Regional Metabolic Alterations in Alzheimer's Disease: An in vitro ¹H NMR Study of the Hippocampus and Cerebellum

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The concentrations of selected metabolites in the hippocampus and cerebellum of 13 Alzheimer's disease (AD) and four nondemented postmortem brains were measured using high resolution ¹H NMR spectroscopy. For both the hippocampal region and the cerebellum, the putative neuronal marker N-acetyl aspartate (NAA) was significantly lower in AD brains relative to the nondemented brains. For the hippocampal region, the NAA concentration correlated inversely with semiquantitative assessments of neuronal loss and neurofibrillary tangles. The γ-aminobutyric acid levels in both hippocampus and cerebellum of an age- and a postmortem interval-matched subset of AD brains were lower than those of the controls. Because the cerebellum is generally thought to be unaffected by AD, the NAA decrease in the Alzheimer cerebellum may be due to lesions of either the Alzheimer or non-Alzheimer type in contralateral cerebrum.

Neuropathologic changes that occur in Alzheimer's disease (AD) include brain atrophy, regional shrinkage and loss of neurons, alterations in the fine structure of innumerable neuronal processes, abnormal fibers in neuronal cell bodies, and a decrease in activity of the enzyme choline acetyltransferase (Bowen et al., 1976; Perry et al., 1978). In histopathologic terms, the appearance of altered cortical neurons and neuronal loss may be more specific for AD than plaques and tangles (Braak et al., 1986; Mann et al., 1984). As the disease progresses, the hippocampal region and the cerebellum, the putative neuronal marker N-acetyl aspartate (NAA) was significantly lower in AD brains relative to the nondemented brains. For the hippocampal region, the NAA concentration correlated inversely with semiquantitative assessments of neuronal loss and neurofibrillary tangles. The γ-aminobutyric acid levels in both hippocampus and cerebellum of an age- and a postmortem interval-matched subset of AD brains were lower than those of the controls. Because the cerebellum is generally thought to be unaffected by AD, the NAA decrease in the Alzheimer cerebellum may be due to lesions of either the Alzheimer or non-Alzheimer type in contralateral cerebrum.

Although in vitro confirmation of the above results is essential, few in vitro ¹H NMR studies of postmortem AD brain tissue have appeared. Klunk et al. (1992) reported a decrease in NAA and γ-aminobutyric acid (GABA), and an increase in Glu in perchloric acid extracts of AD brains relative to controls. There was no change in the other metabolites studied. More recent preliminary results by Klunk and colleagues (1994) suggested increases in Inos, Glu, and aspartate, in partial contradiction to their earlier results. Kwo-On-Yuen et al. (1994) saw reductions of NAA (other metabolites were not studied) in AD in several brain regions, and a correlation of cortical NAA level with severity of dementia. Recently, we (Mohanakrishnan et al., 1995) reported results for 90% methanol extracts of temporoparietal cortex, where NAA, Cr, and GABA were decreased in AD relative to nondemented brain, but no changes were seen for Glu or Inos.

In this report, we present our findings from high-resolution ¹H NMR studies of extracts of the hippocampus...
and the cerebellum of postmortem Alzheimer brains. This study was undertaken in view of the prevailing discrepancies in the literature. The choice of hippocampus as one region of this study is because of its role in cognition and memory. The cerebellum was included as the second region to find if there are any metabolic changes, because cerebellum is considered the reference region to evaluate the hypoperfusion (or hypometabolism) in other brain regions in AD.

