EXCITOTOXICITY IN NEUROLOGICAL DISEASES

New Therapeutic Challenge

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Chapter 13
THE GLUTAMATERGIC SYSTEM IN ALZHEIMER'S DISEASE BRAIN: DYSFUNCTION ASSOCIATED WITH AMYLOID β-PEPTIDE AND OXIDATIVE STRESS

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Abstract: Excitotoxic mechanisms of neuronal death can occur in the presence of excess glutamate. Hence, means to remove or convert extra-neuronal glutamate exist in brain. However, in Alzheimer's disease (AD) brain, the glutamate transporter and glutamine synthetase function with considerably reduced activity. Consistent with the observed oxidative stress in AD brain and the oxidative stress induced by amyloid β-peptide (Aβ), both the glutamate transporter (EAAT2) and glutamine synthetase are oxidatively modified in AD brain. Moreover, Aβ causes EAAT2 and glutamine synthetase to be oxidized, consistent with the notion that excess Aβ in AD brain contributes to the oxidative stress observed. This chapter reviews studies that support the notion that the dysfunctional glutamatergic system in AD brain results from oxidative modifications of key components of this system, and that Aβ plays a role in this oxidative modification. Consequent excitotoxicity could be one means to account for neuronal and synapse loss in AD brain.

Keywords: glutamate, oxidative stress, HNE, lipid peroxidation, amyloid β-peptide.

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1. Introduction

Alzheimer's disease (AD), an age-related neurodegenerative disorder, is characterized clinically by progressive loss of memory and cognition and subsequently speech (Katzman and Saitoh, 1991). Pathologically, AD is characterized by three principal hallmarks: senile (neuritic) plaques, consisting mostly of fibrillar amyloid β-peptide (Aβ) surrounded by dystrophic neurites; neurofibrillary tangles, consisting mostly of hyperphosphorylated tau, a cytoskeletal protein; and loss of synapses (Katzman and Saitoh, 1991). Except for a small percentage of AD patients who inherit the disease in an autosomal dominant manner, the pathogenesis of this dementing disorder is not known.

Oxidative stress could be experimentally measured by assaying protein oxidation (assayed by protein carbonyl levels), lipid peroxidation (assayed by a number of lipid peroxidation end-products, including 4-hydroxynonenal [HNE] and acrolein [2-propen-1-al], free radical formation (assayed often by increased fluorescence of 2',7'-dichlorofluorescein); 3-nitrotyrosine (formed by action of NO with superoxide radical and subsequent reactions of the product, peroxynitrite) and advanced glycation end products (aggregates initiated by binding of a reducing sugar with a protein amino group followed by complex Amadori chemistry).

Oxidative stress is extensive in AD brain (Marksbery, 1997; Butterfield et al., 2001; Butterfield et al., 2002; Butterfield and Lauderback, 2002). Increased protein oxidation (Hensley et al., 1995; Castegna et al., 2002a,b), increased levels of the lipid peroxidation products HNE and acrolein (Marksbery et al., 1998; Lovell et al., 2001), excess 3-nitrotyrosine (Smith et al., 1997), increased advanced glycation end products (Smith et al., 1994), and increased DNA oxidation (Gabbi et al., 1998) are reported for AD brain.

Many researchers agree that Aβ is central to the pathogenesis of AD (Selkoe, 2001). For example, subjects who inherit AD due to mutations in the genes for presenilin-1, presenilin-2, and amyloid precursor protein (APP) always have excessive amounts of the 42-mer, Aβ(1-42). In addition, patients with Down's syndrome always develop AD if they live long enough, and the gene for APP is located on chromosome 21, the locus of the trisomy of Down's syndrome. Transgenic mice that overexpress mutant human APP and mutant tau, develop plaques before developing neurofibrillary deposits. [Reviewed in Selkoe, 2001].

