ABSTRACT

Enormous effort is now being devoted to developing drugs that slow neurodegeneration in Alzheimer’s disease (AD), although insights into AD genetics and molecular pathogenesis only arose in the last 15 years. Acetylcholinesterase inhibitors that temporarily slow loss of cognitive function remain the only approved AD drugs. Discovery of mutations in three genes leading to severe early onset AD was critical in focusing attention on the role of amyloid peptides (Aβ) in neuronal cell death, and enhanced understanding of the biology of these peptides has led to an array of mechanism-based drug discovery strategies. These include inhibitors for Aβ-generating proteases, agents that prevent or reverse Aβ oligomerization, immunotherapies to reduce Aβ in brain and plasma, and drugs to modulate cholesterol-mediated effects on Aβ transport. Strategies are also underway to minimize toxic effects of Aβ fibrils on neurons, and these include antioxidants, blockers of glutamate-mediated excitotoxicity, and modulators of inflammatory responses within the brain. Although several approaches involve new agents for recently discovered targets, many are based on new applications of existing drugs such as statins and nonsteroidal anti-inflammatory drugs. Discovery of abnormally phosphorylated τ protein in neurofibrillary tangles in AD brain has led to strategies for identifying selective inhibitors of τ kinases and central nervous system/brain-permeable drugs that help maintain microtubule integrity. Clearly, a large gap exists between our understanding of the cellular cascades targeted in drug discovery and widespread failure of the nervous system that AD represents. Nevertheless, the pace of recent research clearly supports optimism that slowing progression of AD will soon be possible.

Nearly a century has passed since Alois Alzheimer provided his meticulous description of the impaired cognitive performance and neuropathological analysis of his patient “Auguste”. His observations still guide expanding efforts in both clinical medicine and basic research to uncover the pathogenesis of the brain degeneration and, ultimately, develop therapeutic interventions that prevent or slow progression of Alzheimer’s disease (AD). Clinical manifestations of AD appear first as short-term memory deficits, progressing to language problems, social withdrawal, and deterioration of executive function. Although definitive diagnosis requires a postmortem neuropathological examination, neurologists and neuropsychologists have now developed clinical criteria that often lead to ~90% accuracy in diagnosing AD, making the disease no longer one of exclusion.

Although many therapeutic agents are in various stages of development for several neurodegenerative diseases, the design of clinical trials has been hampered by the difficulty of identifying patients early enough on the disease continuum to test new drugs for effectiveness in slowing the progression of deterioration. Thus, success in pharmacological interventions hinges on developments in both the diagnostic arena and elucidation of the molecular pathogenesis of nerve cell death. Formal characterization of “mild cognitive impairment” (Petersen, 2000) and recent advances in brain imaging are paving the way for drug discovery aimed at “primary prevention” or “disease-modifying” agents rather than symp-
tomatic treatments. The first positron emission tomography images of amyloid plaques in human AD brain generated much excitement at a recent international meeting on AD, as imaging technology such as positron emission tomography and magnetic resonance images may soon contribute to the diagnostic work-up (Engler et al., 2002). The complexity of the disease has presented and continues to present enormous challenges, particularly those of relating end points such as brain lesions to the ultimate neuronal dysfunction that leads to dementia. Nevertheless, the pace of advances occurring at several levels is making it possible to design and test many new therapeutic strategies.

The currently available information about AD is enormous, and many excellent reviews describe the clinical and neuro-pathological characteristics of the disease as well as cellular and molecular cascades involved in neurodegeneration (Selkoe, 2001) and the animal models of the genetic alterations associated with familial AD (Hardy, 1997). This review is focused on current drug discovery strategies, all of which are based on hypotheses derived from molecular analyses of the lesions in human AD brain, from cell and animal models used to characterize the pathogenic cascades or from genetic and epidemiological studies of the incidence of AD. Information is organized around the selective, early demise of cholinergic neurons and the two primary brain lesions amyloid plaques and NFTs. Ongoing approaches to drug development are discussed in the context of these three manifestations of the human disease that appear to reflect a tightly integrated series of events leading to cell death. Nevertheless, the major caveat remains that we have yet to learn how these events begin and how they lead to the progressive behavioral and cognitive demise that characterizes the human disease.

