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Physiopathological modulators of amyloid aggregation and novel pharmacological approaches in Alzheimer's disease*

FERNANDA G. DEFELICE and SÉRGIO T. FERREIRA

Department of Medical Biochemistry, Institute of Biomedical Sciences
Federal University of Rio de Janeiro, 21944-590 Rio de Janeiro, RJ, Brazil

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contributed by SÉRGIO T. FERREIRA***

ABSTRACT

The biological mechanisms underlying the neuropathology of Alzheimer's disease (AD) are complex, as several factors likely contribute to the development of the disease. Therefore, it is not surprising that a number of different possible therapeutic approaches addressing distinct aspects of this disease are currently being investigated. Among these are ways to prevent amyloid aggregation and/or deposition, to prevent neuronal degeneration, and to increase brain neurotransmitter levels. Here, we discuss possible roles of endogenous modulators of $A\beta$ aggregation in the physiopathology of AD and some of the strategies currently under consideration to interfere with brain levels of β -amyloid, its aggregation and neurotoxicity.

Key words: Alzheimer's disease, $A\beta$ peptide, aggregation, neurotoxicity, physiopathological modulators.

1. INTRODUCTION

Alzheimer's disease (AD) is characterized by a slow, progressive decline in cognitive function and behavior. Progression of the disease leads to marked deterioration in memory, judgement, attention and speech and to behavioral changes including depression and psychiatric disturbances. AD poses a significant challenge to health care systems worldwide. It is estimated that about 20 million people currently suffer from dementia caused by AD (Haass and De Strooper 1999), with 20% of the individuals above 75 years old (and ~ 50% of those above 85) at risk of developing the disease. Despite considerable efforts aiming at understanding the molecular basis and physiopathology of AD, there are currently no

effective, clinically accepted treatments to cure it or stop its progression.

The β -amyloid peptide ($A\beta$) plays a central role in the neuropathology of AD (Selkoe 1994, 1999, Yankner 1996, Verbeek et al. 1997). $A\beta$ is a peptide of 39-43 amino acid residues produced by proteolytic cleavage of a large precursor known as the amyloid precursor protein (APP), encoded by a gene located on chromosome 21 in humans (Glenner and Wong 1984) (Fig. 1). APP is an integral membrane glycoprotein, with a short cytoplasmic C-terminal tail and a large extracellular N-terminal domain (Kang et al. 1987). Enzymes known as secretases are responsible for proteolysis of APP and release of $A\beta$ (Verbeek et al. 1997). The first 28 amino acid residues of $A\beta$ originate from the extracellular domain of APP and the remaining 11-15 residues originate from the transmembrane region of APP (Kang et al. 1987). $A\beta$ is released follow-

Correspondence to: Sérgio T. Ferreira
E-mail: ferreira@bioqmed.ufrj.br

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**Member of Academia Brasileira de Ciências

ing cleavage of APP at positions 597 and 637-639 by β - and γ -secretases, respectively (Verbeek et al. 1997, Nunan and Small 2000). β -secretase has recently been identified as an aspartic protease (Vassar et al. 1999, Yan et al. 1999, Sinha et al. 1999, Lin et al. 2000). γ -secretase may cleave APP at the C-terminal end of A β at four different positions, giving rise to A β peptides that are 39-43 amino acids long. The exact position of C-terminal cleavage appears critical to the development of AD, since generation of the more amyloidogenic peptides (such as A β ₁₋₄₂ or A β ₁₋₄₃) is strongly correlated with the development of AD (Scheuner et al. 1996, Small and McLean 1999, Nunan and Small 2000). The precise molecular identity of γ -secretase remains elusive. However, recent evidence suggests that presenilins 1 and 2, acting in association with another protein known as nicastrin, may constitute the γ -secretase complex responsible for the release of A β (Yu et al. 2000).