Our laboratory combined these two concepts: the centrality of Aβ(1-42) to the pathogenesis of AD on the one hand with the oxidative stress under which the AD brain exists on the other into a comprehensive, Aβ(1-42)-centered model for neurodegeneration in AD brain based on the
oxidative stress associated with Aβ(1-42) (Butterfield et al. 2001; 2002; Butterfield and Lauderback, 2002; Varadarajan et al., 2000; Butterfield, 2002). Consistent with the model, Aβ(1-42) induces protein oxidation (Yatin et al., 1999); lipid peroxidation (Lauderback et al., 2001; Butterfield et al., 2002); reactive oxygen species production (Yatin et al., 2000) and many other markers of oxidative stress (Butterfield, 2002). These effects of Aβ(1-42) are blocked by antioxidants.

Glutamate is the principal excitatory neurotransmitter in the mammalian CNS and is essential for synaptic transmission. When glutamate levels increase dramatically, excessive neuronal stimulation causes damage to and subsequent loss of neurons in a process known as excitotoxicity (Maragakis and Rothstein, 2001). Therefore, clearance of excitotoxic glutamate from the synaptic cleft is essential for neuronal survival. Astrocyte-mediated glutamate uptake is the primary mean of maintaining synaptic glutamate concentrations (Anderson et al. 2000; Rothstein et al., 1996). If glutamate transport is inhibited, neurodegeneration and death ensue (Rothstein et al., 1993; Robinson et al., 1993; Lievens et al., 2000), consistent with a role for impaired glutamate transport in excitotoxicity.

Although evidence is scant in some cases, excitotoxicity and subsequent neuronal death have been implicated in several neurodegenerative disorders, including stroke, amyotrophic lateral sclerosis (ALS) and AD (Maragakis and Rothstein, 2001). In AD, glutamate transport is inhibited (Masliah et al., 1996), though until recently (Lauderback et al., 2001), the mechanism for inhibition of glutamate transport in AD brain was unknown (see below).

2. The glutamatergic system is dysfunctional in AD

Glutamate is involved in cognitive processes, (Collingridge and Singer, 1990), possibly involving synaptic protein remodeling (Sheng and Kim, 2002). Synapse-resident enzymes to degrade glutamate are not known. Consequently, as mentioned above, this excitatory neurotransmitter must be cleared from the synapse by high-affinity presynaptic and glial transporters. Five different forms of glutamate transporters have been identified: GLAST (EAAT1), GLT-1 (EAAT2), EAAC-1 (EAAT3), EAAT4 and EAAT5. GLAST and GLT-1 are found exclusively in astroglial cells. GLT-1 is observed throughout maturity, (Furuta, et. al., 1997), while GLAST expression is observed during early stages of development.
Consistent with a role for Aβ(1-42) in AD, recent studies suggest that glutamate-induced NMDA receptor activation stimulates APP processing to produce Aβ peptide (Gordon-Kraicer et al., 2002). Consequently, over-stimulation of the receptor by excess glutamate could lead to excess Aβ(1-42) production with consequent oxidative stress-induced neurotoxicity (Butterfield et al., 2001; 2002; Butterfield and Lauderback, 2002). Similarly, the reported decreased number of NMDA receptors in AD brain (Greenamyre, 1986) may lead to their overstimulation by excessive glutamate. Along with Aβ(1-42), other sources of free radicals upon NMDA receptor activation have been reported (LaFon-Cazal et al., 1993).

Neurodegeneration in AD was recently reported to be associated with expression of EAAT-2 glutamate transporter, which was localized to tangle-bearing neurons (Thal, 2002). The expression of a related transporter, EAAT-1 (GLAST), also was reported for AD brain (Scott et al., 2002).

The EAAT-2 (GLT-1) glutamate transporter system in AD brain has a significant reduction of activity (Mastiah et al., 1996; Scott, et al., 1995), increasing the possibility for excitotoxicity. Table I shows that this transporter is oxidatively modified by HNE, a lipid peroxidation product, in the inferior parietal region of AD brain (Lauderback et al., 2001). HNE is known to bind to proteins by Michael addition (Butterfield and Stadtman, 1997), and in synaptosomes HNE alters the structure of synaptosomal membrane proteins (Subramaniam et al., 1997).

| Table I. Relative percent increase in HNE binding to GLT-1 in AD brain or following exposure of synaptosomes to Aβ(1-42)* |
|--------------------------------------------------|-----------------|-----|--------|
| Mean +/- SEM | N | P-Value |
| AD Brain | 155 +/- 7 | 4-7 | <0.002 |
| Following exposure of synaptosomes to Aβ(1-42) | 182 +/- 6 | 5-6 | <0.002 |

