Pharmacological Mechanisms in Alzheimer's Therapeutics

A. Claudio Cuello

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Forewords

Alzheimer's disease is poised to create a global public health crisis. A 2005 *Lancet* report estimated that 24.3 million people worldwide currently have dementia, and somewhere in the world, someone develops dementia every 7s. The *Lancet* study projected that the global total will nearly double every 20 years, to 42.3 million in 2020 and 81.1 million in 2040. In the USA alone, an estimated 5.1 million Americans have Alzheimer's today, including 200,000–500,000 individuals younger than 65 years with some form of early-onset dementia. That number represents a 10% increase over the previous 2002 estimate. Currently, one in eight Americans aged 65 or older has Alzheimer's, and nearly half of Americans aged 85 or older have the disease.

A major factor in this skyrocketing prevalence is the unprecedented graying of the global population. In 2006, 78.2 million American baby boomers (those born between 1946 and 1964) began turning 60 at the rate of 330 per hour. World Population Ageing, a 2002 United Nations report, found that

- Population aging is a pervasive, irreversible, worldwide phenomenon.
- By 2050, the number of persons aged 60 and older will exceed the number of persons under the age of 15, for the first time in human history.
- In the more-developed countries, one-fifth of the population was aged over 60 in 2000; by 2050, one-third will be 60 or older in these regions. In the less-developed world, 8% of residents are currently aged over 60, and that proportion will reach 20% by 2050.
- The world's fastest-growing age group is "oldest old" individuals aged 80 and older.

These historic demographic shifts not only bring unprecedented numbers of people into the age groups at greatest risk for Alzheimer's but also strain the ability of families and social systems to provide the care, support, and health services they need.

Developing disease-modifying treatments for Alzheimer's will be a critical part of an effective global response to this impending crisis. We are extremely fortunate that a committed international research community is gaining insight into the fundamental neurobiology of the brain and pathological

processes are implicated in Alzheimer's at an unprecedented rate. I applaud the authors and editor of *Pharmacological Mechanisms in Alzheimer's Therapeutics* for this very thorough and informative overview of some of the most promising current pathways to intervention.

The array of therapeutic approaches discussed here represents the kind of potentially broad pharmacological armamentarium we will need to address a complex, multifactorial condition such as Alzheimer's. A truly successful treatment protocol will likely include a mix of agents aimed at several pathological mechanisms rather than a single "magic bullet." The inevitable variation in patient response to any specific agent is another factor driving the need for a full spectrum of therapeutic options.

This compendium also offers a valuable perspective on some of the challenges inherent in identification and clinical testing of new molecular entities for neurodegenerative diseases. One of the biggest hurdles is the reality of the drug development timeline. A typical estimate for the time needed to move from target identification and validation to new drug approval is 12–15 years. The world's rapidly changing demographics demonstrate clearly that time is not on our side. We need to redouble our efforts to sustain the recent pace of identification of promising new targets and to move every promising approach from the laboratory into clinical testing as quickly as possible.

Validation of the first disease-modifying compounds will provide a highly motivating proof of concept, and help us expand our horizons to make prevention part of our conceptual framework for the ideal therapeutic landscape. Another important factor in shifting our priorities to prevention will be the availability of interventions with a safety profile appropriate for use in presymptomatic individuals. Preventing or delaying emergence of symptoms may well be our ultimate therapeutic response to Alzheimer's. As Brookmeyer and colleagues concluded in 1998, delaying onset of Alzheimer's by even 5 years could decrease the prevalence of the disease by 50% over 50 years. The elegant and exciting strategies described here offer potentially vital steps toward our goal of a world without Alzheimer's disease.

William H. Thies, PhD
Vice President,
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Another volume dealing with the neurobiology of Alzheimer's disease might seem superfluous, considering the attention that has been given to this disorder in recent years. Yet, this book provides something new: a highly desirable collection of papers that describe the large variety of attempts at treatment, based on clinical or pathological analysis. We have an evaluation of experience with many targets. The disappearance of much of the acetylcholine from certain brain centers in Alzheimer's disease keeps anticholinesterases in the limelight. But there are other specific targets including muscarinic receptors, glutamate receptors, apolipoprotein E, and other proteins known to play some role in the aging process. Some investigators focus on immunological processes; others look for means of overcoming destructive oxidant reactions that occur in the brain, the downside of our oxygen-dependent lives.

The point is that basic research into cerebral processes is guiding the attempts at therapy. One is reminded of the success attained by laboratory studies in the treatment of another devastating disease of the nervous system, namely, Parkinson disease. In that case, one clue from laboratory research led to another, and ultimately to the introduction of L-DOPA (3,4-dihydroxy-L-phenylalanine) as a specific therapeutic agent, followed by the discovery of substitutes for this amino acid, i.e., the various dopamine agonists. Without minimizing the effort required to achieve a corresponding desirable result in the case of Alzheimer's disease, this analogy is presented as encouragement to investigators of the aging brain.

The approach or approaches that will ultimately prevail in the successful attack on Alzheimer's disease is unpredictable. But the contributions in this volume, as they focus attention on potential therapies, can be expected to aid significantly in finding means to overcome a disease that at present takes such a toll of individuals and of society.