METHOD
The postmortem brain samples were obtained either locally from the autopsy cases at the University of Arkansas for Medical Sciences (cases A, K, L, and N–Q in Table 1) or Veterans Administration hospitals in Little Rock or from the Brain Tissue Resource Center (cases B–J and M) supported by the National Institute on Aging at McLean Hospital, Belmont, MA. Immediately after removal from the body, one half (left or right) of each brain was frozen, stored typically at –70 °C, and warmed to 0 °C just before methanol–water extraction. About 0.7–1.0 g of brain tissue was removed from each region of interest for extraction. The formalin-fixed contralateral side of each brain was used for histologic examination by conventional neuropathologic methods (Mirra et al., 1991; Tomlinson, 1992), as described previously (Mohanakrishnan et al., 1995). The brain tissue samples from the regions of interest were further sliced into thin sections, frozen with liquid nitrogen, powdered, and extracted with ice-cold, 90% aqueous methanol in the manner described by Koller et al. (1984). Methanol in the extract was removed under a thin stream of dry nitrogen, and the concentrate was freeze-dried and resuspended in a mixture of H2O and D2O. The pH of the extracts ranged from 6.85 to 7.0.

The 1H NMR spectra were acquired at ambient probe temperature (typically 23–25 °C) at 300.5 MHz on a General Electric GN-300WB FT NMR spectrometer using a 90° pulse of width 6 μs and a delay of 20 s. The free induction decays (FIDs) were subjected to zero filling and a simple exponential multiplication of 0.1 Hz before Fourier transformation. The residual water signal was attenuated by the application of a 4-s presaturation pulse before data acquisition. Each spectrum was typically the result of 64 co-added FIDs. The chemical shift referencing was relative to the readily identifiable methyl signal of NAA at 2.02 ppm. The metabolite concentrations were estimated relative to a reference of known concentration in a capillary tube concentric to the 5-mm NMR sample tube. The water contents of gray and white matter of human brain are 0.819 and 0.716 ml/g, respectively (Agranoff and Hajra, 1994). To allow for traces of white matter in a gray matter sample, the water content was assumed to be 0.79 ml/g for the cerebellum and the hippocampus. The quantitation procedure was tested and standardized using solutions of known concentrations of metabolites such as Cr, Cho, NAA, and N-acetyl aspartyl glutamate (NAAG).

Figure 1 shows portions (0.8–4.2 ppm region and its expansions) of the 300 MHz 1H NMR spectrum of the methanol-water extract of one such case (case J in Table 1), a 76-year-old woman with AD. The signals originating from some metabolites of interest have been assigned to the appropriate chemical moieties. The signal assignments were based on a comparison to similar studies in several laboratories (Cerdan et al., 1985; Petroff et al., 1988, 1989; Komoroski et al., 1991; Holowenko et al., 1992). The metabolite concentrations were estimated using the signal assignments in Figure 1, as described in detail previously (Mohanakrishnan et al., 1995).

There was no detectable acetate in samples with low postmortem interval (PMI) values. Hence acetate, whenever detected, was assumed to originate from the hydrolysis of NAA, and NAA concentrations were corrected accordingly. This correction did not alter the significance of any statistical analysis performed on the NAA results. Other metabolite concentrations were uncorrected for any loss during extraction and were expressed as absolute concentrations (mM) and not as mol percentages or metabolite ratios. This was necessary since there is no a priori knowledge of quantitative changes in the concentration of each metabolite by the disease process, the PMI, or perimortem factors in each case. The concentrations of metabolites for the Alzheimer (AD) and nondemented (ND) groups were given as a mean ± SD.

Differences in the mean levels of metabolites were assessed using univariate analysis with Bonferroni-adjusted significance levels used to compensate for multiple comparisons. Using Levene’s test, significant heterogeneity of variance was found only for hippocampal GABA and cerebellar inositol; group differences for these two metabolites were assessed using Welch’s t-test. Student’s t-test was used to test the group differences for all other metabolites. Analysis of covariance (ANCOVA) was performed using NAA as a dependent variable, disease status as the grouping variable, and age and PMI as the covariates. Tests for parallelism of slopes and homogeneity of variance indicated that the assumptions of ANCOVA were met. Because age and PMI were not matched between the two groups, the t-test was used to compare the ND group with a subset of AD group consisting of the seven youngest cases. The age and PMI of this subset were matched with those of the control group. NFT, SP, and NL were ranked for the range of severity: 1 for mild, 2 for moderate, 3 for severe, and 4 for very severe. A rank of 0 was assigned to the controls. Spearman correlation coefficients were computed for the association between the metabolite estimates and the numerical ranking of histopathology. The correlations were considered significant when p < .05.