*Lauderback et al., 2001

This alkenal also decreases the activity of numerous transmembrane enzymes and transporters (Butterfield et al., 2002), presumably because covalent modification of the proteins changes their structure and thus their function. Consequently, increased HNE binding to
GLT-1 in AD brain (Lauderback et al., 2001) is likely to be the cause of the loss of activity of this transporter in AD brain (Masliah et al., 1996). Aβ(1-42) added to synaptosomes leads to lipid peroxidation (Butterfield et al., 2002; Lauderback et al., 2001; Butterfield, 2002) and increased HNE binding to GLT-1 (Lauderback et al., 2001), suggesting that, in AD brain, where excess Aβ(1-42) deposition occurs, Aβ-induced lipid peroxidation could account for the increased HNE binding to GLT-1. Inhibition of glutamate transport, coupled with decreased activity of glutamine synthetase in AD brain (see below), might lead to increased extra-neuronal glutamate with consequent stimulation of NMDA receptors and excitotoxic processes involving excess intraneuronal Ca²⁺ accumulation and eventual cell death (Figure 1). In astrocytes, Aβ (Harris et al., 1995) and HNE (Blanc et al., 1998) inhibit glutamate uptake. Their inhibitory effects are modulated by antioxidants or glutathione, respectively.

Recent studies report that, in contrast to the usual case and in certain cells of altered shape, GLT-1 is expressed in neurons (Thal, 2002). A different alkenal, acrolein, which also alters the structure of synaptosomal membranes (Pocernich et al., 2000; 2001), decreased uptake of glutamate in neurons (Lovell et al., 2001). In contrast to the decreased activity of glutamate transport in AD brain (Masliah et al., 1996, Scott et al., 1995), where excess Aβ is observed, and in contrast to the many studies suggesting that Aβ peptides decrease glutamate uptake (reviewed in Butterfield and Pocernich, 2003), one study recently reported that Aβ enhances glial glutamate uptake activity with subsequent decreased synaptic activity (Ikegaya et al., 2002). Impaired learning was predicted if this phenomenon occurred in AD brain.

In glia, glutamate is converted to glutamine by glutamine synthetase (GS). The glutamine is released, taken up into neurons and converted to glutamate by mitochondrial glutaminase. The activity of GS is significantly decreased in AD brain (Smith et al., 1991; Hensley et al., 1995; Butterfield et al., 1997). This loss of activity may result from specific oxidation of GS in AD (see below). Consistent with the loss of GS activity in AD, the levels of glutamate + glutamine (GLX) were substantially reduced in the cingulate cortex of AD patients, and this loss strongly correlated with both the cognitive and functional status of the patients (Antuono et al., 2001). Some reports of GLX in AD revealed no significant difference in the mid-frontal or temporoparietal GLX, (Moats et al., 1994), but an increase in GLX in the occipital lobe of AD patients was found (Ernst et al., 1997).
Figure 1. Glutamatergic system. In AD brain, excess glutamate, resulting from decreased glutamine synthetase (GS) activity and decreased GLT-1 transport function due to oxidative modification in both cases, could lead to excitotoxicity and neuronal death. See text.

