Region-Specific Neurotrophin Imbalances in Alzheimer Disease

Decreased Levels of Brain-Derived Neurotrophic Factor and Increased Levels of Nerve Growth Factor in Hippocampus and Cortical Areas

Christoph Hock, MD; Klaus Heese, PhD; Christine Hulette, MD, PhD; Carlyn Rosenberg, MS; Uwe Otten, MD

Background: Nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), neurotrophin 3 (NT-3), and neurotrophin 4/5 (NT-4/5) are members of the neurotrophin gene family that support the survival of specific neuronal populations, including those that are affected by neurodegeneration in Alzheimer disease (AD).

Objective: To determine whether neurotrophin protein levels are altered in the AD-affected brain compared with control brains.

Methods: We quantitated protein levels of NGF, BDNF, NT-3, and NT-4/5, and calculated neurotrophin/NT-3 ratios in AD-affected postmortem hippocampus, frontal and parietal cortex, and cerebellum, and compared them with age-matched control tissue (patients with AD/controls: hippocampus, 9/9 cases; frontal cortex, 19/9; parietal cortex, 8/5; and cerebellum, 5/7, respectively). We applied highly sensitive and specific enzyme-linked immunosorbent assays in rapid-autopsy–derived brain tissue (mean±SD postmortem interval, 2.57±1.75 h, n=71) to minimize postmortem proteolytic activity.

Results: Levels of BDNF were significantly reduced in hippocampus and parietal cortex (P<.001, and P=.01) as well as BDNF/NT-3 ratios in frontal and parietal cortices (P<.05, and P=.01) in the group with AD compared with the control group. Levels of NGF and NGF/NT-3 ratio were significantly elevated in the group with AD compared with the control group in the hippocampus and frontal cortex (P<.001). Levels of NT-4/5 and the NT-4/NT-3 ratio were slightly reduced in hippocampus and cerebellum in the group with AD compared with the control group (P<.05). In contrast, the levels of NT-3 were unchanged in all brain regions investigated.

Conclusion: Decreased levels of BDNF may constitute a lack of trophic support and, thus, may contribute to the degeneration of specific neuronal populations in the AD-affected brain, including the basal forebrain cholinergic system.

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SUBJECTS AND METHODS

The clinical diagnosis of AD was made according to the National Institute of Neurological and Communicative Disorders and Stroke-Alzheimer's Disease and Related Disorders Association (NINCDS-ADRDA) criteria,10 and was histopathologically confirmed according to the Consortium to Establish a Registry for Alzheimer’s Disease (CERAD) criteria.19 Tissue samples from age-matched patients without neurological disorders were used as controls. Brain tissues from patients with AD and from controls were frozen in dry ice and stored at −85°C. Characteristics of the brain tissue samples investigated are given in the Table 1. Different brain tissue volumes were available for the analysis of the neurotrophin proteins. This fact explains the different sample sizes for the individual measurements. All available brain tissue samples were used for the analyses.

EXTRACTION OF NEUROTROPHINS FROM BRAIN TISSUE

Tissue samples were dissected from the frozen brain sections, weighed, thawed on ice, and supplied with ice-cold homogenization buffer (44 mmol/L of Tris-hydrochloride [pH 7.4], 300 mmol/L of sodium chloride, 2 mmol/L of EDTA, 0.1 mmol/L of phenylmethylsulfonyl fluoride [PMSF], 0.1 mmol/L of benzethonium chloride, 0.1 mmol/L of benzamidine hydrochloride, 1 µg/mL of aprotinin, 0.010% Triton X-100, 0.010% sodium deoxycholate, 0.010% Nonidet P-40, 0.03% bovine serum albumin, 0.025% sodium azide), as described previously.20,21 Following homogenization with a pestle, the solutions were sheared 5 times by a sterile ice-cold needle (1.2 × 10 mm). An additional sonication step occurred for 10 seconds within 2-mL Eppendorf tubes (Eppendorf Inc, Hamburg, Germany) placed in an ice bath. After centrifugation (14000 rpm for 25 minutes at 4°C) the S1 supernatants were collected and stored at −80°C. The pellet-containing tubes were taken and each pellet was resuspended in homogenization buffer with a pH 7.0 via a 3-second sonication step. The probes were subjected to centrifugation (13000 rpm for 30 minutes at 4°C) and the S2 supernatant was mixed with the former supernatant fraction S1. After an additional sedimentation step (14000 rpm for 7 minutes at 4°C), the supernatants were directly placed on the prepared enzyme-linked immunosorbent assay (ELISA) plates coated with the first neurotrophin-specific antibody. Brain tissue samples of about 30 mg wet weight were homogenized in 1 mL of homogenization buffer. Thus, about 6-mg weight wet was analyzed in each of the 120-µL brain supernatants used for the ELISAs. Nonspecific binding was blocked with block and sample 5× buffer (Promega, Madison, Wis.).