**Loss of CNS Cholinergic Innervation**

Loss of central cholinergic neurons of the basal forebrain was the first biochemical observation about the pathogenesis of AD, and cholinergic deficits have been closely linked to altered processing of the amyloid precursor protein (APP) and to cognitive impairment (Roberson and Harrell, 1997). Even to this day, the only Federal Drug Administration (FDA)-approved drugs for symptomatic treatment of AD are the inhibitors of acetylcholinesterase, tacrine, donepezil, rivastigmine, and galantamine. These agents do not stop disease progression, but clinical studies have shown they temporarily stabilize cognitive impairment and help maintain global function, often delaying the need for patient placement in nursing homes by several months. Because prolonging the lifetime of released acetylcholine (ACh) by targeting acetylcholinesterase slows the loss of cognitive function for only a limited time, several other strategies for enhancing cholinergic function have been explored. Efforts to increase ACh synthesis by administration of precursors such as choline, as well as use of nicotinic and muscarinic cholinergic receptor agonists, have not yet proven useful, often due to poor bioavailability, limited efficacy, and various central and peripheral side effects. One cholinesterase inhibitor, galantamine, has been shown also to activate some subtypes of nicotinic ACh receptor-ion channels, however, apparently acting as a positive allosteric modulator and enhancing the receptor response to available ACh and increasing the frequency of ion channel opening (Maelicke et al., 2000). This dual action may be responsible for the promising outcomes of numerous phase III clinical trials reported recently (Wilcock and Truyen, 2002). Most patients in these trials had mild to moderate AD, and ~20% maintained cognitive function at baseline levels for 36 months, with good tolerance for the drug. Thus, therapies that enhance cholinergic function are likely to remain in the multidrug regimens that will one day have an impact on this debilitating disease.

**β-Amyloid and Neurodegeneration**

Although loss of choline acetyltransferase in the nucleus basalis was the first biochemical marker for AD brain, it is the reduction in generation, aggregation, and deposition of amyloid fibrils that has become the major focus for drug development. Prominent star-shaped amyloid fibrils in extracellular plaques and the intracellular NFTs in the brains of patients appeared to hold the key to the pathogenesis, and characterization of the constituents of these lesions was vigorously pursued in the 1970’s and 80’s. Extraction and sequencing of the highly insoluble fibrillar protein in plaques (Masters et al., 1985) revealed it to be identical to the amyloid β protein isolated by Glenner and Wong (1984) from deposits around meningeal blood vessels in brains of patients with AD and Down’s syndrome. Hence, the race was on to clone the gene for amyloid and to determine whether a mutated amyloid protein was associated with AD, particularly in families in which vulnerability to early dementia was documented. Cloning of the gene encoding the 40-42 amino acid peptide (Aβ) in plaques revealed that the peptide was derived from a much larger APP encoded on human chromosome 21 and expressed as three prominent splice variants of ~700 amino acids. Although a small number of early onset AD patients were found to have mutations in this gene, the majority of late-onset cases had no APP mutations, despite widespread Aβ-containing plaques in the brain (Hardy, 1997). The discovery of two more genes in which mutations were associated with highly penetrant early onset familial AD, presenilin 1 (PS1) on chromosome 14 and PS2 on chromosome 1, led to the finding that excess Aβ₄₂ peptide was produced in cells with PS mutations. This finding further supported the “Amyloid Hypothesis of AD”, the idea that the Aβ peptide, with its high propensity to form β-sheets and fibrils, is a primary culprit in the neurodegeneration in both familial and sporadic cases. Despite the fact that we do not know the cause of Aβ plaque formation in sporadic AD, research over the past decade has provided a wealth of information about the origins and properties of Aβ, making it the primary target for drug development. Thus, as shown schematically in Fig. 1, multiple therapeutic strategies are being tested, all with the goal of reducing generation or enhancing clearance of Aβ fibrils.

