Proteomics Analysis of the Alzheimer's Disease Hippocampal Proteome

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Abstract. Alzheimer's disease (AD) is characterized by the presence of intracellular neurofibrillary tangles (NFT), extracellular senile plaques (SP), and synaptic loss. The hippocampus is a region that plays an important role in memory and cognitive function, and it is severely affected in AD. The levels of proteins in the hippocampus may provide a better understanding of the pathological changes known. In the present study we used two-dimensional gel electrophoresis and mass spectrometry techniques to determine changes in protein levels in AD and control hippocampus. We identified 18 proteins with altered protein levels that are involved in regulating different cellular functions. Protein levels were found to be significantly decreased for peptidyl prolyl cis-trans-isomerase (Pin1) (0.6-fold compared to control, p <0.03), dihydropyrimidinase-like protein 2 (DRP-2) (0.74-fold compared to control, p <0.02), phosphoglycerate mutase 1 (PGM1) (0.7-fold compared to control, p <0.01), beta-tubulin (0.34-fold compared to control, p <0.01), and aldolase A (0.87-fold compared to control, p <0.0002), whereas the protein levels were found to be significantly increased for enolase (1.35-fold compared to control, p <0.05), ubiquitin carboxyl terminal hydrolase L1 (UCH L1) (1.31-fold compared to control, p <0.02), triosephosphate isomerase (TPI) (1.38-fold compared to control, p <0.05), carbonic anhydrase II (CAH-II) (1.24-fold compared to control, p=<0.05), heat shock protein 70 (1.14-fold compared to control, p <0.03), fructose bisphosphate aldolase (1.38-fold compared to control, p <0.05), ferritin heavy chain (1.23-fold compared to control, p = 0.05), 2,2'-cyclic nucleotide 3' phosphodiesterase (CNPase) (1.12-fold compared to control, p <0.02), peroxiredoxin I (1.39-fold compared to control, p <0.05), and adenylate kinase I (1.19-fold compared to control, p <0.03). We found 2 proteins spots that were identified as glyceraldehyde-3-phosphate dehydrogenase (GAPDH). One of the spots showed a 1.28-fold increase in protein level compared to control (p <0.01), and the other spot showed a similar 1.26-fold increase in protein level compared to control (p <0.04). Thus, proteomics has provided knowledge of the levels of key proteins in AD brain. We discuss the functions regulated by these proteins with respect to AD pathology.

Keywords: Alzheimer's disease, protein expression, proteomics, glucose metabolism, cell cycle, antioxidant, structural proteins, synaptic process, scaffolding proteins

INTRODUCTION

Alzheimer's disease (AD) is the most common cause of dementia in the elderly [65]. AD is characterized by the presence of intracellular neurofibrillary tangles (NFT), extracellular senile plaques (SP), and synaptic loss [33,55]. NFT consist of paired helical filaments and related straight filaments, which are composed of hyperphosphorylated microtubule associated
protein tau [32]. The main component of SP is amyloid β-peptide (Aβ), a 40–42-amino acid peptide derived from proteolytic cleavage of amyloid-β protein precursor (AβPP) by the action of beta- and gamma-secretases. Aβ is considered to play a causal role in the development and progress of AD [70]. Among the various neurotransmitter systems, cholinergic neurons in the nucleus basalis of Meynert (NBM) are lost early in the course of AD, and the dysfunction of cholinergic neurons is believed to be involved in cognitive deficits in this disease [26].

The etiology of AD is largely unknown, but mutations of the genes for presenilin-1 (PS-1), presenilin-2 (PS-2) and amyloid precursor protein (APP) have been observed in inherited AD. In addition, there is also an association between AD and allele 4 of the apolipoprotein E (APOE) gene, endothelial nitric oxide synthase −3 gene, and alpha-2-macroglobulin. The exact biochemical mechanism of the pathogenesis of AD is still unknown, but several hypotheses have been proposed to explain AD pathogenesis including amyloid cascade, excitotoxicity, oxidative stress, and inflammation [10,11, 36,52,67].

