The PDAPP Mouse Model of Alzheimer’s Disease: Locus Coeruleus Neuronal Shrinkage

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ABSTRACT

Alzheimer’s disease is characterized by neuronal degeneration in the cerebral cortex and hippocampus and subcortical neuronal degeneration in such nuclei as the locus coeruleus (LC). Transgenic mice overexpressing mutant human amyloid precursor protein V717F, PDAPP mice, develop several Alzheimer’s disease-like lesions. The present study sought to determine whether there is also loss of LC noradrenergic neurons or evidence of degenerative changes in these animals. PDAPP hemizygous and wild-type littermate control mice were examined at 23 months of age, at a time when there are numerous amyloid-β (Aβ) plaques in the neocortex and hippocampus. Tissue sections were stained immunohistochemically with an antibody against tyrosine hydroxylase (TH) to identify LC neurons. Computer imaging procedures were used to count the TH-immunoreactive somata in sections through the rostral-caudal extent of the nucleus. There was no loss of LC neurons in the hemizygous mice. In a second experiment, homozygous PDAPP and wild-type mice were examined, at 2 months and 24 months of age. Again there was no age-related loss of neurons in the homozygous animals. In the portion of the LC where neurons reside that project to the cortex and hippocampus, however, the neurons were decreased in size selectively in the 24-month-old transgenic animals. These data indicate that overt LC cell loss does not occur following abundant overexpression of Aβ peptide. However, the selective size reduction of the LC neuronal population projecting to cortical and hippocampal regions containing Aβ-related neuropathology implies that these cells may be subjected to a retrograde-mediated stress. J. Comp. Neurol. 492:469–476, 2005. © 2005 Wiley-Liss, Inc.

Indexing terms: amyloid-β protein; immunohistochemistry; tyrosine hydroxylase

Alzheimer’s disease (AD) is characterized by several hallmark pathologies, including amyloid-β (Aβ)-containing neuritic plaque deposition, neurofibrillary tangles, dystrophic neurites, and synaptic loss in the cerebral cortex (for review see Selkoe, 2001). The neuritic plaques are extracellular masses consisting of Aβ protein, derived from the amyloid precursor protein (APP). The plaques also contain dystrophic neurites with degenerating nerve terminals, along with reactive astrocytes and microglia. In addition, there are intracellular inclusions found within neurons; these neurofibrillary tangles are composed of paired helical filaments (Kidd, 1963). Whether neurodegeneration is related to Aβ deposition and/or neurofibrillary tangle formation is not clear.

In addition to the cortical neuropathology, there is also prominent subcortical neuropathology in AD. For example, there is significant neuronal loss in the locus coeruleus (LC) in AD post-mortem brains (Tomlinson et al., 1981; Iversen et al., 1983; Bondareff et al., 1987; Chan-Palay and Asan, 1989; German et al., 1992). The LC noradrenergic neurons innervate the entire cerebral cor-
text, cerebellar cortex, and certain brainstem nuclei (Moore and Bloom, 1979; Loughlin et al., 1986). In AD postmortem brains, neurofilibrillary tangles and neurodegeneration have been identified in 10 subcortical nuclei, including the noradrenergic LC neurons, which share the common feature that all project to the cerebral cortex (German et al., 1987). It has been speculated that the neuropathology of AD begins in the cerebral cortex and spreads to subcortical regions as a result of retrograde transport of a toxin (e.g., Aβ) and/or lack of transport or support from neurotrophic factor/s (Appel, 1981; Saper et al., 1987; Mufson et al., 1995).

It is uncertain whether Aβ is specifically linked to all of the neuropathology of AD. Several transgenic mouse models have been developed to examine the role of Aβ in AD neuropathology (for review see Higgins and Jacobsen, 2003; German and Eisch, 2004). Games et al. (1995) described transgenic mice by using the platelet-derived growth factor-β promoter to drive a human APP (hAPP) minigene encoding the V717F familial AD mutation (Chartier-Harlin et al., 1991). The APPV717 mutation results in an overproduction of the highly amyloidogenic Aβ1-42 peptide relative to other Aβ peptides (Suzuki et al., 1994). These PDAPP mice develop several AD-like lesions in the cortex and hippocampus, including neuritic plaque formation that increases with age, and gliosis and degeneration of cholinergic nerve terminals (Games et al., 1995; Masliah et al., 1996; Irizarry et al., 1997; German et al., 2003). There is subcortical neuronal loss in the LC in AD, so the present study sought to determine whether there is also loss or degenerative changes of these neurons in the PDAPP mouse model of AD.