As shown in Fig. 1, APP has a single membrane-spanning domain, with a long extracellular N-terminal and short intracellular C-terminal region. Cleavage of the protein by α-secretase in the extra-cellular domain allows for the release of a large fragment (APPα-) from the cell surface and retention of an 83-residue COOH terminal domain in the membrane for further processing. Formation of the Aβ peptide results from alternative cleavage of some APP molecules by β-secretase and further cleavage at one of two sites by...
γ-secretase, the protease that hydrolyzes the 99 amino acid C-terminal fragment left within the transmembrane domain by β-secretase and releases the Aβ peptides (Selkoe, 2001). These elusive proteases presented tantalizing targets for drug development, but the search for the enzymes required more than a decade, and there is still some uncertainty about the exact identity of proteins involved in γ-secretase activity (Vassar and Citron, 2000).

Inhibition of β- and γ-Secretase Activities. The β-secretase (BACE; β-site APP-cleaving enzyme), aka Asp2 or memapsin 2, was initially discovered through an expression cloning strategy to identify genes that altered Aβ production. The properties of BACE as a membrane-bound Asp protease have been further characterized, along with discovery of the very similar BACE2 (Vassar and Citron, 2000). The novel BACE proteases most closely resemble the pepsins, although their in vivo substrates, other than APP, remain unknown. Although BACE activity may not be rate limiting, it is absolutely required for Aβ production. A high-affinity peptide inhibitor was used to isolate BACE1 from brain (Sinha et al., 1999), and the crystal structure of the protein complexed to an eight-residue transition-state inhibitor peptide OM99-2 was reported recently (Hong et al., 2000). Although such large peptides are not likely to be developed as drugs, they are providing lead structures for ongoing design of selective, brain-permeable, small-molecule inhibitors.

Identification of the protein responsible for γ-secretase activity, the enzyme that cleaves APP within the membrane, has been very challenging. Much evidence indicates that the catalytic activity resides in presenilins (PS1/PS2), proteins with multiple transmembrane domains, as mutagenesis of 2 aspartates in PS1 eliminated γ-secretase activity (Wolfe, 2001). Dozens of missense mutations in the PS genes are associated with early onset familial AD, and the mutations result in increased production of Aβ42 over Aβ40, an alteration that appears to be central to the pathogenesis of AD. The γ-secretase activity is associated with a complex of integral membrane proteins that includes, at least, a novel aspartyl protease, presumably PS, and nicastrin, a protein with a single transmembrane domain. Use of difluoroketone-based compounds as γ-secretase inhibitors has provided insights into the proteolytic activity and suggested such inhibition might be a useful therapeutic strategy. Some compounds are currently in phase I clinical trials. Characterization of the γ-secretase as a member of a unique class of proteases that cleave membrane-spanning domains of their substrates, however, revealed a similarity to the cleavage of Notch1, a protein required for transcriptional regulation dur-
ing development (Kopan and Goate, 2000). Deletion of the PS1 gene in mice is lethal in utero, with a phenotype similar to that observed in Notch1-null mutants (Shen et al., 1997). Furthermore, inhibitors of γ-secretase blocked proteolysis of Notch1 by a γ-secretase-like activity designated “S3”, raising significant concerns about the potential in vivo effects of drugs targeting γ-secretase in AD. Cleavage of Notch1 by S3 leads to release of the Notch intracellular domain, the protein fragment that is translocated to the nucleus where it regulates transcription of target genes. Liberation of Notch intracellular domain appears similar to the release of the unstable and initially elusive APP intracellular domain (AICD) by γ-secretase (Sastre et al., 2001). Although transcriptional regulation by AICD has not yet been demonstrated in vivo, Cao and Sudhof (2001) showed that AICD associated with another protein, Fe65. This complex may move to the nucleus, bind to Tip60, a histone acetyl transferase, and influence gene transcription. Several gaps in our understanding of this potential trans-activation pathway still exist. Although one group has reported on secretase inhibitors that did not affect Notch1 cleavage (Petit et al., 2001), it is clear that the design of safe and effective γ-secretase inhibitors will require clear understanding of the role that AICD may play in cell signaling and demonstration of high selectivity to prevent the loss of signaling mediated by other protein products of intramembrane proteases.

