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REVIEW

Familial Alzheimer's Disease: Oxidative Stress, β-amyloid, Presenilins, and Cell Death

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ABSTRACT. 1. The basic etiology of Alzheimer's disease remains unknown, although four genes have so far been involved: β-amyloid precursor protein, presenilin-1, presenilin-2 and apolipoprotein E genes.

2. The largest familial Alzheimer's disease (FAD) kindred so far reported belong to a point mutation in codon 280 that results in a glutamic acid-to-alanine sustitution in presenilin-1 characterized in Antioquia, Colombia.

3. A hypothetical unified molecular mechanism model of cell death in FAD mediated by presenilin-1, β-amyloid, and oxidative stress is proposed as an attempt to explain the mechanisms of neuronal loss in this neurodegenerative disorder. GEN PHARMAC 31;5:675–681, 1998. **© 1998 Elsevier Science Inc.**

KEY WORDS. Familial Alzheimer's disease, presenilin-1, oxidative stress, E280A mutation, RAGE, β-amyloid

INTRODUCTION

Alzheimer's disease (AD) is a devastating neurodegenerative disorder that is the most common cause of dementia. The disorder, as described by Alzheimer in 1907, is a clinical-pathological entity. Clinically, it presents with progressive intellectual deterioration that involves not only memory, orientation, and language functions but other components of higher function as well, such as personality, judgment, problem solving, calculation, and visual–spatial and constructional abilities (Morris *et al.*, 1989; Mirra *et al.*, 1991). The pathological hallmark of AD includes neuroanatomy changes (Van Hoesen *et al.*, 1994) and, on microscopic examination, includes neurofibrillary tangles, which are abnormal neuronal soma in which the cytoplasm is filled with submicroscopic filamentous structures, consisting of filaments approximately 10 nm in diameter that are wound around each other with a helical period of 800 nm, forming the paired intracellular helical filaments (Katzman, 1986). Numerous senile neuritic plaques appear throughout the hippocampus and cerebral cortex. Neuritic plaques consist of clusters of degenerating nerve axonal and dendritic endings with a core that contains extracellular linear filaments that have the ultrastructural characteristic of an amyloid protein. Amyloid, meaning cellulose-like, is composed of 39–43 amino acid β-pleated peptide (Aβ), which forms insoluble fibrils of sheet conformation deriving from polymerization of a normally (Goldgaber *et al.*, 1987; Tanzi *et al.*, 1987; Sipe, 1994), proteolitically soluble protein amyloid precursor protein (APP) (Howlett *et al.*, 1995).

The basic etiology of AD remains unknown, although a highly heterogenous genetic component has been associated with this disorder. Indeed, several observations suggest that AD has a genetic component: (1) Family history is a risk factor for AD (Hirst *et al.*, 1994), (2) AD pathology and dementia are associated with trisomy 21 (Down's syndrome) (Rumble *et al.*, 1989), and (3) the most convincing evidence for a genetic component of AD comes from studies of pedigrees in which the disease segregates in a manner consistent with a fully penetrant autosomal dominant trait (Cornejo *et al.*, 1987; Lopera *et al.*, 1997). Familial AD (FAD) has been clasified on the basis of age of onset into late-onset FAD, with a mean age of onset of disease at age 65 years and older, and early-onset FAD with a mean onset of disease below 65 years of age. Except for earlier age of onset—and in some patients, shorter disease duration—FAD is clinically indistinguishable from sporadic or non-FAD, but neuropathologically, FAD is distinguishable because evidence has shown an increased deposition of Aβ42 levels in different brain regions of FAD patients when compared with sporadic AD patients (Lemere *et al.*, 1996). Genetic studies have so far led to the identification of three genes, encoding β-amyloid precursor protein (APP) localized in chromosome 21 (Tanzi *et al.*, 1987; for review see Schellenberg, 1992), presenelin-1 (PS1) (Clark *et al.*, 1995; Sherrington *et al.*, 1995), and presenelin-2 (PS2) (Levy-Lahad *et al.*, 1995). In addition, the ε4-allele of apolipoprotein E gene is a risk factor for AD (Nicoll *et al.*, 1995). Much of the recent progress in elucidating the pathogenesis of AD has centered on the apparent role of the 40-42 residue amyloid β-protein (Aβ) as a unifying pathological feature of the genetically diverse forms of this complex disorder (Gravina *et al.*, 1995; Sisodia and Price, 1995; Selkoe, 1996). Several lines of evidence suggest that Aβ plays a central role in the disease process. First, as Aβ is derived from proteolytic processing of APP, the AD associated with Down's syndrome and APP FAD missense mutations is likely to be due to abnormal production and depostion of Aβ (Robakis *et al.*, 1987). The majority of early-onset FAD pedigrees, however, are linked to mutations in the PS1 and PS2 genes. Since its discovery in 1992 (Shellenberg *et al.*, 1992), more than 35 different mutations have been found in PS1 gene with over 50 families of different ethnic origins (Lendon *et al.*, 1997). Among these families, the largest FAD kindred so far reported belongs to a point mutation in codon 280 that results in a glutamic acid-to-alanine substitution in PS1, characterized in Antioquia, Colombia (Lopera *et al.*,