MATERIALS AND METHODS

Transgenic PDAPP mice were generated according to previously described methods (Games et al., 1995). Mice were bred from the PDAPP-109 line over several generations on hybrid backgrounds representing combinations of C57BL/6, DBA, and Swiss-Webster strains. Initially, five hemizygous male PDAPP mice and five nontransgenic male littermates were examined, at 23 months of age. In a second experiment, we examined six male homozygous PDAPP and wild-type control mice at 2 months and 24 months of age. The wild-type control animals were from the same hybrid genetic background as the transgenic animals.

Tissue preparation

All experiments were conducted with the approval of the University of Texas Southwestern Medical School's Institutional Animal Care and Use Committee. Mice were killed with Nembutal (120 mg/kg, i.p.), and the brains were rapidly removed and immersion fixed in 10% neutral buffered formalin. Whole brains were fixed for 1–3 days. Prior to cutting the brain, the tissue block containing the LC was placed in 10% formalin/20% sucrose for 24 hours. Coronal sections, 20 μm thick, were cut through the rostral-caudal extent of the LC on a freezing microtome.

Immunostaining

Tissue sections were immunohistochemically stained with a polyclonal antibody against tyrosine hydroxylase (TH; Protos Biotech Corp., 1:4,000) and counterstained with cresyl violet. An antibody against choline acetyltransferase (ChAT; Chemicon International, Temecula, CA; AB144P) was used to examine the relationship between amyloid neuritic plaques and cholinergic nerve terminals. An antibody against human Aβ (12H7; Elan Pharmaceuticals, South San Francisco, CA; 1:1,000) was used to examine the regional distribution of neuritic plaques in the PDAPP mice. In addition, an antibody recognizing hAPP (monoclonal 5E5; Elan Pharmaceuticals) was used to immunostain sections through the LC of wild-type and transgenic hemizygous mice to determine whether either Aβ or hAPP was localized within LC somata in the transgenic animals.

The sections were processed for immunocytochemistry by using the unlabeled peroxidase antiperoxidase (PAP) method, as used previously in our laboratory (German et al., 1987). Sections were immersed in 1) 1% H2O2 in phosphate-buffered saline (PBS), pH 7.4, to eliminate endogenous peroxidase (30 minutes); 2) normal sheep serum (5%) in 0.1 M PBS with 0.3% Triton X-100 (PBST; 30 minutes); 3) rabbit or mouse antibody at room temperature in PBST with 1% normal sheep serum (PBSTSS; 24 hours); 4) sheep anti-rabbit IgG or anti-mouse IgG (Miles Labs, Elkhardt, IN) 1:100 in PBST (30 minutes); 5) rabbit or mouse PAP 1:500 in PBST (1 hour); and 6) 0.035% diaminobenzidine (DAB) with 2.5% nickel ammonium sulfate and 0.003% H2O2 in 0.1 M sodium acetate buffer, pH 6.0 (5–10 minutes). All of the major steps were performed on a shaker table, with three 10-minute washes in PBS between major steps. Sections were mounted on subbed slides, counterstained with cresyl violet, dehydrated, and coverslipped. Control procedures involved omitting the primary antibody, and in the case of each antibody there was no immunostaining.

