Increased pro–nerve growth factor and decreased brain-derived neurotrophic factor in non–Alzheimer's disease tauopathies

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Post mortem brain

ABSTRACT

Alterations in the expression and signaling of brain-derived neurotrophic factor (BDNF) and the precursor to nerve growth factor (NGF), proNGF, play a role in the neuronal and cognitive dysfunction of Alzheimer’s disease. Aggregated amyloid-β has been shown to down-regulate specific BDNF transcripts in Alzheimer’s disease, but the role of tau pathology in neurotrophin dysregulation has not been investigated. We measured levels of BDNF mRNA and protein using real-time quantitative reverse transcription–polymerase chain reaction and enzyme-linked immunosorbent assay and proNGF protein using Western blotting in parietal cortex of subjects with tauopathies, neurodegenerative diseases exhibiting tau pathology without amyloid-β accumulation. We observed a significant increase in the level of proNGF protein in Pick’s disease and a significant decrease in BDNF mRNA and protein levels in Pick’s disease and corticobasal degeneration, but no neurotrophin alterations in progressive supranuclear palsy. The decrease in total BDNF mRNA levels in these tauopathies was predominantly due to down-regulation of transcript IV. These findings implicate tau pathology in neurotrophin dysregulation, which may represent a mechanism through which tau confers toxicity in Alzheimer’s disease and related non-Alzheimer’s dementias.

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1. Introduction

In Alzheimer’s disease (AD), the degree of synaptic loss in basal forebrain cholinergic neurons, entorhinal cortex, hippocampus, and cortical regions of the brain correlate strongly with the severity of dementia (Scheff and Price, 2003; Terry et al., 1991). AD brain exhibits intraneuronal neurofibrillary tangles caused by post-translational modifications to tau, a microtubule binding protein, as well as extracellular senile plaques caused by aggregation of the amyloid-β (Aβ) peptide. The amyloid cascade hypothesis of Alzheimer’s disease proposes that amyloid-β accumulation is the initial insult leading to degeneration of central nervous system neurons (Hardy and Selkoe, 2002). Importantly, recent evidence argues that Aβ toxicity is mediated through tau (Ittner et al., 2010; LaFerla, 2010; Roberson et al., 2007).

Neurotrophins regulate neuronal survival, differentiation, and function in the peripheral and central nervous systems (Huang and Reichardt, 2001). Dysregulation of the levels of neurotrophins or their receptors, or alterations in neurotrophin function or trafficking, can damage neurons, leading to gradual neuronal degeneration (Mufson et al., 2007).

Brain-derived neurotrophic factor (BDNF) promotes neuronal survival, synaptic plasticity, and memory consolidation (Binder and Scharfman, 2004; Fahnestock, 2011; Lu, 2003; Yamada et al., 2002). BDNF mRNA and protein are decreased in post-mortem brain tissue from subjects with mild cognitive impairment (MCI) and AD (Connor et al., 1997; Ferrer et al., 1999; Garzon et al., 2002; Hock et al., 2000; Holsinger et al., 2000; Michalski and Fahnestock, 2003; Peng et al., 2005; Phillips et al., 1991), and the reduction in BDNF correlates with the degree of cognitive impairment (Peng et al., 2005). BDNF administration can rescue synaptic loss and cognitive dysfunction in animal models, implicating loss of BDNF as a contributing factor to AD (Arancibia et al., 2008; Bluton-Jones et al., 2009; Nagahara et al., 2009). Down-regulation of BDNF does not exacerbate Aβ deposition or tau pathology in a 3X transgenic mouse model of Alzheimer’s
disease, further confirming that the decrease in BDNF is not a cause of, but is rather a consequence of, Aβ and tau pathology (Castello et al., 2012). Soluble, oligomeric Aβ42 has been shown to be at least partially responsible for down-regulation of BDNF (Garzon and Fahnestock, 2007; Peng et al., 2009; Tong et al., 2001, 2004). Although BDNF is not down-regulated in tau transgenic mice (Burnouf et al., 2012), whether tau contributes to BDNF down-regulation in human tissue has not been investigated.