**Clearance of Aβ Peptides.** In addition to the substantial efforts devoted to inhibition of Aβ generation as a therapeutic strategy, several diverse approaches are being developed to reduce the presence of Aβ fibrils in the brain and periphery. Although the large Aβ aggregates present in plaques were initially regarded as the culprits responsible for neurodegeneration, recent biological studies on Aβ fibrils indicate that early protofibrillar forms of the peptide may initiate the cell death cascades (Walsh et al., 1999). These findings have raised questions about whether AD, particularly the late-onset sporadic form, results from overproduction of Aβ or from a failure to prevent protofibril formation or to clear the peptide rapidly enough to prevent fibrillization. Attempts to target these processes for therapeutic purposes have resulted in promising results from the use of 1) immune-mediated Aβ clearance, 2) disruption of Aβ fibrils or aggregates, and 3) modulation of the cholesterol-mediated Aβ transport. One of the most novel strategies to enhance clearance of Aβ involved active immunization with Aβ injected in vivo directly into APP transgenic mice that overproduce the peptide. Mice were monitored to determine whether the Aβ equilibrium in the brain could be altered and plaque burden reduced or prevented (Schenk et al., 1999). Despite skepticism regarding penetration of antibodies into the brain, results with transgenic mice that overexpress a mutant form of human APP were quite promising. Immunization of young APP mice led to markedly decreased deposition of Aβ with time, and more remarkably, existing plaque burden was reversed in APP transgenic mice immunized after the neuropathology had developed (Morgan et al., 2000). Immunized mice also showed improved performance on cognitive tests (Janus et al., 2000).

Mechanisms involved in Aβ clearance are not yet clear but may involve some phagocytic activity by brain microglia. Recent reports on the effects of passive immunization with monoclonal antibodies to Aβ showed that circulating antibodies enhanced the efflux of apparently soluble Aβ from brain to plasma. This suggests that the antibodies generate a peripheral sink for efflux of Aβ out of CNS compartments, reducing the potential for aggregation and deposition (DeMattos et al., 2002). Others have suggested that, even in passive immunization, some antibodies enter the CNS and neutralize Aβ in situ (Bard et al., 2000). Several in vivo studies with APP transgenic mice now provide support for the potential use of immune-mediated clearance of Aβ as a therapeutic strategy (Kotilinek et al., 2002). Although the first phase II trials with the active immunization approach were halted due to meningoencephalitis in ~5% of the AD patients, investigators believe that such complications can be overcome and numerous related strategies are still under development.

Efforts to determine the nature of the Aβ species responsible for toxicity at physiological concentrations have indicated that very early protofibrillar intermediates in the process of Aβ fibrillogenesis disrupt membrane ion gradients and that the highly aggregated peptide in plaques is actually a late marker of pathogenic events (Walsh et al., 1999). Oligomers of naturally secreted Aβ, rather than monomers or large fibrils, may form pores in the cell membrane, allowing for influx of ions such as Ca2+, that disrupt neuronal signaling and initiate cell death cascades (Kawahara et al., 2000). The possibility of disrupting the process of fibrillogenesis has attracted much attention and is leading to development of novel strategies to intervene at this step. Small molecules such as Congo red, some antibiotics, and antineoplastics such as doxyrubicin prevent Aβ toxicity, possibly by stabilizing the monomers and preventing oligomerization. Screening for additional compounds led to identification of several leads for potential antifibrillogenic agents.

One older antibiotic, clioquinol, is experiencing a comeback as an Aβ fibril disruptor, apparently due to its effectiveness as a chelator of copper and zinc (Cherny et al., 2001). Heavy metals appear to play roles in promoting Aβ oligomerization and generating free radicals that propagate further toxicity. Clioquinol crosses the blood-brain barrier and, following several weeks of oral administration to APP transgenic mice, appeared to increase brain levels of soluble Aβ and decrease immunohistochemical amyloid plaque surface, with no signs of drug-induced toxicity. Clioquinol was withdrawn from use as an antibiotic due to induction of a vitamin B12 deficiency, but this agent is now being tested in a phase II clinical trial that includes administration of vitamin B12 supplements. Prior experience with this drug made it possible to test the hypothesis about prevention or disruption of Aβ fibrillogenesis as a therapeutic strategy in patients with unusual speed, and answers should be forthcoming soon.

