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Noradrenaline deficiency in brain increases β-amyloid plaque burden in an animal model of Alzheimer's disease

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Abstract

Loss of Locus coeruleus (LC) noradrenergic (NA) neurons occurs in several neurodegenerative conditions including Alzheimer's disease (AD). In vitro and in vivo studies have shown that NA influences several features of AD disease including inflammation, neurodegeneration, and cognitive function. In the current study we tested if LC loss influenced beta amyloid (Aβ) plaque deposition. LC neuronal degeneration was induced in transgenic mice expressing mutant V717F human amyloid precursor protein (APP) by treatment with the selective neurotoxin *N*-(2-chloroethyl)-*N*-ethyl-2-bromobenzylamine DSP4 (5 mg/kg every 2 weeks beginning at age 3 months). At 9 months of age, when control mice show low amyloid load, DSP4-treated mice showed an approximately 5-fold increase in the average number of Aβ plaques. This was accompanied by an increase in the levels of APP C-terminal cleavage fragments. DSP4-treatment increased both microglial and astroglial activation. In vivo, DSP4-treatment decreased expression and activity of the Aβ degrading enzyme neprilysin, while in vitro NA increased phagocytosis of Aβ1-42 by microglia. These findings suggest that noradrenergic innervation from LC are needed to maintain adequate Aβ clearance, and therefore that LC degeneration could contribute to AD pathogenesis.

Keywords: Inflammation; Locus coeruleus; Amyloid; Noradrenaline; Nitric oxide

1. Introduction

The currently accepted hypothesis of AD pathology attributes abnormalities in Aβ metabolism to the deposition of plaques which leads to neuronal death and dementia. AD brains are typically characterized by the presence of β-amyloid (Aβ) plaques [13] and neurofibrillary tangles [11], loss of neurons and glial inflammation [20], and damage to the blood brain barrier [48]. An additional hallmark of AD, as well as Parkinson's disease (PD), is loss of Locus coeruleus (LC) noradrenergic neurons [2,32] and reduced levels of noradrenaline [30,36]. The LC provides noradrenergic input to most regions of the brain including forebrain, cerebellum, brainstem, and spinal cord. AD patients have up to 60% fewer LC neurons compared to normal age matched controls [31], and LC neuronal loss has been correlated with plaque number and the duration and severity of dementia [2]. Since aging is the major risk factor for AD, it is of significance that both the number of LC neurons and brain NA content are reduced with age [33].

NA plays an important role in regulating cognitive and behavioral functions [38], and can influence some symptoms which accompany dementia, such as depression, aggression, agitation, and psychosis [19]. The consequences of NA deficiency have been examined in animal models of neurodegenerative diseases including AD and PD. NA can also protect neurons against various types of injury including that due to inflammatory, metabolic, and oxidative insults [33], and it has been proposed that NA serves as an endogenous suppres-

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sor of brain inflammatory responses [9]. The possible effects of NA loss on amyloid processing or clearance have not been well characterized, although the presence of a CREB binding site in the BACE1 promoter [43] and effects of NA and cAMP on macrophage phagocytosis have been reported [23,35], suggesting that activation of βARs by NA could modify these systems. Findings that amyloid deposition is sensitive to inflammatory mediators [17,28,47] suggest that NA could regulate plaque burden indirectly via its ability to suppress inflammation.

Transgenic APP (TgAPP) mice are used extensively as a model of AD [10]. In general, these mice exhibit some features of AD including plaque formation, cognitive dysfunction [5], and changes in synaptic transmission [21]. The aim of the current study was to assess the importance of NA deficiency on amyloid deposition in an existing TgAPP mouse model of AD. To accomplish this we used the neurotoxin DSP4 which selectively lesions LC noradrenergic neurons [24]. We previously showed that DSP4 treatment of adult rats exacerbated inflammatory responses to intracortical injection of Aβ1-42 [14], including an increase in neuronal expression of the inducible form of nitric oxide synthase (NOS2), a localization that is observed in AD brain [18,26,46]. In a recent study, Heneka et al. reported that in 10-month-old APP23 mice (which harbor the double Swedish mutation), an acute, high dose (50 mg/kg) treatment with DSP4 increased amyloid burden measured 6 months later [16]; however the mechanism(s) responsible for that increase were not established. We now show in TgAPP mice harboring the V717F Indiana mutation that chronic treatment with a lower dose of DSP4 (5 mg/kg) also increases amyloid plaque burden and levels of APP C-terminal cleavage fragments, and is paralleled by a reduction in cortical neprilysin (NEP) expression and activity. Additionally, using primary microglial cells we show that NA increases microglial phagocytosis of Aβ. These results suggest that LC degeneration contributes to increased amyloid deposition due to loss of noradrenergic regulation of amyloid clearance.