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Preface from the Editor

Alzheimer's disease is a serious health concern in developed countries where the population is progressively aging. At the personal level, the diagnosis of the disease represents a devastating scenario for both the sufferer and the caregivers. In recent years, medications have been developed that mitigate somewhat the symptoms and delay, for a while, the progression of the disease. It is expected that in the coming years new medications will be developed that are capable of halting the chain of pathological events and symptoms of the disease. This book covers a wide range of the pharmacological mechanisms underlying the present and potential new therapies. The recent extraordinary advances in our understanding of the cell and molecular biology of Alzheimer's disease allow for an optimistic forecast of innovative therapies. I am glad that Andrea Malacuso, from Springer, asked me to edit a book addressing these issues. The opportunity allows me to contribute a little to the awareness of the pharmacological challenges. I am most grateful to all the contributors who enthusiastically responded to the call. I am particularly gratified in having them as authors of comprehensive reviews as they have made important contributions to the field and, just as important, because of their friendship, which I have had the privilege of enjoying for many years.

I trust that this book will be of value to a wide audience interested in cellular and molecular mechanisms leading to the pathology of Alzheimer's disease and on the multiple, possible, therapeutic opportunities ahead of us. The field of research is enormous, and therefore we have selected the therapeutic targets that seem the most hopeful and for which there is a solid rationale. There are a number of emerging therapeutic targets, such as the inactivation/removal of Aβ peptides, among others, which might have potential applications if specific leading compounds were to be identified.

On a personal note I would like to say how committed I am to this subject of research, both because of its social importance and for the good science it is generating. I would also like to thank all my past and present collaborators and express my gratitude for the friendship of many of the leading actors in this field. I would like to say here also "thank you" to Dr. Alan Frosst and

the Frosst family and Merck-Frosst Canada for their interest in our work and the granting institutions which make it possible, the Canadian Institutes of Health Research, and the US Alzheimer's Association. Finally, I would like to say "thank you" to Martha, my wife and best friend, for her love and fortitude, and to my daughters, Paula and Karina, for bringing us so much happiness, and also for their patient ears to "Papa's dreams."

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1
Overview of the Alzheimer's Disease Pathology and Potential Therapeutic Targets

A. Claudio Cuello

Introduction

This chapter succinctly summarizes some basic aspects of Alzheimer's disease (AD) pathology for the nonexpert reader. The objective is to provide an overview for subsequent chapters that deal with specific current and prospective AD therapeutics. The AD literature is so vast that, unavoidably, it was not possible to cover all aspects of the interesting or exciting issues under investigation. Although this chapter reflects a personal view of the field, I have tried, as much as possible, to bring ideas that have the greatest consensus to the forefront.

Alois Alzheimer's Realization of a Dementia Accompanied with a Defined Brain Pathology

The devastating neurological disorder known today as AD was first clinically recognized in 1901 by Alois Alzheimer, a German clinician working at a Frankfurt hospital. Alzheimer was interested in neurohistology and learned basic staining techniques from his colleague Nissl, around the time of the emergence of Cajal's "neuronal theory." He examined a 51-year-old patient (Auguste D) who had difficulty naming familiar objects, writing complete sentences, and remembering words. She repeated "I have lost myself," was strongly jealous toward her husband, and experienced increasing memory impairments and disorientation. She carried around various objects and hid them, and occasionally felt that someone wanted to kill her and sometimes screamed out loudly. Alois Alzheimer followed the progress of this patient even after he moved to Munich. Auguste D died in 1906, several years after her dementia was diagnosed. Alois Alzheimer performed a postmortem examination of the brain and applied histological staining techniques available at the time. He was the first to describe the characteristic amyloid plaques and neurofibrillary tangles (NTFs), which, even today, are used as the neuropathological signature of the disease. The case was reported in the

form of a lecture in 1906 and a publication followed in 1907 (Alzheimer, Stelzmann, Schnitzlein, & Murtagh, 1995). The gross anatomy and microscopical features of the brain Alois Alzheimer investigated for the first time would have been similar to the one represented schematically in Fig. 1. The middle and lower parts of this figure illustrate a much shrunken Alzheimer's brain with diminished cerebral cortex mass (white) and dilated sulci and fissures (deep gray). On the top, for comparison, a representation of a normal

