

Review

Slow Excitotoxicity in Alzheimer's Disease

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Accepted 12 February 2013

Abstract. Progress is being made in identifying possible pathogenic factors and novel genes in the development of Alzheimer's disease (AD). Many of these could contribute to 'slow excitotoxicity', defined as neuronal loss due to overexcitation as a consequence of decreased energy production due, for instance, to changes in insulin receptor signaling; or receptor abnormalities, such as tau-induced alterations in *N*-methyl-D-aspartate (NMDA) receptor phosphorylation. As a result, glutamate becomes neurotoxic at concentrations that normally show no toxicity. In AD, NMDA receptors are overexcited by glutamate in a tonic, rather than a phasic manner. Moreover, in prodromal AD subjects, functional MRI reveals an increase in neural network activities relative to baseline, rather than loss of activity. This may be an attempt to compensate for reduced number of neurons, or reflect ongoing slow excitotoxicity. This article reviews possible links between AD pathogenic factors such as A β PP/A β and tau; novel risk genes including clusterin, phosphatidylinositol-binding clathrin assembly protein, complement receptor 1, bridging integrator 1, ATP-binding cassette transporter 7, membrane-spanning 4-domains subfamily A, CD2-associated protein, sialic acid-binding immunoglobulin-like lectin, and ephrin receptor A1; metabolic changes including insulin resistance and hypercholesterolemia; lipid changes including alterations in brain phospholipids, cholesterol and ceramides; glial changes affecting microglia and astrocytes; alterations in brain iron metallome and oxidative stress; and slow excitotoxicity. Better understanding of the possible molecular links between pathogenic factors and slow excitotoxicity could inform our understanding of the disease, and pave the way towards new therapeutic strategies for AD.

Keywords: Alzheimer's disease, amyloid- β peptide, tau, excitotoxicity, insulin resistance, phospholipase A₂, cholesterol oxidation products, iron, memantine

INTRODUCTION

Alzheimer's disease (AD) is a progressive neurodegenerative disorder associated with memory

impairment and severe dementia. Neuropathological AD is characterized by accumulation of amyloid- β peptide (A β) neuritic plaques, neurofibrillary tangles, inflammation, and loss of synapses [1, 2]. Although the molecular mechanisms associated with pathogenesis of AD are not clearly understood, A β -mediated oxidative stress, induction of neuroinflammation, and abnormal glutamate metabolism have been implicated

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in onset and progression of the disease [1–5]. AD is a multifactorial disease with contributing factors such as aging, positive family history, unhealthy lifestyle and exposure to environmental toxicity [1, 2]. Although no single lifestyle factor has conclusively been shown to alter the risk of AD, evidence suggests that many risk factors for heart disease could also increase the risk of developing AD. These include lack of exercise, smoking, hypertension, hypercholesterolemia, and diabetes [6]. Other than variations in genes encoding amyloid- β protein precursor (A β PP), presenilin-1 (PSEN-1), and -2 (PSEN-2) that cause early-onset familial AD, a huge effort has been put in identifying gene loci that are associated with increased risk for sporadic AD [7]. It is now well-established that the homozygote apolipoprotein E4 (ApoE4) haplotype is associated with a significantly increased risk for sporadic AD. Moreover, a recent large genome-wide association study in late-onset AD has identified nine novel loci, and the authors of this study estimate genetic components to account for 60–80% of the disease [8]. These include clusterin, phosphatidylinositol-binding clathrin assembly protein (PICALM), complement receptor 1 (CR1), bridging integrator 1 (BIN1), ATP-binding cassette transporter 7 (ABCA7), membrane-spanning 4-domains subfamily A (MS4A cluster), CD2-associated protein (CD2AP), sialic acid-binding immunoglobulin-like lectin (CD33), and ephrin receptor A1 (EPHA1) [8]. As will be discussed below, many of these risk factors could have effects on slow excitotoxicity.

Excitotoxicity is defined as a process by which high levels of glutamate overexcite neurons and brings about cell death [9, 10]. Glutamate or its analogs exert their effect on neurons by interacting with excitatory amino acid receptors on the post-synaptic cell membrane, including N-methyl-D-aspartate (NMDA), α -amino-3-hydroxy-5-methyl-4-isoxazole propionate (AMPA), and kainate (KA) receptors [11, 12]. Excess stimulation of these receptors is accompanied by massive calcium influx initiating a cascade of events involving free radical generation, mitochondrial dysfunction, intracellular signaling cascades, such as p38 MAPK, extracellular signal-regulated kinase (ERK), and c-Jun N-terminal kinase, and activation of many calcium-dependent enzymes, including those involved in the generation and metabolism of arachidonic acid. The enzymes include isoforms of phospholipase A₂ (PLA₂), cyclooxygenase-2 (COX-2), lipoxygenases (LOX), and epoxygenases (EPOX) [12]. Accumulation of oxygenated arachidonic acid metabolites through an uncontrolled “arachidonic acid cascade”, along with lack of energy generation

and abnormal ion homeostasis results in neural cell death.

High levels of glutamate damage glial cells by mechanisms that do not involve glutamate receptor activation, but rather glutamate uptake [14, 15]. The latter is essential for maintaining excitatory postsynaptic currents, and preventing excitotoxicity due to overstimulation of glutamate receptors [16]. Out of five glutamate transporters cloned from brain tissue, at least two transporters, namely excitatory amino acid transporter 1 (EAAT1) and excitatory amino acid transporter 2 (EAAT2), are expressed in astrocytes, oligodendrocytes, and microglial cells. Exposure of astrocyte, oligodendrocyte, and microglial cell cultures to glutamate produces glial cell death by a transporter-related mechanism involving inhibition of cystine uptake, which causes a decrease in the antioxidant glutathione, and makes glial cells vulnerable to free radical injury [17].

The purpose of this article is to review: i) Evidence for slow excitotoxicity in AD, ii) AD pathogenic factors, iii) AD pathogenic factors and slow excitotoxicity, iv) Possible links between novel AD risk genes and slow excitotoxicity, v) Possible links between AD associated metabolic changes and slow excitotoxicity, vi) Possible links between AD associated lipid changes and slow excitotoxicity, vii) Possible links between AD associated glial changes and slow excitotoxicity, viii) Possible links between AD associated iron changes and slow excitotoxicity, and ix) Treatment strategies for AD targeting slow excitotoxicity.

EVIDENCE FOR SLOW EXCITOTOXICITY IN AD

Two types of excitotoxicity are distinguished, a classical or acute, and a slow form. In ‘classical excitotoxicity’, acute elevation of glutamate is the key event inducing neuronal damage in conditions such as stroke, status epilepticus and traumatic brain and spinal cord injury. The increase in glutamate is due to release from damaged neurons or failure of glutamate transporters, and results in overexcitation of neurons. In contrast, ‘slow excitotoxicity’ in chronic neurodegenerative conditions is a consequence of decreased energy production or receptor abnormality [18]. For example, alterations in insulin signaling or disturbance of mitochondrial function can cause an impaired energy state of neurons. This results in decreased intracellular ATP levels and allows glutamate to become neurotoxic at concentrations that normally show no toxicity [19].

ATP is needed for ion pumps to maintain physiological intracellular calcium levels. When the cell membrane is depolarized from its usual -90 mV to between -60 and -30 mV, the voltage-dependent Mg^{2+} block of the NMDA receptor is relieved, leading to its persistent activation. Partial neuronal depolarization induced by inhibitors of either glycolysis (iodoacetate) or oxidative phosphorylation (cyanide) results in NMDA receptor activation, in the absence of any increase in extracellular glutamate concentrations [20]. In addition, aminooxyacetic acid or 1-methyl-4-phenylpyridinium (MPP), which produces impaired mitochondrial energy metabolism, results in excitotoxic striatal lesions that are blocked by either glutamatergic denervation or NMDA receptor antagonists [21, 22]. Besides energy deficits, changes in cellular membranes may underlie receptor abnormalities that lead to slow excitotoxicity. Many Ca^{2+} -dependent enzymes are involved in neural membrane phospholipid, sphingolipid, and cholesterol metabolism and breakdown of neuronal cytoarchitecture. They contribute to elevated levels of phospholipid-, sphingolipid-, and cholesterol-derived lipid mediators, some of which have effects on the nucleus [23, 24]. Moreover, changes in membrane lipids may affect the structural properties of cell membranes and function of NMDA receptors, with possible implications for neuronal excitation and excitotoxicity [25].

