Somatostatin, tau, and β-amyloid within the anterior olfactory nucleus in Alzheimer disease

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Abstract

Impaired olfaction is an early symptom of Alzheimer disease (AD). This likely reflects neurodegenerative processes taking place in basal telencephalic structures that mediate olfactory processing, including the anterior olfactory nucleus. Beta-amyloid (Aβ) accumulation in AD brain may relate to decline in somatostatin levels: somatostatin induces the expression of the Aβ-degrading enzyme neprilysin and somatostatin deficiency in AD may therefore reduce Aβ clearance. We have investigated the expression of somatostatin in the anterior olfactory nucleus of AD and control brain. We report that somatostatin levels were reduced by ~50% in AD brain. Furthermore, triple-immunofluorescence revealed co-localization of somatostatin expression with Aβ (65.43%) with Aβ and tau (19.75%) and with tau (2.47%). These data indicate that somatostatin decreases in AD and its expression may be linked with Aβ deposition.

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Table 1

<table>
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<tr>
<th>Case</th>
<th>Diagnosis</th>
<th>Sex</th>
<th>Age (years)</th>
<th>Weight (g)</th>
<th>PMD (h)</th>
<th>Cause of death</th>
<th>Duration of the disease</th>
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<td>M</td>
<td>61</td>
<td>1310</td>
<td>Encephalopathy</td>
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<td></td>
</tr>
</tbody>
</table>

Diagnosis according to Braak’s stages (Braak and Braak, 1991).

Brain weighted after fixation. PMD: post-mortem delay.
Fig. 1. Coronal sections of the human anterior olfactory nucleus. A, B, Nissl-stained images revealing the olfactory tract (ot) and the medial (AONm) and lateral (AONI) portions of the anterior olfactory nucleus in a control case. C, D, immunostaining for somatostatin in control and AD brain respectively. E–H, triple immunofluorescence for somatostatin (blue; white arrows indicate labeled cells), Aβ (red; arrowheads indicate neuritic plaques), and tau (green; large asterisks indicate neurofibrillary tangles while small asterisks indicate dystrophic neurites). Double-labeled cells for somatostatin and Aβ (yellow arrows) and triple-labeled cells (gray arrows) are indicated. Abbreviations: Acb: nucleus accumbens, AONcp: anterior cortical olfactory nucleus, cortical posterior; molf: medial olfactory radiation; olfr: olfactory radiation. Calibration bar for: A 1600 μm; B–D 400 μm, E–G 80 μm, H 25 μm.
The pathological accumulation of Aβ peptide in AD may be related to changes in somatostatin levels. Somatostatin is known to regulate the level of expression of neprilysin, a peptidase that catalyzes the proteolytic degradation of Aβ. Somatostatin levels decline with normal aging (Lu et al., 2004) and cortical somatostatin immunoreactivity was also found to be systematically reduced in AD brain versus controls (Davies et al., 1980). Decreased somatostatin expression may therefore predispose to Aβ accumulation. This finding has raised the possibility that somatostatin receptor agonists may be of therapeutic value in AD (Hama and Saito, 2005; Iwata et al., 2005; Saito et al., 2005; Saito and Iwata, 2006).

To address the association between somatostatin decline and the deposition of Aβ and tau aggregates we analyzed anterior olfactory nucleus expression levels of somatostatin in AD versus controls. Six AD brain samples and 3 control samples (Table 1) were kindly provided by 2 brain banks (the Banco de Tejidos/Fundación para Investigaciones Neurobiológicas de la Universidad Complutense de Madrid and the Banc de Teixits Neurològics de la Universitat de Barcelona-Hospital Clínic). All procedures were approved by the Ethical Committee for Clinical Research at the University Hospital of Albacete (grant number PI-2006/15). Primary antibodies used for immunodetection were mouse anti-tau (tau 46, 1:800, Cell Signaling Technology, Beverly, MA, USA), rabbit anti-Aβ (1:250, Cell Signaling Technology), and goat anti-somatostatin D-20 (1:1000, Santa Cruz Biotechnology, Santa Cruz, CA, USA). Secondary antibodies were either biotinylated (anti-goat IgG, 1:2000, Vector Laboratories, Burlingame, CA, USA) or fluorescent-labeled (1:200, alexas 488 donkey anti-mouse, 568 donkey anti-rabbit, and 350 donkey anti-goat; Molecular Probes, Invitrogen, Carlsbad, CA, USA). These were used to detect somatostatin, tau, and Aβ in 50 µm coronal sections of the anterior olfactory nucleus obtained using a sliding freezing microtome. Somatostatin-positive cells were charted with an X-Y recording system (AccuStage, Minnesota Datametrics, MN, USA). Two areas measuring each of 12 mm² were analyzed in the medial and lateral portions of the anterior olfactory nucleus and the mean cell number per mm² was calculated (Fig. 2A). The over counting error was assumed since it was similar for all measures (Guillery, 2002). For triple immunofluorescence, sections were visualized under a LSM 710 Zeiss confocal microscope (Carl Zeiss MicroImaging, Barcelona, Spain). Intensities of each fluorochrome were analyzed using the profile tool of the ZEN software (Zeiss) to quantitatively estimate single-, double- and triple-labeled cells (Fig. 2B).

