Neurobiology of Aging 34 (2013) 2694.e13–2694.e18

Contents lists available at SciVerse ScienceDirect

Neurobiology of Aging

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

Cholinergic activity correlates with reserve proxies in Alzheimer's disease

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ARTICLE INFO

Article history:
Received 13 August 2012
Received in revised form 22 April 2013
Accepted 19 May 2013
Available online 29 June 2013

Keywords:
Alzheimer's disease (AD)
Positron emission tomography (PET)
Acetylcholinesterase (AChE) activity
Reserve

ABSTRACT

The clinical expression of Alzheimer's disease (AD) occurs as neuropathology exceeds the brain "reserve capacity." A possible association between the cholinergic system and reserve is suggested by preclinical observations that the cholinergic system allows cortical plasticity and by clinical observations of variable responses to cholinergic treatments depending on the patient's educational level. The aim of this study was to investigate the association of reserve proxies, that is, education and occupation, with acetylcholinesterase (AChE) activity, measured voxelwise by [^{11}C]-MP4A and positron emission tomography (PET), in 9 healthy controls (HC), 7 patients with early probable AD, and 9 subjects with mild cognitive impairment (MCI) at the time of PET imaging, who progressed to AD at follow-up (prodromal AD). The analysis of prodromal and early AD showed positive correlations between education and AChE activity in the hippocampus, bilaterally, and between occupation and AChE activity in the right posterior cingulate gyrus. The significant correlation between AChE activity in structures belonging to the memory network and reserve proxies suggests that the brain reserve in AD is associated with a preserved/stimulated cholinergic neurotransmission.

1. Introduction

The clinical expression of Alzheimer's disease (AD), that is, progressive cognitive dysfunction, is thought to occur as the amount of brain tissue damage, due to the accumulation of neuritic plaques, tangles and synaptic loss, exceeds a critical threshold of "reserve capacity," beyond which normal cognitive function can no longer be sustained (Stern, 2009). Premorbid intellectual activity and higher educational and occupational attainments are associated with a delay in the onset of AD clinical features, possibly allowing highly educated individuals to tolerate greater brain damage before exceeding the reserve threshold. Differences in reserve mechanisms may account, at least in part, for the heterogeneity in the occurrence and timing of AD (Stern, 2009). Neuroimaging studies have so far supported the existence of reserve phenomena in neurodegenerative conditions, and specifically in AD (Garibotto et al., 2008; Kemppainen et al., 2008). The molecular mechanisms sustaining this phenomenon and the implicated neurotransmitter systems, however, are still unknown.

The cholinergic system plays a pivotal role in cognitive symptoms in dementia and, possibly, normal aging. According to the cholinergic hypothesis of geriatric memory dysfunction proposed by Bartus et al. (1982), the impairment of cholinergic activity in the brains of healthy older adults and demented patients is related to memory loss and cognitive impairment (Bartus et al., 1982; Giacobini, 2003). Restoration of cholinergic function may reduce the severity of the cognitive impairment, as shown, for example, by the modest positive effects of cholinesterase inhibitors on cognition in AD patients (Raina et al., 2008).

Positron emission tomography (PET) allows the in vivo non-invasive quantification of acetylcholinesterase (AChE) activity with the tracer Carbon11 labeled *N*-methyl-4-piperidyl-acetate ([^{11}C]-MP4A). AChE is membrane-bound predominantly on presynaptic cholinergic neurons and, to a lesser degree, on postsynaptic cholinoceptive neurons in the cerebral cortex. AChE staining is thus typically reduced when the cholinergic system is

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0197-4580/$ – see front matter © 2013 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.neurobiolaging.2013.05.020

affected by a neurodegenerative process such as AD (Henke and Lang, 1983). For this reason, it has been previously suggested that cortical AChE activity can be considered also a biomarker for the integrity of the ascending cholinergic system (Herholz et al., 2004).

There are 2 main cholinergic nuclei in the brainstem: the basal forebrain, including the nucleus basalis of Meynert and the medial septal nucleus, and the pedunculopontine–lateral dorsal tegmental group (Mesulam et al., 1983). Thalamic AChE activity is thought to reflect the function of the ascending cholinergic systems mainly from the pedunculopontine group, whereas the medial septal nucleus and the nucleus basalis of Meynert are the main origins of cholinergic projections to the hippocampus (Perry et al., 1999).

Impairment of AChE activity has been reported in vivo using PET molecular imaging, in AD and MCI (Marcone et al., 2012). Specifically, in these patients, we observed significant reductions of AChE activity, when compared to controls, in several associative cortices (frontal, parietal, temporal, occipital), in the hippocampus and in the thalamus (Marcone et al., 2012).