A novel approach to destabilizing existing amyloid deposits was recently described by Pepys et al. (2002). These authors targeted a nonfibrillar, proteolysis-resistant plasma glycoprotein, serum amyloid protein (SAP), which binds to amyloid fibrils and blocks their degradation. This protein may contribute to failure to clear amyloid deposits in vivo in several amyloidoses. High throughput screening for inhibitors of SAP binding to Aβ led to identification of a series of agents related to captiopril. The best candidate, CHPIC, is a palindromic derivative of the amino acid proline and a potent cross-linker of SAP, leading to its clearance by the liver and reduced plasma SAP. Following a toxicity-free demonstration
of reduced amyloid load in a mouse model of amyloidosis, the authors tested the drug in patients with systemic amyloidosis and found marked reductions in plasma SAP and indications of reduced SAP in amyloid deposits. This strategy may hold promise for shifting the equilibrium toward removal of Aβ from AD brain.

Another class of widely used drugs, the cholesterol-lowering statins, was found in epidemiological studies to be associated with a reduced risk of AD, possibly due to a role for lipoproteins and their receptors in clearance of Aβ from the brain. Discovery of an increased risk for AD in individuals expressing the apolipoprotein E4 allele and studies with apo-lipoprotein E4 and APP transgenic mice support a link between APP processing and cholesterol homeostasis in the brain, although the molecular events have not yet been elucidated (Poirier, 2000; Refolo et al., 2001). The low-density lipoprotein receptor-related protein, a member of the low-density lipoprotein receptor family, appears to play a role in the effects statins have in reducing Aβ accumulation, and the demonstration of close interactions between receptor-related protein and APP in cell membranes (Kinoshita et al., 2001) suggests this lipoprotein receptor is localized to membrane regions that also contain secretases involved in Aβ production. Despite the complexity of the cellular mechanisms that still need to be resolved, that this class of drugs is already characterized in terms of safety and effectiveness in hyperlipidemias opens up avenues for rapidly testing their potential to reduce neurodegeneration in AD.

Reducing the Cellular Toxicity of Aβ. As indicated in Fig. 1, another important therapeutic strategy is aimed at reducing the toxic cellular events that occur with Aβ accumulation in the vicinity of neurons, particularly those due to inflammatory cascades and free-radical generation. Recent observations on astrocyte activation as part of a neuroinflammatory cascade led to the identification of a novel death-associated protein kinase as a mediator of diverse apoptotic signals (Velentza et al., 2002). Derivatives of 3-amino pyridazine appear to be selective inhibitors of this kinase, leading to the possibility that Aβ activation of neuroinflammatory responses in astrocytes can be modulated with this novel class of agents (Watterson et al., 2002). Resident macrophages in brain, the microglia, are activated in the presence of Aβ oligomers, triggering the complement cascade and release of cytokines that propagate the inflammatory response (McGeer and McGeer, 1995). Again, retrospective epidemiological studies indicated a reduced risk of AD in patients on long-term NSAID therapy for conditions such as arthritis and prompted systematic analyses of anti-inflammatory agents in cell and animal models of AD. It was assumed that the mechanism through which NSAIDs exert beneficial effects was inhibition of cyclooxygenase (COX-1 and -2), enzymes involved in production of mediators of the inflammatory response. It was puzzling, however, that some NSAIDs such as ibuprofen appeared effective in epidemiological studies, but others like aspirin did not even though all agents in the class inhibit COX activity. One recent study showed that, in transfected cells and mutant APP transgenic mice, some NSAIDs actually decreased production of Aβ42 and, quite surprisingly, led to an increase in Aβ43, suggesting that active NSAIDs exert a novel effect on γ-secretase activity (Weggen et al., 2001). These authors also showed that Notch1 cleavage was not altered by NSAIDs, that the drugs did not enhance catabolism of exogenously added Aβ42, and that the effect on Aβ42 was independent of COX inhibition. Clearly, this is a case of a well characterized class of drugs showing novel actions that not only may modulate inflammatory responses in AD but also actually decrease production of the offending stimulus. These findings may explain discrepancies in the epidemiological data and provide additional markers to monitor in future clinical studies with this class of drugs. In considering use of anti-inflammatory agents for AD, however, it is crucial to keep in mind that many inflammatory responses are beneficial. Certainly, the complexity of the process of inflammation and the genetic diversity in inflammatory responses must guide development of these types of drugs for neurodegenerative diseases in general.