Basal forebrain cholinergic neurons (BFCNs) are crucial for learning and memory, and require nerve growth factor (NGF) for survival and function (Gutierrez et al., 1997; Woolf et al., 2001). In AD, BFCNs undergo degeneration that is associated with decreased NGF-immunoreactive protein in BFCN cell bodies and increased NGF precursor (proNGF) in BFCN target tissues such as hippocampus and cortex (Fahnestock et al., 1996, 2001; Mufson et al., 1995; Peng et al., 2004; Salehi et al., 2006; Scott et al., 1995). The high-affinity receptor for NGF, tropomyosin receptor kinase A (TrkA), is reduced in BFCNs in AD (Counts et al., 2004; Ginsberg et al., 2006), which could account for proNGF accumulation in BFCN target tissues due to impaired retrograde transport of this protein (Bradbury, 2005; Reichardt and Mobley, 2004; Salehi et al., 2004, 2006). Tau dysfunction can also contribute to impairment of axonal transport (Cowan et al., 2010; Ittner et al., 2010; Niewiadomska and Baksalerska-Pazera, 2003; Niewiadomska et al., 2005; Vossel et al., 2010), which may lead to proNGF accumulation in BFCN target tissue. To determine whether tau contributes to neurotrophin dysregulation in neurodegenerative diseases, we analyzed proNGF protein and BDNF mRNA and protein levels in post mortem parietal cortex from subjects with tauopathies, a class of neurodegenerative diseases that exhibit tau pathology in the absence of Aβ accumulation.

## 2. Methods

### 2.1. Human brain tissue samples

Postmortem parietal cortex from subjects with tauopathies including Pick's disease (PiD), corticobasal degeneration (CBD), and progressive supranuclear palsy (PSP), and from age-matched controls were provided by Dr Virginia M.Y. Lee (University of Pennsylvania, Philadelphia, PA). There were no differences in age, sex, post-mortem interval, or yield of protein or RNA between groups. Subject characteristics are shown in Table 1.

### 2.2. ProNGF Western blotting

Human normal and tauopathy parietal cortex tissue (n = 6 per group) were used for proNGF quantification. Frozen tissue samples (40–90 mg) were sonicated (Sonic Dismembrator Model 100, Fisher Scientific) in a 1:10 w/v ratio in homogenization buffer (0.05 mol/L Tris-Cl, pH 7.5, 0.5% Tween-20, 10 mmol/L ethylenediaminetetraacetic acid, 2 μg/mL aprotinin, 2 μg/mL pepstatin, 2 μg/mL leupeptin, and 100 μg/mL phenylmethylsulphonyl fluoride). Homogenates were kept on ice for 15 minutes and then centrifuged for 15 minutes at 9500 x g at 4 °C. Equal volumes of supernatants were assayed for total protein using the DC protein assay (Bio-Rad Laboratories, Hercules, CA).

Total protein (30 μg from each sample) was loaded onto 12% sodium dodecyl sulfate–polyacrylamide gels and transferred to polyvinylidene fluoride (PVDF) membranes (Amersham Biosciences, Oakville, ON, Canada). Membranes were blocked for 1 hour in Tris-buffered saline-Tween (TBS-T:50 mmol/L Tris-Cl, pH 8.0, 133 mmol/L NaCl, 0.2% [v/v] Tween-20) with 5% (w/v) nonfat milk powder (Nestle, North York, ON, Canada) and probed with a 1:500 dilution of affinity-purified polyclonal rabbit anti-NGF (H-20, Santa Cruz Biotechnology, Santa Cruz, CA), overnight at 4 °C. The membrane was rinsed in TBS-T and incubated with horseradish peroxidase–conjugated donkey anti-rabbit (1:5000, Amersham) in TBS-T plus 5% nonfat milk powder for 1 hour at room temperature. Bands were visualized by ECL chemiluminescence (Amersham) on Kodak film (Kodak X-OMAT LS, Kodak, Vancouver, BC, Canada). The same membrane was then washed in TBS-T and re-probed with monoclonal mouse anti-β-actin (1:10000 dilution, Sigma, St Louis, MO), a validated housekeeping gene, followed by horseradish peroxidase–conjugated