Several lines of evidence suggest an important role for oxidative stress in the pathogenesis and/or progression of AD [10,13,14,46,52]. Protein oxidation, lipid peroxidation, nucleic acid and carbohydrate oxidation products were found to be elevated in AD brain [13, 14,35,46,52,68]. In addition the levels of antioxidant enzymes were found diminished in AD brain which strongly supports the role of oxidative stress in AD [1, 50]. In AD brain the products of oxidative stress were found in Aβ-rich regions such as cortex and hippocampus, but are not observed in cerebellum, where the Aβ accumulation was found to be negligible [35]. Further, the use of vitamin E in cell cultures diminishes Aβ (1–42)-induced toxicity, supporting a role for Aβ and oxidative stress in AD pathology [8,12,83].

Previously, we used redox proteomics to identify oxidatively modified proteins in several neurodegenerative disorders and model thereof. In AD we found several oxidatively modified proteins including creatine kinase BB (CK), glutamine synthase (GS), ubiquitin carboxy-terminal hydrolase L-1 (UCH-L-1), dihydropyrimidinase related protein 2 (DRP2), α-enolase, phosphoglycerate mutase 1, γ-soluble NSF attachment protein (SNAP), carbonic anhydrase II (CaH-II), peptidyl prolyl cis-trans isomerase (Pin 1), neuropilin peptide h3, triosephosphate isomerase and glyceraldehyde 3-phosphate dehydrogenase [17–19,74,77]. The aim of the current study was to investigate the differential levels of proteins in AD hippocampus using a proteomic approach. Proteomic techniques monitor global protein production and can provide a valuable insight into mechanisms of processes regulating the fate of the cell and also disease pathogenesis. Eighteen protein spots exhibiting a different level of proteins were identified by two-dimensional gel electrophoresis followed by trypsin digestion and mass spectrometric (MS) analysis. These proteins were found be involved in regulating various cellular functions that are discussed in this manuscript.

**MATERIALS AND METHODS**

**Control and AD brains**

Frozen hippocampal samples were obtained from 6 AD patients and 6 age matched controls for the present study. The Rapid Autopsy Program of the University of Kentucky Alzheimer’s Disease Center (UK ADC) provided autopsy samples with average postmortem intervals (PMIs) of 2.1 h for AD patients and 2.9 h for control subjects (Table 1). All AD patients displayed progressive intellectual decline and met NINCDS-ADRDA Workgroup criteria for the clinical diagnosis of probable AD [56]. Hematoxylin-eosin and modified- Bielschowsky staining, amyloid β-peptide (Aβ) antibody (10D5), and alpha-synuclein immunohistochemistry were used on multiple neocortical, hippocampal, entorhinal, amygdala, brainstem and cerebellum sections for diagnosis. Some patients were also diagnosed with AD plus dementia with Lewy bodies, but the results of this study showed no difference between AD patients with or without the presence of Lewy bodies. Control subjects underwent annual mental status testing and semi-annual physical and neurological exams, as a part of the UK ADC normal volunteer longitudinal aging study and did not have a history of dementia or other neurologic disorders. All control subjects had test scores in the normal range. Neuropathologic evaluation of control brains revealed only age associated gross and histopathologic alterations. All procedures to obtain human brain samples were approved by the University of Kentucky Institutional Review Board and experiments to be performed were approved by the Sanders-Brown Center on Aging Core Faculty.
### Table 1
Demographic characteristics of AD and control subjects

<table>
<thead>
<tr>
<th>Sample</th>
<th>Age at Death (Years)</th>
<th>Gender (M/F)</th>
<th>APO E Genotype, If Known (N)</th>
<th>Postmortem Interval (h)</th>
<th>MMSE (Mean of Last Test); Months Prior to Death Test Taken</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>85.8 ± 4.1</td>
<td>4/2</td>
<td>3/3 (3)</td>
<td>2.9 ± 0.23</td>
<td>28 ± 0.8</td>
</tr>
<tr>
<td>AD</td>
<td>84.5 ± 5.2</td>
<td>4/2</td>
<td>3/4 (2)</td>
<td>2.1 ± 0.47</td>
<td>15.7 ± 2.6</td>
</tr>
</tbody>
</table>