The association between the reserve phenomenon and the cholinergic system capacity has not yet been investigated. An association between these 2 factors was, however, suggested by previous preclinical and clinical observations. Preclinical data showed that the cholinergic system is involved in cortical plasticity associated with complex cognitive activity (Ramanathan et al., 2009). For example, animals living in an enriched environment had an increase in neurotransmitters such as acetylcholine (Por et al., 1982). Importantly, hippocampal neural stem cells responded to physiological and pharmacological cholinergic stimulation by proliferating, also in aged animals (Itou et al., 2011).

There are a few clinical studies on the effectiveness of cholinergic treatment, which took into account the reserve proxies, such as education. In particular, 2 recent observations reported a reduced efficacy of cholinesterase inhibitors (ChEI) in highly educated individuals (Wattmo et al., 2011; Weng et al., 2011). Another study reported a better response to ChEI treatment in subjects with more atrophy of a cholinergic structure, the substantia innominata (Tanaka et al., 2003).

The aim of this study was to assess by [^{11}C]-MP4A and PET the correlation of AChE activity and the most commonly used reserve proxies, this is, education and occupational attainment. Our working hypothesis was that education and high levels of occupation might modulate the degeneration of the cholinergic system in AD, contributing to the reserve phenomenon.

2. Methods

2.1. Subjects

Nine healthy controls, 9 subjects with MCI, and 7 subjects with probable AD participated in the study. These subjects have already been described in a previous paper reporting cholinergic activity levels in these patient populations and in controls (Marcone et al., 2012). We included subjects with a diagnosis of multidomain amnestic MCI, according to the Mayo Clinic Criteria (Petersen, 2009), at the moment of the scan, who progressed to probable AD according to the criteria of McKhann et al. (McKhann et al., 1984) within 2 years of follow-up. We included only MCI subjects who had progressed to AD at the clinical follow-up, to exclude MCI of a possible non-degenerative or non-AD etiology. Seven patients had a diagnosis of probable AD at inclusion according to McKhann et al. criteria.

We applied a systematic screening for the list of self-reported medications for each participant, to ascertain that no current treatment could influence the central cholinergic activity, by following the method recently suggested by Whalley et al. (Whalley et al., 2012). All subjects included either were using no medications or drugs with no effect on the cholinergic system.

The study was approved by the institutional Ethical Committees of the San Raffaele Hospital and of the Turku PET Centre. Written informed consent was obtained from each subject and/or a family member prior to the study, in accordance with the Declaration of Helsinki.

All patients underwent a clinical, neuropsychological, and standard neuroradiological evaluation (computed tomography [CT] or magnetic resonance imaging [MRI]) before entering the study.

2.2. Reserve proxies

Education was measured considering the number of completed years of formal education, including university or apprenticeship (only when associated with formal education). Occupational attainment was measured with a score ranging from 1 to 6, from less to more complex occupation, corresponding to the last employment of each subject: 1. no occupation; 2. unskilled laborer; 3. housewife; 4. skilled laborer, tradesman, lower level civil servant, employee, self-employed small business, office or sales personnel; 5. mid-level civil servant or management, head of a small business, academician, or specialist in a subordinate position; 6. senior civil servant or management, senior academic position, self-employed with high degree of responsibility.

Education and occupation of the patients are reported in Table 1.

We tested the correlation between reserve proxies and Mini-Mental State Examination (MMSE) scores to assess the association between reserve and level of cognitive impairment at the time of investigation.

2.3. PET acquisition protocol

To assess local AChE activity, we administered 500 to 700 MBq [^{11}C]MP4A. PET dynamic acquisition was recorded with a PET/CT

Table 1
Demographic and clinical characteristics of the sample

Subject	Diagnosis at time of PET scan	Age	Gender	Education (y)	Occupational score	MMSE
Control 1	HC	69	F	9	4	28
Control 2	HC	55	F	14	4	28
Control 3	HC	68	M	7	4	26
Control 4	HC	62	F	9	6	27
Control 5	HC	60	F	13	5	29
Control 6	HC	64	M	13	4	30
Control 7	HC	67	M	5	1	30
Control 8	HC	58	F	8	4	30
Control 9	HC	67	M	17	4	30
Patient 1	MCI	76	F	7	3	25
Patient 2	MCI	83	F	8	3	26
Patient 3	MCI	64	F	8	3	24
Patient 4	MCI	82	F	5	3	23
Patient 5	MCI	74	M	10	4	22
Patient 6	MCI	66	M	12	2	26
Patient 7	MCI	79	F	8	3	24
Patient 8	MCI	67	M	13	5	27
Patient 9	MCI	69	F	5	3	28
Patient 10	AD	84	F	5	3	22
Patient 11	AD	79	F	8	3	19
Patient 12	AD	72	M	5	2	22
Patient 13	AD	83	M	8	2	24
Patient 14	AD	70	F	5	3	22
Patient 15	AD	61	M	5	2	23
Patient 16	AD	76	F	17	5	21

Key: AD, Alzheimer's disease; F, female; HC, healthy control; M, male; MCI, mild cognitive impairment; MMSE, Mini-Mental State Examination; PET, positron emission tomography.