RESULTS AND DISCUSSION
Because of the difficulty in obtaining tissue samples from neurologically normal brain (free of aging-related episodes such as infarcts, white matter lesions, and stroke), we were able to include only four nondemented controls in this study. The PMIs for the cases in Tables 1 and 2 vary from 1.4 to 16.8 h. Because it was not possible to control the PMIs, the possibility that some metabolite concentrations may have changed during this time cannot be ruled out. Subject age ranged from 63 to 95. Although all ND and only 38% of AD cases were male, no effect is expected for a difference in sex. Among AD cases, no significant gender-related differences were observed for NAA in hippocampus or in cerebellum. Also, no such effect of sex has been reported in numerous in vivo studies. Indeed, a recent study (Charles et al., 1994)
Figure 1. Portions of the 300-MHz proton NMR spectrum of the methanol-water extract of the hippocampus of a 76-year-old female AD patient (case J). Spectrum A is the 0.8–4.2 ppm region; B is the 1.8–2.8 ppm region expanded (vertical gain of 4 x A); and C is the 2.8–4.2 ppm region expanded (vertical gain = 2 x A).
Table 1. Demographic/Neuropathologic Data and Metabolite Concentrations in the Hippocampus of Autopsy Cases

<table>
<thead>
<tr>
<th>Case</th>
<th>Age/Sex</th>
<th>PMI</th>
<th>Side</th>
<th>SP</th>
<th>NFT</th>
<th>NL</th>
<th>NAA</th>
<th>NAAG</th>
<th>Cre</th>
<th>Cho</th>
<th>Glu</th>
<th>Inos</th>
<th>GABA</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>64/F</td>
<td>13.5</td>
<td>L</td>
<td>s</td>
<td>s</td>
<td>u</td>
<td>1.95</td>
<td>0.19</td>
<td>4.69</td>
<td>1.56</td>
<td>6.90</td>
<td>4.06</td>
<td>1.00</td>
</tr>
<tr>
<td>B</td>
<td>94/F</td>
<td>1.4</td>
<td>L</td>
<td>m</td>
<td>s</td>
<td>v</td>
<td>0.30</td>
<td>0.36</td>
<td>5.67</td>
<td>1.45</td>
<td>8.25</td>
<td>3.58</td>
<td>0.88</td>
</tr>
<tr>
<td>C</td>
<td>91/F</td>
<td>5.5</td>
<td>L</td>
<td>s</td>
<td>s</td>
<td>v</td>
<td>1.89</td>
<td>0.36</td>
<td>5.59</td>
<td>2.31</td>
<td>4.61</td>
<td>4.57</td>
<td>0.98</td>
</tr>
<tr>
<td>D</td>
<td>83/F</td>
<td>12.4</td>
<td>R</td>
<td>m</td>
<td>s</td>
<td>s</td>
<td>2.82</td>
<td>0.28</td>
<td>6.00</td>
<td>1.50</td>
<td>7.24</td>
<td>2.15</td>
<td>0.66</td>
</tr>
<tr>
<td>E</td>
<td>82/M</td>
<td>14.0</td>
<td>R</td>
<td>v</td>
<td>s</td>
<td>m-s</td>
<td>2.29</td>
<td>0.25</td>
<td>6.84</td>
<td>2.09</td>
<td>7.78</td>
<td>2.31</td>
<td>0.78</td>
</tr>
<tr>
<td>F</td>
<td>87/M</td>
<td>1.4</td>
<td>L</td>
<td>m</td>
<td>m</td>
<td>m</td>
<td>2.99</td>
<td>0.36</td>
<td>6.00</td>
<td>1.50</td>
<td>7.24</td>
<td>2.15</td>
<td>0.66</td>
</tr>
<tr>
<td>G</td>