**Sample preparation**

Brain samples were minced and suspended in 10 mM HEPES buffer (pH 7.4) containing 137 mM NaCl, 4.6 mM KCl, 1.1 mM KH₂PO₄, and 0.6 mM MgSO₄ as well as protease inhibitors: leupeptin (0.5 mg/mL), pepstatin (0.7 μg/mL), type II soybean trypsin inhibitor (0.5 μg/mL), and PMSF (40 μg/mL). Homogenates were centrifuged at 14,000 g for 10 min to remove debris. Protein concentration in the supernatant was determined by the BCA method (Pierce, Rockford, IL, USA).

**Two-dimensional electrophoresis**

Samples (150 μg) were dissolved in rehydration buffer (8 M urea, 2 M thiourea, 2% CHAPS, 0.2% (v/v) biotin, 50 mM dithiothreitol (DTT), and bromophenol blue). Samples were sonicated in rehydration buffer on ice three times for 20 s intervals and were applied to a Ready Strip IPG (pH 3–10) (Bio-Rad, Hercules, CA, USA). The strip was then rehydrated at 50V for 16 h in a protean IEF cell (Bio-Rad). Isoelectric focusing was performed at 20°C as follows: 800 V for 2 h linear gradient, 1200 V for 4 h slow gradient, 8000 V for 8 h linear gradient, and 8000 V for 10 h rapid gradient. The strips were stored at −80°C until second dimension electrophoresis was performed. Gel strips were equilibrated for 10 min prior to second dimension separation in 50 mM Tris – HCl (pH 6.8) containing 6 M urea, 1% (w/v) sodium dodecyl sulfate, 30% (v/v) glycerol, and 0.5% dithiothreitol, and followed by re-equilibration for 10 min in the same buffer containing 4.5% iodoacetamide in place of dithiothreitol. Linear gradient precast criterion Tris-HCl gels (8–16%; Bio-Rad) were used to perform second dimension electrophoresis, and the electrophoresis was carried out at 200 V for 65 min.

**SYPRO ruby staining and image analysis**

The gels were fixed in a solution containing 10% (v/v) methanol, 7% (v/v) acetic acid for 20 min and stained overnight at room temperature with agitation in 50 ml of SYPRO Ruby gel stain (Bio-Rad). The gels were placed in deionized water overnight and scanned using a UV transiluminator (λex = 470 nm, λem = 618 nm, Molecular Dynamics, Sunnyvale, CA, USA), and the images were saved as Tiff files. PD Quest software (Bio-Rad) was used to compare gels for protein levels between control and AD samples. Average mode of background subtraction was used to normalize intensity value, which represents the amount of protein per protein spot. After completion of spot matching, the normalized intensity of each protein spot from 12 individual gels was compared between groups using statistical analysis.

**Trypsin digestion**

Samples were prepared according to the method described by Thongboonkerd et al. [78]. The protein spots that were identified as significantly different in AD compared to control were excised from the gel with a clean razor blade and transferred to clean 1.5 ml microcentrifuge tubes. The gel pieces were washed with 0.1 M ammonium bicarbonate (NH₄HCO₃) for 15 min at room temperature under a flow hood, followed by addition of acetonitrile and incubation at room temperature for 15 min. The solvents were removed and the gel pieces were allowed to dry. The gel pieces were incubated with 20 μL of 20 mM DTT in 0.1 M NH₄HCO₃ and incubated for 45 min at 56°C. The DTT solution was removed and 20 μL of 55 mM iodoacetamide (IA) in 0.1 M NH₄HCO₃ was added and incubated for 30 min in the dark at room temperature. The liquid was drawn off and the gel pieces were incubated with 200 μL of 50 mM NH₄HCO₃ at room temperature for 15 minutes. Acetonitrile was added to the gel pieces for 15 min at room temperature. The
RESULTS