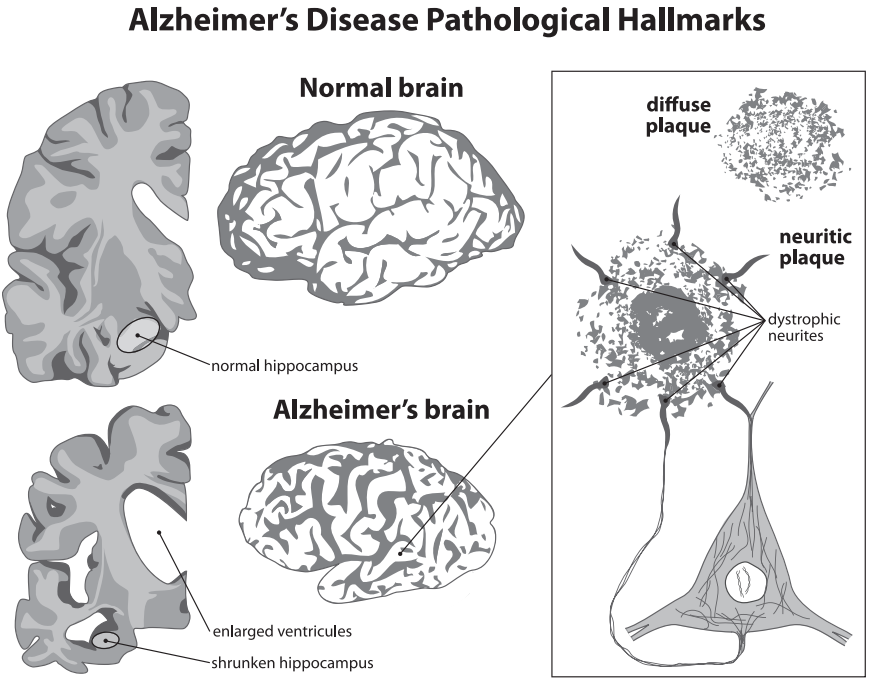

FIG. 1. Simplified, schematic illustration of the pathological hallmarks of Alzheimer's disease; *bottom left* depicts a coronal brain section from a brain with advanced Alzheimer's pathology, showing expanded ventricular spaces and a highly shrunken hippocampal complex as compared with the normal brain, above. Note the overall shrunken state of the Alzheimer's brain (*bottom, center*), the reduced volume of the cortical gyri, and the expanded sulci (gray), also depicted in the coronal sections. The microscopic hallmarks found in the cerebral cortex and other CNS regions are schematically represented in the right panel. In the upper part, the Aβ peptide aggregation in the form of a diffuse amyloid plaque, in the center a mature, neuritic plaque with a dense core (usually Thioflavin-s positive) surrounded by Aβ amyloid material and, more peripherally, a corolla of dystrophic neurites. These are grossly pathological, distorted, neuronal processes originating from neurons (*bottom right*) containing neurofibrillary tangles

brain is presented. On the left, coronal (i.e., perpendicular to the brain's midline) sections of a normal brain (top) and of an Alzheimer's brain showing the diminished cortical gray matter, the expanded sulci and fissures, grossly enlarged ventricular cavities, and a shrunken hippocampus. The hippocampus and the neighboring entorhinal cortex are early and prime targets of the Alzheimer's neuropathology. The inset on the right represents, in a much simplified and schematic manner, diffuse plaques containing aggregated proteins forming an irregular sphere and mature or neuritic plaques with a true amyloid center (Aβ proteins in a fibrillar, β-sheated conformation, and therefore stained with thioflavin S or Congo red) and an irregular aggregation of proteins (predominantly Aβ) surrounded in the periphery by a corolla of so-called dystrophic neurites. These dystrophic neurites are degenerative dendritic or axonal processes engulfed in the plaque pathology. On the bottom right, a neuron is represented (magnified and out of proportion with representation of plaques) containing abundant NTFs in its cell body as well as in dendritic and axonal processes. Following neuronal cell death, these tangles are not enzymatically digested and remain as neuronal cell body or neuritic ghosts.

Key Molecules in the Alzheimer's Pathology

Not much attention was paid to this disease for decades during which time this condition was often referred to as senile dementia. Great confusion existed as to whether the dementia often observed in old age and Alzheimer's disease were the same or different entities. It took nearly a century to define that the plaques were composed primarily of a specific peptide initially named A4 and today referred to as Aβ (Glenner & Wong, 1984; Wong, Quaranta, & Glenner, 1985) and that tangles are composed primarily of hyperphosphorylated forms of tau, a microtubule-associated protein (Grundke-Iqbal, Iqbal, Quinlan, et al., 1986; Grundke-Iqbal, Iqbal, Tung, et al., 1986; Kosik, Joachim, & Selkoe, 1986; Wood, Mirra, Pollock, & Binder, 1986). Tau is a protein known to stabilize microtubules present primarily in axonal processes and involved in axonal transport of subcellular components. The abnormal phosphorylation of this microtubule-related protein leads to molecular protein structures called paired helical filaments (PHFs), which constitute the ultrastructural core of the microscopic structures recognized as NTFs (Goedert, Wischik, Crowther, Walker, & Klug, 1988; Grundke-Iqbal, Iqbal, Quinlan, et al., Kosik, Joachim, & Selkoe, 1986).

Most contemporary research on the molecular basis of the disease has focused chiefly on these two proteins, and the causality of the disease has been attributed to either or both of these proteins. For a while, the two camps of thought were humorously referred to as the Baptists (for Aβ) and the Taoists (for tau).

