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Neurobiology of Aging 31 (2010) 625–635

NEUROBIOLOGY OF AGING

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Dogs with canine counterpart of Alzheimer's disease lose noradrenergic neurons

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Received 6 December 2007; received in revised form 25 March 2008; accepted 18 May 2008

Available online 24 June 2008

Abstract

Degeneration of noradrenergic neurons in the locus ceruleus is a well-described feature of Alzheimer's disease (AD). In spite of extensive utilization of the dog as a model for human degenerative diseases, there is no data on the response to aging of the noradrenergic system in dogs. We have used modern unbiased stereology to estimate the total number of A6–A7 noradrenergic neurons in normal, aged dogs and dogs with the canine counterpart of AD. In small-breed dogs with no cognitive impairments, the total mean number of tyrosine hydroxylase immunolabeled A6–A7 neurons was 17,228 ± 1655, with no differences between young and aged dogs. In contrast, aged dogs with cognitive impairments exhibited a significant reduction in the total number of A6–A7 neurons (13,487 ± 1374; $P = 0.001$). Additionally, we found a negative correlation between the number of A6–A7 neurons and the extent of β-amyloid deposits in the prefrontal cortex. These results suggest that the canine model could be useful in exploring the potential benefits of noradrenergic drugs for the treatment of AD.

Keywords: Amyloid-beta; Unbiased stereology; Aging; Locus ceruleus; A6–A7; Tyrosine hydroxylase; Gyrus proreus

1. Introduction

Degeneration of the locus ceruleus (LC) is a well-described feature of Alzheimer's disease (AD) (Dringenberg, 2000; Grudzien et al., 2007). Numerous studies reported significant loss of LC noradrenergic neurons and diminished noradrenalin (NA) concentrations in AD brains (Bondareff et al., 1982; Busch et al., 1997; Chan-Palay and Asan, 1989a; German et al., 1992; Grudzien et al., 2007; Hoogendijk et al., 1995; Mann et al., 1982; Matthews et al., 2002; Szot et al., 2006; Tomlinson et al., 1981). These findings have been overshadowed by focus on the cholinergic system and hampered by the lack of an acceptable hypothesis to explain how LC neuronal death might influence AD pathogenesis. Interest has recently increased with the finding that the effect of AD on LC neurons was greater than that observed on basal forebrain cholinergic neurons in a study that included a large group of patients (Zarow et al., 2003). In concordance with that finding, Gonzalo-Ruiz et al. (2003) reported that the injection of amyloid-beta (Aβ) in the rat retrosplenial cortex caused a more pronounced neuronal loss in the LC than in the nucleus basalis. More recently, in a particularly enlightening study, Heneka et al. (2006) used transgenic mice that expressed the human amyloid precursor protein (APP) with the AD-linked Swedish mutation (APP23 mice) to demonstrate that the experimental induction of specific noradrenergic neurodegeneration increased glial activation, Aβ load, neuronal cell death in LC projecting areas, and cognitive deficits. Interestingly, these changes did not occur in the non-transgenic control mice, indicating that Aβ was required as a triggering cofactor. This and other works on animal models (Kalinin et al., 2007) provide evidence for a link between noradrenergic neurodegeneration, neuroinflammation, and classic AD pathology.

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0197-4580/$ – see front matter © 2008 Elsevier Inc. All rights reserved.
doi:10.1016/j.neurobiolaging.2008.05.014

The response of LC neurons to aging was addressed in a number of studies with controversial results. Modern unbiased stereological studies failed to find significant differences in the number of LC neurons between young and aged humans without dementia (Mouton et al., 1994; Ohm et al., 1997); in contrast, earlier studies reported up to 50% losses with normal aging (Chan-Palay and Asan, 1989b; Lohr and Jeste, 1988; Manaye et al., 1995; Vijayashankar and Brody, 1979). Interestingly, studies with some transgenic mice models of AD have reported a significant loss of LC noradrenergic neurons at approximately the same age they started to develop Aβ pathology (Guerin et al., 2007; O'Neil et al., 2007). However, the genetic background of the different transgenic mice should be considered a potential confounding factor; for example mice that expressed the transgenes, PDGF promoter expressing amyloid precursor protein (PDAPP) or APP23, did not exhibit this age-related noradrenergic degeneration (German et al., 2005; Heneka et al., 2006). Thus, although the usefulness of transgenic mice is undisputed, we hypothesized that additional relevant information on the mechanisms that link noradrenergic degeneration and AD could be obtained from other species wild-type animal models.