Computer imaging

The number of TH-immunoreactive LC somata was counted in representative sections from rostral to caudal through the nucleus. StereoInvestigator software was used for the computer imaging (MicroBrightField, Inc., Williston VT). TH-immunoreactive neurons were mapped and quantified by using a computer imaging system that has been described in detail previously (German et al., 1992). Briefly, the system utilizes a Leica DMRE microscope with a computer-driven stage to scan a grid containing all neurons of interest within a given section. Lines were drawn around the perimeter of the LC in each tissue section at low power (×100), and all of the TH-immunoreactive cell locations were then marked at higher power (×400) in each section. LC cell counts were made in every fourth to sixth section (80–120 μm between sections) through the nucleus in the first experiment and in every fifth section (100 μm) in the second experiment. From seven to ten sections through the LC were analyzed per animal. TH-immunoreactive cells were defined as somata with an unstained nucleus or as a piece of somata that was larger than half the size of immunoreactive somata with an unstained nucleus. Cell counts were made with the person performing the task blind to the animal’s experimental condition (i.e., wild type, hemizygous, homozygous). In the first experiment, F.L. performed the cell counting, and, in the second experiment, O.N. performed the cell counting and cell size measurements.

LC cell size was measured in three regions of the nucleus. Cell size was measured 1) in the rostral portion of the nucleus, where cells reside that project to the hypo-
thalamus; 2) in the dorsal-central portion of the nucleus, where cells project to the cerebral cortex, hippocampus, hypothalamus, and cerebellum; and 3) in the nucleus subcoeruleus, where cells project to the spinal cord and cerebellum (Loughlin et al., 1986). In the rostral LC, 26–54 cells were measured in each animal, in the dorsal-central LC 27–72 cells were measured per animal, and in the subcoeruleus 20–43 cells were measured per animal (n = 6 animals/group). With ×400 magnification, outlines were traced around the perimeter of each TH-stained neuron with an unstained nucleus. The number of cells measured represents 1) the total number of neurons present in two or three 20-μm-thick sections that reside in the specific region of interest and 2) the neurons with a nucleus that was unstained for TH. Cell measurements represent the cell area in square micrometers. When a difference in cell size was found between animal groups, the cell counts for the specific rostral-caudal location within the LC were corrected for split-cell counting error by using Abercrombie’s correction factor (Abercrombie and Johnson, 1946).

Photographs were taken with a digital camera (DP11; Olympus, Melville, NY). Photo size, brightness, and contrast were modified in Photoshop (Adobe Systems, San Jose, CA).

RESULTS

Neuritic plaques in PDAPP mice

There are numerous amyloid plaques in the cerebral cortex and hippocampus of the aged hemizygous PDAPP mouse. Figure 1 illustrates Aβ immunoreactive plaques in the cerebral cortex of a 23-month-old, hemizygous PDAPP mouse. The plaques are 20–40 μm in diameter, and there
are 10–20 plaques located in the cortex/hippocampus in a single 20-μm-thick section. Irizarry et al. (1997) have reported similar amyloid plaque densities in mice from the same PDAPP transgenic line.

Neuritic plaques are covered with swollen axonal varicosities in the neocortex and hippocampus. The TH-containing nerve terminals are markedly larger than normal in the PDAPP mouse, and they were found in locations that correspond to the size and shape of neuritic plaques (Fig. 1C). Nerve terminals containing the cholinergic marker ChAT are also enlarged in proximity to neuritic plaques (Fig. 1D). The number of amyloid plaques in the homozygous animals than in the hemizygous animals yet the plaque diameter was similar in the two groups. In homoygous mice, there were from 40 to 60 plaques in the cortex/hippocampus in a single 20-μm-thick section. In age-matched wild-type control mice, no Aβ immunostaining was observed.

**LC neurons in PDAPP mice**

The LC cells span a rostral-caudal distance of approximately 900 μm in both transgenic and nontransgenic littersmate mice. Rostrally, the LC somata are loosely packed and situated medial to the mesencephalic nucleus of the trigeminal nerve (Fig. 2A). Caudally, the cells become more densely packed and more numerous. In the dorsal portion of the nucleus (Fig. 2B), neurons project to cortical and hippocampal regions according to retrograde transport studies (Loughlin et al., 1986). Large TH-containing cells located below the LC are the subcoeruleus neurons (Fig. 2C). In the caudal portion of the nucleus, the LC consists of a small cluster of cells situated adjacent to the lateral wall of the fourth ventricle (Fig. 2D).