The Amyloid Hypothesis

The controversy regarding the prime molecular cause of the disease has lasted for a long time and still lingers today. Presently, the predominant theory is that the abnormal accumulation of Aβ peptides provokes the complex pathological cascade that defines AD. This theory is referred to as the Amyloid Hypothesis, and has been championed by a number of very influential investigators such as Dennis Selkoe, John Hardy, Colin Masters, Konrad Beyreuther, and Blas Frangione, among others (Hardy, 2006; Selkoe, 2003). Much of the initial thought was centered around the amyloid burden and the nature of the central nervous system (CNS) deposits forming the characteristic plaques. The amyloid material was initially thought to be systemically derived from serum proteins and characterized as a short peptide (Glenner & Wong, 1984; Wong, Quaranta, & Glenner, 1985), whose sequence was soon confirmed by Masters and Beyreuther (Masters et al., 1985), and was proposed to originate from a membrane CNS amyloid precursor protein (APP) (Kang et al., 1987). These basic tenets provided a suitable platform for the current theory that a dysmetabolism of Aβ is a central causative aspect in the AD pathology. We know, nowadays, that Aβ peptides are produced as a result of the cleavage of the APP lodged in cell membranes (see Fig. 2). The release of Aβ peptides from membranes is achieved by the consecutive action of a β-secretase cleaving APP at the N-terminal site of the Aβ domain, followed by its cleavage at the γ-secretase site at the C-terminal end, thus generating Aβ fragments of diverse lengths, but typically of 40 and 42 amino acids in length. The longer peptide, Aβ 1–42, is more neurotoxic and more prone to aggregation and amyloidogenic. The β- and γ-secretases have been identified and cloned. Two proteins are currently recognized with β-secretase functions. They are named BACE (beta amyloid converting enzyme) 1 and 2, of which BACE 1 appears to be more important for the development of the AD pathology (Vassar & Citron, 2000). The β-secretases release a large peptide which in biochemical jargon is referred to as C99, containing both the Aβ motif and another motif defined as AICD (APP internal C-terminal domain). The γ-secretase site is more complex. Initially, it was proposed that presenilins (mutations of which were already known to cause familial forms of AD) were the actual γ-secretase (Wolfe et al., 1999) (see Fig. 2). Today, there is consensus that the γ-secretase site is composed of an ensemble of proteins, some of which might be responsible for the modulation of the APP-catalytic activity preselinins 1 and 2. This complex has the peculiarity of being capable of a catalytic action in the fairly hydrophobic milieu of cell and organelle membranes. The catalytic activity of the γ-secretase action is ultimately responsible for the liberation of the amyloidogenic Aβ peptide and the AICD fragment (Wolfe, 2006). AICD is suspected to either act as a transcription factor or be involved in cell signaling mechanisms in the CNS, however, its actual biological significance is still being debated (Kimberly, Zheng, Guenette, & Selkoe, 2001; Leissring et al., 2002).

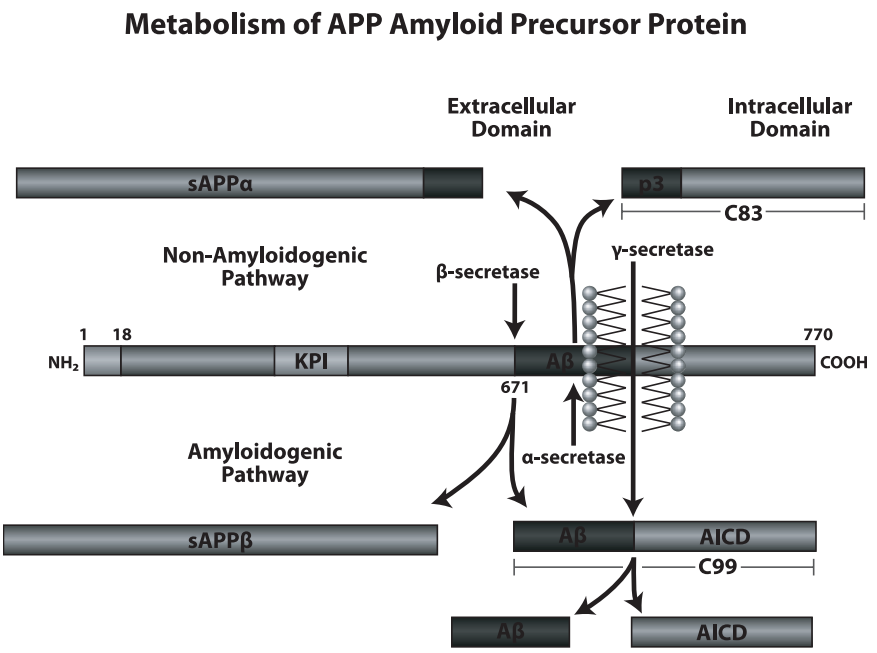

FIG. 2. Schematic representation of the metabolism of the amyloid precursor protein (APP). The Aβ peptide domain is partially embedded in the plasma membrane (or in the membranes of the subcellular organelles). The upper part of the scheme represents the nonamyloidogenic α-secretase processing of APP-releasing soluble APPα (sAPPα), which may display neurotrophic-like properties. The cleavage of APP on the α-secretase site also releases a C-terminal intracellular fragment (C-83). The amyloidogenic pathway requires the consecutive action of a β-secretase releasing a soluble APPβ (sAPPβ) fragment and an intracellular C-99 fragment, which is further cleaved by the γ-secretase to release the amyloidogenic peptide Aβ and an APP internal C-terminal domain (AICD). For further descriptions and key references, see text

No definitive biological role has yet been attributed to the holoprotein APP, but it is thought to have a role in cell-to-cell contact and, perhaps, synaptogenesis. APP can also generate other peptide fragments, which means there is an alternative nonamyloidogenic pathway in the metabolism of APP. This cleavage in the midst of the Aβ domain by the catalytic action of an α-secretase precludes the release of Aβ peptides. The peptide fragment derived from the APP ectodomain is referred to as soluble APPα (sAPPα) whereas the resulting intracytoplasmic C-terminal fragment is referred to as the C-83 peptide (see Fig. 2). Both in vitro and in vivo studies suggest the sAPPα fragment possesses neurotrophic properties (Bell, Zheng, Fahrenholz, & Cuello, 2007; Mattson et al., 1993; Mattson, Guo, & Geiger, 1999; Meziane et al., 1998; Roch et al., 1994). Putatives α-secretases are members of the

ADAM family (a disintegrin and metalloproteinase), of which ADAM-10, ADAM-17 (TACE, tumor necrosis factor-alpha convertase), and ADAM-9 rank as the most probable convertases for this site (Allinson, Parkin, Turner, & Hooper, 2003; Kojro & Fahrenholz, 2005).