Comparison of AD and control hippocampus protein levels was carried out by using PD Quest analysis. Individual protein spots were matched between the 2D-PAGE maps and each spot was normalized to the protein content in the 2D-PAGE. Based on the PD Quest analysis, 18 significantly different protein spots were identified in AD brain. Based on the data obtained from the MASCOT database, these proteins were successfully identified and the gi accession number, Mowse scores, percent coverage, pl and molecular weight of these proteins are described in Table 2. Protein levels were found to be significantly decreased for peptidyl prolyl cis/trans-isomerase (Pin 1, EC 5.2.1.8)) (0.6-fold compared to control, p < 0.03), dihydropyrimidinase-like protein 2 (DRP-2) (0.74-fold compared to control, p < 0.02), phosphoglycerate mutase 1 (PGM1, EC 5.4.2.1) (0.7-fold compared to control, p < 0.01), beta-tubulin (0.34-fold compared to control, p < 0.01), and aldolase A (EC 4.1.2.12) (0.87-fold compared to control, p < 0.0002).

Protein levels were found to be significantly increased for enolase (EC 4.2.1.11) (1.35-fold compared to control, p < 0.05), ubiquitin carboxyl terminal hydrolase L1 (UCH L1) (1.31-fold compared to control, p < 0.02), triosephosphate isomerase (TPI, EC 5.3.1.1) (1.38-fold compared to control, p < 0.05), carbonic anhydrase II (CAH-II, EC 4.2.1.1) (1.24-fold compared to control, p = 0.05), heat shock protein 70 (1.14-fold compared to control, p < 0.03), fructose bisphosphate aldolase (EC 4.1.2.13) (1.38-fold compared to control, p < 0.05), ferritin heavy chain (1.23-fold compared to control, p = 0.05), 2',3'-cyclic nucleotide 3' phosphodiesterase (CNPase, EC 3.1.4.37) (1.12-fold compared to control, p < 0.02), peroxiredoxin II (EC 1.11.1.15) (1.39-fold compared to control, p < 0.05), and adenylyl kinase I (EC 2.7.4.11) (1.19-fold compared to control, p < 0.03). We found 2 proteins that were identified as glyceraldehyde 3-phosphate dehydrogenase (GAPDH, EC 1.2.1.13). One of the spots showed the expected pl of GAPDH, i.e., 8.5, and showed a 1.26-fold increase in protein level compared to control (p < 0.04). An additional spot also was identified as GAPDH with a pl of 8.2 that showed a similar 1.28-fold increase in protein level compared to control (p < 0.01). The appearance of the second GAPDH spot suggested modification in the structure and thereby in the pl of the GAPDH protein.
Table 2
Summary of the protein properties and identification of proteins using MALDI