NMDA receptors interact with various lipids and post synaptic density proteins (PSDs), such as PSD-95, to regulate downstream signaling pathways that mediate synaptic plasticity [26, 27]. Under physiological conditions, low levels of phospholipid-, sphingolipid-, and cholesterol-derived lipid mediators are involved in signal transduction, adhesion, sorting, and trafficking. Under pathological conditions such as AD however, elevation in levels of lipid mediators is closely associated with neuronal injury [28, 29]. Neurochemical changes include glutamate-mediated activation of cytosolic PLA_2 (c PLA_2) and plasmalogen-selective PLA_2 (PlsEtn- PLA_2), decrease in phosphatidylcholine and loss of plasmalogens, and reduction in the presynaptic vesicle protein synaptophysin [30, 31]. It has been proposed that increased cross-talk between excitotoxicity, oxidative stress and neuroinflammation, along with elevated levels of phospholipid-, sphingolipid-, and cholesterol-derived lipid mediators in cytoplasmic and nuclear compartments may result in loss of synapses in AD [1, 2]. Aging, the major risk factor for AD, causes synaptic loss in the dentate region of the hippocampus, but in advanced AD, there is a disproportionately high loss of synapses, which corre-

lates with the degree of dementia [32, 33]. In addition, soluble $A\beta$ oligomers, also referred to as amyloid- β derived diffusible ligands (ADDLs) act as highly specific pathogenic ligands for binding sites at synapses and promote synaptic loss [34]. This binding not only stimulates PlsEtn- PLA_2 [35], but also induces oxidative stress, loss of synapses, and ectopic redistribution of receptors crucial for plasticity and memory [34].

In AD, NMDA receptors are overexcited by glutamate in a tonic rather than a phasic manner. This continuous over-activation may lead to neuronal damage by stimulating Ca^{2+} -dependent enzymes related to lipid, protein and nucleic acid metabolism [36]. Increased hippocampal activity is a possible early indicator of AD-related neurodegeneration. In prodromal AD subjects, functional MRI reveals an increase in neural network activities relative to baseline, rather than loss of activity [37]. This may be an attempt to compensate for lower neuron numbers but could also reflect ongoing 'slow excitotoxicity'. Alternatively, loss of inhibitory neurons may disinhibit neurons [38]. Together, these findings indicate that, in AD, neurons and neural networks do not simply 'get silenced' but may instead be hyperactive, and could interfere with processes underlying learning, memory, and other cognitive functions. Interestingly, magnetic resonance spectroscopy reveals significantly lower levels of glutamate in AD brains compared to controls and patients with mild cognitive impairment [39]. This supports the notion that increased excitation or network activity reflects a decreased capacity to cope with existing glutamate levels, rather than a rise in glutamate level *per se*.

SUMMARY OF AD PATHOGENIC FACTORS

A β PP/A β

$A\beta$ PP is a member of a family of conserved type I membrane proteins and is present in the postsynaptic density and is involved in maintaining active synapses [40]. It may also be involved in iron transport across membranes [41]. $A\beta$ PP is expressed in neurons and glial cells in the brain, and peripheral tissues including muscle, epithelial tissue, and circulating cells, such as platelets. $A\beta$ PP occurs in three isoforms ($A\beta$ PP695, $A\beta$ PP751, and $A\beta$ PP770), generated by alternative splicing of exons 7 and 8 [42]. $A\beta$ PP undergoes proteolytic processing by one of two pathways [43]. It can be processed via the non-amyloidogenic pathway mediated by α -secretase, of which putative candidates belonging to the family of a disintegrin and metalloprotease (ADAM) have been identified: ADAM9,

ADAM10, and ADAM17. Enzymatic cleavage by α -secretase occurs within the A β domain, thereby preventing the generation and release of A β peptide. Alternatively, A β PP can be a substrate for β -secretase, releasing an ectodomain (sA β PP), and retaining the last 99 amino acids of A β PP (known as C99) within the membrane. The first amino-terminal amino acid of C99 is the first amino acid of A β . To eventually release A β , C99 is subsequently cleaved by the γ -secretase complex, consisting of PSEN-1 or PSEN-2, nicastrin, anterior pharynx defective, and presenilin enhancer 2. This cleavage predominantly produces a 40 amino-acid species A β ₄₀, and the more amyloidogenic 42 amino-acid variant A β ₄₂ in a ratio of 10:1. A β ₄₂ aggregates much faster and may be directly related to the pathogenesis of AD [44]. The presence of A β ₄₂ oligomers (also known as ADDLs) correlates well with the severity of dementia in AD patients [45]. Unlike insoluble fibrils, ADDLs are diffusible molecules that bind a variety of targets thus acting as pathologic ligands [46]. Interaction of ADDLs with synapses results in tau hyperphosphorylation and induction of oxidative stress [47], loss of long-term potentiation [48] and deterioration of synapses [49].

Tau

The microtubule-associated protein, tau, is expressed predominantly in neurons and has been implicated in stabilizing microtubules, regulation of motor-driven axonal transport, and postsynaptic scaffolding [50]. Tau becomes hyperphosphorylated at both physiological and pathological sites in AD, and leads to the formation of toxic aggregates, and eventually neurofibrillary tangles [51]. Aberrant phosphorylation detaches tau from microtubules and affects its microtubule-stabilizing functions, resulting in disruption of communication between the neuronal cell body and processes [52]. A series of recent publications suggests tau to be essential for A β to induce downstream toxicity in the pathogenesis of AD [53]. Accordingly, neurons from tau knockout mice show resistance to A β toxicity [54]. In addition, crossing A β PP transgenic with tau knockout mice ameliorates A β -induced water-maze learning and memory deficits, increased exploratory locomotor activity and premature mortality, but does not alter high A β levels [55, 56]. Furthermore, tau-knockout neurons are resistant to amino-terminally truncated pyroglutamylated forms of A β , which are more toxic than A β ₄₂ or A β ₄₀ and possibly involved in initiation of AD pathology [57].

ApoE

ApoE takes up lipids generated after neuronal degeneration and redistributes them to cells requiring lipids for proliferation, membrane repair, or remyelination of new axons [58, 59]. It is well established that the ApoE4 isoform is associated with increased risk of developing AD [60, 61]. Studies in ApoE-deficient mice expressing mutant A β PP demonstrate that ApoE is required for the formation of fibrillar amyloid plaques [62, 63]. ApoE is associated with amyloid plaques, and lipid-free ApoE3 and ApoE4 can form stable complexes with A β , with ApoE4 forming complexes more rapidly and effectively [64]. C-terminally truncated ApoE4 also increases tau phosphorylation and formation of intracellular neurofibrillary tangle-like inclusions in cultured neuronal cells and transgenic mice [58, 59]. Moreover, ApoE4 is associated with mitochondrial dysfunction in AD patients [65]. ApoE4 fragments target neuronal mitochondria, leading to mitochondrial dysfunction and neurotoxicity [66, 67].

Presenilin

Presenilin polymorphism is associated with an increased risk of sporadic AD. PSEN-2 mutations increase A β generation and oxidative stress [68]. PSEN-1 is a component of the γ -secretase complex involved in A β PP processing. To date, more than 100 pathogenic mutations in the PSEN-1 gene have been identified, and their main biochemical effect is to increase the production of the 'long form' of A β ₄₂ [69]. However, the PSEN-1 mutation insR352 is associated with a frontal temporal dementia phenotype, and decreases A β production by inhibiting γ -secretase cleavage of A β PP [70].

AD PATHOGENIC FACTORS AND SLOW EXCITOTOXICITY

A β PP/A β

Prolonged activation of extrasynaptic NMDA receptors results in increased expression of neuronal Kunitz protease inhibitory domain (KPI) containing A β PP (KPI-A β PP), a shift from α -secretase to β -secretase-mediated A β PP processing, and increased neuronal production of A β [71]. A β in turn enhances neuronal sensitivity to glutamate and increases the activity of synaptic networks, resulting in excitatory potentials and Ca²⁺ influx [72]. This could occur in several ways: i) A β increases Ca²⁺ influx into neurons by

inducing an oligomeric pore in the membrane, and triggers multiple pathways involving the signal transduction mediators protein kinase A, MAPK, Akt, and cFos [73]. This leads to increased intracellular calcium concentration and glutamate release from axon terminals, and predispose neurons to excitotoxicity [74, 75]. ii) A β oligomers generate reactive oxygen species (ROS) and membrane-associated oxidative stress that impairs the function of ion-motive ATPases and glutamate and glucose transporters [76, 77]. Reduced level of ATPases and glutamate or glucose transporters could lead to depolarization of neurons, calcium influx, and excitotoxicity. iii) A β induces activation of NMDA receptors that leads to damage of cellular components and excitotoxicity [78, 79], and hippocampal neurons that express A β PP695 show increased damage after glutamate treatment [80]. iv) A β and NMDA receptors together induce endoplasmic reticulum (ER) stress that leads to alterations in calcium homeostasis. A β -induced ER stress and hippocampal dysfunction are prevented by ifenprodil, an antagonist of NMDAR2B (GluN2B) subunits [81]. v) A β induces transcriptional and/or translational regulation of the serine racemase gene, and release of low levels of NMDA receptor co-agonists including glutamate and D-serine [82]. vi) A β reduces glutamate uptake at the synaptic cleft [83, 84]. vii) A β affects tau, resulting in changes in neuronal signaling. viii) A β induces neuronal insulin resistance and lead to energy deficits that predispose to slow excitotoxicity. ix) A β affects mitochondria, resulting in neuronal injury (vi–ix are further discussed below).