Tau Pathology in Alzheimer's

As discussed above, in Alzheimer's disease, abnormally phosphorylated tau forms the so-called PHFs, which is the macromolecular assembly forming the core of the NFTs (Friedhoff, von, Mandelkow, & Mandelkow, 2000)(see also the chapter by Grundke-Iqbal in this book). NFTs invade the entirety of neurons forming fairly compacted and contorted filamentous structures that remain in the extracellular space after cell death. These NFTs have a clear-cut temporal and topographic distribution across brain areas as the disease progresses. The best staging of such structures has been provided by Braak and Braak (Braak & Braak, 1998). Interestingly, the earliest Braak stage is the occurrence of NFTs in the entorhinal cortex in the absence of an obvious deposition of Aβ material in this brain region. This observation has been used as an argument to dissociate tau from Aβ pathology in AD. The causal relation between these two molecular pathologies remains uncertain. However, relatively recent observations in transgenic animals indicate that either the transgenic overproduction of Aβ or the injection of Aβ peptides facilitates the formation of NTF-looking microscopic structures in mice overexpressing mutated forms of tau analogous to those present in frontotemporal dementia (Gotz, Chen, van, & Nitsch, 2001; Lewis et al., 2001).

There is a controversy regarding the development of PHFs and NFTs. It has been proposed that the first modification of tau in AD is a conformational change in the molecule presenting epitopes, which would not be evident otherwise (Weaver, Espinoza, Kress, & Davies, 2000). We have also observed that tau epitopes, which were later recognized as conformation dependent, appear first and that tau epitopes of the microtubule binding region appear later as the AD dementia progresses (Mena, Wischik, Novak, Milstein, & Cuello, 1991). It has been suggested that this final arrangement of PHFs are the truncated forms of tau (Zilka et al., 2006). Although the evolution of tau into accelerated form of PHFs is not fully resolved, there is a general consensus that abnormal phosphorylation as initially proposed by the Iqbals (Grundke-Iqbal, Iqbal, Quinlan, et al., 1986a) is a necessary step. Several kinases have been postulated as key in the AD-related abnormal tau phosphorylation. Of these, GSK-3β (glycogen synthase kinase-3β) is regarded as an important therapeutic target. These and other tau-related therapeutic targets and their rationale are dealt with in detail in the chapter by Iqbal and Grundke-Iqbal later in this book.

Additional Components of the Alzheimer's Pathology

In an Alzheimer's diseased brain, the abnormalities of the key proteins Aβ and tau result in a complex pathological cascade. The relative importance of the various components and the sequential evolution of this cascade is the subject of ongoing investigations. The simplified scheme of Fig. 3 attempts to highlight some of the most notable participants of this pathological cascade. Neurons possess APP molecules in their cell membrane and also in membranes of cell organelles (typically rough endoplasmic reticulum, Golgi complex, endosomes). In the cell surface the action of α-secretases (1) releases soluble APPα fragments which are regarded as neurotrophic molecules (Fig. 3). This is the APP nonamyloidogenic pathway, precluding the formation of Aβ peptides. The APP amyloidogenic pathway (3) involves the sequential cleavage of APP in its β and γ sites releasing Aβ peptides. This process apparently involves an intracellular cycle and some of the Aβ material accumulates abnormally in neurons in AD and Down syndrome (8) (Fig. 3). The extracellular soluble Aβ material is progressively oligomerized (5) forming highly neurotoxic peptides (Fig. 3). A possible outcome of this toxicity is that neuronal cell mechanisms are perturbed unleashing the formation of tau PHFs (7), which eventually provokes the microscopically visible NTFs (Fig. 3). The toxic Aβ oligomers are capable of disrupting synaptic function (Walsh et al., 2002) and memory mechanisms (Lesne et al., 2006). Extracellular Aβ peptides further aggregate into compact fibrils and conglomerate in the well-known amyloid plaques (6) which are named senile or neuritic when they are surrounded by dystrophic neuronal processes. NFTs cause functional impairments in axonal transport and generate dystrophic neurites. The massive NFT (7) or Aβ (8) intracellular accumulation might lead to neuronal cell death (9) either by necrosis or apoptosis (active/programmed cell death) (Fig. 3). Aβ peptides in oligomeric, aggregated, and fibrillar forms provoke complement deposition and the production of inflammatory mediators (McGeer & McGeer, 2001) with the consequent activation of microglia (10), which actively remove Aβ and cellular debris, including degenerating synapses (Fig. 3). Glial cells are also involved in the production of apoliprotein E (11) which supports the mobilization of cholesterol for membrane recycling and also the removal of Aβ material, in particular by endothelia cells (not illustrated). Basal forebrain cholinergic neurons are rather vulnerable to the Aβ burden becoming atrophic and losing synaptic contacts both in the cerebral cortex and hippocampus. The release of transmitter acetylcholine (ACh) stimulates M1 and M3 receptors, which in turn stimulates the nonamyloidogenic pathway of APP, via the activation of protein kinase C (13) (Fig. 3). The current cholinergic therapy in AD is based on the inhibition of enzymes responsible for the breakdown–inactivation of AChE (circle, AChE-inhibition, acetylcholinesterases) (See Fig. 3 and chapter by Gordon Wilcock and Serge Gauthier).

