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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 (Aβ) found in the Alzheimer's disease (AD) brain. There is evidence for oxidative stress in the AD brain, and Aβ has been found to generate reactive oxygen species (ROS), thus adding to the stress or possibly initiating it. The oxidative inhibition of the glutamate transporter protein by Aβ increases the vulnerability of glutamatergic neurons, and by rendering them susceptible to the excitotoxic insult that results from impaired glutamate uptake, Aβ can be directly connected to the neurodegeneration that follows.

Key Words: β-amyloid, free radicals, glutamate uptake, excitotoxicity

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 initiated by stimulation of glutamate receptors, especially of the N-methyl-D-aspartate (NMDA) 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 consequent loss of Ca^{2+} homeostasis results in an activation of a complex cascade of enzymes, messengers, and intracellular reactive oxygen species (ROS) that lead to cell death (4,5-10). Evidence continues to accumulate that links glutamate toxicity to neurodegenerative disorders such as Alzheimer's disease (AD). Glutamate is a major excitatory transmitter in regions of the brain involved in the pathophysiology of AD (11-14), and the loss of neurons that use glutamate as a transmitter or have receptors for glutamate is a hallmark of the AD brain (11,14). There is a correlation between neuronal vulnerability and expression of neuronal calcium binding proteins in human postmortem tissue (4), suggesting a role for glutamate toxicity and its disturbance of neuronal Ca^{2+} homeostasis.

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The distribution of senile plaques (SP) in the AD brain correlates with the distribution of glutaminergic synapses (15,16), while the Aβ 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 Ca^{2+} concentrations (17,19). Aβ is thought to play a central role in AD pathogenesis (20). Further, oxidative stress is evident in the AD brain (21,22). Aβ-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, which is responsible for the removal of glutamate from the synapse and prevention of excitotoxic damage by high affinity uptake of glutamate. Removal of or damage to this transport system would result in an increased vulnerability of the cell to excitotoxic damage or death. Rothstein et al. (23) has shown the importance of glial glutamate transporters, as opposed to neural transporters, by using chronic antisense oligonucleotide administration to reduce synthesis and expression of the respective transporters. By using transport knockout systems in cell cultures, it was determined that glial glutamate transport removes the majority of synaptic glutamate. When glial tranporters were missing, excessive glutamate concentrations in the synapse caused repeated depolarization and a disruption of neural circuitry. Hence, the loss of glial glutamate 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 [^{3}H]-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 where oxidative stress is evident.

Aβ and Glutamate Uptake Inhibition

Glutamate uptake was measured in primary hippocampal astrocytes while monitoring its inhibition by Aβ(25-35) (26-28). Before incubation with the cells, Aβ was shown to yield an EPR signal in spin trapping experiments, indicating radical generation by the peptide (28,29). 100μM Aβ(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 substrate/inhibitor of glutamate uptake, pyrrolidine-2,4-carboxylate (PDC). Further, Aβ induced a 3-fold increase in protein carbonyls as measured by the biotin-4-amidobenzoic 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 Aβ inhibits glutamate uptake in synaptosomes (31). Aβ 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 Aβ (34). HNE forms covalent adducts with proteins (35), and recent studies have shown that HNE binds to GLT-1, a glial glutamate transporter, and inhibits glutamate uptake (36-38).

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 Aβ could potentially disrupt glutamate uptake. Aβ has been shown to inhibit the activity of this enzyme (19,26); however, inactivation by specific inhibitors does not lead to a decrease in glutamate uptake (26). Loss of membrane integrity by Aβ-induced lipid peroxidation could also disrupt the clearance of glutamate from the synapse. By measuring LDH release from astrocytes treated with Aβ, it has been shown that the astrocytic membrane is intact, ensuring that cell lysis is not the mechanism of uptake inhibition (26). Aβ 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 Aβ (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 Aβ 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 transporter function (26). These results do not rule out the fact that Aβ indirectly inhibits the transporter through the actions of HNE. Covalent linkage of HNE to the transporter would also 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, Aβ 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 transport induces excitotoxicity and neuronal degeneration (23,24,26). Aβ has been shown to be neurotoxic in the absence of glutamate (43) in addition to its ability to potentiate excitotoxic 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 Aβ to generate free radicals spontaneously in solution (29) may link these phenomena.