<td>81/F</td>
<td>7.0</td>
<td>L</td>
<td>m</td>
<td>m</td>
<td>m</td>
<td>2.00</td>
<td>0.16</td>
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<td>8.20</td>
<td>4.00</td>
<td>1.10</td>
</tr>
<tr>
<td>H</td>
<td>79/F</td>
<td>4.8</td>
<td>L</td>
<td>m</td>
<td>m</td>
<td>m</td>
<td>2.06</td>
<td>0.25</td>
<td>5.82</td>
<td>2.61</td>
<td>8.64</td>
<td>6.92</td>
<td>1.09</td>
</tr>
<tr>
<td>I</td>
<td>95/F</td>
<td>4.7</td>
<td>R</td>
<td>m</td>
<td>l</td>
<td>s</td>
<td>2.22</td>
<td>0.25</td>
<td>6.04</td>
<td>2.31</td>
<td>6.59</td>
<td>3.22</td>
<td>0.77</td>
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<tr>
<td>J</td>
<td>76/F</td>
<td>7.9</td>
<td>R</td>
<td>m</td>
<td>s</td>
<td>s</td>
<td>2.40</td>
<td>0.30</td>
<td>7.40</td>
<td>2.79</td>
<td>8.86</td>
<td>7.05</td>
<td>1.14</td>
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<tr>
<td>K</td>
<td>69/M</td>
<td>12.0</td>
<td>L</td>
<td>v</td>
<td>s</td>
<td>u</td>
<td>2.39</td>
<td>0.26</td>
<td>7.62</td>
<td>2.49</td>
<td>8.06</td>
<td>8.39</td>
<td>0.95</td>
</tr>
<tr>
<td>L</td>
<td>84/M</td>
<td>11.0</td>
<td>R</td>
<td>m</td>
<td>s</td>
<td>u</td>
<td>1.02</td>
<td>0.08</td>
<td>3.85</td>
<td>1.26</td>
<td>3.46</td>
<td>5.06</td>
<td>0.67</td>
</tr>
<tr>
<td>M</td>
<td>78/M</td>
<td>1.9</td>
<td>L</td>
<td>m</td>
<td>s</td>
<td>v</td>
<td>3.67</td>
<td>0.46</td>
<td>9.32</td>
<td>2.16</td>
<td>10.52</td>
<td>8.95</td>
<td>1.98</td>
</tr>
<tr>
<td>N</td>
<td>74/M</td>
<td>16.8</td>
<td>nondemented</td>
<td></td>
<td></td>
<td></td>
<td>3.57</td>
<td>0.35</td>
<td>3.41</td>
<td>1.26</td>
<td>6.16</td>
<td>2.83</td>
<td>1.00</td>
</tr>
<tr>
<td>O</td>
<td>63/M</td>
<td>13.5</td>
<td>nondemented</td>
<td></td>
<td></td>
<td></td>
<td>3.22</td>
<td>0.18</td>
<td>6.75</td>
<td>1.54</td>
<td>6.48</td>
<td>3.26</td>
<td>0.87</td>
</tr>
<tr>
<td>P</td>
<td>70/M</td>
<td>9.0</td>
<td>nondemented</td>
<td></td>
<td></td>
<td></td>
<td>4.16</td>
<td>0.51</td>
<td>10.07</td>
<td>2.49</td>
<td>9.58</td>
<td>7.04</td>
<td>1.82</td>
</tr>
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</table>

Notes: The neuropathology in Tables 1 and 2 is for the contralateral side. The metabolite concentrations in Tables 1–3 are in m mol per liter of tissue water. PMI = postmortem intervals (in hrs); SP = plaques; NFT = neurofibrillary tangles; NL = neuronal loss; NAA = N-acetyl-aspartate; NAAG = N-acetyl aspartyl glutamate; Cre = creatine; Cho = choline; Inos = inositol; GABA = γ-aminobutyric acid; nd = not detectable. The letter designations F, M, R, L, l, m, s, u, and v are for female, male, right, left, mild, moderate, severe, undetermined, and very severe, respectively.