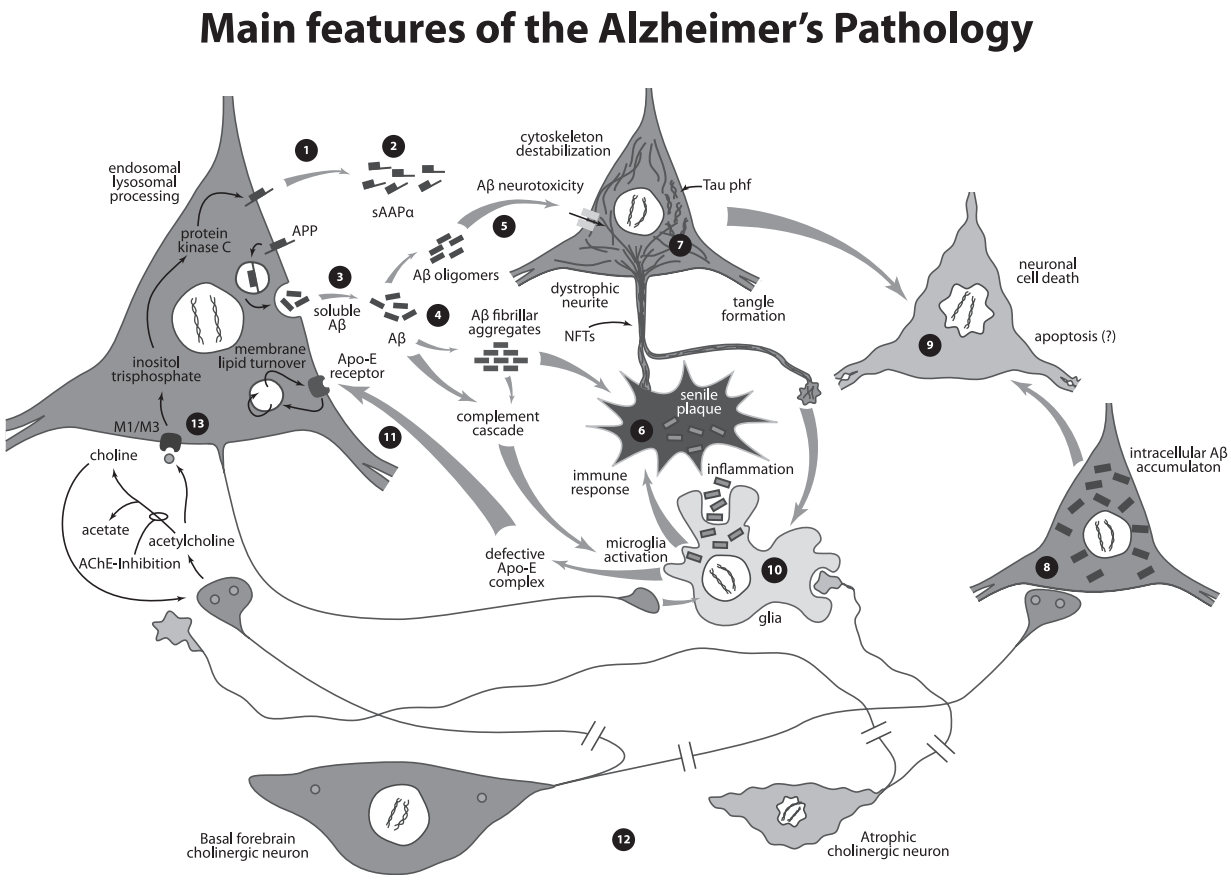

FIG. 3. This scheme represents some of the most prominent features resulting from or accompanied by the progressive accumulation of Aβ peptides intra- and extracellularly. For detailed description see text

Clinical Evolution and Diagnosis of Alzheimer's Disease, a Synopsis

Alzheimer's disease is the main cause of dementia in the aged population. The earliest symptom is a gradual loss of memory, followed by increasing impairment of language abilities and other cognitive functions, such as mathematical abilities. AD sufferers develop impairments in naming objects and people, and have difficulty with word finding, often paraphrasing to define an object. At later stages, both verbal and written communication become compromised. Visual–spatial impairments result in objects being lost and to physical disorientation, for example, finding the way home. At advance stages, analytical capabilities are seriously compromised and one or more of the following behavioral symptoms, disinhibition, aggressiveness, agitation, delusions, hallucinations, or paranoia, are also exhibited. In the final stage, patients experience feeding difficulties, profound weight loss, ambulatory difficulties, motor dysfunction, and incontinence.

