# GENETIC ABERRANCIES AND NEURODEGENERATIVE DISORDERS

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ADVANCES IN CELL AGING AND GERONTOLOGY

*Series Editors:* PAOLA S. TIMIRAS
E. EDWARD BITTAR

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## ADVANCES IN CELL AGING AND GERONTOLOGY

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# PREFACE

Recent advances in epidemiological investigations, molecular genetics, and methods in molecular biology have led to compelling evidence that gene mutations and polymorphisms make major contributions to most, if not all, neurodegenerative disorders. The purpose of organizing this volume was to bring together, under one cover, fundamental information concerning the roles of inherited traits in the pathogenesis of different neurodegenerative disorders. In addition to providing a catalogue of the known genetic alterations that are linked to specific neurodegenerative disorders, we have attempted to convey the current state of understanding of the cellular and biochemical mechanisms whereby the genetic aberrancies lead to neuronal dysfunction and degeneration. This latter realm of investigation is only in its infancy and, indeed, in most cases we have very little clue as to the precise sequence of events that lead from a genetic defect to nerve cell degeneration. Nevertheless, the emerging pictures of each disorder, painted by pathological, biochemical, and molecular brushes, share several important features, including increased levels of oxidative stress, perturbed ion homeostasis, mitochondrial dysfunction, apoptotic proteolytic cascades, and protein aggregation. The existence of these common themes of the neurodegenerative process provides the opportunity to design experiments (in cultured nerve cells and transgenic mice, for example) that can establish the precise pathogenic mechanism of a specific mutation or genetic risk factor. The value of this approach is exemplified by recent studies of how mutations in Cu/Zn-superoxide dismutase cause amyotrophic lateral sclerosis

(ALS) and how presenilin mutations result in early-onset Alzheimer's disease (AD).

Some neurodegenerative disorders arise solely from a genetic defect, with Huntington's disease (HD) being an example in which the disorder is caused by trinucleotide expansions in the huntingtin gene. The genetic contributions to many other prominent neurodegenerative disorders are quite complex, with some cases being caused by gene mutations and other cases being influenced by polymorphisms that can be considered susceptibility or risk factors. The major risk factor for many of the disorders covered in this volume is increasing age. The aging process is still not well understood, but clearly involves both environmental factors such as progressive accumulation of free radical-mediated damage to cellular constituents and genetic factors such as apolipoprotein E genotype. It is of considerable interest and importance that events that occur during aging predispose neurons to genetic aberrancies that promote degenerative cascades, and that specific genetic defects exert their influence on certain populations of neurons in a disorder-specific manner. Why do presenilin mutations results in age-related degeneration of neurons in the entorhinal cortex and hippocampus, whereas polyglutamine expansions in Huntington render striatal neurons vulnerable and Cu/Zn SOD mutations afflict mainly lower motor neurons? The answer to such questions has proven elusive because of the fact that, in most cases, the defective gene is expressed at similar levels in both vulnerable and nonvulnerable populations of neurons. It is hoped that the chapters in this volume will stimulate readers to generate new hypotheses concerning the pathogenic mechanisms of genetic aberrancies that can be experimentally tested.

The scope of the subject of this book is certainly too vast to fully cover in detail in one volume. However, I expect the reader will find each chapter information-rich and will appreciate the relative lack of redundancy that often occurs in such edited volumes. The organization of the book is rather simple—each chapter covers a given disorder or class of disorders. AD is an intriguing disorder because its genetics are quite complex, and the cellular and molecular neurodegenerative mechanisms that appear to be operative involve a set of regulatory systems that normally function in neuronal development and adaptive synaptic plasticity. In the chapter on AD, I have attempted to provide a view of how genetic causal and risk factors interact with age-related changes in the brain to promote degenerative biochemical cascades. The trinucleotide repeat disorders are covered by P. Hemachandra Reddy and Danilo Tagle. The latter have several intriguing features including "anticipation," in which the number of trinucleotide repeats in the affected gene increase in successive generations resulting in enhance voracity of the clinical phenotype. The fact that such trinucleotide repeats are responsible for degeneration of different neuronal populations depending upon the disorder provides a novel opportunity to address basic mechanisms underlying selective neuronal death. Neil Kowall and