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1994, 1997). In this review, we establish a correlation between FAD caused by the E280A-PS1 mutation, a high aggregation of β-amyloid, and the important role played by this peptide in neurodegeneration mediated by oxidative stress.

OXIDATIVE STRESS IN THE PATHOLOGY OF ALZHEIMER'S DISEASE: ROLE OF Aβ

Oxygen is a "double-edged" molecule. On one hand, it is required by all aerobic cells for the efficient production of energy. On the other hand, it can produce oxygen derived free radicals as by-products of respiration and oxidative metabolism. By defintion, a free radical is any species capable of independent existence that contains one (or more) unpaired electron(s) (Halliwell and Gutteridge, 1989). Thus, acceptance of a single electron by an O_2 molecule forms the superoxide anion-radical, O_2, which is converted rapidly to O_2 and H_2O_2 by superoxide dismutase (Fridovich, 1975). The H_2O_2 in turn can either be enzymatically decomposed by catalase and glutathione peroxidase in O_2 and/or H_2O, or react nonenzymatically with metal (ferrous or cupric) ions to produce a more reactive oxygen species, hydroxyl radical, OH, which can nick DNA, damage essential enzymes, and provoke uncontrolled chain reactions, such as lipid peroxidation or autoxidation reactions (Halliwell and Gutteridge, 1989; Winterbourn, 1995). A normal cell maintains the status quo in a balance between oxidative events and antioxidative forces. When the normal balance is upset, either by loss of reducing agents such as tocopherol (vitamin E), ascorbate (vitamin C) or protective enzymes, by increased production of oxidizing species (hydrogen peroxide and the oxyradicals), or by both events simultaneously; the tissue is considered to be under oxidant stress. It has become clear that oxidative stress is an accumulative process that progressively damages the brain, particularly by formation of reactive oxygen species (ROS), damaging sensitive proteins, lipid peroxidation of membranes, and by break-down of nucleic acids under toxic stress (Halliwell and Guterridge, 1990).

The mechanism by which Aβ causes neuronal degeneration remains poorly defined. However, one nascent theory of AD etiology holds that Aβ is involved in creating microenvironments of neuronal oxidative stress. Initially, Behl and co-workers (1992) showed evidence that Aβ peptide (β 1–42 and β 25–35) were capable of promoting toxicity directly to cultured nerve cells involving oxidative stress and free radical generation because vitamin E protected cells from damage (Behl *et al.*, 1992). These results were supported later by the finding that Aβ 25–35 generated free radicals by itself in aqueous media, in a metal-independent reaction but oxygen-dependent mechanism (Hensley *et al.*, 1994), and that Aβ 25–35 peptide was a potent lipoperoxidation initiator (Butterfield *et al.*, 1994). These authors also demonstrated that hydrogen peroxide mediates Aβ toxicity (Behl *et al.*, 1994). Moreover, Aβ induced the activity of the transcription nuclear factor (NF-kB), thought to be regulated by oxidative stress. Indeed, catalase and reagents that inhibit flavin oxidases blocked the production of H_2O_2 and Aβ toxicity (Behl *et al.*, 1994).