Inspection of Nissl-stained coronal sections of the basal frontal lobe revealed the cytoarchitecture of the cortical anterior olfactory nucleus including the medial and lateral subdivisions on both sides of the olfactory tract (Figs. 1A, B). Immunostaining for somatostatin in control (Fig. 1C) and AD (Fig. 1D) sections revealed labeled cells throughout the nucleus. The distribution pattern of positive cells was similar in AD versus controls, but the number of positive cells was reduced by half (one-way ANOVA, post hoc Bonferroni test, p<0.05) (Fig. 2A).

Triple label experiments were used to simultaneously detect somatostatin-positive cells (white arrows in Fig. 1E), neuritic Aβ plaques (arrowheads in Figs. 1E–H), and neurofibrillary tangles (big asterisks in Fig. 1E) as well as dystrophic neurites (small asterisks in Fig. 1E) associated with abnormal forms of tau. This revealed that some somatostatin-positive cells were associated with Aβ deposits (yellow arrows in Figs. 1E, H); and with Aβ deposits and tau aggregates (gray arrows in Figs. 1F, G). In contrast, there was almost no association between somatostatin-positive cells and tau aggregates (not shown). Quantitative observations reveal that most somatostatinergic cells are associated with Aβ deposits (65.43%) or with Aβ deposits plus tau aggregates (19.75%); whereas single somatostatin-labeled cells (12.35%) or associated with tau (2.47%) are less abundant (Fig. 2B).

These results indicate that levels of somatostatin, a multifunctional peptide widely distributed in the central nervous system (Epelbaum, 1986; Gillies, 1997; Viollet et al., 2008), are reduced by approximately 50% in the anterior olfactory nucleus of AD cases compared to controls (Fig. 2A). This reduction is in agreement with previous data regarding normal aging and AD (Davies et al., 1980; Ferrier and Leake, 1990; Lu et al., 2004; Burgos-Ramos et al., 2008). We cannot exclude the possibility that differences in age between the AD and control patient samples (mean age in years ± standard deviation: AD, 80.17 ± 6.18; controls, 63.0 ± 5.29) might partly contribute to the somatostatin decline reported here. The distribution of neuropathological markers in the anterior olfactory nucleus of AD patients is substantially in agreement with previous reports (Ohm and Braak, 1987; Braak and Braak, 1991; Price et al., 1991). Because the anterior olfactory nucleus plays a central role in human olfactory processing (Price, 1990), both reduced somatostatin levels and Aβ and tau deposition may underlie to the olfactory impairment associated with AD (Koss et al., 1988; Doty, 1991; Doty et al., 1991; Hughes et al., 2001; Djordjevic et al., 2008). On the other hand, it has been reported that the reduction in somatostatin provokes the downregulation of neprilysin that may be a trigger for Aβ accumulation leading to late-onset sporadic AD suggesting that somatostatin receptors may afford a pharmacological target for AD treatment (Saito et al., 2005). The present findings that somatostatin expression is associated with sites of Aβ deposition but not with tau pathology, as previously reported (van de Nes et al., 2006), are broadly consistent with this hypothesis.

![Fig. 2. A, Mean numbers ± typical error of the mean of somatostatin-labeled cells per mm² in control (4.84 ± 0.22) versus Alzheimer’s disease (2.73 ± 0.43) cases (p<0.05). Two areas each of 12 mm² were analyzed in the medial and lateral portions of the anterior olfactory nucleus and the mean cell number per mm² was calculated. B, Percentages of co-localization (somatostatin, Aβ and tau) of single-, double-, and triple-labeled cells ± typical error of the mean.](image-url)
Acknowledgments

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.expneurol.2009.06.010.

References