colleagues cover the seemingly straightforward genetics of HD, and the less straightforward mechanisms whereby polyglutamine repeats in the huntingtin protein lead to degeneration of striatal neurons. Recent findings suggesting that aggregation of abnormal huntingtin may be involved in its neurodegenerative effects, and may provide mechanistic links between this disease and AD and PD, two other disorders that exhibit abnormal aggregations of amyloid β-peptide and synuclein, respectively. The identification of mutations in Cu/Zn SOD as causal for some cases of familial amyotrophic lateral sclerosis (ALS) brought the free radical theory of neurodegenerative disorders to center stage; Ed Kasarskis and Daret St. Clair cover the genetics of ALS and possible mechanisms underlying the pathogenic actions of Cu/Zn SOD mutations, which appear not to be as simple as originally thought. Mutations in prion proteins result in formation and amplification of amyloid protein aggregations, which appear to be the key events in the infective feature of these disorders. Bernardino Ghetti and Pierluigi Gambetti describe the genetic alterations responsible for several different types or prion disorders and emphasize the role of these mutations in protein conformation and protein-protein interactions.

Epilepsy has a remarkably complex genetics, probably because there are so many factors that can alter neuronal excitability. Carl Stafstrom, John Slevin, and coauthors provide a view of this complexity and its implications for mechanisms of disease pathogenesis. As described by LaRoy Penix and Doug Lanska, stroke is a leading cause of disability and death that has important genetic predisposition and causal factors. The inherited disorder CADASIL was recently shown to be caused by mutations in Notch-3, which raises very interesting questions concerning its pathogenic mechanism, and suggests possible mechanistic links between AD and stroke, since presenilin-1 appears to be involved in the Notch signaling pathway. Damage to oligodendrocytes resulting in demyelination in the central nervous system is a central feature of multiple sclerosis (MS). While accumulating data suggest an autoinflammatory response plays a role in MS, the causes are unkown. Robert Bell presents emerging evidence that is revealing genes which predispose to MS. Because many other neurodegenerative disorders involve inflammation-like processes, a better understanding of genetic influences on cytokine cascades and leukocyte physiology is clearly of interest.

Mitochondrial DNA mutations are increasingly recognized as playing important roles in an array of age-related neurodegenerative disorders. Gordon Glazner describes the central role of mitochondria in free radical metabolism and calcium homeostasis, and provides a view of how disruption of these processes may be a central consequence of mitochondrial DNA mutations. The pivotal role of mitochondria in apoptosis, and the likely involvement of this form of cell death in many different neurodegenerative disorders, emphasizes the importance of understanding how abberancies of the mitochondrial genome arise and how they lead to nerve cell

degeneration. There exists a quite remarkable array of hereditary neurodegenerative disorders that arise from alterations in metabolic pathways. Zeynep Tümer and Nina Horn describe inherited disorders of copper metabolism, including their unusual clinical manifestations and their biochemical bases. Robert Jolly and colleagues present the lysosomal storage diseases classified as ceroid-lipofuscinoses, a complex set of related disorders often characterized by electroencephalogram abnormalities and retinal degeneration. The genetic alterations responsible for the latter disorders are beginning to be identified, and it is expected that definition of the protein alterations that lead to perturbed lysosomal metabolism will soon follow.

We have attempted to capture the excitement and optimism of the current era of genetic and molecular biology as applied to neurodegenerative disorders, and hope that this volume spurs interactive research efforts aimed at identifying mechanisms that are shared among neurodegenerative disorders.

**MARK P. MATTSON**

## ACKNOWLEDGEMENTS

We, as authors, are indebted to Sally Malley who has been the key person involved in organizing and editing the manuscripts for this volume. We also thank the many outstanding coworkers who contributed to the basic research described in the chapters.

Chapter 1

# Genetic Contributions to the Pathogenesis of Alzheimer's Disease

MARK P. MATTSON



## INTRODUCTION

Alzheimer's disease (AD) is a progressive degenerative disorder characterized by nerve cell dysfunction and death in brain regions involved in learning and memory processes, including the hippocampus, entorhinal cortex, and basal forebrain. Examination of postmortem brain tissue from AD patients reveals striking abnormalities including degenerated neurons and dystrophic neurites containing abnormal accumulations of insoluble straight and twisted filaments comprised of a cytoskeletal protein called tau (see Selkoe, 1991, for review). Tau normally functions in the modulation of microtubule polymerization, thereby regulating adaptive