Although metal ions have also been implicated in toxicity of Aβ, the mechanism by which they mediated oxidative injury has not been determined. However, aluminium, iron, and zinc may increase oxidative stress by promoting peptide Aβ aggregation (Mantyh *et al.*, 1993; Bush *et al.*, 1994; Kawahara *et al.*, 1994), and aluminium potentiated Fe-induced oxidative stress (Mundy *et al.*, 1995; Xie *et al.*, 1996) or by producing severe impairment of nerve cells by entering cell nuclei (Yumoto *et al.*, 1995). It is not known to what extent metals may react with Aβ-produced H_2O_2 and contribute to cell damage. However, there is compelling experimental data on AD brains that show iron and zinc ions increased in some cerebral regions correlated with severe histopathological alterations (Deibel *et al.*, 1996).

β-AMYLOID AND CELL DEATH

Cell death can take place by two quite distinct mechanisms, necrosis and apoptosis. Necrosis occurs when cells are exposed to extreme variance from physiological conditions that may result in organelle damage, cell swelling, and plasma membrane breakdown. Necrotic cell death is virtually instantaneous. Apoptosis, in contrast, is a mode of cell death that occurs under normal physiological conditions in which the cell becomes an active participant in its own demise ("cellular suicide"). The term apoptosis—derived from a Greek word, αποπτοσισ, meaning the process of leaves falling from trees or petals falling from flowers—was introduced by Kerr *et al.* (1972) to describe the morphological features of cells undergoing degeneration in a variety of either physiological or pathological conditions (Schwartzman and Cidlowski, 1993; Majno and Joris, 1995). Apoptotic cells display a distinct morphology characterized by nuclear condensation and the fragmentation of DNA but a preservation of most intracellular organelles. Biochemically, apoptotic cells present degradation of the genomic DNA. In many living systems, this DNA fragmentation has been shown to result from activation of an endogenous Ca2+/Mg2+ dependent nuclear endonuclease generating mono- and oligonucleosomal fragments of 180–200 pairs, giving the familiar ladder seen by agarose gel electrophoresis (Willie, 1980; Bortner *et al.*, 1995).

Animal models as well as neuronal cell lines have been used to investigate the toxic effect of Aβ peptide (Kowall *et al.*, 1991, 1992; Emre *et al.*, 1992; Rush *et al.*, 1992; Baker *et al.*, 1993; Gschwind and Hubert, 1995). PC12 cells, a clonal catecholaminergic cell line derived from rat pheochromocytoma that responds to nerve growth factor (NGF) by undergoing differentiation into a sympathetic-like neuronal phenotype (Green and Tischer, 1976), have been particularly fruitful for the study of Aβ toxicity (Behl *et al.*, 1992, 1994; Gschwind and Hubert, 1995). A particularly striking observation was that Aβ interacts with a cell surface receptor for advanced glycation end (RAGE) products; it interacts in neurons (PC12 cells), microglia, and endothelial cells (Yan *et al.*, 1996). Their data demonstrate that Aβ induce oxidative stress to PC12 cells when exposed to Aβ manifesting generation of thiobarbituric acid-reactive substances and activation of NF-kB. These effects could be prevented with antioxidants proburol/N-acetylcysteine or blocking direct access to RAGE. Indeed, elevated thiobarbituric acid-reactive substances and antioxidant enzyme activity have been reported as increased in all brain regions in AD patients (Lovell *et al.*, 1995). It has been shown that RAGE-mediated induction of cellular oxidant stress triggers a cascade of intracellular signals involving the oncogenic p21ras- and mitogen activated protein kinase, culminating in transcription factor activation (Lander *et al.*, 1997).