### Table 1

<table>
<thead>
<tr>
<th>Samples analyzed for proNGF</th>
<th>Clinical diagnosis</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Normal (n = 6)</td>
<td>PiD (n = 6)</td>
</tr>
<tr>
<td>Age at death, y Mean ± SD</td>
<td>72.3 ± 2.4</td>
<td>68.3 ± 6.8</td>
</tr>
<tr>
<td>Range</td>
<td>69–75</td>
<td>59–76</td>
</tr>
<tr>
<td>Males, n (%)</td>
<td>3 (50%)</td>
<td>4 (67%)</td>
</tr>
<tr>
<td>Yield of protein, μg/mg tissue</td>
<td>44 ± 5</td>
<td>40 ± 5.8</td>
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</tbody>
</table>

### Table 2

<table>
<thead>
<tr>
<th>Subject characteristics</th>
<th>Value</th>
</tr>
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<tr>
<td>Age at death, y Mean ± SD</td>
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</tr>
</tbody>
</table>

Data are expressed as mean ± standard deviation (age and protein yield), range (age and yield of RNA), and percentage of males. p Values were determined by 1-way analysis of variance.
sheep anti-mouse IgG secondary antibody (1:5000 dilution, Amersham).

Each Western blot contained a standard curve consisting of 5, 7.5, 10, 15, 20, 30, and 60 μg of total protein from a single human cortex sample, common to all blots. Standard curves for proNGF and β-actin were used to normalize pixel values between blots and to ensure that blots were not oversaturated and spanned the linear range of detection for both targets. Recombinant proNGF and 2.5S (mature) NGF were used as positive controls in all blots to confirm efficacy of the antibodies. Furthermore, we assessed specificity of the bands by blocking with 5-fold molar excess of NGF peptide. The pixel value of the immunoreactive bands was determined by densitometry of films using a Hewlett Packard Scanjet scanner and Scion Image beta 4.01 software, with local background subtracted.

Samples were analyzed 3 times in independent experiments, and the mean pixel values from the 3 experiments were used for further statistical analysis. One outlier in the control group (outlier defined as more than 1.5 quartiles below the first quartile or above the third quartile) was excluded from subsequent analysis. Between-group differences were analyzed by 1-way analysis of variance (ANOVA), followed by the Dunnett post-hoc test, using SPSS version 17 software (SPSS Inc, Chicago, IL).

Table 2

<table>
<thead>
<tr>
<th>Target</th>
<th>Product size</th>
<th>Forward primer (5'→3')</th>
<th>Reverse primer (5'→3')</th>
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<tr>
<td>β-Actin (NM_001101.3)</td>
<td>109 bp</td>
<td>CTCGTCACGCTTCGCTTC</td>
<td>TGTGGCTGAAGCGAGCTT</td>
</tr>
<tr>
<td>Total BDNF (EF889809)</td>
<td>249 bp</td>
<td>AACACTCGGAGCAGAGGC</td>
<td>AGAGAGGAGGCTCCTCAGAAGC</td>
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<tr>
<td>BDNF exon I (EF868021)</td>
<td>286 bp</td>
<td>GCGGTGTTAGGCGTTGAGG</td>
<td>ACCCTGTCCTCGGATTTTC</td>
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<tr>
<td>BDNF exon II (NM_00143806.1)</td>
<td>148 bp</td>
<td>GCCGGTGTAGGCGTTGAGG</td>
<td>ACCCTGTCCTCGGATTTTC</td>
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<tr>
<td>BDNF exon III (NM_00143807.1)</td>
<td>487 bp</td>
<td>TTAGAAGGGTTCCCGCTTT</td>
<td>GACCTCTGATGACGTCGTCG</td>
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<tr>
<td>BDNF exon IV (NM_170733.3)</td>
<td>382 bp</td>
<td>GAGATTACACCTGGGACG</td>
<td>ATTCAGGCTTCCGAGCG</td>
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<tr>
<td>BDNF exon V (EF868011)</td>
<td>219 bp</td>
<td>CATGGCTGTCCTGCTCAT</td>
<td>ATTCAGGCTTCCGAGCG</td>
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<tr>
<td>BDNF exon VI (EF868014)</td>
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<td>GCCGTTGACACCTGCCGAA</td>
<td>GACCTCAGTGGATGTGCCC</td>
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<tr>
<td>BDNF exon VII (EF868017)</td>
<td>198 bp</td>
<td>ATCCGCGGCGGACGACG</td>
<td>ATTCAGGCTTCCGAGCG</td>
</tr>
<tr>
<td>BDNF exon IXabed (NM_170735.5)</td>
<td>597 bp</td>
<td>TTTCTGCAGCAGCTAGA</td>
<td>CTGCTTACCAAGCACGTCCTTTCTTAC</td>
</tr>
</tbody>
</table>