<table>
<thead>
<tr>
<th>Proteins (gln accession number)</th>
<th>No. of peptides matched in the identified protein</th>
<th>Percentage coverage of the matched peptides (%)</th>
<th>pL Mr (KDa)</th>
<th>MOWSE</th>
<th>Fold increase/decrease</th>
<th>P values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heat shock 70 protein (5729877)</td>
<td>23</td>
<td>45</td>
<td>5.37, 71</td>
<td>163</td>
<td>1.14</td>
<td>0.03</td>
</tr>
<tr>
<td>Tuba (2130312)</td>
<td>10</td>
<td>21</td>
<td>4.8, 50</td>
<td>89</td>
<td>0.34</td>
<td>0.01</td>
</tr>
<tr>
<td>3-oxoacid CoA-transferase</td>
<td>16</td>
<td>38</td>
<td>7.4, 56</td>
<td>133</td>
<td>1.50</td>
<td>0.03</td>
</tr>
<tr>
<td>Fructose bisphosphate aldolase</td>
<td>9</td>
<td>28</td>
<td>6.4, 39</td>
<td>82</td>
<td>1.38</td>
<td>0.05</td>
</tr>
<tr>
<td>Peptidyl Prolyl</td>
<td>5</td>
<td>32</td>
<td>7.8, 21</td>
<td>60</td>
<td>0.60</td>
<td>0.03</td>
</tr>
<tr>
<td>Cit/Trans-trans-isomerase</td>
<td>Q13526</td>
<td>8</td>
<td>5.3, 21</td>
<td>87</td>
<td>1.23</td>
<td>0.05</td>
</tr>
<tr>
<td>Ferritin heavy chain (FRIHL Human)</td>
<td>13</td>
<td>28</td>
<td>8, 74</td>
<td>105</td>
<td>1.12</td>
<td>0.0002</td>
</tr>
<tr>
<td>2',3'-Cyclic nucleotide 3' phosphodiesterase (38570991)</td>
<td>12</td>
<td>33</td>
<td>8, 39</td>
<td>132</td>
<td>0.87</td>
<td>0.01</td>
</tr>
<tr>
<td>Aldolase A (229074)</td>
<td>8</td>
<td>25</td>
<td>8, 23, 6</td>
<td>73</td>
<td>1.28</td>
<td>0.04</td>
</tr>
<tr>
<td>Glyceraldehyde 3-phosphate dehydrogenase (31645)</td>
<td>12</td>
<td>37</td>
<td>8, 5, 36</td>
<td>111</td>
<td>1.26</td>
<td>0.05</td>
</tr>
<tr>
<td>Peroxiredoxin II (P32119)</td>
<td>11</td>
<td>46</td>
<td>5, 8, 22</td>
<td>141</td>
<td>1.39</td>
<td>0.05</td>
</tr>
<tr>
<td>Dihydropyrimidinase-like protein 2DPR-2 (Q16855)</td>
<td>11</td>
<td>75</td>
<td>6, 1, 26</td>
<td>75</td>
<td>0.74</td>
<td>0.02</td>
</tr>
<tr>
<td>Carbonic anhydrase II (P00918-00-01-00)</td>
<td>9</td>
<td>44</td>
<td>6, 8, 9, 29</td>
<td>75</td>
<td>1.24</td>
<td>0.05</td>
</tr>
<tr>
<td>Adenylate kinase I (4502011)</td>
<td>8</td>
<td>42</td>
<td>8, 7, 21</td>
<td>76</td>
<td>1.19</td>
<td>0.03</td>
</tr>
<tr>
<td>Phosphoglycerate mutase I (P18669)</td>
<td>8</td>
<td>39</td>
<td>6, 7, 28</td>
<td>81</td>
<td>0.70</td>
<td>0.01</td>
</tr>
<tr>
<td>Alpha Enolase (P06733)</td>
<td>18</td>
<td>47</td>
<td>6, 9, 47</td>
<td>194</td>
<td>1.35</td>
<td>0.05</td>
</tr>
<tr>
<td>Triose Phosphate isomerase (P00174-00-00-00)</td>
<td>10</td>
<td>28</td>
<td>6, 5, 26</td>
<td>65</td>
<td>1.38</td>
<td>0.05</td>
</tr>
<tr>
<td>Ubiquitin carboxyl terminal hydrolase L-1 (P09935-00-01-00)</td>
<td>14</td>
<td>72</td>
<td>5, 3, 25</td>
<td>165</td>
<td>1.31</td>
<td>0.02</td>
</tr>
</tbody>
</table>

**DISCUSSION**

In this study, we identified several proteins that showed a differential protein levels in AD hippocampus using two-dimensional electrophoresis coupled with mass spectrometry. These proteins are grouped together or on a functional basis in Table 3. Previous studies reported an alteration of the synaptic proteins [53] in AD brain but did not look at the total proteome. Many of these proteins were reported by our group and others to have altered protein levels and to be oxidized, suggesting the impairment of various cellular pathways leading to the observed AD pathology [2,13,24,38,48,66, 74,77]. It is conceivable that the observed alterations in the protein levels in AD hippocampus could be related to AD pathology such as neuronal loss and atrophy, since hippocampus is one of the first brain regions to be affected in AD. However, until now, it has not been clear what causes the loss of synaptic connections and neurons in the AD brain.