The LC noradrenergic neurons were counted in sections spanning the rostral-caudal extent of the nucleus. In AD there is a greater loss of LC neurons that are located in the portion of the nucleus that projects to the cerebral cortex, compared with LC neurons that project to noncortical regions (German et al., 1992), so cell counts were taken from all portions of the nucleus for comparative analysis. There was no difference in the number of LC neurons in control and transgenic hemizygous PDAPP mice (ANOVA, F = 0.16, P = 0.68; Fig. 3). Likewise, the cell numbers were similar in both transgenic and age-matched, nontransgenic littersmate controls across the rostral-caudal levels of the nucleus, but they did not contain evidence of either hAPP or Aβ accumulation. Instead, there was diffuse immunostaining for both antibodies within the tissue sections (data not shown).

### DISCUSSION

The present data indicate that overexpression of AD-related mutant APP in the mouse, which causes the deposition of Aβ immunoreactive neuritic plaques in the neocortex and hippocampus, is not sufficient to cause a loss of cells in the LC. In the hemizygous PDAPP mouse, Aβ-laden neuritic plaques are found in the cingulate cortex, entorhinal cortex, and hippocampus, and plaques develop in an age-dependent manner to reach a density of 20–50% of the neuropil by 18 months of age (Irizarry et al., 1997). However, the mutant APP expression that caused the neuritic plaque pathology does not cause a loss of LC noradrenergic neurons in either the 2-month-old hemizygous mouse or the 24-month-old hemizygous mice. In the homozgyous PDAPP animals, there is also no loss of basal forebrain cholinergic neurons, which also innervate the cerebral cortex and hippocampus (German et al., 2003). In AD, both the LC and the basal forebrain cholinergic neurons degenerate (Tomlinson et al., 1981; Whitehouse et al., 1982; Rasool et al., 1986; Chan-Palay and Asan, 1989; German et al., 1992), and it appears that the LC neuro-
Fig. 2. The LC neurons change in number, size, and density across the rostral-caudal extent of the nucleus. Sections are illustrated from rostral (A) to caudal (D) within the LC immunostained for TH (black) and counterstained with cresyl violet (gray cells) in a 24-month-old homozygous PDAPP mouse. LC cells are illustrated on the right side of the brain, and each section is 140 μm apart. A: In the rostral portion of the nucleus, cells are relatively few and are not densely packed. B: In the midcentral portion of the nucleus, the LC neurons are more densely packed, with smaller cells located dorsally and larger cells located ventrally. Arrow in B illustrates the mesencephalic tract of V cells, lateral to the LC (Me5). C: More caudally in the LC, the large subcoeruleus (SC) neurons are located ventral to the LC. D: In the caudal LC, there are small numbers of densely packed TH-stained somata located in the lateral wall of the fourth ventricle (4V). Scale bar = 80 μm in D (applies to A–D).
Degeneration is of greater magnitude than that of the basal forebrain cholinergic neurons (Zarow et al., 2003). Furthermore, recent evidence suggests that degeneration of LC neurons may allow the degeneration of neurons in other brain regions in AD (Marien et al., 2004). Mouse models of AD that express both mutant APP and tau exhibit neuritic plaques and neurofibrillary pathology (Lewis et al., 2001; Oddo et al., 2003); future studies will determine whether these AD mouse models exhibit loss of subcortical neurons like the LC.

