Behavioural Brain Research 274 (2014) 30–42

Contents lists available at ScienceDirect

Behavioural Brain Research

journal homepage: www.elsevier.com/locate/bbr

Research report

Interactions between Aβ oligomers and presynaptic cholinergic signaling: Age-dependent effects on attentional capacities

Vinay Parikh*, Carcha S. Bernard, Sean X. Naughton, Brittney Yegla

Department of Psychology and Neuroscience Program, Temple University, Philadelphia, PA 19122, United States

HIGHLIGHTS

- Soluble Aβ produced subtle decrements in SAT performance irrespective of age.
- Performance under distracting conditions robustly declined in Aβ-infused aged rats.
- Soluble Aβ disrupted the capacity of cholinergic synapses to clear choline.
- Depolarization-evoked ACh release declined in aged rats infused with Aβ oligomers.
- Aging but not soluble Aβ reduced the expression of presynaptic cholinergic proteins.

ARTICLE INFO

Article history:
Received 19 May 2014
Received in revised form 14 July 2014
Accepted 25 July 2014
Available online 4 August 2014

Keywords:
Attention
Cholinergic
Presynaptic
Soluble amyloid-beta
Aging
Alzheimer's disease

ABSTRACT

Substantial evidence suggests that cerebral deposition of the neurotoxic fibrillar form of amyloid precursor protein, β-amyloid (Aβ), plays a critical role in the pathogenesis of Alzheimer's disease (AD). Yet, many aspects of AD pathology including the cognitive symptoms and selective vulnerability of cortically projecting basal forebrain (BF) cholinergic neurons are not well explained by this hypothesis. Specifically, it is not clear why cognitive decline appears early when the loss of BF cholinergic neurons and plaque deposition are manifested late in AD. Soluble oligomeric forms of Aβ are proposed to appear early in the pathology and to be better predictors of synaptic loss and cognitive deficits. The present study was designed to examine the impact of Aβ oligomers on attentional functions and presynaptic cholinergic transmission in young and aged rats. Chronic intracranial infusions of Aβ oligomers produced subtle decrements in the ability of rats to sustain attentional performance with time on task, irrespective of the age of the animals. However, Aβ oligomers produced robust detrimental effects on performance under conditions of enhanced attentional load in aged animals. *In vivo* electrochemical recordings show reduced depolarization-evoked cholinergic signals in Aβ-infused aged rats. Moreover, soluble Aβ disrupted the capacity of cholinergic synapses to clear exogenous choline from the extracellular space in both young and aged rats, reflecting impairments in the choline transport process that is critical for acetylcholine (ACh) synthesis and release. Although aging *per se* reduced the cross-sectional area of BF cholinergic neurons and presynaptic cholinergic proteins in the cortex, attentional performance and ACh release remained unaffected in aged rats infused with the control peptide. Taken together, these data suggest that soluble Aβ may marginally influence attentional functions at young ages primarily by interfering with the choline uptake processes. However, age-related weakening of the cholinergic system may synergistically interact with these disruptive presynaptic mechanisms to make this neurotransmitter system vulnerable to the toxic effects of oligomeric Aβ in robustly impeding attentional capacities.

1. Introduction

Alzheimer's disease (AD) is a progressive neurodegenerative disorder characterized by irreversible cognitive deterioration. Although there is a widespread decline in various neurotransmitter-containing cell bodies and axonal terminals in end-stage AD, the most consistent losses are seen in the cortical projections of basal forebrain (BF) cholinergic neurons [1]. The activation of BF cholinergic neurons and consequent release of acetylcholine (ACh) in the cortex mediate attentional processes and capacities [2–5]. As attentional impairments constitute the core components of global cognitive decline in AD subjects [6],

* Corresponding author. Tel.: +1 215 204 1572.
E-mail address: vinay.parikh@temple.edu (V. Parikh).

http://dx.doi.org/10.1016/j.bbr.2014.07.046
0166-4328/

abnormally regulated cortical cholinergic transmission may underlie attentional dysfunction associated with this disorder.

The progressive accumulation of the extracellular neurotoxic fibrillar form of amyloid-beta (Aβ) protein is known to play a central role in the genesis of AD. Yet, many aspects of AD pathology including the cognitive symptoms and selective vulnerability of BF cholinergic neurons are not well explained by this hypothesis. Studies involving transgenic mice harboring mutations in AD-associated genes including amyloid precursor protein (APP) and presenilin-1, have provided insights into possible reciprocal interactions between cholinergic markers and Aβ [7–10]. However, the cause and effect relationship between Aβ accumulation and cholinergic dysfunction is not established. Specifically, it is not clear why cognitive decline appears early when the loss of cortically-projecting BF cholinergic neurons and plaque deposition are manifested late in AD pathology. Therefore, delineation of mechanisms that determine how neuropathological markers of AD disrupt cholinergic transmission is likely to provide gainful insights in understanding the neurobiological basis of cognitive decline in AD.