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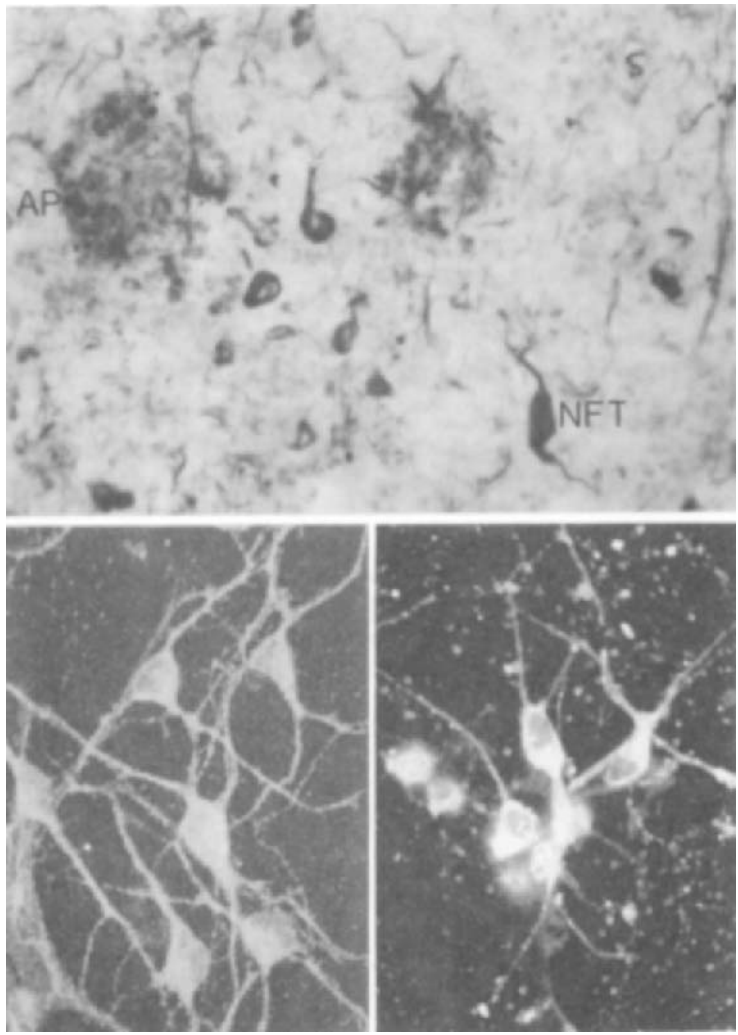


***Figure 1.*** Association of amyloid deposition with neuronal degeneration in Alzheimer's disease (AD). The upper panel shows the two overt abnormalities observed upon microscopic examination of brain tissue from AD patients—the neurofibrillary tangle (NFT) consisting of intracellular accumulations of insoluble filaments of the microtubule-associated protein tau in the nerve cell body, and amyloid plaques (AP) comprised of extracellular aggregations of amyloid β-peptide (Aβ) often associated with degenerated neurites. The lower panels show an example of the damaging effect of Aβ on cultured hippocampal neurons—one culture was exposed to a control peptide (Control) and the other to the fibril-forming Aβ. The cells were then reacted with an antibody that recognizes phosphorylated tau (white color). Aβ caused acummulation of phosphorylated tau filaments and neuron degeneration.

changes in neuronal structure and physiology. In AD tau becomes hyperphosphorylated and self-aggregates, and microtubules depolymerize which might contribute to neurite dysfunction and degeneration. Immunohistochemical and ultrastructural analyses have shown that extensive synapse loss occurs in the relatively early stages of AD (see Lassman, 1996, for review). Another prominent abnormality in AD brain is the accumulation of extracellular amyloid plaques which are spherical structures comprised mainly of a protein called amyloid β-peptide (Aβ) (Figure 1). Aβ is a 40- to 42-amino acid peptide generated as a proteolytic product of a much larger amyloid precursor protein (APP) (see Mattson, 1997a, for review). Plaques manifest as either a diffuse form in which Aβ is in an unaggregated state not associated with neuronal degeneration, and a compact form in which Aβ forms antiparallel fibrils with a β-pleated sheet structure that exhibit birefringence under polarized light. The fibrillar Aβ deposits are often associated with degenerated neurites. Cognitive deficits are strongly correlated with density of neurofibrillary tangles, amyloid burden, and synapse loss suggesting that neurodegeneration is responsible for cognitive dysfunction and that amyloid accumulation is linked to the neurodegenerative process.