Since Aβ has been shown to induce apoptosis in several cell lines (Gschwind and Hubert, 1995) displaying the typical 180 bp DNA ladder (Cotman *et al.*, 1994), calcium has been implicated as a critical second messenger in excitotoxicity and glutamate-mediated cell death in the presence of Aβ (Mattson, 1994). Specific and dose-dependent activation of cytoplasmic calcium by β 1–40 and β 25–35 was observed in PC12 cells (Joseph and Han, 1992; Fukuyama *et al.*, 1994). These peptides also cause selective reduction of Na+/K+ ATPase activity in synaptosomes from postmortem human hippocampus. The impairment of this enzyme was sufficient to induce ele-

vation of [Ca2+] and apoptosis (Mark *et al.*, 1995). It has been shown that Aβ 1–40 can incorporate into liposomes and lipid bilayer membranes and form cation-selective channels that conduct Ca2+ and other cations (Arispe *et al.*, 1993a, 1993b); zinc 2+ can block the open channel in a dose-dependent manner and also can modulate channel gating and conductance from one side of the channel (Arispe *et al.*, 1996). Moreover, Aβ may contribute to increase neuronal vulnerability to glutamate because ROS derived from Aβ peptide inhibited glutamate uptake (Harris *et al.*, 1995), resulting in deregulation of Na+, K+, and Ca2+ ions by glutamate ionotrophic and metabolotrophic receptors (Hoffman and Heinemann, 1994).

Taken together, these data support the hypothesis that the mechanism of neurotoxicity of Aβ appears to be mediated by oxidative stress (Buttke and Sandstrom, 1994) generating oxy-radicals (Jacobson, 1996), impaired Na+/K+ ATPase, altered Ca2+ homeostasis (Mattson, 1994), and apoptosis. Althought direct evidence of oxidative stress as a cause of neuronal cell death in AD brains is lacking, Smale and colleagues (1995) reported an elevated incidence of apoptosis neurons and astrocytes from hippocampus of AD postmortem brain. A similar result has been reported in frontal and hippocampal cortices of AD patients when compared with controls (Troncoso *et al.*, 1996; Li *et al.*, 1997). Laferla and co-workers (1995) have reported that Aβ peptide induces extensive neuronal degeneration and apoptotic cell death in transgenic mice. These findings suggest that apoptosis may be the principal underlying cellular feature of Alzheimer's disease. Moreover, immunocytochemical studies demonstrated a correlation of the increased expression of proapoptotic protein (Bax, a Bcl-associated X protein), DNA damage, and brain pathology in neurons and microglia from hippocampal brain region as compared with controls (Su *et al.*, 1997). These data lead to the proposal that Bax protein may have an important role in regulating early event associated with neuronal death. However, the relationship between oxidative stress and the molecular regulators of cell death pathways is awaiting experimental evidence.

β-AMYLOID AND PRESENILINS

Most autosomal dominant inherited forms of early onset familial Alzheimer's disease are caused by two novel highly homologous (67%) genes in the presenilin 1 gene on chromosome *14* (Sherrington *et al.*, 1995) and presenilin 2 gene on chromosome *1* (Levy-Lahad *et al.*, 1995) members of an evolutionary conserved gene familiy. Sequence analysis predicts PS1 (467 amino acids) and PS2 (448 amino acids) as integral membrane proteins that contain (1) seven membrane-spanning domains, (2) a short hydrophilic amino and carboxyl-terminal tail (Boteva *et al.*, 1996; Prihar *et al.*, 1996) expressed throughout the brain and localized mainly in endoplasmic reticulum, (3) Golgi complex (Kovacs *et al.*, 1996), and (4) cell membrane (Dewji and Singer, 1996). The mutations in presinilins that result in FAD are missense that affect the splicing without affecting the coding of the protein, suggesting a gain of function rather than loss of function. The mechanism(s) by which presinilin mutations promote neuron degeneration in AD are not yet fully understood. However, *in vivo* (Duff *et al.*, 1996), *in vitro* (Weideman *et al.*, 1997), or both (Borchelt *et al.*, 1996; Citron *et al.*, 1997) studies suggest that all known early-onset FAD act to foster Aβ deposition, particulary the Aβ 42-residue form. The mutation E280A PS1 has been demonstrated to induce massive deposition of Aβ 42 in different human brains regions (Lemere *et al.*, 1996). Moreover, mutant PS1 has been shown to alter proteolytic processing of the β-amyloid precursor protein (Mercken *et al.*, 1996; Lemere *et al.*, 1996).

