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BRAIN RESEARCH

Research Report

Aβ peptides can enter the brain through a defective blood–brain barrier and bind selectively to neurons

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ABSTRACT

We have investigated the possibility that soluble, blood-borne amyloid beta (Aβ) peptides can cross a defective blood–brain barrier (BBB) and interact with neurons in the brain. Immunohistochemical analyses revealed extravasated plasma components, including Aβ42 in 19 of 21 AD brains, but in only 3 of 13 age-matched control brains, suggesting that a defective BBB is common in AD. To more directly test whether blood-borne Aβ peptides can cross a defective BBB, we tracked the fate of fluorescein isothiocyanate (FITC)-labeled Aβ42 and Aβ40 introduced via tail vein injection into mice with a BBB rendered permeable by treatment with pertussis toxin. Both Aβ40 and Aβ42 readily crossed the permeabilized BBB and bound selectively to certain neuronal subtypes, but not glial cells. By 48 h post-injection, Aβ42-positive neurons were widespread in the brain. In the cerebral cortex, small fluorescent, Aβ42-positive granules were found in the perinuclear cytoplasm of pyramidal neurons, suggesting that these cells can internalize exogenous Aβ42. An intact BBB (saline-injected controls) blocked entry of blood-borne Aβ peptides into the brain. The neuronal subtype selectivity of Aβ42 and Aβ40 was most evident in mouse brains subjected to direct intracranial stereotaxic injection into the hippocampal region, thereby bypassing the BBB. Aβ40 was found to preferentially bind to a distinct subset of neurons positioned at the inner face of the dentate gyrus, whereas Aβ42 bound selectively to the population of large neurons in the hilus region of the dentate gyrus. Our results suggest that the blood may serve as a major, chronic source of soluble, exogenous Aβ peptides that can bind selectively to certain subtypes of neurons and accumulate within these cells.

1. Introduction

Alzheimer's disease (AD) is a neurodegenerative disease of the elderly that results in progressive and dramatic memory loss, cognitive decline, changes in behavior, reactive gliosis, inflammation and extensive destruction of neurons and their synapses in the cerebral cortex, entorhinal area, hippocampus, ventral striatum and basal forebrain (Braak and

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Braak, 1991; Dickson, 1997; Felician and Sandson, 1999; Gomez-Isla et al., 1997; Hardy and Allsop, 1991; Kasa et al., 1997; Mirra et al., 1993; Scott et al., 1991; Selkoe, 2002; Wisniewski et al., 1997; Wisniewski and Wen, 1985). The alarming rise in the incidence of this disease is coincident with recent increases in the average lifespan, with a nearly 40% incidence in individuals over the age of 85 years (Hebert et al., 2003). The deposition of amyloid beta (Aβ) peptides, predominantly the 42 amino acid form (Aβ42), within neurons and amyloid plaques in brain tissue appears early in the course of the disease and is a well-known hallmark of AD (D'Andrea et al., 2001; Masters et al., 1985; Nagele et al., 2002; Selkoe, 2002). Aβ42 is generated by the sequential cleavage of the amyloid precursor protein (APP) by beta- and gamma-secretases, respectively, and is capable of self-assembling into nondegradable fibrils that persist within the brain tissue (Koo and Squazzo, 1994; Masters et al., 1985; Wilson et al., 1999).

An understanding of the factors and conditions that lead to the deposition of Aβ42 in the brain is crucial to the development of effective treatment methods. Some proposed therapeutic strategies for AD are currently aimed at reducing or eliminating the deposition of Aβ42 in the brain. The achievement of this will most likely require a reduction in the generation of Aβ42 from APP and/or some means of lowering existing Aβ42 levels from sources that directly contribute to the deposition of this peptide in the brain (De Felice and Ferreira, 2002). Surprisingly, the source(s) of the Aβ42 that accumulates within neurons and plaques in AD brains has not yet been clearly identified. One possibility is that this peptide is generated endogenously by the same neurons that later accumulate large quantities of Aβ42 within their perikarya (Hardy and Allsop, 1991). If so, then these neurons should express APP as well as the beta- and gamma-secretase enzymes that cleave it into Aβ peptide fragments. Indeed, several studies have indicated that all of these proteins are expressed in neurons, suggesting that these cells may produce Aβ peptides throughout life and carefully regulate the balance between their production and clearance (Selkoe, 1996). Why these Aβ peptides accumulate to a pathology-inducing level within neurons and amyloid plaques in the elderly is a question of central importance. A partial list of potential, aging-associated causative factors in the development of sporadic AD includes a shift in the balance between Aβ peptide production and its clearance from neurons that favors intracellular accumulation, increased secretion of Aβ peptides by neurons into the surrounding extracellular space, increased levels of oxidative damage to these cells and global brain hypoperfusion and the associated compensatory metabolic shifts in affected neurons (Cohen et al., 1988; Higgins et al., 1990; Kalaria, 2000; Nalivaevaa et al., 2004; Teller et al., 1996; Wen et al., 2004).