In addition to the neuronal degeneration and Aβ deposition present in brains of AD victims, there are numerous cellular and biochemical alterations that suggest the presence of an inflammation-like process (see McGeer and McGeer, 1995, for review). Reactive astrocytes and microglia are associated with neuritic plaques, with astrocytes surrounding the plaques and microglia being concentrated within the plaques. Local increases in several cytokines have been described in association with neuritic plaques including interleukin-1β, interleukin-6, and tumor necrosis factor-α. Moreover, immunohistochemical studies indicate the presence of complement proteins such as C1q in association with neuritic plaques. By analogy with inflammatory responses in other tissues, the glial and immune alterations in AD brain most likely represent a secondary response to a primary neurodegenerative process. Nevertheless, such secondary responses may accelerate the neurodegenerative process. Indeed, recent epidemiological data suggest that nonsteroidal anti-inflammatory drugs may be beneficial in delaying the onset of the symptoms of AD (Breitner et al., 1994). Although genetic contributions to AD may act at the level of the primary neurodegenerative process, it is important to consider the roles of inflammatory processes in the progression of the disease.

## MUTATIONS LINKED TO EARLY-ONSET INHERITED ALZHEIMER'S DISEASE

There are families in which AD is inherited in an autosomal dominant manner such that all affected family members develop AD symptoms at an early age, usually when they are in their 30s, 40s, and 50s. Such familial AD (FAD) cases account for approximately 15% of all AD cases (see Finch and Tanzi, 1997, for review). The remaining 85% of cases are not caused by a specific genetic defect, and are

***Table 1.*** Genetic Causal and Risk Factors for Alzheimer's Disease

| | *Gene* | *Chromosome* | *Age of Onset (yrs)* |
|---|---|---|---|
| Causal Factor | Amyloid precursor protein | 21 | 45–65 |
| | Presenilin-1 | 14 | 28–50 |
| | Presenilin-2 | 1 | 40–55 |
| Risk Factor | Apolipoprotein E4 | 19 | 65–85 |
| | α2-macroglobulin | 12 | 65–85 |
| | Bleomycin hydrolase | 17 | 65–85 |

characterized by a relatively late age of onset, typically in the range of 65 to 85 years of age; the sporadic forms of AD are, however, influenced by genetic polymorphisms that can be considered susceptibility or risk factors. During the past 10 years, tremendous progress has been made in identifying the genetic defects responsible for FAD (Table 1). At least five different chromosomes harbor defective genes including chromosomes 1, 12, 14, 17, and 21. The first gene linked to FAD was the β-amyloid precursor protein (APP) located on chromosome 21 (see Mullan and Crawford, 1993, for review). Three years ago, two homologous genes were identified as harboring mutations linked to the most vigorous (earliest age of onset) forms of AD. The genes are now called presenilin-1 (chromosome 14) and presenilin-2 (chromosome 1) (see Hardy, 1997, for review). The defective genes located on chromosomes 12 and 17 have yet to be identified, although recent findings suggest that α2-macroglobulin or the low-density lipoprotein–related receptor (LRP), or both, may be the culprit(s) on chromosome 12 (Blacker et al., 1998) and that tau is the affected gene on chromosome 17 (Poorkaj et al., 1998).

## APP MUTATIONS RESULT IN ABERRANT PROTEOLYTIC PROCESSING OF APP, LEADING TO OXIDATIVE STRESS AND PERTURBED CALCIUM REGULATION IN NERVE CELLS

APP is a large transmembrane protein that is expressed in neurons and glial cells throughout the nervous system, as well as in many non-neural tissues including vascular smooth muscle and endothelial cells (see Mattson, 1997a, for review). In neurons APP is axonally transported and accumulates in presynaptic terminals and growth cones. APP mutations in FAD cases are missense in nature resulting in either a single amino acid change at codon 717 ("London" mutation) or a two amino acid substitution at codons 670 and 671 ("Swedish" mutation). The mutations are located immediately N- (Swedish mutation) or C-terminal (V717F) to the Aβ sequence (Figure 2). Additional families harbor mutations at codons 692 ("Flemish" mutation) or 693 ("Dutch" mutation), which lie within the Aβ sequence. A cleavage of APP in the middle of the Aβ sequence, effected by an enzyme called