Tau

Tau has also been implicated in excitotoxicity [53, 55]. KA-induced excitotoxicity leads to transient dephosphorylation of tau, followed by sustained hyperphosphorylation of tau at multiple sites in the mouse brain [85]. The initial dephosphorylation of tau is due to activation of protein phosphatase 2 (PP2A), whereas sustained hyperphosphorylation may result from activation of cyclin-dependent kinase 5 and down-regulation of PP2A during the later phase [85]. Tau is critical in mediating excitotoxicity via NMDA receptors [53]. It is important in postsynaptic targeting of the Src kinase Fyn which phosphorylates the NMDA receptor, thus linking it to downstream signaling. Mis-sorting of tau in transgenic mice expressing truncated tau or absence of tau in knockout mice disrupts postsynaptic targeting of Fyn [86]. Decreased expression of tau uncouples NMDA

receptor-mediated excitotoxicity and A β toxicity but interestingly, tau overexpression with hyperphosphorylation leads to increased postsynaptic Fyn levels and NMDA receptor phosphorylation [56]. Whether increased mortality in A β -forming mice on a tau transgenic background is due to increased excitotoxicity is unknown, but it is clear that reducing endogenous tau levels prevents behavioral deficits in transgenic mice expressing human A β PP, without altering their high A β levels. Tau reduction also protects transgenic and non-transgenic mice against excitotoxicity [56]. Immunization strategies targeting tau ameliorates the tau associated pathology, and could be a potential strategy for treatment of AD [87–89].

ApoE

ApoE attenuates glutamate excitotoxicity in neurons after exposure to NMDA [90]. ApoE treatment has no effect on H₂O₂ stimulated glutamate release, but increases the rate of glutamate uptake via high affinity glutamate transporters [91]. Increased ApoE immunoreactivity is observed in astrocytes after excitotoxic injury induced by the glutamate analog, KA [92]. Astrocyte-derived ApoE4 is excitoprotective, while neuronal expression of ApoE4 leads to neuronal death after excitotoxic challenge [93]. ApoE4 is associated with decreased cerebral glucose metabolism in both AD patients and non-demented subjects [94]. This could lead to reduced energy production and failure of ion pumps, and predispose neurons to excitotoxicity.

Presenilin

PSEN-1 has an effect on the threshold for excitotoxicity. Primary neurons overexpressing mutated PSEN-1 show increased vulnerability to excitotoxic damage. Accelerated neuronal death is also found in the hippocampus of mice overexpressing mutated PSEN-1 after KA induced excitotoxicity [95]. In comparison, mice with mutant PSEN-2 show decreased expression of cyclooxygenases COX-1 and COX-2 in the hippocampus, and reduced seizure activity after KA injury [96].

Kynurenine metabolism

Decrease in serum levels of tryptophan correlates with level of cognitive impairment, increase in serum kynurenine (KYN), and increase in KYN/tryptophan ratio in AD [97]. Moreover,

acute tryptophan deficiency worsens cognitive functions in AD [98]. Microglial cells degrade KYN into 3-hydroxykynurenine and quinolinic acid. 3-Hydroxykynurenine is neurotoxic because of its ability to generate oxidative radicals, while quinolinic acid acts as an agonist for NMDA receptor, and increases tau phosphorylation and neurofibrillary tangle formation [99].

POSSIBLE LINKS BETWEEN NOVEL AD RISK GENES AND SLOW EXCITOTOXICITY

Clusterin (Table 1)

Clusterin is a lipoprotein that modulates the membrane attack complex and prevents the inflammatory response associated with complement activation after protein aggregation. Clusterin expression is increased in AD, where it is associated with A β plaques [100]. It co-precipitates with A β from cerebrospinal fluid and protects against A β neurotoxicity [101]. Clusterin maintains A β solubility [102] and the clusterin-A β complex is associated with plasma high-density lipoprotein fractions [103]. Many oxidants (e.g., ethanol, tert-butylhydroperoxide or H₂O₂) induce an immediate secretory clusterin mRNA and protein upregulation [104], which has a protective effect against oxidative stress [105, 106]. Secretory clusterin localizes to mitochondria and suppresses apoptosis by inhibiting Bax activation [107], modulating p53 stress

signals and stabilizing the cytosolic Ku70-Bax protein complex [108]. It is therefore possible that alterations in clusterin could lead to an increase in inflammatory response or oxidative stress in neurons.

PICALM

Single nucleotide polymorphisms (SNPs) in the PICALM gene are associated with AD [109]. In addition, PICALM mRNA is elevated in the frontal cortex of AD patients [110]. PICALM increases A β PP internalization and A β production. It recruits clathrin and AP-2 (adaptor protein 2) to the plasma membrane, and plays a role in fusion of synaptic vesicles with the presynaptic membrane [111]. Mice deficient in PICALM show dysfunctional haemopoiesis and abnormal iron metabolism [112]. Recent studies using yeast have indicated a role of PICALM in A β toxicity [113, 114]. Changes in adaptor proteins decrease clathrin mediated receptor endocytosis and sensitizes neurons to glutamate receptor excitotoxicity [115]. Together, these findings suggest that alterations in PICALM may affect iron metabolism, processing of A β PP, endocytosis of receptors or exocytosis of neurotransmitters, with effects on neuronal excitation and excitotoxicity.

CR1

CR1 is a multifunctional receptor that binds to C1q, other C opsonins (C4b, C3b, iC3b), and MBL. It is found on circulating monocytes, neutrophils, and B-lymphocytes. C1q binding protein (C1qbp) is located in mitochondria [116]. C1q knockout mice show neuroprotection coupled with attenuated oxidative brain injury, and resistance to oxygen-glucose deprivation. In contrast, post-ischemic exposure to exogenous C1q increases both mitochondrial ROS production and neuronal injury [117]. C1q receptor overexpression in cells results in caspase-3 activation, mitochondrial dysfunction, and apoptosis [118]. Another study, however, shows that siRNA knockdown of C1qbp increases sensitivity to H₂O₂-induced mitochondrial membrane permeability transition and cell death [119]. C1q knockout mice exhibit enhanced synaptic connectivity and frequent seizures [120]. Thus, variants of CR1 may not only induce activation of the adaptive immune response and microglia, but also affect multiple cell signal pathways related to neuronal excitation and excitotoxicity.

Table 1

Role of novel genetic-associated AD risk factors in excitotoxicity

Gene	Possible mechanism [Reference]
Clusterin	A β neurotoxicity [101] A β solubility [102] Oxidative stress [105, 106] Mitochondria dysfunction [107, 108]
PICALM	A β production [109] Iron metabolism [111] Synaptic dysfunction [112] A β toxicity [113, 114]
CR1	Immune response and microglia activation, mitochondrial function and oxidative stress [116, 117] Synaptic dysfunction, seizures [120]
BIN1	Synaptic dysfunction [121]
ABCA7	Lipid metabolism [122] A β secretion [123]
MS4A	Ca ²⁺ homeostasis [124]
CD2AP	Production of A β [127]
CD33	Inflammation and levels of A β [130]
EPHA1	Glutamate receptor modulation [132, 133]

BIN1

BIN1 encodes members of the BAR (Bin/amphiphysin/Rvs) adapter family that have been implicated in membrane dynamics, such as vesicle fusion and trafficking, specialized membrane organization, and actin organization. Loss of amphiphysin 1 causes a reduction of BIN1 in the brain, defects in synaptic-vesicle recycling, learning deficits and increased mortality from rare irreversible seizures [121]. Variants of BIN1 may thus affect the endocytosis/exocytosis cycle in axon terminals, with effects on neuronal excitation and excitotoxicity.

ABCA7

ABCA7 is a member of the ABC superfamily of transporters that is expressed in cortical neurons, and transports substrates across cell membrane. It is involved in efflux of lipids from cells to lipoprotein particles, and may affect the actions of ApoE and clusterin [122]. ABCA7 regulates A β PP processing, inhibits A β secretion, and modulates phagocytosis of apoptotic cells by macrophages mediated through C1q on the apoptotic cell surface [123]. Variations in ABCA7 may therefore affect the lipid environment of the cell membrane, with effects on membrane receptors and excitotoxicity.

MS4A

The MS4A family, including at least 16 paralogues, has transmembrane domains and N- and C-terminal cytoplasmic domains [124]. MS4A12 is a store operated calcium channel in intestinal cells [125]. This suggests that the protein could have a role in calcium homeostasis.

CD2AP

CD2AP is important in regulating vesicular trafficking to the lysosome, particularly during the formation of multivesicular bodies [126]. The protein is also reported to be involved in modulation of A β PP processing and production of A β [127].

CD33

CD33, also known as Siglec (sialic acid-binding Ig like lectin)-3, encodes a cell-surface receptor on cells of monocytic or myeloid lineage. CD33 controls the innate immune system of the brain and levels of A β

[128]. The Siglec family of lectins binds sialic acid and regulates the innate immune system via the activation of caspase-dependent and caspase-independent cell-death pathways [129]. It is recently reported that activation of certain Siglecs results in induction of apoptosis. For example, Siglec-8 induces apoptotic cell death in eosinophils, and Siglec-9 triggers apoptosis in neutrophils [128–130]. Although the mechanism of Siglec-mediated apoptosis is not fully understood, recent studies have indicated that Siglec-8 and -9 induce apoptosis largely through ROS dependent processes [128–130].