A β is the major protein constituent of the senile plaques found in the brains of AD patients (Glenner and Wong 1984, Masters et al. 1985). A β forms characteristic non-covalent fibrillar aggregates both *in vitro* and *in vivo*, and its aggregation and ensuing amyloid deposition in the brain have been related to AD neurotoxicity (Pike et al. 1993, Lorenzo and Yankner 1994, Geula et al. 1998) (Fig. 1). *In vitro*, amyloid fibril formation can be influenced at various stages by factors that either stimulate or inhibit aggregation. Such factors include peptide concentration, changes in its primary sequence, pH and interactions with various biomolecules (for examples, see Levy et al. 1990, Wisniewski et al. 1991, Fraser et al. 1991, 1992, 2001, Inouye et al. 1993, Evans et al. 1995). It is likely that the development of amyloid plaques *in vivo* also depends on the combined actions of at least some of these components. Thus, identifying physiological factors involved in A β aggregation and the interactions that are important for amyloid stability may reveal possible targets for therapeutic intervention and prevention of amyloid aggregation and toxicity.

2. PHYSIOPATHOLOGICAL MODULATORS OF AMYLOID AGGREGATION

Many different biomolecules (including proteins, proteoglycans, lipids, metals and other small molecules) have been reported to be associated with amyloid plaques in AD brains. While it is possible that some of these molecules may be related to secondary events in amyloid deposition, *in vitro* and *in vivo* studies have demonstrated that many of them may actually regulate A β aggregation (Table I). It should be noted that a delicate balance exists between A β production, aggregation and clearance in the brain, so that even agents that have a relatively small effect on A β aggregation *in vitro* may play significant roles in the regulation of those events *in vivo*.

It has been shown that certain plasma proteins, at physiological concentrations, control A β polymerization (Bohrmann et al. 1999). Albumin, α_1 -antitrypsin, IgG, and IgA are potent inhibitors of A β fibrillogenesis, with IC₅₀ values substantially lower than their plasma concentrations (Bohrmann et al. 1999). However, these proteins are present at low concentrations in cerebrospinal fluid, and possibly have little or no effect on A β aggregation. For example, although albumin is the most abundant protein in cerebrospinal fluid, it is present at a concentration below its IC₅₀ value, suggesting that it might cause only partial inhibition of A β polymerization (Bohrmann et al. 1999). The acute phase response protein, α_1 -antichymotrypsin, is up-regulated as a result of inflammatory processes and belongs to the serpin family of serine protease inhibitors. Co-localization of α_1 -antichymotrypsin with amyloid deposits has been reported exclusively in Alzheimer's disease (Abraham et al. 1988), suggesting a specific interaction with A β . When present at high concentrations, α_1 -antichymotrypsin enhances amyloid aggregation (Ma et al. 1994, Janciauskiene et al. 1996). By contrast, at low concentrations α_1 -antichymotrypsin inhibits amyloid formation and disaggregates previously formed aggregates (Fraser et al. 1993, Eriksson et al. 1995,

