β to produce a variety of inflammatory mediators (El Khoury et al. 1996; Goodwin et al. 1995; Klegeris et al. 1994). Some of these mediators like TNF- α and IL-1 β synergize with A β to enhance neuronal degeneration (Perini et al. 2002). Thus, by interfering with A β binding to p75^{NTR} in glial cells, p75^{NTR} antagonistic cyclic peptide may prevent their activation and thus decrease the inflammatory response.

Regardless of mechanism, our studies suggest that inhibiting the binding of A β to p75^{NTR} may have the beneficial effect of slowing the progression of AD.

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Fig. 1. p75^{NTR} antagonistic cyclic peptide decreases the size of Aβ-induced brain inflammation. About 14–16-week-old C57BL/6 mice were injected with Aβ and diluent (**a**) or Aβ and p75^{NTR} antagonistic cyclic peptide (**b**). Representative sections of the cerebral cortex stained with CV show that the inflammatory infiltrate, apparent along the injection track, is more extensive and more intense in (**a**) than in (**b**) (original magnification $\times 100$). (**c**) C57BL/6 mice were injected with Aβ and p75^{NTR} antagonistic cyclic peptide or Aβ and scrambled peptide. Graphic representation of lesion size averaged over all mice. There is a statistically significant difference in lesion size between the hemispheres injected with Aβ and scrambled (control)

peptide versus A β and p75^{NTR} antagonistic (active) peptide ($P < 0.025$). Mean $\pm$ SEM are displayed