Production of damaging free radicals has been demonstrated both in AD brain and numerous experimental models of the disease, possibly due to oxidative properties of the Aβ peptide or inflammatory reactions by microglia and astrocytes or both (Fig. 1). Induction of oxidative stress leads to excess release of the excitatory transmitter L-glutamate and over-activation of the NMDA subtype of glutamate receptors. Dysregulation of NMDA receptor activity leads to significant “excitotoxicity”, a process that may contribute to neuronal cell death. Thus, the use of antioxidants and development of effective modulators of NMDA receptors are two additional strategies for reducing neuronal damage. Most antioxidants currently used as dietary supplements are believed to be safe, and several clinical studies with agents such as vitamins C and E in AD patients are ongoing, with results expected in the near future.

Blockade of NMDA receptors in clinical conditions associated with severe oxidative stress led to serious side effects that limited the use of such inhibitors. Although no NMDA receptor blockers are currently approved for use in the U.S., the drug memantine is being tested in clinical trials to assess both safety and efficacy. Memantine is a moderate affinity, uncompetitive NMDA receptor antagonist that blocks NMDA receptor channels in the resting state, similar to the physiological blocker Mg2+, and dissociates from the channel upon activation. This rapid blocking and unblocking by memantine differs from the kinetics of high-affinity antagonists that produced adverse effects in earlier trials, and this seems to have dramatically improved patient tolerance and safety with this agent. Initial placebo-controlled studies in the U.S. revealed statistically significant benefit for up to 40 weeks in cognitive, daily living, and global assessments in patients with moderate and severe dementia (Ferris et al., 2001). These promising results suggest that modulation of NMDA receptor activity may become a valuable component of a therapeutic regimen.

Neurofibrillary Pathology

Despite substantial evidence for the Amyloid Hypothesis, it has yet to be proven that Aβ initiates the degenerative cascade in AD (Delacourte and Buee, 2000). Skepticism arises in part from the fact that severe plaque deposition in APP and PS mutant mice does not lead to either the formation of intracellular NFTs or extensive neurodegeneration. The NFTs are composed of highly phosphorylated aggregates of the microtubule (MT)-associated protein τ, self-associated into paired helical filaments (PHF-τ). τ pathology develops...
slowly with increasing age in a large percentage of the population, and this may help explain why age is the major risk factor AD. The discovery that mutations in the gene encoding the \( \tau \) protein are associated with severe dementias linked to chromosome 17, demonstrated that \( \tau \) dysfunction leads to neuronal cell death, presumably due to failure of the self-assembled \( \tau \) to regulate the MT dynamics essential for cell survival (Lee et al., 2001). Demonstration that A\( \beta \) in the vicinity of neurons enhanced \( \tau \) phosphorylation in vitro in neuronal cultures and in vivo in brain suggested a link between the two lesions (Busciglio et al., 1995; Geula et al., 1998). This was further supported in a recent article showing that double transgenic mice expressing mutant human \( \tau \) (P301L) and mutant APP developed significant neurofibrillary pathology and degeneration in cortical and subcortical brain regions (Lewis et al., 2001). In addition, neurons from \( \tau \) knockout mice show no significant degeneration in the presence of A\( \beta \) (Rapoport et al., 2002). Although the in vivo sequence of presentation of the earliest forms of the two classical lesions is not yet known, a mechanistic link between the two in AD is emerging. Thus, drugs targeted to preventing neurofibrillary pathology may help slow progression of cell death. Identification of such agents is still in very early stages, but some efforts are focused on agents that might decrease abnormal phosphorylation of \( \tau \) and/or prevent the loss of MT structure.