Forward primer sequences for BDNF exons Vh and IX, and reverse sequences for exons III, Vh, and IX were taken from Pruunsild et al. (2007). All other primers were designed using Primer3 version 4.0 (online version, Massachusetts Institute of Technology) using the NCBI reference number list. Location of primers in specific exons was confirmed by referring to the intron-exon boundaries identified previously (Pruunsild et al., 2007). For transcripts that were detected before a cycle threshold of 32, a dissociation curve confirmed that only 1 major product was amplified.

Key: BDNF, brain-derived neurotrophic factor.
standard curve using recombinant BDNF protein was run on each ELISA plate.

3. Results

3.1. Increased proNGF protein in Pick’s disease

ProNGF levels in post mortem parietal cortex samples from non-AD tauopathies were compared to those of age-matched controls. A representative Western blot is shown in Fig. 1A. β-Actin did not differ between groups (p = 0.6, 1-way ANOVA) and was used to normalize proNGF pixel values between samples. Mature NGF was not detectable in human cortical tissue, as previously reported (Fahnestock et al., 2001). A 1-way ANOVA showed an overall significant effect of group (p = 0.007) in the level of 34kDa proNGF (normalized to β-actin). This was followed by a post hoc Dunnett test that revealed a statistically significant increase in proNGF in Pick’s disease (PiD) compared to that in age-matched controls (p = 0.02). The increase represents a 50% elevation in proNGF protein in PiD relative to control values and an effect size of 1.60. This increase was not observed in corticobasal degeneration (CBD, p = 0.68) or progressive supranuclear palsy (PSP, p = 0.83) (Fig. 1B).

3.2. Decreased BDNF mRNA in Pick’s disease and corticobasal degeneration

BDNF mRNA was measured in post mortem parietal cortex of subjects with PiD (n = 8), CBD (n = 11), and PSP (n = 13) and age-matched controls (n = 11) by quantitative real-time reverse transcription—polymerase chain reaction (RT-PCR). A total of 6 independent RT products, with 3 PCR reactions per RT, were analyzed for total BDNF and β-actin copy numbers. β-Actin mRNA did not differ between groups (p = 0.35) and was used to normalize BDNF and transcript levels. A 6-level repeated-measures ANOVA (p = 0.02) followed by a Dunnett post-hoc test revealed a statistically significant down-regulation of total BDNF mRNA in PiD (p = 0.03) and CBD (p = 0.04) compared to values in the age-matched control group (Fig. 2). In both groups, this represents approximately a 55% reduction in total BDNF/β-actin copy number ratio when compared with controls, with an effect size for PiD versus controls of 1.19 and for CBD versus controls of 1.05. There was no reduction in total BDNF mRNA in PSP subjects compared to controls (p = 0.86).

3.3. BDNF transcript down-regulation

In AD, BDNF transcripts I, II, IV, and VI are specifically down-regulated (Garzon et al., 2002). As in AD, expression of BDNF transcript IV was down-regulated in the 2 tauopathy groups exhibiting total BDNF down-regulation, PiD (n = 8) and CBD (n = 11) compared to control (n = 11) (Fig. 3, 1-way ANOVA, p = 0.006 followed by Dunnett post-hoc test, p = 0.01 and p = 0.03, respectively). These results represent a BDNF transcript IV/β-actin copy number ratio of approximately 75% below control levels in both the PiD and CBD groups. Effect sizes are 1.33 for PiD versus control and 1.14 for CBD versus control. No significant change was observed in the level of BDNF transcript IV in PSP compared to control (p = 0.88) (Fig. 3). In contrast to AD, there were no transcript-specific differences in PiD and PSP when compared to age-matched controls for transcripts I, II, or VI (Fig. 4A–C, 1-way ANOVA, p > 0.05). Similar to AD, transcript VII was not down-regulated in PiD or CBD (Fig. 4D, 1-way ANOVA, p > 0.05). Combining PiD
and CBD groups did not alter the results. Transcripts III, V, Vh, and IX fell below the limit of detection, defined as the amount of product obtained from 50 ng of standard transcript cDNA.