**Energy-related enzymes**

Glucose metabolism is the main source of energy for brain under normal conditions, and it also plays an important role in maintaining normal synaptic function [79,80]. The necessity for glucose in brain function had been considered solely due to ATP production. Any alteration in the protein levels that regulate the glucose metabolism may lead to cellular dysfunction...
such as impaired ion-motive ATPase to maintain potential gradients, operate pumps, and maintain membrane lipids asymmetry, etc. dysfunction of which are physiological hallmarks of AD [17-19,37,76]. Such changes could lead to exposure of phosphatidylserine to the outer membrane leaflet, a signal for apoptosis [20,59].

In the present study, we observed a significant decrease in the levels of PGM1, and aldolase A, whereas, enolase, TPI, fructose bisphosphate aldolase, 3-oxoacid CoA-transferase, GADPH, and adenyate kinase protein levels were found to be significantly increased. All these proteins are involved in glucose metabolism and play a role directly or indirectly in ATP production. The observed increased protein levels of some of these proteins, e.g., enolase, TPI, 3-oxoacid CoA-transferase, and GADPH did not correlate with the activity of these enzymes in AD brain. It is tempting to speculate that the modification of these proteins might hinder their function, leading to reduced glucose metabolism and thereby energy production in AD [58,79]. Previous studies from our laboratory showed the oxidation of creatine kinase BB, enolase, GADPH, TPI, and PGM1 in AD brain that, with the exception of TPI, correlate well with the altered activities of these proteins [18,19, 39,54,74,76,77]. Further, in AD it has been reported that ATP diminution induces hyperthermia which would lead to abnormal tau hyperphosphorylation through differential inhibition of kinase and phosphatase activities, ion pumps, electrochemical gradients, cell potential, voltage-gated ion channels [64]. Our results are consistent with previous findings that reported a decreased protein level of PGM1 in AD brain [39,57]. PGM1 is also identified as an oxidized protein that would be one of the reasons for the reported decrease in PGM1 activity [39,57]. In a recent study, using immunoblotting with AD sera, aldolase A was identified as a major autoantigen in AD patients [60]. No change in TPI and aldolase activity was observed in AD brain [39]. Accumulation of glyceraldehyde-3-phosphate dehydrogenase along with α-enolase and γ-enolase has been shown in AD brain [69]. Additionally, reduced activity of glyceraldehyde-3-phosphate dehydrogenase has been reported in AD [54]. Meier-Ruge et al. [57] reported a significant decrease in enolase activity in AD brain compared to the age-matched control [57]. Using a redox proteomics approach, we reported α-enolase as a specifically oxidized protein in inferior parietal of AD brain [18,74,77]. However, no change in TPI activity was observed [58,74]. PGM1, enolase, TPI, and GADPH were previously found to be oxidized proteins in AD brain and that could contribute to the accumulation of these proteins in addition to the reported decrease in proteasomal activity [17,24,74].

Adenyate kinase catalyzes the reversible reaction of MgATP with AMP leading to the formation of MgADP and ADP. Three isoforms of AK have so far been characterized [30]. AK1 is present in cytosol of brain, erythrocytes, skeletal muscles [41]. Brain requires large amounts of ATP to support neurotransmission, the main function of the brain, and hence has high levels of AK1 protein [62]. The increase in the protein level of AK1 protein in AD brain suggests a compensatory mechanism for the decrease in ATP production. In fact, AK1 may serve as an integral component of phosphotransfer networks, along with CK and glycolysis, effectively coupling ATP-generating with ATP-consuming or ATP-sensing intracellular sites [40]. The importance of AK1 as a putative extracellular signaling molecule has been established [29]. ATP and ADP are released in a regulated manner from neurons and other cells and interact with cell surface receptors, which in turn can promote a variety of functional responses including neurotransmission.