\( \tau \) is predominantly a neuronal protein encoded in a single gene, with six splice variants expressed in adult brain, primarily in axons (Buee et al., 2000; Lee et al., 2001). Depending on the splicing of exon 10, the carboxy terminal of the expressed \( \tau \) contains either three or four MT-binding regions composed of repeats of a highly conserved 18 amino acid motif (3R-\( \tau \) or 4R-\( \tau \)). The ratio of 3R-\( \tau \) to 4R-\( \tau \) is \( \sim 1.0 \) in human brain. The 4R-\( \tau \) forms bind MTs with higher affinity and are more efficient in promoting MT assembly. Many of the more than 20 pathogenic mutations in \( \tau \) lead to an increase in 4R- versus 3R-\( \tau \), although the mechanisms by which this leads to neuronal dysfunction are not known. In AD, the neurofibrillary pathology is not due to mutations in the \( \tau \) gene but rather to some cellular cascade that results in abnormal phosphorylation of \( \tau \) proteins that causes them to assemble into filaments. These filaments occupy space in the cytosol and also prevent normal \( \tau \) regulation of MT structure and activities such as axonal transport. Identification of the kinases involved in \( \tau \) phosphorylation is being actively pursued, as such enzymes are potential therapeutic targets.

Although \( \tau \) has as many as 79 Ser/Thr sites and is phosphorylated by numerous kinases in vitro, fewer than 30 sites have been found in PHF-\( \tau \) and 13 of those are adjacent to prolines (Lau et al., 2002). Consequently, inhibition of proline-directed kinases has become a major focus for drug development (Fig. 2). Of the proline-directed kinases, glycogen synthase kinase (GSK3\( \beta \)) and cyclin-dependent kinase 5 (cdk5) are the primary targets for drug discovery efforts.

\[ \begin{align*}
A\beta & \rightarrow A\beta \text{ Protofibrils} \\
\text{MT-Stabilizing} & \text{Drugs} \\
\text{MT-Stabilizing} & \text{Kinase Inhibitors}
\end{align*} \]

**Fig. 2.** Schematic representation of A\( \beta \)-induced activation of the \( \tau \) kinases GSK3\( \beta \) and cdk5 and potential sites for drug intervention. The binding of cdk5 to p35 is believed to keep cdk5 near its physiological substrates in the plasma membrane. In the presence of A\( \beta \), a rise in free intracellular \([Ca^{2+}]_i\) can activate the high- and low-affinity forms of calpain, leading to the cleavage of p35 to p25, a strong activator of the kinase. The p25/cdk5 complex can move away from the plasma membrane and phosphorylate \( \tau \) near the MTs. GSK3\( \beta \) appears to associate closely with MTs, and its kinase activity is regulated by its own phosphorylation state.
because of their association with MTs, their phosphorylation of $\tau$ at AD-relevant epitopes, and their involvement in apoptotic cascades in various models (Lau et al., 2002).

Exposure of primary neurons to A$\beta$ activates GSK3$\beta$, increases $\tau$ phosphorylation, and leads to cell death. Several experiments using LiCl as a GSK3$\beta$ inhibitor revealed that activation of this kinase may be a consequence of a variety of toxic stimuli that initiate apoptosis, although LiCl is apparently not yet being pursued as a disease-modifying therapeutic strategy. The $\tau$ kinase cdk5 is also activated by A$\beta$ in cultured neurons and in AD (Dhavan and Tsai, 2001). As illustrated in Fig. 2, cdk5 activity is regulated by a 35-kDa myristoylated membrane-attached protein, p35. When p35 undergoes calpain-mediated cleavage to p25, kinase activity is greatly enhanced. The cdk5/p25 complex appears to be delocalized from the plasma membrane, possibly leading to nonphysiologic phosphorylation of substrates such as $\tau$. If this scenario is correct, inhibition of cdk5 as well as calpain would be expected to decrease $\tau$ pathology in AD.