Table 2. Neuropathologic Data and Metabolite Concentrations in the Cerebellum of Autopsy Cases

<table>
<thead>
<tr>
<th>Case</th>
<th>Cerebellum</th>
<th>Cerebrum</th>
<th>NAA</th>
<th>NAAG</th>
<th>Cre</th>
<th>Cho</th>
<th>Glu</th>
<th>Inos</th>
<th>GABA</th>
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<tbody>
<tr>
<td>A</td>
<td>Autolysis of granular layer</td>
<td>Infarcts in thalamus, frontal cortex, striatum</td>
<td>1.46</td>
<td>0.28</td>
<td>5.49</td>
<td>1.13</td>
<td>5.74</td>
<td>3.47</td>
<td>0.55</td>
</tr>
<tr>
<td>B</td>
<td>Mild neuronal loss, gliosis</td>
<td>Infarcts in thalamus, frontal cortex, striatum</td>
<td>3.86</td>
<td>0.39</td>
<td>10.87</td>
<td>2.43</td>
<td>9.66</td>
<td>4.63</td>
<td>1.53</td>
</tr>
<tr>
<td>C</td>
<td>Mild atrophy</td>
<td>Striatal infarcts</td>
<td>1.68</td>
<td>0.26</td>
<td>4.76</td>
<td>0.67</td>
<td>5.50</td>
<td>0.64</td>
<td>0.51</td>
</tr>
<tr>
<td>D</td>
<td>Scattered rod-like microglia</td>
<td>Mild cortical hypoxic ischemic episodes</td>
<td>2.58</td>
<td>0.42</td>
<td>7.62</td>
<td>1.23</td>
<td>8.26</td>
<td>3.52</td>
<td>1.35</td>
</tr>
<tr>
<td>E</td>
<td>Infarct-cavitated globus pallidus</td>
<td>Infarct-cavitated</td>
<td>1.56</td>
<td>0.24</td>
<td>5.43</td>
<td>0.75</td>
<td>2.73</td>
<td>2.17</td>
<td>0.56</td>
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<tr>
<td>F</td>
<td>Mild atrophy</td>
<td>Cortico-subcortical infarcts</td>
<td>2.78</td>
<td>0.18</td>
<td>4.42</td>
<td>1.23</td>
<td>4.47</td>
<td>2.14</td>
<td>0.56</td>
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<tr>
<td>G</td>
<td>Mild atrophy</td>
<td>Hydrocephalus</td>
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<td>0.47</td>
<td>5.83</td>
<td>1.13</td>
<td>6.09</td>
<td>3.02</td>
<td>0.91</td>
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<tr>
<td>H</td>
<td>Mild atrophy</td>
<td>Hydrocephalus</td>
<td>1.76</td>
<td>0.25</td>
<td>5.93</td>
<td>1.21</td>
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<td>1.10</td>
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<tr>
<td>I</td>
<td>Mild atrophy</td>
<td>Carcinoma in hippocampus and substantia nigra</td>
<td>2.16</td>
<td>0.33</td>
<td>7.84</td>
<td>2.32</td>
<td>9.83</td>
<td>4.37</td>
<td>1.22</td>
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<td>9.80</td>
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<td>Carcinoma in hippocampus and substantia nigra</td>
<td>2.70</td>
<td>0.29</td>
<td>10.99</td>
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<td>0.95</td>
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<td>2.96</td>
<td>0.38</td>
<td>10.78</td>
<td>2.21</td>
<td>7.29</td>
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<td>1.15</td>
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<td>M</td>
<td>Mild atrophy</td>
<td>Carcinoma in hippocampus and substantia nigra</td>
<td>1.31</td>
<td>0.33</td>
<td>2.34</td>
<td>0.30</td>
<td>3.68</td>
<td>2.34</td>
<td>0.30</td>
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<td>N</td>
<td>Mild atrophy</td>
<td>Carcinoma in hippocampus and substantia nigra</td>
<td>5.16</td>
<td>0.49</td>
<td>11.87</td>
<td>1.90</td>
<td>10.22</td>
<td>5.70</td>
<td>1.94</td>
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<td>O</td>
<td>Mild atrophy</td>
<td>Carcinoma in hippocampus and substantia nigra</td>
<td>3.86</td>
<td>0.36</td>
<td>4.00</td>
<td>1.13</td>
<td>6.74</td>
<td>2.06</td>
<td>1.03</td>
</tr>
<tr>
<td>P</td>
<td>Mild atrophy</td>
<td>Carcinoma in hippocampus and substantia nigra</td>
<td>2.40</td>
<td>0.34</td>
<td>7.87</td>
<td>1.23</td>
<td>6.27</td>
<td>2.33</td>
<td>0.91</td>
</tr>
<tr>
<td>Q</td>
<td>Mild atrophy</td>
<td>Carcinoma in hippocampus and substantia nigra</td>
<td>4.69</td>
<td>0.78</td>
<td>8.72</td>
<td>1.44</td>
<td>14.52</td>
<td>5.44</td>
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</table>