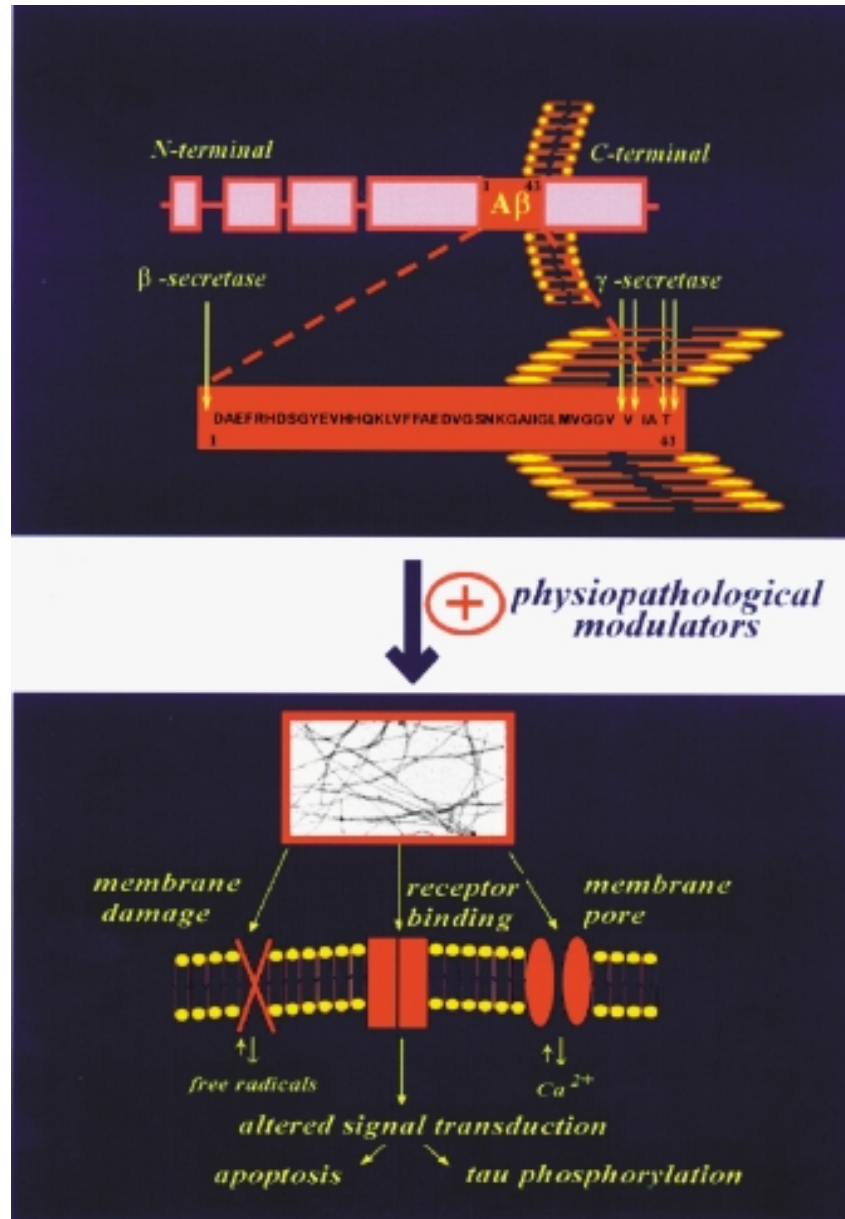


Fig. 1 – Proposed topology of APP in the plasma membrane, A β production and possible mechanisms of neurodegeneration. *Upper panel:* The plasma membrane-anchored APP (shown in pink) is cleaved by β - and γ -secretases (vertical arrows) to yield the 39-43 amino acid residue A β peptide fragment (red). Aggregation of A β is stimulated by physiopathological modulators (as discussed in the text). *Lower panel:* Aggregated A β leads to neurodegeneration through a number of possible mechanisms, including membrane damage, binding to cell-surface receptors and activation of intracellular signal transduction events and/or alteration of ion homeostasis. These events ultimately lead to hyperphosphorylation of tau and cell death.

TABLE I
Physiopathological modulators of A β aggregation¹

Modulator	A β secondary structure	Effect on A β aggregation	A β interaction domain
Plasma proteins			
albumin	n/d	↓	1-28
α_1 -antitrypsin	n/d	↓	11-28
IgG	n/d	↓	n/d
IgA	n/d	↓	n/d
α_1 -antichymotrypsin	random	↑ / ↓	11-28, 29-42
α_2 -macroglobulin	random	↓	11-28
serum amyloid A	n/d	↑	1-28
Glicosaminoglycans			
heparan sulfate	β -sheet	↑	13-16
keratan sulfate	β -sheet	↑	n/d
dermatan sulfate	β -sheet	↑	n/d
chondroitin sulfate	β -sheet	↑	13-16
Apolipoproteins			
apoE	β -sheet	↓ / ↑	29-42
apoJ	β -sheet	↓ / ↑	29-42
apoA-1	n/d	↓	n/d
Complement C1q	n/d	↑	1, 3, 7, 11
Acetylcholinesterase	n/d	↑	n/d
Laminin	n/d	↓	n/d
Entactin	random	↓	n/d
Phospholipids ²			
PS	random	↑	29-42
PI	β -sheet	↑	29-42
PC	random	no change	n/d
PE	random	no change	n/d
PA	random	↑	29-42
IS	α -helix	stabilize small aggregates	n/d