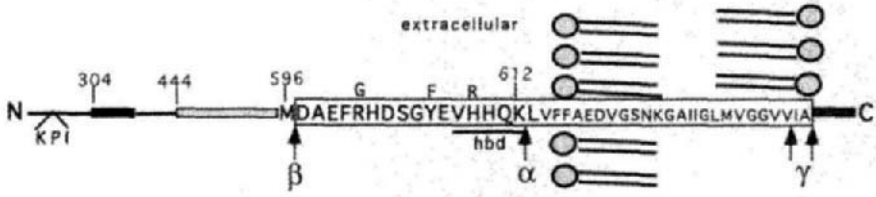


***Figure 2.*** Structure, proteolytic cleavage sites, and functional domains of human APP. APP consists of a large extracellular domain of approximately 616 amino acids, a hydrophobic transmembrane domain, and a short cytoplasmic C-terminus. The numbering of amino acids in the diagram is based on the 695 amino acid isoform; additional isoforms of APP (APP751 and APP770) contain a kunitz protease inhibitor (KPI) domain near the N-terminus. The amino acid sequence of Aβ is indicated in the boxed portion of the diagram. APP is proteolytically cleaved at at least four different sites. α-Secretase cleaves between amino acids 612 and 613, which lies within the Aβ sequence (this cleavage releases sAPPα from the cell surface). β-Secretase cleaves at the N-terminus of Aβ (between amino acids 596 and 597), releasing sAPPβ from the cell surface and leaving a C-terminal membrane-associated fragment containing intact Aβ. γ-Secretase cleaves at the C-terminus of Aβ in at least two different sites, resulting in release of intact Aβ1-40 or Aβ1-42. There are several functional domains in sAPPα including a region just N-terminal to the β-secretase cleavage site that is involved in modulating neuronal excitability and survival, and a heparin-binding domain (hbd) at the C-terminus. Within the Aβ domain, the amino acid sequences of human and rodent Aβ differ by three amino acids (G, F, and R are present in rat Aβ as indicated in the diagram).

α-secretase, results in release of a secreted form of APP called sAPPα from axon terminals. This secretory cleavage prevents production of intact, and therefore potentially amyloidogenic, Aβ. Interestingly, the secretory cleavage of APP is induced when neurons are electrically active and studies of the effects of sAPPα on neuronal activity and synaptic function suggest that sAPPα may play an important role in learning and memory processes (Doyle et al., 1990; Huber et al., 1993; Roch et al., 1994; Furukawa et al., 1996a, 1996b; Ishida et al., 1997; Furukawa and Mattson, 1998). A striking biological activity of sAPPα is its ability to protect neurons from being damaged and killed by conditions relevant to the pathogenesis of AD including exposure oxidative and metabolic insults (Mattson et al., 1993; Smith-Swintosky et al., 1994; Furukawa et al., 1996b). An alternative cleavage of APP at the N-terminus of the Aβ sequence (by β-secretase activity) leaves a membrane-associated fragment that contains intact Aβ and can be further cleaved at the C-terminus (by γ-secretase activity) resulting in the release of Aβ. APP mutations result in altered APP processing in a manner that increases production of Aβ and decreases production of sAPPα.

There are at least two mechanisms whereby APP mutations may promote neuronal degeneration in AD (see Mattson, 1997a, for review). The first involves increased Aβ production leading to excessive accumulation of fibrillar Aβ, which causes cell damage and death. The second mechanism involves reduced production of sAPPα; it has been shown that sAPPα can prevent death of cultured neurons and of brain neurons in adult rodents exposed to metabolic and oxidative insults relevant to the pathogenesis of AD, suggesting that APP mutations may result in loss of a neuroprotective function of APP.

When mutated forms of APP are expressed in cultured cells and transgenic mice, there is increased production of Aβ, particulary the longer 42 amino acid form of the peptide (Aβ1-42). Aβ1-42 exhibits an increased propensity to self-aggregate and form amyloid fibrils; this property of Aβ1-42 is correlated with increased toxic activity toward cultured neurons. Exposure of cultured hippocampal neurons to Aβ results in an increase in levels of various oxyradicals, and consequent free radical–mediated damage to membrane lipids, proteins, and DNA. Aβ itself may generate free radicals upon interaction with certain metals such as iron ($Fe^{2+}$); such generation of peptide-associated radicals may play an important role in covalent crosslinking of Aβ to form amyloid fibrils (Dyrks et al., 1992; Hensley et al., 1994; Mattson, 1995). Alternatively (or coincidentally) Aβ may induce oxidative stress by engaging receptor-mediated pathways. For example, data suggest that Aβ binds to the receptor for advanced glycation end products (RAGE), and that this interaction results in increased nitric oxide production in cells such as microglia that express RAGE (Mattson and Rydel, 1996; Yan et al., 1996).