Proteasome-related and scaffolding proteins

The levels of heat shock protein 70 and ubiquitin carboxyl terminal hydrolase L-1 were found to be increased in AD hippocampus. The former protein serves as a protein chaperone, maintaining the structure of
the damaged or aggregated proteins. The latter protein serves several functions related to repair or elongation of neurites [17,18,24,28,74]. Increased levels of these proteins are assumed to help the cells tightly regulating the protein structure and degradation of any damaged proteins [17,18,24,28,74]. In contrast, a previous study showed a decreased level of UCHL-1 protein in AD brain [24]. This may be due to the use of different solubilization buffers during sample preparation. However, previous studies reported oxidation and loss of activity of these proteins. Heat shock protein 70 (HSP 70) is up-regulated in AD and is also associated with protein misfolding and decreased protein turnover [84]. One of the major consequences of aberrant UCH L-1 activity is an impaired ubiquitination/de-ubiquitination machinery, causing formation of protein aggregates, synaptic deterioration, and degeneration in AD hippocampus [17,24,38,74]. Consistent with this notion, a recent in vitro study showed that the hydrolyase activity of recombinant UCH L-1 was decreased by treatment with 4-hydroxy-6-nonenal, a lipid peroxidation product that is elevated in AD brain and formed by Aβ-induced lipid peroxidation [71]. These different lines of evidence support a role for dysfunction of the ubiquitin-proteasome pathway in the pathogenesis of AD.

**Structural proteins**

Tubulin beta, and dihydropyrimidinase-like protein 2 (DRP-2) play important roles in neuronal connections and communication, repair and regeneration of adult neurons. Tubulin is a core protein of microtubules, which play a role in cytoskeletal maintenance. Additionally, tubulin has been shown to be involved in the transport of membrane-bound organelles and is required for extension and maintenance of neurites. We previously reported a non-significant trend toward oxidation of tubulin in AD brain and a model of AD [9]. DRP2 is a member of the dihydropyrimidinase-related protein family. This protein is involved in axonal outgrowth and path-finding through the transmission and modulation of extracellular signals. DRP-2 has been reported to be oxidatively modified [18,74], and our results are consistent with a previous finding that showed decreased levels of DRP-2 in AD and fetal Down's syndrome (DS) brain [18,49]. A decrease in the levels of these proteins may interfere with synaptogenesis and
neuronal differentiation and migration [25]. It is thus conceivable that oxidation of tubulin, and DRP-2, could be related to impaired axonal transport mechanisms and loss of cell shape, and may lead to the degeneration of affected neurons as observed in AD brain. In AD brain, DRP-2 is associated with neurofibrillary tangles, and these findings have been associated with abnormal neuritic and axonal outgrowth of tangle-bearing neurons and thus acceleration of neuritic degeneration, due to the depletion of the soluble, cytosolic DRP-2 pool [49].

Cell Cycle, Tau phosphorylation, Abeta production

Peptidyl – prolyl cis/trans isomerase (PPIase) protein level is found to be significantly decreased in AD hippocampus and these results are consistent with previous studies [48,73]. Pin1 plays an important role as a chaperone protein and also in cell cycle regulation [47]. Pin 1 interacts with a number of proteins that are involved in cell cycle regulation [61,85]. Recent studies reported that cell cycle machinery is altered in AD, MCI (Mild cognitive impairment) and by Aβ [7,75,82].

Pin1 also catalyzes the isomerization of tau, a neuronal cytoskeleton protein, which is hyper-phosphorylated AD brain [45]. In addition, Lu et al., showed that Pin1 could restore the function of tau protein in AD [48]. Pin 1 regulates the dephosphorylation of phosphorylated tau by interacting with protein phosphatase 2A [15].

In addition, Pin 1, by interacting with APP, has recently been reported to modulate Aβ production [63]. Oxidation of this protein could be associated with diminished function [46,48,73]. A recent study from our laboratory showed oxidation and decreased activity of Pin1 in MCI and AD brain [16,48,73]. Therefore, the decrease in Pin1 protein level could be one of the initial events that trigger tangle formation, Aβ accumulation, and prevention of neurons from exiting mitosis in the cell cycle in AD.