Substantial evidence suggests that synaptic loss predicts the degree of severity of cognitive deterioration in AD [11–13]. Moreover, the soluble oligomeric forms of Aβ1–42 disrupted synaptic plasticity [14,15], and cognitive dysfunction in early AD correlated well with Aβ oligomers but not with plaques [16,17]. These findings supported the notion that intraneuronal or extracellular accumulation of soluble Aβ oligomers may produce synaptic and cognitive dysfunction in early stages of AD. Aβ oligomers disrupt synaptic plasticity and may produce cognitive dysfunction without producing neuronal cell death in early AD [18]. Moreover, attentional deficits appear early during the course of AD pathology [19,20] implicating dysregulation of cortical cholinergic transmission in early AD.

In BF neuronal cultures, the toxic effect of soluble Aβ appeared more rapidly in the cholinergic axon terminal than in cell bodies [21], exemplifying that disruption in synaptic cholinergic transmission and associated cognitive deficits may precede cholinergic cell loss in AD. A recent postmortem study showed a correlation between the levels of Aβ oligomers and reductions in choline acetyltransferase (ChAT) activity in the brains of AD subjects [22], substantiating detrimental effects of Aβ oligomers on the cholinergic system. How Aβ oligomers modulate presynaptic cholinergic activity and influence attentional functions in the absence of cholinergic cell loss remains unknown.

The present study was designed to examine the impact of Aβ oligomers on presynaptic cholinergic transmission in the cortex and attentional functions that depend upon the integrity of these BF cholinergic projections. As aging is a well-recognized risk factor for AD, and the aging cholinergic system is more vulnerable to degeneration [1,23], we also explored how aging might influence the interactions between soluble Aβ and cortical cholinergic function.

2. Materials and methods

2.1. Animals

Male Wistar rats aged 2–3 months (young) or 10–12 months (middle aged; retired breeders) were acquired from Charles River Laboratories (Malvern, PA, USA). The animals were housed in a temperature- and humidity-controlled facility with a 12-hour light/dark cycle (lights "on": 7:00 AM) and had free access to food and water. Retired breeders were maintained until 22 months of age following which training in an operant attentional task was initiated (see behavioral procedures). Operant training took place 6 days/week. Rats were handled extensively prior to behavioral training and were partially water-deprived by restricting access to a 10-min period in the home cage following each behavioral session. On non-training days, water access was increased to a 30-min period. Rats were individually housed and food was available *ad-libitum* throughout the behavioral training and testing. All experiments were conducted in accordance with the National Institute of Health guidelines and were approved by the Institutional Animal Care and Use Committee at Temple University.

2.2. Behavioral training and testing

2.2.1. Apparatus

Rats were trained in operant chambers encased in sound-attenuating boxes, each containing a fan to provide ventilation and low-level background noise (Med Associates Inc., St. Albans, VT). Each chamber was equipped with two retractable levers, a central panel consisting of three panel lights (2.8 W each), a liquid receptacle attached to a water dispenser, and a house light (2.8 W) located on the rear wall. All events including the signal delivery, lever presentations, and water dispense were transmitted using programs written in Medstate notation via SmrtCtrl™ interface running through MED-PC software on a Dell Optiplex 960 computer.

2.2.2. Operant sustained attention task (SAT)

Young and aged rats were trained on an operant sustained attention task (SAT) as described previously [24–27]. Briefly, rats were initially autoshaped on a FR-1 schedule of reinforcement to attain the lever press response and subsequent reward (0.02 mL water). To deter a side bias, lever presses on the dominant lever (i.e. the lever with ≥5 presses) ceased to be reinforced until the discrepancy was reduced. Once the rats made 120 lever presses within a session, they were moved to the next phase of training, which required discrimination between signal (illumination of the central panel light for 1 s) and non-signal (no illumination) events. Each event was followed by the presentation of two levers 2 s later; levers remained extended for 4 s or until a lever press occurred. If no response was made during the 4 s lever presentation, an omission was recorded and the intertrial interval (ITI; 12 ± 3 s) was reinstated. On signal trials, a left lever press was scored as a "hit" and rewarded; an incorrect response (depression of the right lever) was deemed a "miss". During non-signal trials, a right lever press was scored as a "correct rejection" and reinforced, while a left lever press was considered a "false alarm." The animals were not rewarded for incorrect responses. The presentation of signal and non-signal trials were pseudo-randomized. Half of the animals in a group were trained with the reverse set of rules.

After attaining 70% correct responses to signal and non-signal trials for three consecutive days, animals progressed to the *final* stage of training, during which the duration of signals was decreased to 25, 50, or 500 ms. Moreover, the ITI was reduced to 9 ± 3 s and the house light remained illuminated throughout the session. These events are known to constrain rats' behavior for continuous monitoring of the central panel [27]. Each behavioral session consisted of a pseudo-randomized sequence of 81 signal (27 per signal duration) and 81 non-signal trials (total 162 trials). Sessions were divided into three blocks of 54 trials (27 signal trials and 27 non-signal trials) with each signal type presented 9 times per block. Stable criterion performance was characterized by signal duration-dependent hit rates, ≥70% hits to 500 ms signals, ≥70% correct rejections, and a relatively low number of omissions (<10% of all trials; equal distribution among trial types) for at least 3 consecutive sessions. At this stage, animals were exposed to a distractor session (dSAT) that involved the presentation of distractors (flashing house light @ 0.5 Hz) in the second block [28]. This procedure was adopted to minimize the novelty effects of distractors