There are a number of instruments to diagnose Alzheimer's and assess the deterioration of memory, cognitive functions, and the patient's ability to cope with the challenges of daily living of suspected and clinically diagnosed Alzheimer's patient (see the chapter by Gauthier in this book). Currently, the diagnosis of AD is essentially made by clinical examination. There are not, as yet, universally accepted biological tests for an unequivocal diagnoses of the disease, although the presence of Aβ peptides and tau (phosphorylate/unphosphorylated) in plasma and the cerebrospinal fluid has been proposed and investigated in some centers. These tools are not yet widely available and have not been shown to be of unequivocal diagnostic value (Andreasen & Blennow, 2005; Galasko, 2005; Golde, Eckman, & Younkin, 2000). Considerable effort has been made to establish imaging protocols to determine the loss of brain matter and expansion of ventricles, as well as for the application of tracer molecules capable of crossing the blood–brain barrier, which are able to assess the extent of the brain's Aβ amyloid load, the brain's blood flow, and the abundance of key transmitter markers by positron emission tomography (PET) (Archer et al., 2006; Engler et al., 2006; Mosconi et al., 2005; Price et al., 2005). Very recently, PET studies using molecules binding both aggregated tau and Aβ amyloid have been able to discriminate individuals who are not cognitively impaired from mild cognitive impairment (MCI) and AD patients (Small et al., 2006). Imaging techniques have also revealed that the overall brain size in AD patients shrink considerably, at the rate of ~2.8% annually (Chan et al., 2003), whereas the annual atrophy of the hippocampus can be well over 10% annually (Laakso, Lehtovirta, Partanen, Riekkinen, & Soininen, 2000).

The disease progresses inexorably with accelerated loss of brain and body functions ending with death, usually in 4–9 years. The patient remains independent for a relatively short period, requiring increased assistance from

family members and health care professionals. As the population of advanced societies lives longer, the absolute and relative numbers of Alzheimer's sufferers are presently much greater than in any other period in history. The social cost of the disease in the USA alone two decades ago was calculated to be approximately $30 billion per year (Hay & Ernst, 1987). An important development in recent years has been the recognition that there is a clinical entity that could be considered in most cases as prodromic stage of AD. For many years, clinicians have struggled to define the line separating normal age-related cognitive decline from incipient forms of AD. In recent years, it has become evident that there is an abnormal and early state of cognitive impairment, which is prodromal of several dementias, AD in particular. Although a debate continues as to the precise definition, there is generalized acceptance that sporadic AD is preceded by a phase of MCI, which often converts into AD after a few years (Bennett, 2004; Chertkow, 2002).

Genetic and Nongenetic Risk Factors in AD

Two forms of Alzheimer's disease are recognized nowadays: the familial and the sporadic forms. Familial Alzheimer's disease (FAD) is of very early onset (as early as 35 years old) and is entirely due to genetic causes (Bertram & Tanzi, 2004). FAD accounts for only a minority of the AD cases, the majority of which are regarded as the sporadic form of AD, which has a much later onset (usually after 65 years of age). The neuropathology is similar in all forms except for the time of onset and velocity of progression, which is usually faster in FAD. In the familial form of AD, a number of fully penetrant and causal mutations have been identified in three genes. These mutated genes share a common feature: their occurrence facilitates the brain expression of Aβ peptides. These are mutations in the gene encoding for APP (chromosome 21) and in genes encoding for the presenilins 1 and 2 (PS1; PS2) (chromosomes 14 and 1, respectively). Mutations in the APP gene flanking the N-terminal Aβ domain, such as the so-called Swedish double mutation ($APP_{K670N, M671L}$), facilitates the β-secretase cleavage with the consequent increased production of Aβ 1–40 and Aβ 1–42 (Citron et al., 1992). Mutations in the APP gene flanking the C-terminal domain at position 717 (γ-secretase cleavage site) cause an elevation of the longer and more amyloidogenic forms of the peptides Aβ 1–42 and Aβ 1–43 (Cai, Golde, & Younkin, 1993; Citron et al., Suzuki et al., 1994). Mutations in PS1 (such as $PS1_{M146L}$) lead to the elevation of Aβ 1–42(43), by an as yet unknown mechanism (Borchelt et al., 1996; Citron et al., 1997; Duff et al., 1996; Scheuner et al., 1996), which assumes a gain of function of the γ-secretase activity. However, this view has recently been challenged as the loss of a "protective" presenilin function (Wang et al., 2006).

It was noticed early on that there was an association between sporadic AD and the incidence of particular types of ApoE alleles (Poirier et al., 1993; Strittmatter et al., 1993). It is now well established that in the more common

late-onset, sporadic forms of AD this is, so far, the only undisputed partially penetrant genetic risk factor. The dosage of the ApoE 3 and, more importantly, ApoE 4 alleles provide a distinctive proclivity to develop AD and to the conversion from MCI to AD (see the chapter by Poirier in this book).

Most investigators agree that there are additional susceptibility genes in both the early- and late-onset AD. A number of putative AD loci have been proposed and consistently replicated in follow-up analyzes by a number of laboratories (Bertram & Tanzi, 2004; Hardy, 2004). Finally, it is highly possible, and largely unexplored, that a number of epigenetic factors could be at play in unleashing the AD neuropathology. Such a possibility is dramatically illustrated by changes in the DNA methylation patterns in maternally deprived rodents (Meaney & Szyf, 2005).

Nongenetic Risk Factors

A good number of nongenetic risk factors have been identified or proposed for the sporadic form of the disease. Of these, unequivocally, aging is the most relevant.