Membrane lipid peroxidation appears to be a critical early event that results in a cascade of events induced by Aβ that increases the vulnerability of neurons to degeneration (Mattson, 1998). Lipid peroxidation causes release of an aldehyde called 4-hydroxynonenal (HNE), which covalently binds to proteins at cysteine, lysine, and histidine residues. Studies of cultured primary neurons, astrocytes, and synaptosomes have shown that Aβ can impair the function of membrane proteins involved in the regulation of ion homeostasis and energy metabolism, and that 4-hydroxynonenal plays a key role in these actions of Aβ (Figure 3). Exposure of cultured rat hippocampal neurons or human cortical synaptosomes to Aβ impairs the function of the $Na^+/K^+$-adenosine triphosphatase (ATPase) and the $Ca^{2+}$-ATPase, two membrane enzymes critical for maintenance of resting membrane potential and intracellular calcium levels (Mark et al., 1995). The latter effects of Aβ are blocked by antioxidants that suppress membrane lipid peroxidation, and are mimicked by 4-hydroxynonenal (Mark et al., 1995, 1997a; Keller et al., 1997a, 1997b). Additional studies have shown that both Aβ and HNE can impair the function of neuronal and synaptosomal glucose transporters (Mark et al., 1997b; Keller et al., 1997a), and astrocytic glutamate transporters (Keller et al., 1997b; Blanc et al., 1998). The discovery that Aβ impairs the function of these membrane transporters via an oxyradical-mediated mechanism may explain why only neurons, and not glial cells, degenerate and die in AD. Neurons that degenerate in AD express

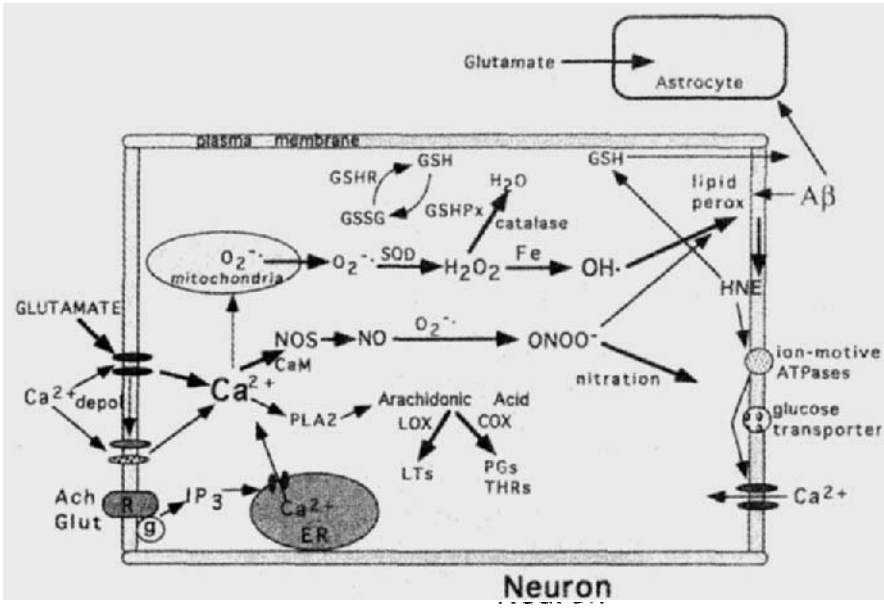


***Figure 3.*** Mechanisms whereby Aβ and activation of glutamate receptors induce oxidative stress and disrupt ion homeostasis in neurons. Aβ can induce membrane lipid peroxidation (MLP), resulting in the production of 4-hydroxynonenal (HNE) an aldehyde that covalently modifies membrane transporters ($Na^+/K^+$-ATPase, $Ca^{2+}$-ATPase, glucose transporter, and glutamate transporter) and thereby impairs their functions. These adverse effects of MLP promote membrane depolarization and excessive activation of glutamate receptors resulting in excitotoxicity. Oxidative stress also perturbs ion homeostasis in endoplasmic reticulum (ER) and mitochondria. Activation of glutamate receptors results in calcium influx which, in turn, promotes oxyradical production in several different ways including compromising mitochondrial calcium homeostasis and membrane potential resulting in increased production of superoxide anion radical ($O_2^-$·). Superoxide dismutases (SOD) convert $O_2^-$· to $H_2O_2$ which, in the presence of $Fe^{2+}$, generates OH·. $O_2^-$· also interacts with nitric oxide (NO) to form peroxynitrite. Both OH· and peroxynitrite induce MLP. Calcium also promotes arachidonic acid production the activities of cyclooxygenases (COX) and lipoxygenases (LOX) with resulant generation of $O_2^-$·.