Ephrins

Ephrins signal via EphA and EphB receptor tyrosine kinases. Eph/ephrin signals play important roles in boundary formation, cell migration, axon guidance, and growth cone development. Increased EphB2 expression in the dentate gyrus of human A β PP transgenic mice reversed deficits in NMDA receptor-dependent long-term potentiation and memory impairments [131]. Ephrin receptors not only initiate new synaptic contacts and recruit/stabilize glutamate receptors at nascent synapses, but also regulate dendritic spine morphology and contribute to long-term changes in synaptic strength and neuroplasticity. EphB receptors are also involved in the regulation of glutamate receptor trafficking, and have been linked to the pathophysiology of AD [132]. EphrinB rescues primary cortical neuronal cultures from necrotic cell death induced by glutamate excitotoxicity through a mechanism involving EphB receptors and PSEN-1 [133].

POSSIBLE LINKS BETWEEN AD ASSOCIATED METABOLIC CHANGES AND SLOW EXCITOTOXICITY

Insulin (Fig. 1)

Brain insulin resistance is an early feature of AD [134]. This is accompanied by insulin-like growth factor-1 resistance and is closely associated with insulin receptor substrate 1 (IRS-1) dysfunction, potentially triggered by A β oligomers. The hippocampal formation and, to a lesser degree, the cerebellar cortex in AD cases without diabetes, exhibit markedly reduced responses to insulin signaling in the insulin receptor/IRS-1/PI3K signaling pathway with greatly reduced responses to insulin-like growth factor-1. Levels of IRS-1 serine phosphorylation and their

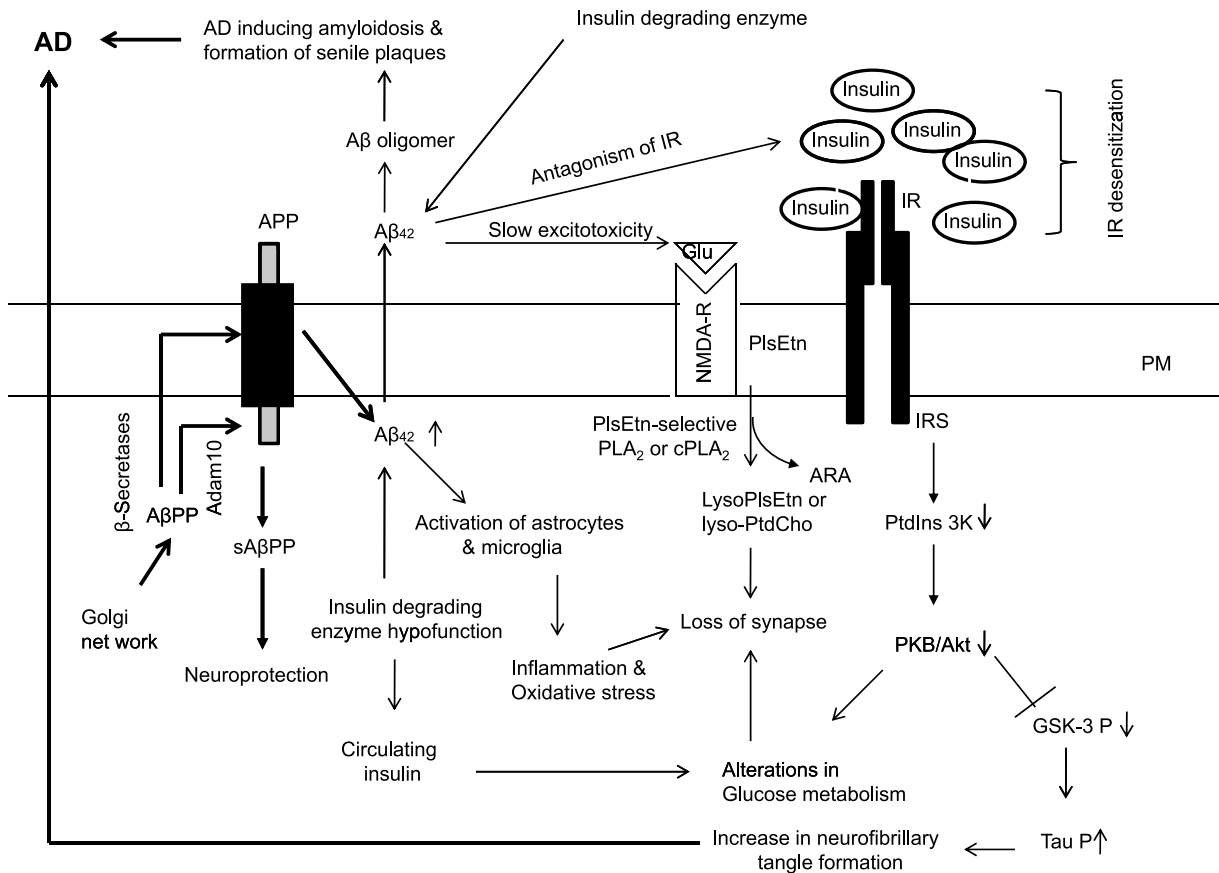


Fig. 1. Abnormal insulin receptor signaling in slow excitotoxicity and increased risk of Alzheimer's disease (AD). Insulin receptor (IR); insulin receptor substrate (IRS); phosphatidylinositol 3 kinase (PtdIns 3K); glycogen synthase kinase 3 (GSK-3); amyloid- β protein precursor (A β PP); soluble amyloid- β protein precursor (sA β PP); amyloid- β (A β); serine/threonine protein kinase (Akt); glutamate (Glu); *N*-methyl-D-aspartate receptor (NMDA-R); plasmalogen-selective phospholipase A₂ (PlsEtn-PLA₂); arachidonic acid (ARA); lyso-phosphatidylcholine (Lyso-PtdCho); plasma membrane (PM); protein kinase B (PKB); upward arrow indicates increase and downward arrow indicates decrease.

activated kinases correlate positively with those of oligomeric A β plaques, and are negatively associated with episodic and working memory [135]. In addition, A β oligomers significantly increase phosphorylation of IRS-1, which triggers its degradation, in cultured hippocampal neurons [136, 137]. These alterations of IRS-1 signaling may, in part, mediate the effects of A β on cognition. It is proposed that brain insulin deficiency and resistance induces neuronal death due to trophic factor withdrawal, deficits in energy metabolism, and inhibition of insulin-responsive gene expression, including those required for acetylcholine homeostasis [138]. Decreased glucose utilization as a result of defect in insulin receptor signaling may lead to reduced energy production and failure of ion pumps, with consequences on excitotoxicity. Metabolic inhibition leads to the progressive elevation of extracellular glutamate and aspartate levels in the hippocampus,

which correlates with decreased content of EAAT2 and diminished glutamate uptake. Increased phosphorylation and protein content of the NR2B subunit of the NMDA receptor are also observed [139]. High levels of insulin limit A β degradation by substrate competition for insulin degrading enzyme (IDE). The latter is a 110-kDa thiol zinc-metalloendopeptidase that cleaves small proteins of diverse sequences, many of which share a propensity to form β -pleated sheet-rich fibrils such as A β , insulin, glucagon, amylin, atrial natriuretic factor, and calcitonin. A close relationship between IDE with A β is supported by recent evidence of decreased IDE expression in the AD brain, and a negative correlation between IDE activity and A β content [140–142].

Brain insulin resistance could promote AD onset by raising the level of A β , tau phosphorylation, oxidative stress, proinflammatory cytokines, advanced glyca-

tion end products (AGEs), dyslipidemia, and apoptosis [143]. Insulin depletion results in persistent tau phosphorylation in mice [144], while high insulin level as well as complete lack of insulin could cause an imbalance in insulin-regulated tau kinases and phosphatases, and increase in tau phosphorylation [145]. Moreover, high insulin level exacerbates inflammatory responses and increases markers of oxidative stress [146]. Type 2 diabetes mellitus and AD patients show accumulation of AGEs. The latter is a normal process of aging that is accelerated in AD, and involves the non-enzymatic glycosylation and cross-linking of proteins that act through the receptor for AGE (RAGE) to induce oxidative stress, neuronal injury, and neuroinflammation [147].

Mitochondria

The activities of mitochondrial complex III (ubiquinol-cytochrome c reductase, EC 1.10.2.2) and complex IV (cytochrome c oxidase EC 1.9.3.1) are reduced by 70% in AD. Inhibition of complex III activity by 60–90% results in a major increase in the rate of calcium independent glutamate release from synaptosomes depolarized with 4-aminopyridine or KCl and may be a factor leading to excitotoxic cell death [148]. A β can localize to mitochondria, and this interaction has been suggested to contribute to its cytotoxic effect [149, 150]. Abnormalities in insulin signaling or mitochondria could lead to generation of ROS (superoxide and hydroxyl anions) and reactive nitrogen species (NO and ONOO⁻), which may lead to loss of synapses in AD [151].

