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Neurobiology of Aging 34 (2013) 286–297

NEUROBIOLOGY OF AGING

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Age-related appearance of dendritic inclusions in catecholaminergic brainstem neurons

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Received 20 October 2011; received in revised form 27 January 2012; accepted 27 February 2012

Abstract

We identified p62-immunoreactive inclusions in dendrites of catecholaminergic brainstem projection neurons using antibodies against p62, ubiquitin, α-synuclein, hyperphosphorylated tau, and tyrosine hydroxylase in 100-μm sections through the brainstem dorsal vagal area, locus coeruleus, and substantia nigra of 149 autopsy cases staged for intraneuronal Alzheimer's and Parkinson's disease-associated lesions. The inclusions resembled Marinesco bodies within cell nuclei of catecholaminergic neurons as well as the dot-like structures previously described by Dickson in specific neuropil areas in humans. The p62-positive inclusions were confined to dendrites of catecholaminergic neurons, lacked neuromelanin granules, and were tau- and α-synuclein-negative. Their immunoreactivity for ubiquitin varied and their prevalence significantly increased with advancing age. The presence or absence of Alzheimer's and/or Parkinson's disease-associated pathology did not influence their existence. There was a strong association between the presence of p62-positive inclusions and Marinesco bodies ($p < 0.0001$). Our results reveal a hitherto unknown alteration within specific neuronal types of the human brainstem that may be independent of the sequestosome-ubiquitin-proteasomal pathway and unrelated to proteinaceous aggregate-formation of neurodegenerative diseases.

Keywords: Aging; Catecholamines; Locus coeruleus; Marinesco bodies; p62; Sequestosome-ubiquitin-proteasomal pathway; Substantia nigra; α-synuclein; Tau

1. Introduction

Relatively little is known about structural changes within human nerve cells that consistently accompany and characterize normal brain aging (Anderton, 1997, 2002; Dickson et al., 1990, 1991; Hof et al., 1996; Keller et al., 2004; Mrak et al., 1997). Historically, these changes include the appearance of lipofuscin and neuromelanin granules, which, in general, accumulate with advancing age and display a variety of distribution patterns in specific neuronal types (Benavides et al., 2002; Braak, 1984; Brunk and Terman, 2002; Double et al., 2008; Gray and Woulfe, 2005; Gray et al., 2003; Halliday et al., 2006; Porta, 2002; Terman and Brunk, 2004; Terman et al., 2007, 2010). Additional but less widespread morphological changes have in common immunoreactivity for ubiquitin. Ubiquitin-positive Marinesco bodies occur within the cell nuclei of catecholaminergic nerve cells (Alladi et al., 2010; Beach et al., 2004; Dickson et al., 1990; Kanaan et al., 2007; Schwab et al., 2012; Yuen and Baxter, 1963), whereas dot-like deposits are thought to develop in dystrophic neuronal processes in neuropil areas between the cellular islands of layer II in both the entorhinal and transentorhinal regions (Dickson et al., 1990, 1991; Pappolla et al., 1989).

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10.1016/j.neurobiolaging.2012.02.031

Morphological changes of this type are considered as being distinct from the structural intraneuronal abnormalities that characterize Alzheimer's (AD) and Parkinson's (PD) diseases. Incidental and early (prodromal) lesions related to both disorders do not suffice to produce clinically observable symptoms and, in fact, elderly nonsymptomatic individuals frequently display an admixture of disease- and aging-related structural brain changes (Anderton, 2002; Dickson et al., 1991; Price et al., 2009) (Table 1).

The widely expressed and conserved stress-responsive protein p62 consists of 440 amino acids and is thought to contribute to the sequestration of misfolded, aggregation-prone proteins and their autophagic or ubiquitin/proteasomal clearance (Gal et al., 2007; Moscat et al., 2007; Olanow and McNaught, 2006; Pan et al., 2008; Seibenhener et al., 2004, 2007; Wooten et al., 2006). In addition, p62 shows a still unexplained propensity to become entrapped in both age- or disease-related protein inclusions (Arai et al., 2003; Kuusisto et al., 2001, 2002, 2003, 2008; Mizuno et al., 2006; Nagaoka et al., 2004; Scott and Lowe, 2007).

The present study calls attention to a hitherto unknown age-related structural change in dendrites of human catecholaminergic projection neurons that is distinctly p62- and variably ubiquitin-immunopositive.