Aging as a Risk Factor

It has been proposed that AD might reflect a continuum of the aging process (Brayne & Calloway, 1988). In other words, given the opportunity, every individual should eventually succumb to AD. This view can be supported by the undeniable fact that aging is the most important of the nongenetic risk factors as shown by the ever growing incidence of AD with aging. On the other hand, the prevalent notion is that the incidence of AD is influenced by a multitude of risks factors (as discussed below) in addition to aging, which might act in a cooperative manner. The extent of the life span could also be regulated by the genetic background interacting with environmental as well as lifestyle aspects (Finch & Tanzi, 1997). The role of genetics in determining the life span is complex and paradoxical. In short, the prevalent view is that for the sporadic form of AD, it is not necessarily all in the genes but rather an interplay with the life experience of that particular individual. The molecular mechanisms of brain aging remain elusive. Several molecular events are suspects in the age-related downfall of brain function, which might be linked to the earlier appearance of AD. Among these are the gradual increase in oxidative stress and inflammation, and decrease in the expression of sex hormones and growth factors, which maintain the neuronal phenotype. Some of these are discussed in subsequent chapters relating to possible therapies for AD. Some attention has also been paid to low levels of vitamin B complex and the plasma elevation of homocysteine, as being responsible for age-related

cognitive deficits and unleashing the AD pathology (Seshadri, 2006; Smith, 2002). This issue remains unresolved, however, it is worth noting that epidemiological studies have shown elevations in plasma homocystein preceding the development of dementia and that the folate pathway is key to DNA methylation and therefore implicated in epigenetic mechanisms. As a result, the administration of complex B vitamins and homocysteine-lowering treatments have been recommended for the preservation of cognition in the early stages of MCI and AD (Seshadri, 2006). As aging is such a prominent AD risk factor, an obvious way to delay the aging process, such as low-calorie intake (Mattson, 2003), exercise, and sensory stimulation, can also delay the onset of AD.

High Plasma Cholesterol

In the early 1990s, high cholesterol was found to be associated with the presence of ApoE4 alleles in clinically diagnosed Alzheimer's disease (Czech et al., 1994). In particular, high levels of LDL cholesterol resulted in a higher risk of dementia and stroke and the question posed was whether the administration of statins could diminish the incidence of these conditions (Moroney et al., 1999). The influential epidemiologic study of Wolozin and collaborators (Wolozin, Kellman, Ruosseau, Celesia, & Siegel, 2000) demonstrated that the patients taking the cholesterol-lowering drugs, which act as inhibitors of 3-hydroxy-3-methylglutaryl coenzyme A reductase, better known as statins, show a 60–73% lower prevalence of probable AD. These findings were followed by numerous studies regarding the impact of high cholesterol in creating favorable conditions for the generation of Aβ peptides and further clinical investigations on the impact of statins (Sjogren & Blennow, 2005; Wolozin, 2004). This issue has been reassessed in this book in the closing chapter by Benjamin Wolozin in the context of interpreting AD clinical trials.

Hypertension

Investigating clinical records from a fairly large population during 1960–1974, Kokmen and collaborators (Kokmen et al., 1991) have shown that out of the 20 risk factors studied, hypertension along with episodic depression and personality disorders were the only risk factors to have statistically significant associations for potential clinical risk factor to develop AD. Since then, there have been a number of extensive studies largely supporting the notion that hypertension is an important risk factor for AD. However, the mechanistic interactions between hypertension and the AD neuropathology are far from clear. For a discussion on these interactions, the reader could consult a recent review by Skoog and Gustafson (Skoog & Gustafson, 2006).

There is no antihypertensive therapeutic strategy for the prevention of AD at the present time. Furthermore, hypertension as a target is complicated by the observation that blood pressure often falls when AD is clinically diagnosed (Birkenhager & Staessen, 2006). However, the treatment of hypertension is advisable for midlife high blood pressure, and in particular, low diastolic pressure and very high systolic pressure, which shows a high association with subsequent development of dementia and Alzheimer's disease. Unfortunately, randomized clinical trials have not provided strong evidence for a protective role of antihypertensives to prevent dementia and stroke-related cognitive decline (Qiu, Winblad, & Fratiglioni, 2005).

Oxidative Stress as a Risk Factor

It is possible that the age-dependent progressive increases in brain oxidative stress contributes or facilitates AD lesions. This hypothesis would indicate the convenience of effective measures to prevent and treat brain oxidative stress. However, until now, there have not been conclusive studies demonstrating efficacy of vitamin C and E in arresting or significantly delaying the onset of AD. More recently, the use of agents capable of crossing the blood-brain barrier, such as lipoic or dehydroascorbic acids, has been suggested (Harman, 2006). There is a large list of antioxidant compounds that have been suggested as beneficial to prevent or delay AD including defined chemical entities or natural products such as green tea, ginkgo biloba, red wine, blueberries, etc. Some effort is being made to define the efficacy of defined extracts (e.g., from blueberries or spinach) and of assessing their effects in suitable cell and animal models (Joseph, Shukitt-Hale, & Casadesus, 2005). Although there are some uncertainties, oxidative stress is no doubt a component of the AD pathology, the study of which might lead to suitable main or adjunct therapies in AD. The brain sources of oxidative agents, their contribution to neurodegeneration, and the potential applications of antioxidants in AD therapy are discussed by George Perry's laboratory in the chapter authored by Moreira and collaborators.

Education, Physical Activity, and Brain Trauma and the Onset of Alzheimer's

Epidemiological investigations would indicate that higher level of formal education and early brain stimulation would delay the onset of AD (Katzman, 1993; Terry & Katzman, 2001). Likewise, exercise and multisensory environmental stimulation appear to provide an increase resistance to the development of age-related cognitive problems (Briones, 2006). These observations have been confirmed by experimental evidence in AD-like transgenic