



AMYLOID β -PEPTIDE INHIBITS Na^+ -DEPENDENT GLUTAMATE UPTAKE

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Summary

The purpose of this review is to summarize much of the work on the inhibition of the astroglial glutamate transporter in relation to excitotoxic neurodegeneration, in particular, inhibition of uptake by the β -amyloid peptide (A β) found in the Alzheimer's disease (AD) brain. There is evidence for oxidative stress in the AD adding to the stress or possibly initiating it. The oxidative inhibition of the glutamate transporter protein by A β increases the vulnerability of glutamatergic neurons, and by rendering them susceptible to the excitotoxic insult that results

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Glutamate is the most abundant amino acid and a major excitatory neurotransmitter in the mammalian CNS (1-3). There is considerable evidence for the neurotoxic effects of glutamate and its role in neurodegeneration. Glutamate signal transduction at the post-synaptic terminal is subtype (2). Binding of glutamate to the NMDA receptor initiates an influx of Ca^{2+} through NMDA-gated channels. Excitotoxic action of glutamate binding is mediated by a neuronal intracellular Ca^{2+} overload due to the prolonged stimulation of the NMDA receptor (1-3). The subsequent loss of Ca^{2+} homeostasis results in an activation of a complex cascade of enzymes such as Alzheimer's disease (AD). Glutamate is a major excitatory transmitter in regions of the brain involved in the pathobiology of AD (11,14) and the loss of function of glutamate correlation between neuronal vulnerability and expression of neuronal calcium binding proteins

The distribution of senile plaques (SP) in the AD brain correlates with the distribution of glutaminergic synapses (15,16), while the A β peptide, which is the major constituent of these SP, has been shown to exacerbate glutamate excitotoxicity in cultured neurons (17,18), and to increase intracellular Ca²⁺ concentrations (17,18). A β is thought to play a central role in AD pathogenesis (20). Further, oxidative stress is evident in the AD brain (21,22). A β -associated free-radical oxidative stress (22) may be the link between these two observations.

Astrocytes help regulate the brain microenvironment with their various transport systems for ions and amino acids. One such transport system expressed in astrocytes is the glutamate transporter

al. (23) has shown the importance of glial glutamate transporters, as opposed to neural

transport was sufficient to cause excitotoxicity. Also, cultures where astrocytes had been mitotically inhibited exhibited a 100-fold increase in vulnerability to glutamate toxicity compared to cultures with a normal astrocyte population (24). Neurons in astrocyte-poor cultures were killed extensively by concentrations of glutamate that are comparable to glutamate levels normally present extracellularly in the hippocampus. These data demonstrate the vulnerability of neurons to glutamate-mediated excitotoxicity when glial glutamate transporters are missing or damaged

Free radical damage has been demonstrated to inhibit glutamate uptake in primary astroglial cell cultures (25). A xanthine/xanthine oxidase (X/XO) system was used for free radical generation while determining the uptake of [³H]-glutamate from the culture medium. A large decrease (~65%) in glutamate uptake was observed after a 30 minute incubation of the cells with X/XO. This effect could be almost completely prevented by the addition of the free radical scavenging enzymes SOD and catalase. These results implicate the damaging effects of increased oxidative stress in relation to glutamate excitotoxicity and infer similar effects in disorders such as AD

A β and Glutamate Uptake Inhibition

Glutamate uptake was measured in primary hippocampal astrocytes while monitoring its

inhibition by A β (25-26) (26-28). Following incubation with the cells, A β was found to inhibit

100pM A β (25-35) was found to inhibit glutamate uptake by ~50% in 30 minutes. This effect was prevented by the free radical scavengers trolox and dithiothreitol (DTT), and a competitive a 3-fold increase in protein carbonyls as measured by the biotin-4-aminobenzoyl hydrazide histochemical technique (26), and decreased the activity of the astrocyte-specific enzyme glutamine synthetase (GS) as shown previously (29,30). Others have confirmed that A β inhibits glutamate uptake in synaptosomes (31). A β is known to initiate lipid peroxidation of brain membrane systems (32,33), and a major lipid peroxidation product, 4-hydroxynonenal (HNE), is formed upon incubation of cultured hippocampal neurons with A β (34). HNE forms covalent adducts with proteins (35) and has been demonstrated that HNE inhibits GLUT1-mediated

The high-affinity uptake of glutamate by astrocytes depends on Na⁺ concentration gradients across the astrocytic plasma membrane. Thus, oxidative damage to the Na⁺/K⁺ ATPase by A β

glutamate uptake (26). Loss of membrane integrity by A β -induced lipid peroxidation could also disrupt the clearance of glutamate from the synapse. By measuring LDH release from astrocytes treated with A β , it has been shown that the astrocytic membrane is intact, ensuring that cell lysis is not the mechanism of uptake inhibition (26). A β induces protein oxidation, a marker of increased oxidative stress, and can be measured by protein carbonyl levels. Oxidation decreases the activity of enzymes by causing conformational changes and modifications to amino acids critical to proper function (39). Increases in protein carbonyls have been reported in astrocytes following treatment with A β (26-28), which is consistent with the ROS-generating capacity of the peptide, and may reflect losses in astrocytic enzyme activities. The loss of glutamate uptake in the presence of A β alone was inhibited by free radical scavengers as well as with PDC (26). Protection of damage by PDC, a competitive inhibitor of glutamate uptake, is speculated to be due to a blocking of the active site and other oxidatively sensitive amino acids that are critical to

increase protein carbonyls, by way of its aldehyde moiety. HNE has been shown to induce protein conformational changes in synaptosomes (40) and to inhibit glutamate uptake (36-38). Thus, A β damage to the glutamate transporter may be due to its effects on lipid peroxidation.

Discussion

It has been suggested that inhibition of glutamate uptake by astrocytes would result in postsynaptic receptor desensitization and an inhibition of further glutamate release (41,42), thus preventing excitotoxic damage. However, several studies have shown that loss of glutamate

damage to neurons in the presence of glutamate (17,18). Since the glutamate transport system is sensitive to oxidative damage by ROS (25), the ability of A β to generate free radicals spontaneously in solution (29) may link these phenomena.

The accumulation of A β in senile plaques of the AD brain has been proposed as a source of increased oxidative stress (22). This oxidative stress in the synaptic region is likely to do general damage to synapses as well as the surrounding astroglia. In this event, damage to glial transport

synaptic degeneration and cell death. If these results reviewed here in culture are typical in the AD brain, then one mechanism of neurotoxicity may be partially explained. Indeed, decreased inactivation remains unclear. Nonetheless, it is evident that there is a relationship among A β -induced free radical toxicity, glutamate-associated excitotoxicity, disruption of Ca²⁺ homeostasis

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