Metabolic syndrome

Metabolic syndrome (MetS) is a condition characterized by central adiposity, dyslipidemia, impaired glucose tolerance, insulin resistance, and hypertension. MetS is a risk factor for the development of AD [138]. Both MetS and AD are accompanied by marked elevation in levels of lipid mediators and cytokines/adipokines [138, 152], which disturbs signaling networks and leads to loss of communication among glutamate, dopamine, and serotonin receptors. MetS and AD are also associated with mitochondrial dysfunction, decrease in ATP production, oxidative stress, and chronic inflammation. Abnormalities in signal transduction networks due to elevated levels of lipid mediators may affect neuronal lipid and protein homeostasis, resulting in neuronal injury [138, 152].

Leptin

Leptin is a 16-kDa adipokine that controls energy balance and food intake by acting on brain centers within the hypothalamus that control satiety and body weight. Lower circulating levels of leptin have been reported in patients with AD [153]. Leptin modulates A β production and tau hyperphosphorylation *in vivo* and *in vitro* [154], and enhances NMDA receptor function [155, 156]. Since synaptic plasticity is the cellular basis of memory formation, it is suggested that leptin may reduce memory loss in animal models of AD. Chronic leptin administration has been reported to reduce A β levels in Tg2576 mice or improve cognitive performance in CRND8 mice models of AD [157–159].

POSSIBLE LINKS BETWEEN AD ASSOCIATED LIPID CHANGES AND SLOW EXCITOTOXICITY

Phospholipid metabolism (Fig. 2)

Alterations in membrane lipids play a key role in many neurological and neuropsychiatric disorders including AD. Arachidonic acid is found in high concentrations in brain phospholipids, and is released as a second messenger during neurotransmission, and much more so during neuroinflammation and excitotoxicity. Upregulated brain arachidonic acid metabolism associated with neuroinflammation has been imaged in rodent and AD brains [160, 161]. Increased content of phosphomonoesters and phosphodiesterases, which may be the result of increased cPLA₂ activity, is also found in the AD brain [162]. Conversely, PLA₂ reduction ameliorates cognitive deficits in a mouse model of AD [163]. Low levels of cPLA₂ are normally present in the rat forebrain [164], but excitotoxic injury by KA induces neuronal injury and increases cPLA₂ mRNA expression in the damaged hippocampus [165, 166]. The increased cPLA₂ is immunolocalized to injured neurons and reactive astrocytes and accompanied by elevated levels of the lipid peroxidation product, 4-hydroxynonenal (4-HNE) in damaged neurons [167]. cPLA₂ inhibitors decrease neuronal injury after KA treatment, indicating an important role of the enzyme in mediating neuronal death after excitotoxic injury [168]. Another isoform of PLA₂, sPLA₂-IIA, is also upregulated in AD brains compared to non-demented elderly controls. sPLA₂-IIA-immunoreactive astrocytes are found in association with amyloid plaques in the AD hippocam-

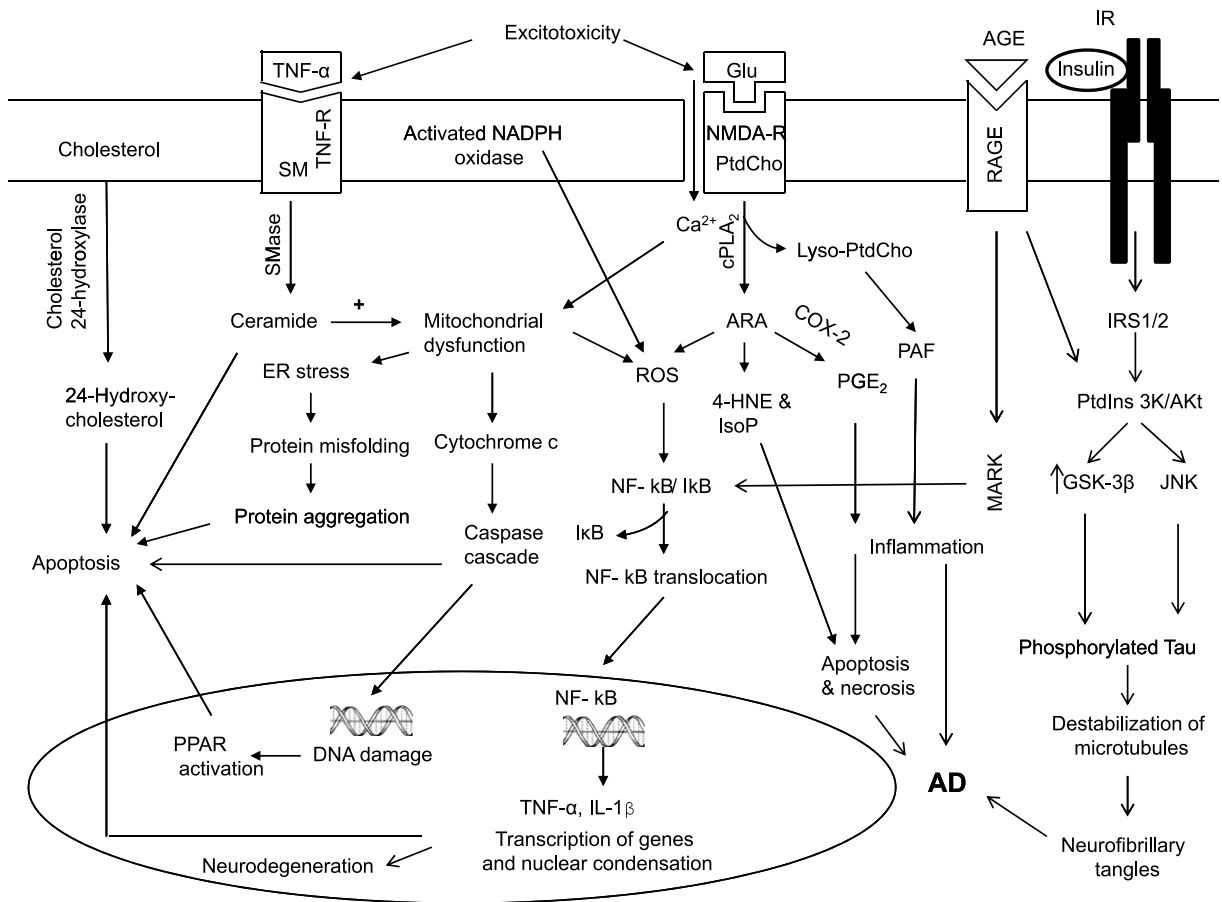


Fig. 2. Hypothetical diagram showing generation of lipid mediators in slow excitotoxicity, oxidative stress, inflammation, and abnormal insulin signaling in Alzheimer's disease (AD). Glutamate (Glu); advanced glycation endproducts (AGE); insulin receptor (IR); receptor for advanced glycation endproducts (RAGE); *N*-methyl-D-aspartate receptor (NMDA-R); tumor necrosis factor receptor (TNF-R); sphingomyelin (SM); sphingomyelinase (SMase); cytosolic phospholipase A₂ (cPLA₂); phosphatidylcholine (PtdCho); arachidonic acid (ARA); lyso-phosphatidylcholine (Lyso-PtdCho); reactive oxygen species (ROS); cyclooxygenase-2 (COX-2); 4-hydroxynonenal (4-HNE); prostaglandin E₂ (PGE₂); platelet activating factor (PAF); isoprostane (IsoP); endoplasmic reticulum (ER); nuclear transcription factor-kappaB (NF-κB); tumor necrosis factor-α (TNF-α); interleukin-1β (IL-1β); insulin receptor substrate 1/2 (IRS1/2); phosphatidylinositol 3-kinase (PtdIns 3K); glycogen synthase kinase 3 kinase (GSK-3β); c-Jun N-terminal kinases (JNK); inhibitor of kappaB (IκB); mitogen-activated protein kinase (MAPK); peroxisome proliferator-activated receptor (PPAR).

pus and inferior temporal gyrus [169]. Expression of sPLA₂-IIA is induced by oxidative stress and pro-inflammatory cytokines [170], and increased sPLA₂ activity is detected in the hippocampus after KA injury [171]. sPLA₂-IIA is packaged in fusion-competent vesicles and released in a regulated manner after KA receptor stimulation [172]. The enzyme itself induces exocytosis and neurotransmitter release in neuroendocrine cells and neurons [173]. Together, these findings suggest that sPLA₂-IIA may play an important role in facilitating neurotransmitter release, with possible effects on excitotoxicity. A related isoform, sPLA₂-III, enhances soluble AβPP secretion through

its action to increase membrane fluidity and recruitment of AβPP at the cell surface [174]. PLA₂ mediates Aβ-induced mitochondrial dysfunction [175]. In addition, activation of PLA₂ results in the release of fatty acids and lysophospholipids, which are capable of altering membrane microdomains and physical properties. Recent studies have linked aberrant PLA₂ activity to oxidative signaling pathways involving NADPH oxidase that underlie the pathophysiology of neurodegenerative diseases [176]. NADPH oxidase generates superoxide by transferring electrons from NADPH inside the cell and coupling these to molecular oxygen to produce superoxide anion (a free radical).