The subcortical neurodegeneration in AD has been speculated to result from retrograde changes to the neurons that have axonal communication with cortical and hippocampal areas containing AD neuropathology (Appel, 1981; Saper et al., 1987). Such a retrograde mechanism would explain why so many cortical-projecting subcortical nuclei are affected in AD. In the present study, and in studies of cholinergic degeneration in AD mouse models (Wong, 1999; Jaffer et al., 2001; Boncristiano et al., 2002; German et al., 2003), there is evidence that cholinergic and noradrenergic nerve terminals in the cerebral cortex and hippocampus have been altered by the presence of the Aβ-containing deposits in neuritic plaques. Axons expressing both neurotransmitters, when in proximity to neuritic plaques, are swollen to at least two or three times the normal size, as are axons from the entorhinal cortical area (Phinney et al., 1999). Ultrastructural analysis of the hippocampus of hemizygous PDAPP animals also indicates synaptic alterations, with distended and enlarged synaptic endings (Masliah et al., 1996). However, these nerve terminal alterations were not accompanied by obvious retrograde transport or accumulation of Aβ in the LC, indicated by a lack of immunostaining with antibodies against hAPP or Aβ. In other mouse models of AD, however, there is intracellular accumulation of Aβ that has been correlated with neurodegeneration (Oddo et al., 2003; Casas et al., 2004). It is interesting, however, that the size of the LC somata that project to the cortex and hippocampus, based on retrograde transport studies in the rat (Loughlin et al., 1986), are significantly smaller (31%) in the aged homozygous PDAPP mouse, whereas LC neurons in other portions of the nucleus that do not project to these cortical regions are normal in size in the aged transgenic mice. Although the nerve terminals in proximity to neuritic plaques are normal size, as are axons from the entorhinal cortex, these axons have access to insufficient amounts of neurotrophic factors to maintain their normal somata and axons to maintain sufficient levels of neurotrophic factors to maintain their normal somata and axons to maintain sufficient levels of neurotrophic factors to maintain their normal somata (Quintero et al., 2004; Matsunaga et al., 2004). Such cell shrinkage has been observed in the basal forebrain cholinergic neurons following lesions of their nerve terminals in the cerebral cortex of the rat (Sofroniew et al., 1983).

It is possible that overexpression of Aβ alone cannot cause neuronal loss in the mouse. There are several rea-

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**Table 1. LC Cell Size**

<table>
<thead>
<tr>
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<th>Rostral</th>
<th>Dorsal-central</th>
<th>Subcoeruleus</th>
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<tbody>
<tr>
<td>2-Month control</td>
<td>173 ± 22</td>
<td>144 ± 14 2</td>
<td>264 ± 24 2</td>
</tr>
<tr>
<td>24-Month control</td>
<td>170 ± 6</td>
<td>134 ± 13</td>
<td>250 ± 17</td>
</tr>
<tr>
<td>2-Month PDAPP</td>
<td>174 ± 10</td>
<td>156 ± 17</td>
<td>244 ± 26</td>
</tr>
<tr>
<td>24-Month PDAPP</td>
<td>155 ± 15</td>
<td>107 ± 9</td>
<td>225 ± 14</td>
</tr>
</tbody>
</table>

*LC cell size in μm² + SD, n = 6/group.*

1Dorsal-central cell sizes are different among the four groups, F = 14.6, P < 0.001. The cells in the old PDAPP animals are smaller than in the other three groups (P < 0.05 for each comparison).

Subcoeruleus cell sizes were different among the four groups, F = 3.51, P = 0.03. However, the only group that was different was the old PDAPP animals had smaller cells than the young controls (P < 0.05). There was no difference between the young and old PDAPP cell sizes.

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**Fig. 3.** There is no loss of LC neurons in the PDAPP mouse model of AD. The number of LC neurons/20-μm-thick section was plotted across the rostral-caudal extent of the nucleus in wild-type littermate (n = 5) and in 23-month-old hemizygous PDAPP mice (n = 5; top). The number of LC neurons was also examined in control mice at 2 months and 24 months of age (n = 6/group; middle) and in 2-month and 24-month-old homozygous PDAPP mice (n = 6/group; bottom). Cell numbers are from rostral (0) to caudal (9), with each level separated by 100 μm. Each data point represents the mean ± SD cell number.
sons why this may be true. First, the mouse might not live long enough for the Aβ-related pathology to cause overt neurodegeneration. In patients with Down’s syndrome, there is evidence to suggest that amyloid plaques are present for over 20 years before marked AD-like neuropathology occurs (Rumble et al., 1989). To increase the amyloid burden, the present study utilized the oldest hemizygous PDAPP animals studied to date, as well as 2-year-old homozygous mice. Still there was no subcortical neuronal loss in either group of mice. The lack of overt cell loss in the PDAPP mice also might be due to the animals having severalfold higher expression levels of hAPP than endogenous mouse APP (Games et al., 1995; Rockenstein et al., 1995). Such hAPP overexpression has been found to be neuroprotective in transgenic mice (Mucke et al., 1994, 1996) and could potentially interfere with the neurodegenerative effects of Aβ. Finally, the APPSw mouse model of AD has been found to express neuroprotective molecules, such as transthyretin and insulin-like growth factor 2, which block the neurodegenerative effects of Aβ (Stein et al., 2004). Once these molecules were inactivated, tau phosphorylation and neurodegeneration was observed in the CA1 region of the hippocampus of these animals.