The accumulation of Aβ 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 systems could occur, resulting in a decreased glutamate uptake. The result of such damage is increased perisynaptic glutamate concentrations and enhanced activation of glutamate receptors, followed by a massive influx of Ca^{2+}. The loss of Ca^{2+} homeostasis that results activates enzymes and generates ROS that damage intracellular components and eventually leads to 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 glutamate uptake has been recently demonstrated in the AD brain (44), but the mechanism of inactivation remains unclear. Nonetheless, it is evident that there is a relationship among Aβ-induced free radical toxicity, glutamate-associated excitotoxicity, disruption of Ca^{2+} homeostasis and neuronal degeneration.

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References

1. DW CHOI Science **258** 241-243 (1992).
2. S.M. ROTHMAN and J.W. OLNEY Trends in Neurosci. **10** 299-302 (1987).
3. B. MELDRUM and J. GARTHWAITE Trends in Pharmacol.Sci. **11** 379-386 (1990).
4. M.P. MATTSON, K. FURUKAWA, A.J. BRUCE, R.J. MARK, and E. BLANC in *Molecular Models of Dementia* (W. Wasco and R.E. Tanzi, eds.) Humana Press, Totowa, NJ pp.103-143 (1997).
5. M. LAFANZ-CAZAL, S. PIETRI, M. CULCASI, and J. BOEKAERT Nature **364** 535-537 (1993).
6. B. HAMMER, W.D. PARKER, and J.P. BENNETT Neuroreport **5** 72-74 (1993).
7. P.G. GUNASEKAR, A.G. KANTHASAMY, J.L. BOROWITZ, and G.E. ISOM J. Neurochem. **65** 2016-2021 (1995).
8. E. LANCELOT, J. CALLEBERT, M. PLOTKINE, and R.G. BOULU Neuroreport **6** 1033-1036 (1995).
9. J.B. SCHULZ, D.R. HENSHAW, D. SIWEK, B.G. JENKINS, R.J. FERRANTE, P.B. CIPOLLONI, N.W.KOWALL, B.R. ROSEN, and M.F. BEAL J. Neurochem. **64** 2239-2247 (1995).
10. E. LANCELOT, L. LECANU, M-L. REUAUD, R.G. BOULU, M. PLOTKINE, and J. CALLEBERT Neurosci. Lett. **242** 131-134 (1998).
11. R.M. CLARK and G.G.S. COLLINS J. Physiol. (London) **262** 384-400 (1976).
12. R.W. BAUGHMAN and C.D.GILBERT J. Neurosci. **1** 427-439 (1981).
13. W.F. WHITE, J.V. NADLER, A. HAMBERGER, C.W. COTMAN, and J.T. CUMMINS Nature **270** 356-367 (1977).
14. T.P. HICKS and R.C.A. GEDDES Can. J. Physiol. Pharmacol. **59** 893-896 (1981).
15. R.C.A. PEARSON, M.M. ESIRI, R.W. HIORNS, G.K. WILCOCK, and T.P.S. POWELL Proc. Natl. Acad. Sci. USA **82** 4531-4534 (1985).
16. J. ROGERS and J.H. MORRISON J. Neurosci. **5** 2801-2808 (1985).
17. M.P. MATTSON, B. CHENG, D. DAVIS, K. BRYANT, I. LEIBERBURG, and R.E. RYDEL J. Neurosci. **12** 376-389 (1992).
18. J-Y. KOH, Y.L. YANG and C.W. COTMAN Brain Res. **533** 315-320 (1990).
19. R.J. MARK, K. HENSLEY, D.A. BUTTERFIELD and M.P.MATTSON J. Neurosci. **15** 6239-6249 (1995).
20. D.J. SELKOE J. Neuropathol. Exp. Neurol. **53** 438-447 (1994).
21. W.R. MARKESBERY Free Rad. Biol. Med. **23** 134-147 (1997).
22. D.A. BUTTERFIELD Chem. Res. Toxicol. **10** 495-506 (1997).
23. J.D. ROTHSTEIN, M. DYKES-HOBERG, C.A. PARDO, L.A. BRISTOL, L. JIN, R.W. KUNEL, M.A. HEDIGER, Y. WANG, J.P. SCHIELKE, and D.F. WELTY Neuron **16** 675-686 (1996).
24. P. ROSENBERG and E. AIZENMAN Neurosci. Lett. **103** 162-168 (1989).
25. A. VOLTERRA, D. TROTTI, C. TROMBA, S. FLORIDI, and G. RACAGIN J. Neurosci. **14** 2924-2932 (1994).
26. M.E. HARRIS, Y. WANG, N.W. PEDIGO, JR., K. HENSLEY, D.A. BUTTERFIELD, and J.M. CARNEY J. Neurochem. **67** 277-286 (1996).
27. A. PARPURA-GILL, D. BEITZ, and E. VEMURA Brain Res. **754** 65-71 (1997).
28. M.E. HARRIS, J.M. CARNEY, P.S. COLE, K. HENSLEY, B.J. HOWARD, L. MARTIN, P. BUMMER, Y. WANG, N.W. PEDIGO, JR., and D.A. BUTTERFIELD Neuroreport **6** 1875-1879 (1995).
29. K. HENSLEY, J.M. CARNEY, M.P. MATTSON, M. AKSENOVA, M.E. HARRIS, J.F. WU, R.A. FLOYD, and D.A. BUTTERFIELD Proc. Natl. Acad. Sci. USA **91** 3270-3274 (1994).