Both NMDA and oligomeric A β ₁₋₄₂ could induce ROS production from cortical neurons through activation of NADPH oxidase. ROS derived from NADPH oxidase leads to activation of ERK1/2, phosphorylation of cPLA₂, and arachidonic acid release. This effect is prevented by the NMDA receptor antagonists d(-)-2-amino-5-phosphonopentanoic acid and memantine, suggesting the participation of NMDA receptors [177]. Besides arachidonic acid release, ROS induces a more molecularly ordered astrocytic membrane and changes in membrane structure through a mechanism involving phosphorylation of p38 MAPK (mitogen-activated protein kinase) and ERK1/2, and the action of cPLA₂ [178]. A β -induced ROS production, NADPH activation, ERK1/2 activation, and cPLA₂ phosphorylation are inhibited by an antibody to RAGE in cultured brain endothelial cells and astrocytes. Together, these findings indicate the importance of AGE receptors, NADPH, and ERK in mediating oxidative stress and cPLA₂ activation in AD [179].

cPLA₂ and sPLA₂ release arachidonic acid from the *sn*-2 position of glycerophospholipids. Arachidonic acid can undergo lipid peroxidation and decompose to yield the toxic product 4-HNE. The latter is very reactive and forms adducts with membrane proteins, including those crucial for maintaining ATP levels, membrane potential, and extracellular glutamate levels [180]. Decreased activity of glucose transporter and Na⁺/K⁺ ATPase affects ATP production and the ability of ion pumps to maintain ionic balance in neurons, while decreased activity of glutamate transporters results in reduced ability to maintain extracellular glutamate concentration, and predispose neurons to excitotoxicity [181, 182]. cPLA₂ inhibitors prevent an increase in 4-HNE, and significantly reduces neuronal injury after KA-induced excitotoxic injury [168]. Further metabolism of arachidonic acid to eicosanoids and conversion of lysophospholipid to platelet activating factor may contribute to the initiation and intensification of neuroinflammation. Arachidonic acid is metabolized to prostaglandins by COX enzymes. COX-mediated neuronal injury may be due to downstream effects of one or more prostaglandins (PGs) including PGE₂, PGD₂, PGF₂ α , PGI₂ (prostacylin), and TXA₂ (thromboxane) that effect cellular changes through activation of specific PG receptor subtypes and second messenger system. Inhibition of COX activity with non-steroidal anti-inflammatory drugs reduces inflammation and A β accumulation in a mouse model of AD [183]. The other product of PLA₂ action, lysophospholipids, is not only converted into proin-

flammatory platelet activating factor but acts as a detergent at the cell membrane, leading to disturbance in ion homeostasis [184]. Lysophospholipids may also function as signaling molecules [185]. Some lysophospholipids, such as lysophosphatidylinositol, trigger an increase in intracellular calcium and exocytosis in PC12 cells, with possible consequences on excitotoxicity [186].

A third isoform of PLA₂, calcium-independent PLA₂ (iPLA₂), is decreased in AD brain [187]. iPLA₂ activity of platelets from AD patients are also markedly lower than that of control subjects [188]. About five-fold higher mRNA expression of iPLA₂ than cPLA₂ are found in the normal brain [189], and the enzyme is essential for the release of docosahexaenoic acid (DHA), which has anti-inflammatory and anti-oxidative stress effects. Neuroprotective actions of DHA may result from downregulation of AMPA receptors in hippocampal membranes, and through the generation of resolvins and protectins, which have neuroprotective properties [190–192]. A recent study shows that iPLA₂ γ deficiencies accentuate AMPA receptor destabilization and tau phosphorylation, which suggests that this isoform may be a therapeutic target for tau-related disorders, including AD [193]. Together, the results indicate activation or upregulation of cPLA₂ and sPLA₂, and increase in arachidonic acid and phospholipid metabolites that aggravate neuronal injury; but downregulation of iPLA₂, and decrease in DHA and metabolites that reduce neuronal injury, in brain tissue of AD.

Cholesterol (Fig. 2)

There are conflicting reports on the role of cholesterol in AD, although most studies point to an association between cholesterol with AD pathogenesis. While a link is known between serum cholesterol and brain levels of A β in the AD brain [194], there have been few studies that address the question of brain cholesterol levels in areas of ongoing damage within the AD brain. A recent study using an original method to quantify cholesterol distribution using time-of-flight secondary ion mass spectrometry imaging shows that cholesterol overload appears a new and independent alteration of AD cerebral cortex. In this study, the mean cholesterol signal was found to be higher in the lower half of the cortex in AD samples, compared to controls [195]. Studies on the effect of high cholesterol or 27-hydroxycholesterol on human neuroblastoma SH-SY5Y cells and organotypic slices

from rabbit hippocampus indicate that cholesterol and 27-hydroxycholesterol produce AD-like pathology by increasing A β production and triggering apoptotic cell death [196]. The C99 transmembrane carboxyl-terminal domain of A β PP is cleaved by γ -secretase to release A β , and nuclear magnetic resonance and electron paramagnetic resonance spectroscopy of C99 reveals a binding site for cholesterol, providing a mechanistic explanation to how cholesterol promotes amyloidogenesis [197]. *In situ* Raman analysis of tissue sections in AD mice provides distinct spectra useful for distinguishing AD from normal tissues, and biochemical changes including deposition of A β , increase in cholesterol, and hyperphosphorylated tau are detected in hippocampal tissues from AD mice [198].

A large increase in total cholesterol content of up to two fold is detected by GC-MS in the rat hippocampus, 1–2 weeks after excitotoxic injury induced by KA. This is accompanied by early increases in cholesterol biosynthetic precursors, lanosterol, desmosterol, and 7-dehydrocholesterol at 1 day post-KA injection, presumably reflecting increased neuronal biosynthesis in areas of ongoing injury. In contrast, levels of precursors are reduced in the mature glial scar. Elevated levels of potentially toxic cholesterol oxidation products or oxysterols such as 24-hydroxycholesterol, 7 β -hydroxycholesterol, and 7-ketocholesterol are also detected from days to weeks after injury, and loss of expression of the cholesterol transporter, ABCA1 is detected in neurons [199–201]. Together, these findings indicate profound changes in brain cholesterol metabolism in areas of ongoing damage, and increased formation of cholesterol oxidation products in degenerating brain tissue after excitotoxicity [202].

Cholesterol stimulates the insertion of A β PP into phospholipid monolayers, and directly or indirectly promotes A β PP processing in favor of insoluble A β deposition [203]. However, it remains controversial whether cholesterol enhances or reduces A β polymerization, and the association between cholesterol homeostasis and AD may not be simply through the regulation of A β fibrillogenesis [203, 204]. The level of cholesterol in the cell membrane has a prominent effect on trafficking of synaptic vesicles; reduction in cholesterol impairs vesicle movement, while increased cholesterol facilitates vesicle movement and exocytosis of neurotransmitters in neuroendocrine cells [205]. Together, these findings suggest that increased brain cholesterol level after neuronal injury could serve as a positive feedback cycle to further propagate slow excitotoxicity.

Cholesterol oxidation products/oxysterols

A β and A β PP have been shown to oxidize cholesterol to form 7 β -hydroxycholesterol and cholesterol-derived aldehydes that are pro-apoptotic at nanomolar concentrations. 7 β -hydroxycholesterol blocks the secretion of soluble A β PP from cultured rat hippocampal neurons [206]. In addition, cholesterol-derived aldehydes accelerate the early stages of amyloidogenesis through modification of A β and promotion of Schiff base formation [207]. As noted above, significant increase in cholesterol oxidation products is found in damaged hippocampal tissues after KA treatment. This could lead to further injury through sustained increase of cytosolic-free calcium, and dephosphorylation of the Bcl-2-associated death promoter protein. PC12 cells exposed to 7-ketocholesterol show nuclear damage, decrease in mitochondrial transmembrane potential, cytosolic accumulation of cytochrome c, activation of caspase-3, and oxidative stress [208, 209]. Moreover, increased intracellular calcium and exocytosis is observed in cells after treatment with cholesterol oxidation products [210]. Together, these findings indicate that increases in cholesterol oxidation products could induce a rise in intracellular calcium concentration and exocytosis, with possible effects on excitotoxicity.

Ceramides (Fig. 2)

Accumulation of ceramide and perturbation of sphingomyelin metabolism are key events in the dysfunction and degeneration of neurons in AD [211]. Marked increase in levels of ceramide, and increased expression of acid sphingomyelinase (ASMase) and acid ceramidase are found in the AD brain [212, 213]. Significant correlations are also observed between brain ASMase activity and levels of A β and hyperphosphorylated tau protein. Treatment of neural cell cultures with A β oligomers not only activates ASMase, but also increases ceramide [214]. Ceramide induces oxidative stress, insulin resistance, impairment in energy metabolism, and apoptotic cell death [214, 215]. Increased ceramide accumulation occurs in degenerating brain tissues after KA injury [216], partly due to increased expression of serine palmitoyltransferase, the first enzyme in the ceramide biosynthetic pathway, in reactive astrocytes [217]. Treatment of hippocampal slice cultures with the SPT inhibitor ISP-1 (myriocin) or L-cycloserine modulates the increases in 16:0, 18:0, and 20:0 ceramide species, and reduces

KA-induced cell death [217]. Several short chain- and C18 ceramides produce an increase in intracellular calcium, and enhance exocytosis in PC12 cells [218]. Together, these findings indicate increased ceramide levels in AD, and a potential role of ceramides in neurotransmitter release and excitotoxicity.