NEUROTROPHIN ELISAs

After a probe/standard-incubation period of 20 hours at 4°C, ELISA plates were washed 5 times and incubated with the second antineurotrophin antibody. Before color reaction occurred, wells were incubated with a third enzyme-linked antibody followed by addition of a chromogenic substrate.

Brain tissue levels of NGF were measured by an ELISA as described recently.22 Black 96-well microplates (Nunc Inc, Wiesbaden, Germany) were coated with monoclonal anti-β (2.5S, 7S) NGF antibodies (Ab) (clone 847).

RESULTS

NEUROTROPHIN CONCENTRATIONS

Brain tissue levels of NGF were significantly elevated in the group with AD, as compared with the control group, in the hippocampus and frontal cortex (P<.001, and P<.01, respectively) (Figure 1). Nerve growth factor concentrations in the hippocampus of the group with AD amounted to 32.2±4.4 pg/mg (mean±SEM, n=9), compared with 5.6±1.1 pg/mg for the control group (n=9). Nerve growth factor levels in the frontal cortex of the group with AD were 13.8±1.2 pg/mg (n=19), compared with 3.5±0.7 pg/mg for the control group (n=9). Nerve growth factor levels in the parietal cortex were slightly increased compared with the control group, but this difference did not reach statistical significance (Table 2). Nerve growth factor levels in the cerebellum were not different between the group with AD and the control group (Table 2). In the hippocampus and parietal cortex, levels of BDNF were significantly reduced in the group with AD as compared with the control group (P<.001, and P<.01, respectively). Brain-derived neurotrophic factor concentrations in the hippocampus of the group with AD were 41.4±3.8 pg/mg (n=9), compared with 71.2±5.5 pg/mg in the control group (n=9). Brain-derived neurotrophic factor levels in the parietal cortex of the group with AD were 10.4±1.5 pg/mg (n=8), compared with 21.9±3.1 pg/mg in the control group (n=5). Brain-derived neurotrophic factor levels in the frontal cortex were slightly decreased for the group with AD compared with the control group, but this difference did not reach statistical significance, and BDNF levels in the cerebellum were not different between the group with AD and the control group (Table 2). Levels of NT-4/5 were slightly decreased in the hippocampus and cerebellum in the group with AD compared with the control group (AD, 42.0±6.1 pg/mg, n=9; control, 65.2±6.9 pg/mg, n=9; P<.05 [t test], P=.06 [Mann-Whitney test]; AD, 29.8±2.4 pg/mg, n=5; control, 41.8±3.5 pg/mg, n=7; P<.05) (Table 2). Levels of NT-3 were unchanged in all brain regions investigated.

NEUROTROPHIN/NT-3 RATIOS

Nerve growth factor/NT-3 ratios were significantly elevated in hippocampus and frontal cortex in the group with AD compared with the control group (0.34±0.06 and 0.06±0.01, P<.001; 0.5±0.07 and 0.19±0.0, P<.001).
Figure 2 and Table 3). In contrast, BDNF/NT-3 ratios were significantly reduced in frontal and parietal cortices in the group with AD group compared with the control group (0.67±0.09 and 1.08±0.14, P<.05; 0.30±0.04 and 0.71±0.14, P<.01) (Table 3). Brain-derived neurotrophic factor/NT-3 ratio in the hippocampus was apparently decreased in the group with AD compared with the control group, but this difference did not reach statistical significance (P=.056, t test; P=.058, Mann-Whitney test). Finally, NT-4/NT-3 ratio was significantly reduced in the AD-affected hippocampus compared with the control group (0.39±0.03 and 0.66±0.05, P<.001) (Table 3).