Alternatively, the Aβ42 that deposits within neurons and plaques could also originate from outside of the neurons (exogenous Aβ42) during AD pathogenesis. In normal healthy brains, the soluble Aβ peptide levels within the interstitial space of the brain tissue are usually extremely low (Andreasen and Blennow, 2002; Seubert et al., 1992). This suggests that, in the normal state, any Aβ peptides generated internally by neurons either remain within these cells and are eventually degraded or, if released, are rapidly cleared from the extracellular matrix and possibly returned to the venous blood with the CSF through the arachnoid villi. On the other hand, levels of soluble Aβ peptides in the blood are known to be much higher than in the interstitial space and CSF in the brains of healthy individuals (Seubert et al., 1992), raising the possibility that the blood could be a potential source of exogenous Aβ peptides that eventually deposit in the AD brain (Zlokovic et al., 1993). However, except for trace amounts of Aβ that are actively transported across endothelial cells, it is well-known that access of blood-borne Aβ peptides to brain tissue in normal healthy individuals is effectively blocked by the integrity of the blood–brain barrier (BBB) (Kandimalla et al., 2005; Poduslo et al., 1999). The BBB is a complex structure composed of cerebral endothelial cells resting on a basal lamina that is further supported by the foot processes of local astrocytes (Gloor et al., 2001; Risau et al., 1998). It closely regulates the passage of blood components into the brain tissue and is highly impermeable to nearly all proteins and other macromolecules while, at the same time, allowing the selective entry of essential molecules (Mayhan, 2001). Much evidence has revealed that aging is associated with degenerative changes to blood vessels that may compromise the integrity of the BBB. For example, a number of relatively common neurodegenerative diseases in the elderly, including stroke, vascular dementia and AD originate, at least in part, from cerebrovascular pathologies that develop within the microvasculature of the brain (Breteler, 2000; Buee et al., 1997; de la Torre, 1997; Esiri et al., 1999; Kalaria et al., 1996). Although stroke is most often attributed to defects within larger vessels, smaller vessels of the brain microvasculature are also involved in these pathologies and are the leading cause of lacunar stroke, vascular dementia and intracerebral hemorrhage (Esiri et al., 1997; Greenberg, 2006). Additional testimony for a link between the neurovasculature and neurodegenerative disease is the well-known fact that Alzheimer pathology, including amyloid plaques and neurofibrillary tangles, develops subsequently within the vicinity of stroke lesions (Jellinger, 2002; Kalaria, 1996, 2002; Natte et al., 1998). In view of the expected high incidence of cerebrovascular pathology and BBB breakdown in AD patients, it is critical to determine if the defective cerebral microvasculature represents an important source of the Aβ peptides that contribute to amyloid deposition in AD brains.

In the present study, we have investigated the possibility that disruption of the integrity of the BBB can lead to a chronic influx of plasma components, including soluble Aβ peptides, into the brain. We have also examined the fate of key soluble exogenous Aβ peptides, Aβ42 and Aβ40, within the brain parenchyma. To accomplish this, we tracked the fate of fluorescein isothiocyanate (FITC)-labeled Aβ peptides introduced via tail vein injection into mice in which the BBB had been compromised by prior exposure to pertussis toxin. Results show that blood-borne FITC-labeled Aβ peptides can indeed traverse a defective BBB, enter into the brain parenchyma, bind selectively to the surfaces of certain neurons and, in the case of Aβ42, accumulate selectively within the same subtype of neurons that are known to exhibit prominent Aβ42-immunopositive deposits in AD brains. In view of the high incidence of BBB compromise in nearly all patients with AD, as supported here by direct detection of extravasated plasma components in AD brains, we propose that the blood repre-