Neurosteroids

Reduction of neurosteroids, which are derived from cholesterol in the brain, has been found in sporadic AD. Neurosteroids include compounds such as pregnenolone, DHEA, and estrogen that are endogenously produced in the brain, and provide protection from excessive neuronal excitation [219]. Decreased level of pregnenolone is found in the rat hippocampus after KA injury, and may increase the vulnerability of remaining neurons to excitotoxicity [220].

POSSIBLE LINKS BETWEEN AD ASSOCIATED GLIAL CHANGES AND SLOW EXCITOTOXICITY

Microglia

Senile plaques in AD contain large numbers of reactive microglia/macrophages in addition to potentially neurotoxic aggregates of A β [221]. These glial cells are activated by glutamate excitotoxicity in a number of ways, including the release of proinflammatory cytokines, nitric oxide, reactive oxygen intermediates, proteinases, and complement proteins [222, 223]. Microglia/macrophages are capable of generating an 'oxidative burst' to produce superoxide radicals, which react with nitric oxide (NO) to produce peroxynitrite. Superoxide or peroxynitrite can then react with membrane lipids to affect the function of glutamate receptors, with effects on excitotoxicity [224, 225]. In addition, NO overproduction by inducible NO synthase compromises cellular signaling via aberrant protein S-nitrosylation. This can contribute to progression of AD and neuronal cell injury through alterations in mitochondrial dynamics and synapse loss, and ER protein folding [226, 227]. Microglia may also cause a rise in extracellular glutamate levels through the cystine-glutamate antiporter system, which facilitates the cellular intake of cystine in exchange for release of glutamate. Increased expression of the cystine-glutamate antiporter is found in reactive microglia in a mouse model of AD, and its inhibition leads to reduced A β neurotoxicity [228]. Recent studies also

show that microglia express NMDA receptors, and it is possible that these cells may react to high levels of extracellular glutamate, resulting in propagation of neuronal damage [229]. NMDA receptor stimulation triggers microglia activation and secretion of factors that induce cell death of cortical neurons, and in turn, damaged neurons activate microglial NMDA receptors to aggravate neurologic disease [230].

Astrocytes

Astrocytes are activated in AD and contribute to an inflammatory cascade. By transforming from a basal to a reactive state, astrocytes neglect their neurosupportive functions including uptake of glucose and release of lactate; uptake of glutamate and release of glutamine; and uptake of glutathione precursors and release of glutathione. This results in disruptions in synaptic connectivity, imbalance in neurotransmitter homeostasis, and neuronal death through increased excitotoxicity [231, 232]. Oxidative stress induced by A β leads to a reduction in glutamate transporter activity, with EAAT2 being affected more than EAAT1. A β induces activation of MAP kinases, ERK, p38, and c-Jun N-terminal kinase, and these pathways differentially modulated the glutamate transporters activity/levels [84]. The effect of A β astrocyte glutamate transporters and uptake is dependent on adenosine 2A receptors (A2AR). A β enhances expression and density of astrocytic A2AR and decreases EAAT1 and EAAT2 expression in astrocytes from wild type, but not A2AR knockout mice [233]. Expression of astrocyte EAAT2 in the hippocampus is reduced in ApoE mutant mice, suggesting inefficient glutamate uptake by astrocytes [234]. Together, these findings suggest that glial cells could contribute to excitotoxicity by increasing glutamate release in the case of microglia, or through reducing glutamate uptake in reactive astrocytes.

POSSIBLE LINKS BETWEEN AD ASSOCIATED IRON CHANGES AND SLOW EXCITOTOXICITY

Iron accumulation occurs in and around A β plaques and neurofibrillary tangles in AD. This is accompanied by abnormal interactions between iron transport or binding proteins (transferrin and ferritin) and their regulatory elements, and disruption in the sequestration and storage of iron by ferritin [235, 236]. High levels of iron enhance amyloidogenic processing of

A β PP [237], promote phosphorylation of tau protein [238], and increase formation of ROS [239]. Iron may promote the formation of free radicals by A β , via the methionine 35 residue of A β [240]. A late increase in iron level is detected in the degenerating hippocampus after KA induced excitotoxicity, using the quantitative method of nuclear microscopy [241]. In addition, there is a shift in the oxidation state of iron, with more cells becoming positive for ferrous iron at a later time (months). The latter is a state of iron that is particularly effective in catalyzing free radical reactions [242]. The level of iron binding protein, ferritin, increases in tandem with the increase in iron up to one month post-KA lesion, but a later stage, there is decreased ferritin expression which could lead to more iron being present in an unbound form, that is capable of catalyzing free radical reactions [243]. Increased mRNA and protein expression of a ferrireductase, duodenal cytochrome B (DCYTB) (Ong WY, unpublished observations) and increased protein expression of iron transporter, divalent metal transporter-1 (DMT1) are found in reactive astrocytes and their end-feet around brain capillaries after KA injury [244, 245]. Increased expression of these proteins at the blood-brain interface suggests that they could be important in brain iron transport. A recent study shows that uptake of dietary iron is much higher than previously thought. Although only a very small amount of dietary iron entered the brain, this amount is considerable relative to the total brain iron content ($9.19 \pm 0.71\%$), and is comparable in percentage uptake to other tissues [246].

Increase in brain iron could also occur as a result of hypercholesterolemia, probably due to generalized damage to endothelial cells, including those at the blood-brain barrier. New Zealand white rabbits that were treated with a high cholesterol diet for 8 weeks show increased number of iron laden macrophages and oligodendrocytes around microvessels in the cerebral cortex and subcortical white matter [247]. Similar changes are found in the liver, where hypercholesterolemia is associated with elevated levels of oxidative stress and increased number of iron-laden macrophages around microvessels [248]. It is postulated that high levels of serum cholesterol may subtly affect endothelial cells, resulting in increased transport of iron into the brain [247, 249]. Increased iron leads to free radical formation via the Haber-Weiss or Fenton reactions and damage to cellular components. Indeed, many studies show that iron is very epileptogenic and has great potential to induce neuronal excitation, with consequences for excitotoxicity [250].

TREATMENT STRATEGIES FOR AD TARGETING SLOW EXCITOTOXICITY

Memantine (Fig. 3)

Memantine is a non-competitive NMDA receptor antagonist that has been approved by the FDA for treatment of patients with moderate to severe AD [251]. It slows the progression of mild and moderate-to-severe AD and possibly vascular dementia, though its effects are limited [252]. Memantine acts as a non-competitive low-affinity modulator of NMDA receptors, and results in prevention of neuronal necrosis induced by glutamatergic calcium neurotoxicity, but not neuronal apoptosis resulting from oxidative stress [253]. It blocks excessive NMDA receptor activity, while leaving normal function relatively intact [254]. Memantine does not alter the conformation or internalization of A β_{42} and is unable to attenuate A β -induced potentiation of extracellular glutamate levels, but protects neurons by attenuating tau-phosphorylation and its associated signaling mechanisms [255]. Memantine could also have a beneficial effect on mitochondrial function by modulating the reduction in mitochondrial membrane potential and mitochondrial reductase activity after excitotoxic injury [256]. Memantine has good tolerability, low side-effect profiles, and a positive, though limited, therapeutic impact in moderate to severe AD patients, alone, and in conjunction with donepezil [257, 258]. Low levels of memantine have been found to promote neuroplasticity and memory formation [259, 260]. More recently, second generation memantines (NitroMemantine) have been synthesized, which use memantine as a homing signal to target NO to hyperactivated NMDA receptors, in order to avoid systemic side-effects of NO such as hypotension. The NitroMemantines have enhanced neuroprotective efficacy *in vitro*, and in animal models of neurological disorders [261, 262].

Metformin (Fig. 3)

Metformin is a biguanide anti-hyperglycaemic drug that is used to treat type 2 diabetes mellitus. Metformin suppresses gluconeogenesis and enhances glucose uptake and insulin sensitivity. Metformin protects against neurological complications of type 2 diabetes mellitus, including cognitive impairment and cerebral vascular disease [263]. Recently, metformin has been used in a clinical trial for AD, and it is reported that this drug can produce beneficial effects in AD patients [264]. When administered with insulin, metformin