Further analyses revealed that there was no apparent correlation of brain tissue levels of neurotrophins or neurotrophin/NT-3 ratio with APOE genotype, age, sex, or neuropathological staging.
This study demonstrates decreased levels of BDNF in hippocampus and parietal cortex, and increased levels of NGF in hippocampus and frontal cortex in AD-affected brain tissue compared with control brain tissue measured by sensitive and specific ELISAs. We selected these brain regions because they are differentially affected by the histopathological changes during neurodegeneration in AD. Usually, the hippocampal area, the perforant path, and the entorhinal cortex exhibit neurofibrillary tangles earliest in the course of AD. Among cortical association areas, the parietal cortex shows deposition of B-amyloid plaques, neurofibrillary tangles, and neuronal loss earlier in the course of AD.
Figure 2. Brain tissue levels of neurotrophins expressed as neurotrophin/NT-3 ratios; nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), neurotrophin 3 (NT-3), and neurotrophin 4/5 (NT-4/5) in patients with Alzheimer disease (AD)/age-matched healthy control subjects in the hippocampus (Hipp), frontal cortex (FCx), parietal cortex (PCx), and cerebellum (Cbl) were as follows: hippocampus, 9/9; frontal cortex, 19/9; parietal cortex, 8/5; and cerebellum, 5/7, respectively. Ratios are given as mean ± SEM. Asterisk indicates P < .001, Mann-Whitney test; dagger, P < .05, Mann-Whitney test; and double dagger, P < .01, Mann-Whitney. Statistical significance was P < .05.

Table 3. Brain Tissue Levels of Neurotrophins Expressed as Neurotrophin/NT-3 Ratios

<table>
<thead>
<tr>
<th>Neurotrophin/NT-3 Ratio</th>
<th>Hippocampus</th>
<th>Frontal Cortex</th>
<th>Parietal Cortex</th>
<th>Cerebellum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients With AD (n = 9)</td>
<td>controls (n = 9)</td>
<td>Patients With AD (n = 19)</td>
<td>controls (n = 9)</td>
<td>Patients With AD (n = 8)</td>
</tr>
<tr>
<td>NGF/NT-3</td>
<td>Mean ± SEM 0.34 ± 0.06*</td>
<td>0.06 ± 0.01</td>
<td>0.54 ± 0.07*</td>
<td>0.19 ± 0.05</td>
</tr>
<tr>
<td></td>
<td>Range 0.08-0.64</td>
<td>0.01-0.11</td>
<td>0.23-1.49</td>
<td>0.04-0.48</td>
</tr>
<tr>
<td>BDNF/NT-3</td>
<td>Mean ± SEM 0.48 ± 0.09</td>
<td>0.77 ± 0.10</td>
<td>0.67 ± 0.09†</td>
<td>1.08 ± 0.14</td>
</tr>
<tr>
<td></td>
<td>Range 0.11-1.04</td>
<td>0.37-1.42</td>
<td>0.25-1.49</td>
<td>0.39-1.58</td>
</tr>
<tr>
<td>NT-4/5/NT-3</td>
<td>Mean ± SEM 0.39 ± 0.03*</td>
<td>0.66 ± 0.05</td>
<td>1.11 ± 0.13</td>
<td>1.22 ± 0.12</td>
</tr>
<tr>
<td></td>
<td>Range 0.24-0.62</td>
<td>0.47-0.86</td>
<td>0.30-2.17</td>
<td>0.38-1.66</td>
</tr>
</tbody>
</table>

Asterisk indicates P < .001, Mann-Whitney test; dagger, P < .05, Mann-Whitney test; and double dagger, P < .01, Mann-Whitney test.