Notes: The neuropathology is for the contralateral side. The metabolite concentrations are in m mol per liter of tissue water. NAA = N-acetyl-aspartate; NAAG = N-acetyl aspartyl glutamate; Cre = creatine; Cho = choline; Inos = inositol; GABA = γ-aminobutyric acid.

reported that there were no significant differences for in vivo localized 1H NMR estimates of choline, creatine, and NAA between men and women.

Table 1 summarizes the demographic and neuropathologic data of the autopsy cases studied and the concentrations of NAA, NAAG, Cre, Glu, Cho, and Inos in the hippocampal region. It should be noted that the neuropathologic information came from one side of each brain, while the estimation of metabolite concentrations was performed using the contralateral side. Although the distribution of neuro-
pathologic changes of AD was assumed to be bilaterally symmetric, it is conceivable that the extent of neuropathology in some of these cases is asymmetric (Haxby et al., 1990), which is a limitation of the study. Also, the assessments of the amounts of NFT, SP, and NL can only be regarded as semiquantitative. Neuropathologic and metabolite results for the cerebella are listed in Table 2.

In comparing our concentrations with those in the literature (Petroff et al., 1989), it should be noted that the following factors can affect our measurements: the efficiency (~85%) of the extraction procedure, the decrease caused by aging, the likelihood of underestimation due to adherent water in frozen brains, the PMI, and perimortem factors. The concentrations may thus be as low as 70% of those for biopsy specimens from normal adults or in vivo estimates. In a recent study comparing in vivo and in vitro estimates for canine brain (Barker et al., 1994), the extraction efficiency of the perchloric acid method was found to be 65–70%.

The mean concentration of each metabolite is reduced in AD relative to control for both brain regions (Table 3) although statistically significant differences were found only for NAA in both areas. The NAA differences between the two groups remained statistically significant ($p = .007$ for hippocampus and $p = .018$ for cerebellum) and are statistically significant after adjustment for age and PMI. Comparison of the age- and PMI-matched AD subset to the control group using $t$-test showed that the concentrations of NAA and GABA of both hippocampus ($p < .003$ and $p < .04$, respectively) and cerebellum ($p < .01$ and $p = .03$, respectively) of the AD cases are significantly lower than those of the ND group. For the complete dataset we do not find a significant difference for GABA in the hippocampus.