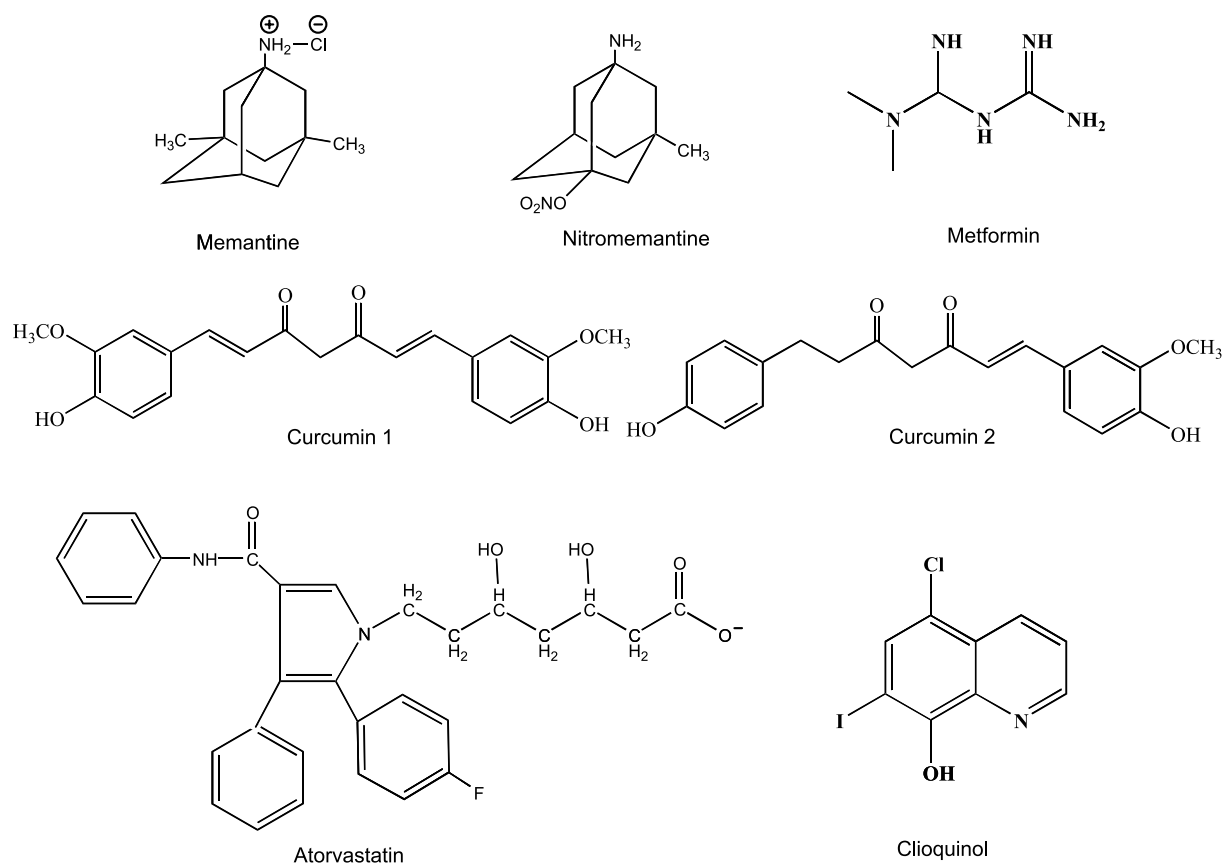


Fig. 3. Chemical structures of therapeutic agents used for the treatment of AD in animal and cell models.

provides significant neuroprotection in that A β PP-A β levels including A β PP-A β neuritic plaques, and oligomeric A β PP-A β -mediated downregulation of the insulin receptor are reduced [265]. Metformin plus insulin may benefit patients in the early stages of AD by significantly improving cognitive performance and slowing the rate of neurodegeneration [146]. Recently, an anti-diabetes agent, exendin-4, has also been reported to prevent impaired A β -induced neuronal pathologies such as axonal transport in animals [266].

Statins

Statins or cholesterol lowering drugs reduce cholesterol levels in the blood. Lovastatin protects cortical neurons in a concentration-dependent manner against glutamate-mediated excitotoxicity. Lovastatin significantly reduces KA-induced neuronal damage *in vivo* and *in vitro* [198, 267]. It increases TNF receptor 2 (TNFR2) expression and protects neurons against ischemic or excitotoxic insults [268], and may have

therapeutic potential in the treatment of neurodegenerative diseases involving excitotoxicity [198, 267]. Besides lovastatin, another brain permeable statin, simvastatin, is also effective in neuroprotection in KA excitotoxicity- and mouse AD models. Simvastatin (3-6 months, 40 mg/kg/d) rescues cerebrovascular reactivity, basal endothelial nitric oxide synthesis, and activity-induced neurometabolic and neurovascular coupling in adult (6 months) and aged (12 months) transgenic mice overexpressing A β PP mutations, and restores short- and long-term memory in adult AD mice [267, 269].

Besides a direct effect on modulation of neuronal cholesterol biosynthesis, it is possible that reduction of serum cholesterol by statins could have a beneficial effect on endothelial cells at the blood-brain-barrier. We postulate that one of the beneficial effects of statins, besides reducing excessive brain cholesterol biosynthesis after excitotoxic damage, is reduction of damage at the blood brain interface, thus preventing excessive influx of metals, and oxidative stress in the brain [249].

Acetylcholinesterase inhibitors

The mainstay of drug treatment for AD at the moment is acetylcholinesterase (AChE) inhibitors, which act to increase the level of acetylcholine in the brain. Donepezil, a potent AChE inhibitor that is used for the treatment of AD, shows neuroprotective effects against A β ₄₂ toxicity in cultured rat septal cholinergic neurons [270]. Donepezil also decreases neuronal damage in cultured neurons after NMDA treatment [271]. The level of NR1 on the cell surface and glutamate mediated Ca²⁺ entry is reduced by donepezil, which might contribute to its neuroprotective effects [272]. Another AChE inhibitor, tacrine, reverses declines in mitochondrial membrane potential, ATP production, and neuronal cell death induced by glutamate [273].

Curcumin

Curcumin, a hydrophobic polyphenol, is the yellow pigment in the Indian spice turmeric (curry powder), derived from the rhizome of the herb *Curcuma longa*. Epidemiological studies in India have indicated that consumption of curcumin in the daily diet is associated with significant reduction in the prevalence of AD [274]. Curcumin can exert anti-inflammatory, antioxidant, and neuroprotective actions; it is reported to decrease oxidative damage and A β deposition in a mouse model of AD, and reverse A β -induced cognitive deficits and neuropathology in rats [275]. Curcumin protects cultured neurons against glutamate-induced excitotoxicity by a mechanism requiring TNFR2 activation, suggesting that therapeutic approaches against cognitive decline designed to selectively enhance TNFR2 signaling are likely to be more beneficial than use of anti-inflammatory drugs *per se* [276]. This action of curcumin is very similar to one proposed for statins, noted above.

Antioxidants

Increased oxidative stress in brain tissue is an important feature in the pathophysiology of AD [277–279]. Conversely, clinical studies have shown antioxidant treatment to be effective in ameliorating symptoms of the disease. For instance, treatment with α -tocopherol reduces neuronal damage and slows the progression of AD [280]. Some traditional herbal antioxidants also exhibit potential for AD treatment. Apocynin, a constituent of the Himalayan medicinal herb *Picrorhiza kurroa*, has been reported to exhibit anti-AD effects

through NADPH-oxidase inhibition to suppress ROS and reactive nitrogen species [281].

Metal chelators, e.g., clioquinol

Clioquinol is an antibiotic with strong metal chelating properties. It crosses the blood-brain barrier and binds Cu²⁺, Zn²⁺, and Fe³⁺ ions that are critically involved in A β aggregation and toxicity [282]. Clioquinol reverses impairment in working memory, significantly decreases A β plaque burden, and attenuates astrogliosis in the cortex and hippocampus in a mouse model of AD [282].

CONCLUSION AND PERSPECTIVE

There is a large body of evidence that supports the role of A β in slow excitotoxicity in the pathogenesis of AD. Possible mechanisms by which A β could cause slow excitotoxicity may involve the ability of A β to reduce glutamate uptake or induce glutamate release, and several existing risk factors for AD have been found to have effects on excitotoxicity. Induction of A β -mediated slow excitotoxicity in the pathogenesis of AD is supported by recent studies demonstrating interactions among glycerophospholipid-, sphingolipid-, and cholesterol-derived lipid mediators. Moreover, the low affinity open channel NMDA receptor antagonist, memantine, slows the progression of AD. Thus, growing evidence supports the view that A β -mediated slow excitotoxicity may be critically involved in neurodegeneration in AD. More recent evidence supports that tau, the second hallmark protein in AD, is also directly involved in excitotoxicity. In particular, it is necessary for A β to mediate its toxicity, and hence, tau depletion or truncation protects mice from A β -induced deficits, including memory deficits. Therefore, slow excitotoxicity in AD may be orchestrated by A β and tau, and new therapeutic strategies should consider this interplay. Further studies will reveal the exact molecular mechanisms that tau contributes to in the post-synapse.

Many of the other novel genetic risk factors for sporadic AD and cellular responses that have been shown to be involved in AD may also contribute to slow excitotoxicity. Here, future research will reveal the exact molecular links. As discussed, possible approaches including maintenance of insulin signaling and energy metabolism, modulation of glial cell function, inhibition of iron accumulation and neuroinflammation, modulation of lipid mediators, and reducing oxidative stress may hold promise in the future to block slow

excitotoxicity. It is hoped that better understanding of the molecular links between pathogenic factors and slow excitotoxicity could inform our understanding of the disease, and pave the way toward new therapeutic strategies for AD.

ACKNOWLEDGMENTS

This work was supported by the National Medical Research Council of Singapore and the National Health and Medical Research Council of Australia. L.M.I. is a National Health and Medical Research Council Senior Research Fellow, Australia.

Authors' disclosures available online (<http://www.j-alz.com/disclosures/view.php?id=1680>).

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