Levels of BDNF transcripts other than transcript IV were not analyzed in PSP. Although specific BDNF transcripts other than transcript IV may change in PSP, because transcript IV comprises more than 50% of the total BDNF mRNA in cortical tissue (Garzon and Fahnestock, 2007), the physiological relevance of the contributions of other transcripts with no change in total BDNF mRNA or protein is considered minor.

3.4. Decreased BDNF protein in Pick's disease and corticobasal degeneration

BDNF protein was measured by ELISA in post mortem parietal cortex of subjects with PiD (n = 9), CBD (n = 12), and PSP (n = 13), and age-matched controls (n = 12). A 1-way ANOVA (p = 0.01) followed by a Dunnett post-hoc test revealed a statistically significant down-regulation of BDNF protein in PiD (p = 0.04) and CBD (p = 0.04) compared to the age-matched control group (Fig. 5). This represents a 44% reduction in BDNF protein in PiD and a 41% reduction in BDNF protein in CBD when compared with controls, confirming the mRNA data. There was no reduction in BDNF protein in PSP subjects compared to controls (p = 1.0). There was a highly significant correlation between levels of BDNF protein measured by ELISA and levels of BDNF Transcript IV mRNA (Pearson correlation, r² = 0.501, p = 0.001), further confirming that mRNA levels are representative of BDNF protein levels.

4. Discussion

This is the first study to implicate tau as a potential mediator of neurotrophin dysregulation in human subjects with tauopathies. We show that proNGF protein is elevated in the parietal cortex of individuals with PiD, but not in those with CBD or PSP compared to age-matched controls. We also show that total BDNF mRNA and protein are decreased in PiD and CBD, but not in PSP, and that this decrease is predominantly attributable to reduced BDNF transcript IV.

A decrease in BDNF mRNA as well as mature and proBDNF protein in AD and MCI brain tissue has been identified previously in several studies (Connor et al., 1997; Ferrer et al., 1999; Garzon et al., 2002; Hock et al., 2000; Holsinger et al., 2000; Michalski and Fahnestock, 2003; Peng et al., 2005; Phillips et al., 1991). Soluble, oligomeric Aβ is sufficient to induce BDNF down-regulation in vitro.
Brain-derived neurotrophic factor (BDNF) protein is decreased in corticobasal degeneration and Pick’s disease compared to controls. Control, n = 12; Pick’s disease (PiD), n = 9; corticobasal degeneration (CBD), n = 12; progressive supranuclear palsy (PSP), n = 13. Horizontal line represents the mean of each group. A significant decrease in BDNF protein as measured by enzyme-linked immunosorbent assay was observed in PiD (p < 0.04) and CBD (p < 0.04), but not in PSP (p = 1.0), when compared to controls. *p < 0.05, 1-way analysis of variance (p = 0.01) and post hoc Dunn test.

Fig. 5. Brain-derived neurotrophic factor (BDNF) protein is decreased in corticobasal degeneration and Pick’s disease compared to controls. Control, n = 12; Pick’s disease (PiD), n = 9; corticobasal degeneration (CBD), n = 12; progressive supranuclear palsy (PSP), n = 13. Horizontal line represents the mean of each group. A significant decrease in BDNF protein as measured by enzyme-linked immunosorbent assay was observed in PiD (p < 0.04) and CBD (p < 0.04), but not in PSP (p = 1.0), when compared to controls. *p < 0.05, 1-way analysis of variance (p = 0.01) and post hoc Dunn test.
consistent with the hypothesis that amyloid-β down-regulates BDNF transcript IV via tau. Our findings indicate that pathologically modified tau may confer toxicity through down-regulation of BDNF and accumulation of proNGF protein, suggesting a novel role for tau-mediated neurotrophin dysregulation in human neurodegenerative disorders.

Disclosure statement

The authors have no actual or potential conflicts of interest.

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