lister than the frontal cortex, whereas the cerebellum is usually preserved and may serve as a negative control. We used rapid-autopsy–derived brain tissue to minimize postmortem proteolytic modifications and assayed for the 4 neurotrophins in parallel under well-standardized conditions. In addition, to control for potential effects of alterations in tissue wet weight on neurotrophin concentrations, we calculated neurotrophin/NT-3 ratios. We used NT-3 as the denominator, because NT-3 levels did not show changes in brain distribution in this study. Significant decreases of BDNF protein levels were detected in the hippocampus and parietal cortex in AD-affected brain tissue compared with control brain tissue. Brain-derived neurotrophic factor/NT-3 ratio analysis confirmed the observed decrease in the parietal cortex and indicated a further decrease in the frontal cortex in the group with AD compared with the control group. Brain-derived neurotrophic factor/NT-3 ratio in the hippocampus was also decreased in the group with AD compared with the control group, but this difference did not reach statistical significance in that type of analysis. Brain-derived neurotrophic factor protein and its signaling receptor trkB are widely expressed in the developing and adult central nervous system in a number of neuronal subpopulations including basal forebrain cholinergic and γ-aminobutyric acid neurons, mesencephalic γ-aminobutyric acid neurons, substantia nigra dopaminergic neurons, cerebellar granule cells, as well as central nervous system neurons of the striatum, hippocampus, and trigeminal mesencephalic nucleus. Brain-derived neurotrophic factor not only acts on hippocampal pyramidal and dentate granule cells, but these neurons also produce BDNF to act on innervating basal forebrain cholinergic neurons. Similar to NGF, BDNF protects basal forebrain cholinergic neurons from degenerative changes after axotomy in the adult brain. In addition to the basal forebrain cholinergic system, neurons containing 5-hydroxytryptamine are also reduced in the AD-affected brain and 5-hydroxytryptamine deficiencies were associated with depression and behavioral disturbances in AD. Our finding is in line with a previous report of reduced BDNF messenger RNA levels in the hippocampus, measured by in situ hybridization, with reduced BDNF expression in the parietal cortex measured by room temperature–polymerase chain reaction and with reduced BDNF protein levels in the entorhinal cortex in the brain of patients with AD. Thus, diminished BDNF production may contribute to a reduced neurotrophic support of cholinergic and 5-hydroxytryptamine-containing neurons during neurodegeneration in AD. Our finding of increased levels of NGF as well as NGF/NT-3 ratios in the hippocampus and frontal cortex is in agreement with previous reports where NGF protein levels were shown to be increased in cortical and subcortical brain areas including the frontal and parietal cortex and the hippocampus. In contrast to BDNF, NGF messenger RNA levels were reported to be unchanged in...
AD-affected cortex, hippocampus, and septum/nucleus basalis area. Increased brain tissue levels of NGF in various brain regions in AD may be caused by both reduced uptake and retrograde transport of NGF to NGF-sensitive cell bodies. Recently, it was shown that expression and protein levels of the specific NGF high-affinity receptor trkA were reduced in target regions of basal forebrain cholinergic neurons, such as the cortical association areas. In addition, inflammatory signals may induce an additional production of NGF in activated microglial cells.

In agreement with previous reports, we found no differences in NT-3 levels between patients with AD and controls in regions affected by the characteristic neuropathology of AD, including the hippocampus, entorhinal, and parietal cortex. In contrast, NT-4 measurements and calculation of NT-4/NT-3 ratios indicated a slight decrease of NT-4 in the AD-affected hippocampus. Surprisingly, we also found a significant reduction in NT-4/5 levels in the cerebellum of patients with AD. It is unclear in which cell types these changes are manifest. Recently, Skaper et al32 reported that neurotrophins, including BDNF and NT-4/5, rescued cerebellar granule neurons from oxidative stress-mediated apoptotic death. However, a potential regulatory role of NT-4 in the hippocampus and cerebellum of patients with AD remains to be investigated.

We demonstrated decreased levels of BDNF and increased levels of NGF in hippocampus and cortical areas in AD-affected brain tissue compared with control brain tissue. Decreased levels of BDNF may be associated with lack of trophic support and may contribute to the degeneration of specific neuronal subpopulations in the AD-affected brain, including the basal forebrain cholinergic system. Elevated levels of NGF may reflect a reduced uptake and retrograde transport by the NGF high-affinity receptor trkA.

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