2. Methods

2.1. Study population

A total of 149 autopsy cases were studied (52 females, 97 males; age range 6–96 years; mean age ± SD: 57 ± 22.4 years). This retrospective study was performed in compliance with university ethics committee guidelines and German federal law governing human tissue usage. Sample demographics and relevant clinical data obtained from medical records of participating university clinics (Hamburg, Bonn, Frankfurt am Main, Mainz) and municipal hospitals (Enschede, The Netherlands; Offenbach am Main, Germany; The Parkinson's Institute, Sunnyvale, CA, USA) as well as from the autopsy reports appear in Table 1. Of the cases in the study cohort, including controls, 119 belonged to consecutive autopsy series performed between the years 2003 and 2007. Exclusionary criteria for the present study were the presence of (1) schizophrenia, (2) dementia with Lewy bodies (DLB) (McKeith et al., 2005), and (3) severe cerebrovascular disease (Román et al., 1993). Upon admission to hospital, nondemented ("controls") and demented individuals were examined between 1 and 4 weeks prior to death using standardized protocols, including neurological status. The majority of these protocols included assessment of cognitive function and the ability to perform activities of daily living (ADLs) independently within the hospital setting. Clinical dementia rating (CDR) scores (Hughes et al., 1982) were examined retrospectively for 7 cases (70, 75, 90, 124, 128, 130, and 145) (Table 1). The data were also used to determine whether these patients met the clinical Diagnostic and Statistical Manual of Mental Disorders (DSM)-IV criteria for dementia (American Psychiatric Association, 1994), which all fulfilled.

AD was diagnosed when dementia was observed and when the degree of AD-related neuropathology indicated the existence of at least a moderate likelihood for AD according to the National Institute on Aging-Reagan criteria (The National Institute on Aging, 1997). PD diagnoses had been made during life using the United Parkinson's Disease Rating Scale (UPDRS) or Hoehn and Yahr scales by hospital neurologists for 10 cases and were confirmed neuropathologically (Braak et al., 2003a; Dickson et al., 2009; Gelb et al., 1999). At autopsy, the brains of 13 individuals whose brains displayed the presence of Lewy body pathology but failed to fulfill the clinicopathological criteria for PD or dementia with Lewy bodies were diagnosed as having incidental Lewy body disease (ILBD). Of these, 1 case (128) also received the diagnosis AD, and 2 cases (134, 146) received the neuropathological diagnoses of PD and AD (Table 1), whereby clinically the leading diagnosis had been Parkinson's disease dementia (PDD).

The majority of these were from individuals with no history of dementia or movement disorders (127 cases: 41 females, 86 males; age range 6–96 years; mean ± SD: 54.2 ± 22.4 years). Eleven cases had suffered from clinically documented sporadic PD (3 females, 8 males; age range 68–88 years; mean age ± SD: 77.5 ± 6.4 years) and 12 brains were from demented individuals with sporadic AD (9 females, 3 males; age range 62–89 years; mean age ± SD: 73.7 ± 8.4 years).

The postmortem interval (PMI), defined as the time between death and autopsy, ranged from 4 to 312 hours (median: 48 hours). Both hemispheres were examined macroscopically in approximately 1 cm thick coronal slices and macroscopic findings noted. Brains were fixed in a 4% aqueous solution of formaldehyde for 11–20 days prior to standardized neuropathological assessment and dissection.

2.2. Tissue dissection, embedding, sectioning, and storage

A set of 3 tissue blocks was dissected, embedded in polyethylene glycol (PEG 1000; Merck, Darmstadt, Germany) (Smithson et al., 1983), and sectioned at 100 μm perpendicular to the brainstem axis of Meynert as described previously (Braak et al., 2003a, 2006). Because our primary focus is neuroanatomy rather than molecular biology, we have found that PEG embedding and the unconventional section thickness, which allows for the superimposition of large numbers of biological structures (Braak et al., 2003b), including nerve cells with their entire dendritic tree (Braak and Del Tredici, 2011), provide sufficient epitope preservation for morphological studies (Klosen et al., 1993, Braak et al., 2006, 2007).

The first tissue block included the medulla oblongata at the latitude of the dorsal motor nucleus of the vagus nerve, the second block ran through the pontine tegmentum and