Recently, the mutation in PS1-L286V (substitution at codon 286 Leu to Val) and PS2- N141L (substitution at codon 141 Asn to Ile) have been shown to increase susceptibility to apoptosis in PC12 induced by trophic factor withdrawal and Aβ (Wolozin *et al.*, 1996; Guo *et al.*, 1996) or by overexpression of PS2 to hydrogen peroxide (Deng *et al.*, 1996). It has also been demonstrated that increases in oxidative stress and intracellular calcium levels induced by the apoptotic stimuli were exacerbated in cells expressing the PS1 mutation (Guo *et al.*, 1997). These observations suggest that PS1 and PS2 may share a common mechanism in inducing apoptotic cell death by interacting with Aβ-sensitizing neurons that promote generation of oxidative stress and altering calcium homeostasis in FAD.

UNIFIED MOLECULAR MECHANISM OF CELL DEATH IN FAD

Based on the accumulated experimental information, a hypothetical molecular mechanism model to explain cell death in FAD mediated by presenilin-1, β-amyloid, and oxidative stress is depicted in Figure 1. In this model, PS1 mutant E280A alter the proteolitic processing of the APP, most likely at secretase activity, inducing Aβ [1–42] overexpression and plaque formation. Aβ either binds to RAGE, which in turn produces hydrogen peroxide or reacts with molecular oxygen to produce ROS, such as superoxide radical and hydrogen peroxide. This last compound may be able to activate p21-ras protein directly (Lander *et al.*, 1995). Once activated, p21-ras lead to activation of a serine/threonine MAP-kinase. This kinase may activate the nuclear factor NF-kB (Lander *et al.*, 1997). Since it has been shown that NF-kB activates the transcriptional factor p53 through transcription regulation (Wu and Lozano, 1994) and that p53 activates the human proapoptotic protein Bax (Miyashita and Redd, 1995), one atractive posibility is that p53 up-regulated Bax in PS1-FAD, leading the neuron to cell death by apoptosis (Fig. 1). Alternatively, Aβ toxicity may contribute by inducing lipid peroxidation, calcium deregulation, and impairment of Na+/K+ ATPase pump.

β-AMYLOID AND NEUROPROTECTION

The treatment of FAD remains in its initial state. So far, several experimental approaches have been tested, including anti-amyloidogenic antibiotics (Camilleri *et al.*, 1994; Tomiyama *et al.*, 1994, 1996), silicates (Kuroda and Kawahara, 1994; Fasman *et al.*, 1995), cholinergic grafts in primates (Baker and Ridley, 1995), inhibition of lipid peroxidation by Aβ 1–42 (Walter *et al.*, 1997), calcium channel blockers (Weiis *et al.*, 1994), intranasal administration of a fatty neuropetide (Gozes *et al.*, 1996), and neurotrophic factor therapy, (Hefti, 1995) but only two drugs have been marketed, tacrine (Cognex) and donepezil.

One of the most promising approaches to treating FAD comes from the molecular observations that vitamin E protects nerve cell *in vitro* from oxidative stress Aβ protein toxicity (Behl *et al.*, 1992, 1994). These reports has been supported by additional work including protection by a synthetic free radical scavenger FUK-8 (Bruce *et al.*, 1996); protection by antioxidants such as catalase, butylated hydroxyanisole, and *N*-acetyl-L-cysteine in Down's syndrome neurons (Busciglio and Yankner, 1995), and protection by the potent hydroxyl radical scavenger, melatonin (Tan *et al.*, 1993). Recently, Sato and co-workers (1997) reported an extensive clinical trial with the anti-parkinsonian drug selegiline alone or in combination with vitamin E. This study showed that, in patients with moderately severe impairment from AD, treatment with either selegiline or alpha-tocopherol slowed the progression of disease. Taken together,