The average concentration of NAA, the putative neuronal marker, in human cerebrum is 5–8 mM, the NAA content of gray matter being about 2 mM higher than that of white matter; the in vitro estimate of the NAA content of cerebellum is slightly lower than that of gray matter for human brain (Barker and Oldendorf, 1989). Assuming up to a 30% loss due to different factors, the NAA estimates for the hippocampus of nondemented cases N–Q are in reasonable agreement with those in the literature (for example, Petroff et al., 1989). The reduction seen here for the hippocampal NAA is in agreement with the reductions seen in vivo, and the reduction seen previously by us for temporoparietal cortex (Mohanakrishnan et al., 1995) and by Klunk et al. (1992, 1994) for frontal or temporal cortex.

Unlike Klunk et al. (1992), we found no significant differences for Glu. For Glu our average concentration in AD was lower than control supporting the in vivo work of Moats et al. (1994). Whereas Moats et al. saw a significant increase in Inos in vivo in AD relative to control, we found no difference. One possible explanation for the discrepancy is that in vitro results are more likely to emphasize the end stage of the disease rather than results acquired in vivo. However, an in vitro analysis at mid- to high-magnetic field strength will not suffer from the formidable problems of resonance overlap and baseline and other artifacts associated with an in vivo study.

Because the cerebellum is considered unaffected in AD, the NAA estimates for AD cases should be comparable to those of nondemented cases. It is evident from the results in Table 2 that this is not the case. Lesions in contralateral cerebrum may have contributed to the decreases in some metabolite concentrations in addition to age differences and perimortem factors (see below). However, the metabolite concentrations did not depend on atrophy. The use of segmentation methods with MRI and spectroscopic imaging data (Meyerhoff et al., 1995) also showed that the metabolic changes in AD in vivo were not due to atrophy. It should be noted that except for cases A and K, the AD cases of this study were age 75 and above, whereas nondemented cases were all below age 75. Klunk et al. (1994) have reported significant elevations in glycerophosphocholine and glycerophosphoethanolamine levels in AD cerebella compared to non-AD ones. This result suggests substantial cell (perhaps neuronal) damage in the cerebella of AD cases.

Cerebellar dysmetabolism in the contralateral side may accompany supra-tentorial lesions (Kushner et al., 1987). A recent single-photon emission computed tomography study suggests the existence of an inverse cerebrocerebellar functional relationship (crossed-cerebellar diaschisis) mediated through networks such as the corticoponto-cerebellar tract (Hanyu et al., 1993). Nondemented case P, who had a reduced NAA concentration in cerebellum, had a history of cerebrovascular accident with residual right-sided weakness, coronary artery disease, congestive heart failure, hypertension, and insulin-dependent diabetes mellitus. Decreased NAA concentration is often observed in instances of hypoxia, anoxia, ischemia, stroke, and diabetes (Kreis and Ross, 1992). The low concentrations of several metabolites (especially NAA) for case P are consistent with the case history. Admittedly, the explanation for the trend in the metabolite concentrations is not simple owing to variation in amounts of these metabolites in different cell types (Urenjak et al., 1992, 1993).

Correlations were computed for the concentration of each metabolite in the hippocampus vs the numerical ranks assigned to the assessments of neuropathologic parameters.
given in Table 1. The only significant correlations were for NAA vs NL (R = -0.87, p = 0.000045) and NAA vs NFT (R = -0.58, p = 0.015). The correlation of NAA with SP was close to significance (p = 0.068). Klunk et al. (1992) observed a correlation of NAA concentration with NFT and SP. Our results suggest that the NAA decrease is correlated with histopathologic parameters like NL and NFT in Alzheimer hippocampus. The correlation of NAA concentration with NL is consistent with the role of NAA as a neuronal marker.

In summary, the findings of this study are that NAA and possibly GABA are decreased in both hippocampus and cerebellum of AD brains compared to controls. The NAA levels of hippocampus were correlated with qualitative assessments of neuronal density/loss and the amounts of neurofibrillary tangles.

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REFERENCES


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