30. M.Y. AKSENOV, M.V.AKSENOVA, M.E. HARRIS, K. HENSLEY, D.A. BUTTERFIELD, and J.M. CARNEY J. Neurochem. **65** 1899-1902 (1995).
31. J.N. KELLER, Z. PANG, J.W. GEDDES, J.G. BEGLEY, A. GERMEYER, G. WAEG, and M.P. MATTSON J. Neurochem. **69** 273-284 (1997).
32. D.A. BUTTERFIELD, K. HENSLEY, M.E. HARRIS, M.P. MATTSON and J.M. CARNEY Biochem. Biophys. Res. Comm. **200** 710-715 (1994)
33. T. KOPPAL, R. SUBRAMANIAM, J. DRAKE, M.R. PRASAD, H. DHILLON, and D.A. BUTTERFIELD Brain Res. **786** 270-273 (1998).
34. R.J. MARK, M.A. LOVELL, W.R. MARKESBERY, K. UCHIDA, and M.P. MATTSON J. Neurochem. **68** 255-264 (1997).
35. D.A. BUTTERFIELD and E.R. STADTMAN in *Advances in Cell Aging and Gerontology* **2** 161-191 (1997).
36. J.N. KELLER, R.J. MARK, A.J. BRUCE, E. BLANC, J.D. ROTHSTEIN, K. UCHIDA, G. WAEG, and M.P. MATTSON Neurosci. **80** 685-696 (1997).
37. E. BLANC, J.N. KELLER, S. FERNANDEZ, and M.P. MATTSON Glia **22** 149-60 (1998).
38. J.E. SPRINGER, R.D. AZBILL, R.J. MARK, J.G. BEGLEY, G. WAEG, and M.P. MATTSON J. Neurochem. **68** 2469-76 (1997).
39. E.R. STADTMAN Science **257** 1220-1224 (1992).
40. R. SUBRAMANIAM, F. ROEDIGER, B. JORDAN, M.P. MATTSON, J.N. KELLER, G. WAEG, and D.A. BUTTERFIELD J. Neurochem. **69** 1161-1169 (1997).
41. R. MAKI, M.B. ROBINSON, and M.A. DICTER J. Neurosci. **14** 6754-6762 (1994).
42. G. TONG and C.E. JAHR Neuron **13** 1195-1203 (1993).
43. J. BUSCIGLIO, J. YEH, and B.A. YANKNER J. Neurochem. **61** 1565-1568 (1993).
44. E. MASLIAH, M. ALFORD, D. SALMAN, R. DETERESA, J. M. MALLORY, and L. HANSEN Ann. Neurol. **40** 759-766 (1997).