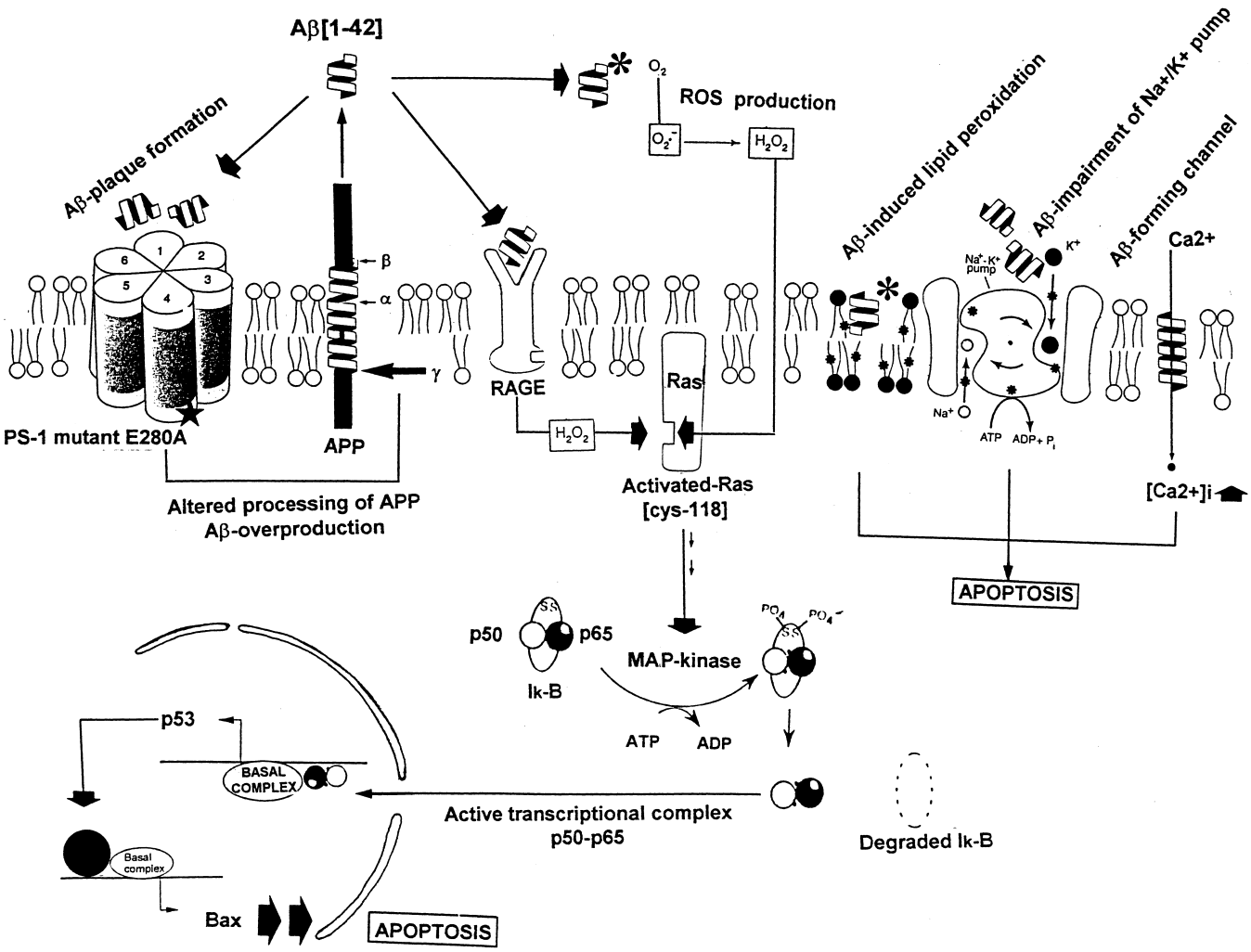

FIGURE 1. Hypothetical molecular mechanism model for presenilin PS-1[E280A] and Aβ protein toxicity according to various authors (see text). In this model, PS1 mutant E280A alter the proteolitic processing of the APP, most probably at secretase activity, inducing Aβ[1-42] overexpression and plaque formation. Aβ either binds to RAGE, which in turn produces hydrogen peroxide, or reacts with molecular oxygen to produce ROS such as superoxide radical and hydrogen peroxide. This last compound may be able to activate p21-ras protein directly. Once activated, p21-ras lead to activation of a serine/threonine MAP-kinase. This kinase may activate the nuclear factor NF-kB. This factor activates the transcriptional factor p53, which in turn activates the proapoptotic protein Bax. Thus, Bax is up-regulated in PS1-FAD, leading the neuron to cell death by apoptosis. Alternatively, Aβ-toxicity may contribute to cell death, inducing lipid peroxidation, calcium deregulation, and impairment of Na+/K+ ATPase pump.