A large number of compounds such as indirubins and paulolones have been shown to be potent inhibitors of GSK3$\beta$ and cdk5 but their effects on A$\beta$-induced $\tau$ phosphorylation and in vivo toxicity have not yet been reported. Most known kinase inhibitors act at the ATP-binding site, leading initially to concern that design of highly selective inhibitors might be difficult. Success in crystallizing several kinases with and without inhibitors bound to the catalytic site, however, has provided structural insights into the diversity of the ATP pocket in different kinases and indicated that selectivity is achievable (Davies et al., 2002; Sausville, 2002). The combination of a hydrophobic binding region and unique hydrogen bonding possibilities across multiple types of kinases provide a rich source for selective binding potential for inhibitors. In addition, because kinases are components of signal amplification pathways, a small level of inhibition upstream may be magnified into a larger biological response. These properties have already been utilized in design of selective inhibitors of cyclin-dependent kinases associated with abnormalities in cell proliferation in cancer (Sausville, 2002), suggesting that $\tau$ kinases and other kinases like the death-associated protein kinase family are important and novel potential targets in AD.

In studies designed to determine whether MT-stabilizing drugs could protect neurons in culture against A$\beta$ toxicity, we found that nanomolar concentrations of paclitaxel (Taxol; Bristol-Myers Squibb Co., Stamford, CT) and related agents enhanced cell survival and markedly reduced A$\beta$-induced apoptosis (Michaelis et al., 1998). In addition, MT-stabilizing drugs effectively blocked both A$\beta$-induced $\tau$ phosphorylation by cdk5 in an in vitro kinase assay and the calpain-mediated cleavage of p35 to p25 (Michaelis et al., 2002; Li et al., 2003). The MT-stabilizing drugs did not directly inhibit the activity of either cdk5 or calpain, suggesting the involvement of other cellular components in the protection. Although we expected that preserving MT structure would help neurons survive the presence of A$\beta$, the mechanism through which MT-stabilizing drugs prevent A$\beta$-induced activation of the calpain-p25/cdk5 complex pathway is still under investigation. Nevertheless, these observations suggest that drugs that protect the integrity of the cytoskeletal network can have significant and novel effects on signaling events in specific cellular contexts.

References


Summary and Conclusions

Just 10 years ago, very few studies were in progress to test new therapeutic strategies for AD, principally due to the dearth of information about the molecular pathogenesis of the disease. The remarkable pace of discoveries over the past decade has led to an impressive array of mechanism-based approaches to therapeutic interventions. Advances in understanding many of the molecular events leading to neurodegeneration and the genetics of early onset AD have uncovered totally new drug targets. Identification of the secretase families and their protein partners is certainly a case in point. Although new classes of drugs are now being developed to modulate the activities of recently discovered targets, it is quite interesting that many of the therapeutic strategies under intensive investigation involve the use of older pharmacological agents. The statins, NSAIDs, antioxidants, and metal chelators certainly show promise as disease-modifying agents that may become part of multidrug regimens to slow clinical progression of the disease. At the same time, the work with such agents in the context of AD pathogenesis has provided unanticipated insights into the pharmacological activities of these well known drugs. For example, efforts to understand the mechanism for the beneficial effects of statins has led to several discoveries about the trafficking of cholesterol-containing particles into and out of the CNS. The synergy occurring between basic cell and molecular studies in AD models and efforts to come up with therapeutic agents, either old or new, is also driving the development of better strategies for future clinical trials. There is a great need for reliable diagnostic indicators that permit patient identification prior to extensive cell death, if drugs for primary prevention are to become a reality. Most clinical trials are conducted in patients with moderate AD, and the criteria for effectiveness currently involve standard assessments of cognitive and global functioning over time. New brain imaging technology for early detection and, especially for the monitoring of disease progression under experimental drug regimens, appears to be on the horizon and will greatly improve the entire drug discovery enterprise directed against this devastating disease.


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