high levels of receptors for the excitatory neurotransmitter glutamate. Neurons that express glutamate receptors are vulnerable to being killed by a mechanism termed "excitotoxicity" in which activation of glutamate receptors under adverse conditions (e.g., when the cells are subjected to oxidative and metabolic stress) results in massive calcium influx and disruption of various structural components and metabolic pathways in the neurons. Exposure of cultured hippocampal neurons to Aβ (Mattson et al., 1992) and 4-hydroxynonenal (Mark et al., 1997a) greatly

increases their vulnerability to glutamate toxicity. Central to the mechanism whereby Aβ increases neuronal vulnerability to excitotoxicity is lipid peroxidation-mediated impairment of ion-motive ATPases, and glucose and glutamate transporters (Keller et al., 1997b; Mark et al., 1997a, 1997b). Such experimental data are consistent with analyses of the human brain which revealed that neurons that degenerate in AD, such as those in the hippocampus and entorhinal cortex, express very high levels of glutamate receptors. Consistent with the oxidative stress–perturbed calcium hypothesis of neuronal degeneration in AD are data showing that insults that induce oxidative stress and disrupt calcium homeostasis in neurons also induce alterations in the microtubule-associated protein tau similar to those seen in neurofibrillary tangles in AD (Mattson, 1990). 4-Hydroxynonenal may play a role in the latter process by promoting crosslinking of tau and preventing its dephosphorylation (Mattson et al., 1997a). Although it remains to be established if and how tau hyperphosphorylation and crosslinking contributes to the neuronal cell death process in AD, the recent finding that mutations in tau account for some cases of frontotemporal dementia (Poorkaj et al., 1998) suggests this possibility.

A prominent abnormality in AD patients is that the ability of their brain cells to transport glucose is severely compromised. This abnormality has been repeatedly documented in brain imaging studies in which the uptake of radiolabeled glucose into brain cells is quantified. Moreover, a deficit in glucose transport can be detected prior to clinical symptoms in patients at risk for AD. Oxidative stress resulting from Aβ deposition and age-related changes likely plays an important role in the impairment of glucose uptake in neurons (Mark et al., 1997b). Aβ also impairs glucose uptake in vascular endothelial cells (Blanc et al., 1997b); these cells provide the main route of transport of glucose from blood to brain. The adverse effect of Aβ on glucose transport can be prevented by treating the neurons and vascular endothelial cells with antioxidants such as vitamin E, glutathione ethyl ester, and 17β-estradiol (Blanc et al., 1997b; Keller and Mattson, 1997; Mark et al., 1997b).

Exposure of cultured cortical neurons to sublethal levels of Aβ resulted in impaired muscarinic cholinergic signaling, analogous to the cholinergic alterations documented in brain tissue from AD patients (Kelly et al., 1996). Detailed analyses indicated that the defect in the signaling pathway involved impaired coupling of the muscarinic receptors to the guanosine triphosphate (GTP)-binding protein $G_{q11}$. The adverse effect of Aβ on cholinergic signal transduction was mimicked by exposure of cells to $Fe^{2+}$, and prevented in cells treated with vitamin E, suggesting a role for lipid peroxidation (Kelly et al., 1996). 4-Hydroxynonenal may mediate lipid peroxidation-induced impairment of muscarinic signal transduction, possibly by covalently crosslinking $G_{q11}$ (Blanc et al., 1997a). Other signaling pathways involving GTP-binding proteins such as those activated by metabotropic glutamate receptors (Blanc et al., 1997a) and thrombin receptors (Mattson and Begley, 1996) may also be adversely affected by Aβ via a lipid peroxidation-mediated mechanism.

Deficits in mitochondrial function and increased oxidative damage to mitochondria have been documented in studies of AD patients (see Benzi and Moretti, 1997;