GS is sensitive to oxidative stress (Aksenov et al., 1995; 1997; Butterfield et al., 1997). Novel electron paramagnetic resonance (EPR) experiments suggested that GS isolated and purified from AD brain was more oxidized than that isolated and purified from control brain (Butterfield et al., 1997). This suggestion was confirmed by direct determination using proteomics that GS is specifically oxidized in AD brain (Castejón et al., 2002a). The decreased enzymatic activity of GS activity in AD hippocampus and neocortex (Hensley et al., 1995) contributes to the increased possibility of excitotoxicity being an important mechanism for neurodegeneration in AD brain. Aβ(1-42) significantly
decreased GS activity in cytosolic fractions of brain homogenates and in cultured hippocampal neurons and astrocytes (Varadarajan et al., 2000; Butterfield, 1997), suggesting the possibility for connecting the decreased activity of GS with Aβ(1-42)-induced oxidative modification of the enzyme in AD brain. In support of this suggestion, Aβ added to GS caused increased protein carbonyl on the enzyme (Aksenov et al., 1997). Fibrils are normally formed upon incubation of Aβ; however, in the presence of GS, small, non-fibillar aggregates of Aβ, that were more toxic than a solution of Aβ alone, were formed (Aksenov et al., 1995). Recent in vivo studies showed that small aggregates of Aβ(1-42) induced protein oxidation in a living animal (Drake et al., 2003), in accord with the notion that these small aggregates of Aβ(1-42) may be the toxic species of this peptide (Ota et al., 1995; Aksenov et al., 1995; Lambert et al., 1998; Walsh et al., 2002). Consistent with oxidative stress associated with both Aβ and excitotoxicity, treatment of non-toxic levels of Aβ potentiates excitotoxic effects of glutamate, suggesting synergy between these two sources of oxidative stress (Mattson et al., 1993).

Diagnosis of AD is confirmed by histological examination at autopsy. Definitive diagnosis of living AD patients, though much improved in recent years, is still largely by elimination of other sources of dementia and is subject to a modest error rate (Katzman and Saitoh, 1991). Consequently, definitive diagnosis of AD requires some sort of biomarker. One such biomarker proposed for AD is the observation that GS is found in CSF and not able to be photolabeled by an azido-ATP photoprobe (Gunnersen and Haley, 1992). This lack of photolabeling may reflect the finding that GS is a specifically oxidized protein in AD brain (Castegna et al., 2002a).

Glutamate is also converted to γ-aminobutyric acid (GABA), an inhibitory neurotransmitter, by glutamate decarboxylase (GD). The AD messenger RNA for GD reportedly is increased in the caudate nucleus and putamen (Boissiere et al., 1998). In contrast, no alteration was found in the AD ventral striatum, suggesting that GABAergic neurotransmission may be increased in the dorsal striatum but not in the ventral striatum. Consistent with the increased messenger RNA in AD brain, GD expression is increased, potentially resulting in increased GABA levels and cell death (Hertz et al., 1988).

As noted above, neurons produce glutamate from glutamine by the enzyme glutaminase. In AD temporal cortex, no significant changes were seen in the levels of phosphate-activated glutaminase (Haug et al., 1996), suggesting that neurons in the cortex area of AD still have the ability to produce glutamate. In contrast, glutamate and glutaminase immunoreactive pyramidal neurons in the hippocampal dentate gyrus were decreased in
number in AD brain (Kowall and Beal, 1991). These same neurons were found to contain neurofilibrillary tangles. This regional difference in glutamatergic neurons conceivably could mirror the role of the hippocampus in processing memory, which is severely diminished in AD patients.

3. Conclusions

Oxidative stress occurs in AD brain and is associated with Aβ(1-42) (Butterfield et al., 2001; 2002; Butterfield and Lauderback, 2002; Butterfield, 2002). This oxidative stress leads to formation of reactive alkenals, like HNE, and protein oxidation (Butterfield and Stadtman, 1997; Lauderback et al., 2001). Oxidative stress and its sequelae provide mechanisms for the oxidative modification and subsequent loss of activity of the glutamate transporter EAAT-2 [GLT-1] and GS in AD brain (Masaliah, et al., 1996; 2000; Hensley et al., 1995; Lauderback et al., 2001; Butterfield et al., 1997; Castegna et al., 2002a). Neurons, exposed to the resulting excess glutamate, could undergo excitotoxicity (Figure 1) with subsequent cell death.

A number of pharmacological interventions to stimulate glutamate transport and prevent excitotoxicity are being pursued (reviewed in Butterfield and Pocernich, 2003). However, given that a major cause of the underlying glutamatergic dysfunction in AD brain might result from oxidative modification of important components of the glutamatergic system in AD, another therapeutic strategy to be considered is to increase the endogenous and exogenous antioxidant levels in AD patients (Butterfield et al., 2002b,c).

Excitotoxicity may be an important contributor to neuronal degeneration in AD brain. Strategies to significantly modulate, if not eliminate, excitotoxicity in AD should be beneficial to patients suffering from this devastating dementing disorder.

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