these data place in closer corelation the hypothesis of oxidative stress as a common mediator of neurodegeneration in Alzheimer's and Parkinson's disease (Jimenez Del Rio *et al.*, 1995; Ebadi *et al.*, 1996; Velez-Pardo *et al.*, 1997, 1998). In this respect, future research efforts regarding the oxidative stress hypothesis include attempts to test and determine the role and integrity of several antioxidants systems with high affinity for different effectors involved in FAD-apoptosis (Fig. 1). Moreover, the key finding that Aβ binds to RAGE receptor opens new avenues for developing drugs that will potentiate novel and more effective therapeutic approaches to AD.

CONCLUSION

Alzheimer's is a complex disease entity. Perhaps it is wise to recall that FAD accounts only for 5% of the cases in AD and that the percentile of sporadic cases of AD is increasing steadily. What is becoming clear is that, although β-amyloid production is necessary to the pathology, it may not in itself be suffient. It is likely that additional environmental factors play a role in the cascade of events leading to neurodegeneration and the subsequent dementia. Actually, the central quest of research on AD, especially the Colombian ("paisa") mutation E280A presenilin1-FAD because of its socioeconomic implication, is to identify potential antioxidant drugs that would slow or effectively prevent the disease. We are presently performing experiments with cultured cells as a model system to gain further insight into this issue.

SUMMARY

The etiology of Alzheimer's disease remains unknown. However, the most convincing evidence for a genetic component of AD comes from studies of pedigrees in which the disease segregates in a manner consistent with a fully penetrant autosomal dominant trait (Cornejo *et al.*, 1987; Lopera *et al.*, 1997). Four genes have so far been involved in this disorder: β-amyloid precursor protein, presenilin-1, presenilin-2, and apolipoporotein E genes. The largest familial

Alzheimer's disease kindred so far reported belong to a point mutation in codon 280 that results in a glutamic acid-to-alanine substitution in PS1, characterized in Antioquia, Colombia (Lopera *et al.*, 1994, 1997). The E280A mutation increased the β-amyloid (Aβ 1–42) deposition and severe cerebrellar pathology in AD (Lemere *et al.*, 1996). Aβ has been involved in oxidative stress (Behl *et al.*, 1992, 1994) and cell death (Yan *et al.*, 1996). Moreover, heavy metals have been shown to increase oxidative stress by promoting Aβ aggregation (Mantyh *et al.*, 1993; Bush *et al.*, 1994; Kawahara *et al.*, 1994). Despite the accumulated experimental evidence of Aβ cytotoxic, an integrated picture of the cytotoxic Aβ pathway has not yet been described. Therefore, in this review, we establish the relationship between presenilin-1 mutant E280A, Aβ peptide, oxidative stress, and cell death. Moreover, a hypothetical unified-molecular mechanism for PS1 (E280A) and Aβ that induces cell death mediated by oxidative stress is proposed, in which the cascade of toxic events leading to apoptosis involve RAGE (Yan *et al.*, 1996), ROS production (Hensley *et al.*, 1996), p21-ras (Lander *et al.*, 1995, 1997), NF-kB (Lander *et al.*, 1997), and p53 activation (Wu and Lozano, 1994). Taken together, these data place in closer corelation the hypothesis that oxidative stress is a common mediator of neurodegeneration in Alzheimer's and Parkinson's disease (Jimenez Del Rio *et al.*, 1995; Ebadi *et al.*, 1996; Velez-Pardo *et al.*, 1997, 1998). These entities may also share a common transduction pathway (Kaltschmidt *et al.*, 1997; Hunot *et al.*, 1997). Actually, the core research on AD, especially the Colombian ("paisa") mutation E280A-FAD because of its socioeconomic implication, is to identify potential antioxidant drugs that would slow or effectively prevent the disease.

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