Along these lines, the dog has been pointed out as an especially appropriate model for the study of human brain aging and neurodegenerative diseases. Increasing interest in this model is based on the fact that the dog may naturally develop an age-related cognitive dysfunction that reproduces several aspects of AD (Cummings et al., 1996b; Head et al., 2002; Head, 2007; Opii et al., 2008; Siwak-Tapp et al., 2008). Thus, numerous studies with dog cohorts that were submitted to several behavioral paradigms have revealed subsets of aged dogs that had learning and memory impairments (Adams et al., 2000; Head et al., 1995; Siwak et al., 2001, 2005; Tapp et al., 2003). This cognitive damage correlated with the extent of βA deposits in the cerebral cortex (Cummings et al., 1996a; Head et al., 1998, 2000). Interestingly, a similar correlation was reported in domestic pet dogs based on cognitive status evaluated from questionnaires that were answered by their caregivers (Colle et al., 2000; Pugliese et al., 2006a; Rofina et al., 2006). Histopathological studies on this canine counterpart of AD showed that the earliest and more consistently affected areas were the prefrontal cortex, including the gyrus proreus and the hippocampus (Head et al., 2000; Hou et al., 1997; Yoshino et al., 1996). As in AD, the Aβ deposition in the dog appeared to progress from deep to superficial cortical layers and from diffuse patches to more dense, better delimited plaques (Satou et al., 1997). However, dense core neuritic plaques and neurofibrillary tangles have not been consistently demonstrated in the dog (Papaioannou et al., 2001; Pugliese et al., 2006b; Wisniewski et al., 1996). The canine counterpart of AD is also particularly suitable from a molecular point of view, because the dog's amyloid precursor protein and most of the enzymatic machinery for its processing bear extensive homology with their human analogs (Sarasa et al., unpublished results; see GenBank accessions AY926579, AY926580, AY926581, AY926582, AY926587, AY926590, AY926591, AY926592, AY926593, AY926594, AY926595, and AY926596).

However, in spite of the dog's relevance as a wild-type model for the study of AD, there is a dearth of data regarding the response to aging in the noradrenergic and other neurotransmitter systems in this species. In particular, to the best of our knowledge, there is not a single work that investigated the possible alterations in the noradrenergic system in dogs with the canine counterpart of AD. In the present work, we used modern unbiased stereological methods to investigate variations in the number of A6–A7 noradrenergic neurons in relation to aging and cognitive impairment in the dog.

2. Materials and methods

2.1. Animal characteristics

The brains of 8 young (<7 years) and 11 aged dogs (>10 years) were used for this study. Young dogs were divided into two groups of light-weight (young-L, small breeds, body weight < 16 kg, $n = 4$) and heavy-weight animals (young-H, large breeds, body weight > 19 kg, $n = 4$). This division was made in order to check whether the number of neurons in a given nucleus varied between heavy- and light-weight dogs. All the aged animals were light-weight animals (body weight < 16 kg). Five aged animals were presented at the clinical facilities of the Veterinary Faculty of the University of Santiago with severe cognitive impairments related to their advanced age (aged-CI). The other aged animals were free of any neurological symptoms (aged-NCI) based on a systematic clinical examination of the nervous system performed in our hospital. The diagnosis of the canine counterpart of Alzheimer's disease was confirmed from a questionnaire answered by the owners and the immunohistological demonstration of extensive Aβ deposits in the cerebral cortex (see below). The questionnaire was based on those published by Colle et al. (2000) and Rofina et al. (2006), but was considerably simplified. We assessed disorientation, interaction with owners, sleep/awake cycle, and loss of housetraining habits with the following 15 "yes" or "no" questions. Disorientation: (1) Does the dog appear disoriented on daily walks? (2) Does the dog appear disoriented at home? (3) Does the dog forget daily habits? (4) Does the dog wander aimlessly around the house? (5) Does the dog gaze at nothing for long periods of time? Interaction: (6) Has the dog lost interest in greeting? (7) Has the dog stopped looking for attention? (8) Has the dog lost interest in playing with owners or other dogs? (9) Has the dog stopped responding to incitation? Sleep/awake cycle: (10) Does the dog sleep during the day and remain restless at night? (11) Does the dog wander around during the night? (12) Does the dog bark during the night? Loss of housetraining habits: (13) Does the dog ask to go outside when necessary? (14) Does the dog urinate indoors? (15) Does the dog forget known commands? All the aged dogs with cognitive impairment included in this experiment scored more