pH regulation

Carbonic anhydrase II plays an important role in regulating cellular pH, CO₂ and HCO₃⁻ transport, and maintaining H₂O and electrolyte balance [72]. CAH-II is also involved in the production of cerebrospinal fluid (CSF) and the synthesis of glucose and lipids [31,51]. In the present study, we found a significant increase in CAH-II protein level. A deficiency in CAH-II could lead to imbalance in pH, which could favor the formation of protein aggregates and may eventually lead to cognitive defects varying from disabilities to severe mental retardation [72]. Though the protein level of CAH-II is increased in hippocampus, it is reported to be oxidatively modified and has diminished activity in AD brain compared to age-matched control brain [57, 74,77]. The increased protein level conceivably could be a compensatory response to oxidative dysfunction of CAH-II. Consequently, both the extracellular and intracellular pH may be altered and may lead to loss of enzyme activities, mitochondrial dysfunction and may eventually lead to progression of AD.

Synaptic processes

CNPase is associated with oligodendrocytes and therefore myelination [4]. CNPase selectively cleaves 2',3'-cyclic nucleotides to produce 2'-nucleotides in vitro [43]. Although physiologically relevant substrates with 2',3'-cyclic termini are still unknown, numerous cyclic phosphate containing RNAs occur transiently within eukaryotic cells. The protein level of 2',3'-cyclic nucleotide-3'-phosphodiesterase (CNPase) in AD hippocampus is found to be increased, in contrast to a previous study that showed a decrease in CNPase [81]. Recent data indicate that myelin can be directly damaged by oligomerized Aβ [21,42]. Furthermore, myelin breakdown has been demonstrated in the older compared to younger normal individuals and in AD patients compared to normal older subjects [5]. Myelin integrity is disrupted by the products of lipid peroxidation [22]. CNPase in AD hippocampus might be elevated to regulate the levels of myelin and thereby plays an important role in synaptic function.

Antioxidant function

An increase in the protein levels of several antioxidant proteins is observed in the AD brain, which agrees with the premise of oxidative stress in the pathogenesis of the disorder [3,14,44,69]. An elevation in the protein levels of the enzymes involved in the removal of these cytotoxic intermediates suggests an adaptive mechanism of up-regulation to counteract the accumulation of these species [3]. Peroxiredoxins II (Prx-II) belong to a group of mammalian peroxiredoxins that plays an important antioxidant role in the protection of neurons from damage induced by hydrogen peroxide. In human brain, Prx II protein was exclusively found in neurons and predominantly localized in cytosol. Our finding confirmed a previous study that showed a significant increase in the levels of Prx II in AD compared to that of controls in brain [44].
Ferritin is a ubiquitous and highly conserved protein which plays a major role in iron sequestration, detoxification and storage [23,34]. Heme oxygenase-1 regulates the protein levels of ferritin heavy chain (H-ferritin). HO-1 cleaves the heme porphyrin ring releasing Fe^{2+}, which induces the protein levels of the Fe^{2+} sequestering protein ferritin. By limiting the ability of Fe^{2+} to participate in the generation of free radicals, since brain is rich in oxygen, the availability of high levels of Fe^{2+} may initiate the Fenton reaction, but the presence of ferritin can act as an anti-oxidant by binding and storing iron ions [5]. H-ferritin has been shown to protect cultured endothelial cells from apoptosis induced by a variety of stimuli [27]. One possible explanation for the observed increase in H-ferritin and Prx II could be that a compensatory mechanism might be initiated by surviving neurons to protect themselves from apoptosis. The increased protein levels of the ferritin heavy chain (H-ferritin) and peroxiredoxin II proteins are consistent with a role of ROS in the pathogenesis of AD.

In summary, our study found altered levels of some proteins in AD hippocampus compared to that in control tissue. Despite an increase in the level of protein of some of the enzymes that are involved in ATP regulation, the energy status is found to be low in AD brain and also the proteasomal activity is low in addition to other altered functions that are mentioned in this paper. All these points link oxidative stress to the altered protein levels and may be important for synaptic loss, plaque and tangle formation, and may be related to cognitive impairments in AD.

ACKNOWLEDGEMENTS

The authors thank the University of Kentucky ADC Clinical and Neuropathology Core for providing the brain specimens used for this study. This work was supported, in part, by grants from NIH [AG-05119; AG-10836].

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