2. Materials and methods

2.1. Animals

Transgenic mice (H6) overexpressing the Indiana V717F mutation of human APP under control of the PDGFR promoter [34] were obtained from Dr. Lennart Mucke (J. David Gladstone Institute, San Francisco, CA). Male hemizygous H6 mice were bred to wild type C57BL6 (Charles River Breeding) females to obtain hemizygous progeny. Genotype was verified by PCR analysis of tail DNA using the forward primer 5′-GGT GAG TTT GTA AGT GAT GCC-3′ and reverse primer 5′-TCT TCT TCT TCC ACC TCA GC-3′. Animals were housed in groups of four under standard conditions with full access to food and water.

2.2. Lesion of the Locus coeruleus

Hemizygous male H6 mice (aged 3–4 months) were injected with vehicle or with DSP4 (5 mg/kg; i.p., every 2 weeks) and all mice were sacrificed at age 9 months. This protocol (a low, chronic dose) results in 70% loss of LC noradrenergic neurons. Brains were dissected, the left hemispheres were fast frozen in isopentane at −30 °C and stored at −80 °C for subsequent protein and RNA studies. The right hemispheres were processed for immunohistochemistry.

2.3. Histochemistry

Hemibrains were fixed overnight in 4% paraformaldehyde in 0.1 M phosphate buffer, then overnight in 10% sucrose in 0.1 M phosphate buffer, and then frozen in isopentane at −30 °C. Serial sections (35 μM thick) were prepared on a cryostat, and stored in cryoprotective solution (0.1 M phosphate buffer:ethyleneglycol:glycerol 30:40:30) at −20 °C. For Aβ1-42 staining, sections were treated with formic acid (95%, 3 min), incubated overnight at 4 °C with primary antibody directed against Aβ1-42 (1:4000 dilution, Calbiochem FCA3542 rabbit polyconal antibody raised against a synthetic peptide corresponding to amino acids 706–713 of human APP), washed, then incubated with anti-rabbit biotinylated secondary antibody (Vector BA-1000, diluted 1:400) and visualized using an ABC kit (Vector). For quantification of Aβ plaques, for each animal ($n = 6$ vehicle-treated and $n = 6$ DSP4-treated mice) eight stained, serial sections separated by 210 μM and spanning approximately 1.5 mm between Bregma points −1.50 mm to −3.00 mm were mounted onto glass slides and imaged with a Zeiss Axiophot 2 microscope, equipped with an Axiocam B&W MRm camera. Sections were viewed at 100× using a 10× objective, and the average plaque number and % area stained (relative to the total section area of 21 mm^2) per section determined. The threshold of brightness intensity (range black = 0 to white = 255) used for selection of plaques was established by determining average pixel intensity from three areas devoid of staining, and then adjusting that value to maximize inclusion of all visibly-stained plaques while minimizing inclusion of background areas. This value was used for all sections (all stained at the same time). For all sections, visual inspection following automated counting was done to ensure exclusion of meninges, ventricular space, or other artifacts.

For Mac-1 staining, sections were incubated with rat anti-mouse Mac-1 (1:1000 dilution, Serotec MCA711) overnight at 4 °C, washed, then incubated with biotinylated goat anti-rat antibody (Serotec STAR80B. diluted 1:40) and staining visualized with ABC kit. For GFAP staining rat monoclonal anti-GFAP (2.2B10) [27] was used at dilution 1:500, followed by anti-rat FITC labeled secondary antibody (1:400 dilution), (Jackson labs). Serial sections 210 μm apart were stained, mounted, and slides examined with a Zeiss Axiophot 2 microscope, equipped with Axiocam MRm camera. Staining was quantified using software provided by the manufacturer