Alternatively, it may be that neurofibrillary tangle formation is important for neurodegeneration in AD. Neurofibrillary tangles are composed of paired helical filaments, the major constituent being the hyperphosphorylated microtubule-associated protein tau. The tangles appear to accumulate within vulnerable neurons and may eventually kill the cell and leave behind only a ghost tangle and no neuron (Saper et al., 1985). Gene mutations in tau have been shown to cause neurodegeneration and dementia in the case of frontotemporal dementia and Parkinsonism linked to chromosome 17 (FTDP-17; for reviews see Goedert et al., 1998; Hardy et al., 1998). Cases with FTDP-17 have circumscribed atrophy of the frontal and temporal lobes along with subcortical changes, with neurons that contain neurofibrillary tangles like those found in AD; the brains, however, do not show Aβ-plaque pathology. Insofar as the PDAPP mice do not have neurofibrillary tangles (Games et al., 1995; Masliah et al., 1996), it is possible that the lack of neurodegeneration is related to the absence of tangle formation in these animals. This would be consistent with data from AD patients, where there is no correlation between neuritic plaque density and neuronal loss (Wilcock and Esiri, 1982; Braak and Braak, 1991; Terry et al., 1991). Several neurodegenerative diseases are characterized by the presence of neurofibrillary tangles but lack Aβ-plaque pathology, such as Niemann-Pick type C disease (Horoupian and Yang, 1978; Love et al., 1995), progressive supranuclear palsy (Steele et al., 1964; Tellez-Nagel and Wisniewski, 1973), postencephalitic Parkinsonism (Hallevorden, 1933, 1935), myotonic dystrophy (Kiuchi et al., 1991), and dementia pugilistica (Corsellis et al., 1973). It is possible that the absence of filamentous tau pathology in the PDAPP mice may be related to the lack of neurodegeneration in cortical and subcortical structures. On the other hand, neurodegeneration is present in a mouse model of Niemann-Pick C, but, unlike the case in human Niemann-Pick C disease, there are no neurofibrillary tangles (German et al., 2001). Although recent data suggest that neurofibrillary tangles are not directly correlated with neurodegeneration in mice expressing nonmutant human tau isoforms (Andorf et al., 2005), with the development of transgenic mice that express Aβ plaques and NFTs (see, e.g., Oddo et al., 2003) it will be possible to study the role of NFTs in neurodegeneration in an AD mouse model.

In conclusion, the present experiment demonstrates that the aged PDAPP mouse exhibits much of the cortical neuropathology of AD. Overexpression of Aβ42 in these mice is associated with neuritic plaque formation, gliosis, synapse loss (Games et al., 1995; Irizarry et al., 1997), and degeneration of cortical cholinergic nerve terminals (German et al., 2003). However, there is no subcortical cell loss in either the LC or the basal forebrain cholinergic neurons (German et al., 2003). Selective shrinkage of LC cells projecting to areas of AD-like pathology suggests that specific mediators of neuronal stress are affecting these neurons. Further experiments will be required to determine whether a complete model of AD will be found in mice expressing multiple AD-related gene mutations (see, e.g., Lewis et al., 2001; Oddo et al., 2003; Schmitz et al., 2004).

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LITERATURE CITED