Lukacs and Christianson 1996). In the latter case, independent studies have shown that interactions between α_1 -antichymotrypsin and A β sequences 11-28/29-42 are involved in inhibition of fibrillogenesis (Lukacs and Christianson 1996, Janciauskiene

et al. 1998). Recent work has also shown that transgenic mice expressing human APP and over-expressing α_1 -antichymotrypsin develop a significantly higher number of amyloid plaques, and at earlier ages than mice expressing only human APP

TABLE I (continuation)

Modulator	A β secondary structure	Effect on A β aggregation	A β interaction domain
Gangliosides			
GM1	β -sheet/ α -helical	↓	n/d
GM2	random	no change	n/d
GM3	random	no change	n/d
GD1a	random	no change	n/d
GT1b	random	no change	n/d
Glycerol/TMAO	β -sheet	↑	n/d
Metals			
Zn ²⁺	β -sheet	↑	His13, His14
Cu ²⁺	β -sheet	↑	His13
Fe ³⁺	β -sheet	↑	His13

¹Appropriate references are cited in the text (Section 2). ²PS, phosphatidylserine; PI, phosphatidylinositol; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PA, phosphatidic acid; IS, inositol stereoisomers.

(Nilsson et al. 2001). Another acute phase protein, α_2 -macroglobulin, associates with A β , prevents fibril formation (Hughes et al. 1998) and attenuates β -amyloid peptide neurotoxicity in cultured rat fetal cortical neurons (Du et al. 1998).

Different types of glycosaminoglycan (GAG) chains, including heparan sulfate (Snow et al. 1988), keratan sulfate (Snow et al. 1996), dermatan sulfate (Snow et al., 1992) and chondroitin sulfate (DeWitt et al. 1993) are found in association with amyloid plaques in AD. A number of studies have shown that GAGs promote formation and/or stabilize amyloid fibrils (Fraser et al. 1992, 2001, Buee et al. 1993a, b, Brunden et al. 1993, Snow et al. 1995, Castillo et al. 1997, Watson et al. 1997, Gupta-Bansal and Brunden 1998, Cotman et al. 2000). The effects of GAGs on fibrillogenesis appear to be mediated by electrostatic interactions between A β and the highly sulfated chains of GAGs (McLaurin et al. 1999). These interactions take place at early stages during the process of fibril formation and result in the structural conversion of A β to β -sheet structures (Sipe

1992, McLaurin et al. 1999). The importance of sulfated groups in amyloid aggregation was highlighted by experiments showing a decrease in fibril formation in the presence of desulfated heparan sulfate (Castillo et al. 1999). Thus, it is believed that understanding the interactions between A β and sulfated GAGs may lead to effective inhibitors of amyloid aggregation (Fraser et al. 2001).

Apolipoprotein E (ApoE) isoforms appear to differentially influence A β aggregation and neurotoxicity, either facilitating or inhibiting aggregate formation *in vitro* (Wood et al. 1996, Moir et al. 1999, Drouet et al. 2001). Metal-induced aggregation of A β has been studied in the presence of purified ApoE2, ApoE3, and ApoE4 (used at the concentrations at which they are found in cerebrospinal fluid) (Moir et al. 1999). This study showed that metal-induced aggregation of A β was highest for both zinc and copper in the presence of ApoE4. A recent study has shown that ApoE2 and ApoE3, but not ApoE4, protect cortical neurons against neurotoxicity induced by A β (Drouet et al. 2001). Apolipopro-

tein J (clusterin) is a multifunctional apolipoprotein made by cells in the brain and many other locations and is associated with aggregated A β in senile and diffuse plaques of Alzheimer's disease (AD). It has been shown that ApoJ partially blocks the aggregation of A β (Oda et al. 1995, Matsubara et al. 1996). In addition, when complexed to ApoJ, A β is more resistant to proteolysis by trypsin and chymotrypsin (Matsubara et al. 1996). Apolipoprotein A-I (ApoA-I), a constituent of high-density lipoprotein complexes, has recently been shown to directly interact with the amyloid precursor protein (APP) and to inhibit A β aggregation and toxicity (Koldamova et al. 2001).

Activation of the complement pathway has been proposed as one of the mechanisms of neurodegeneration in Alzheimer's disease. This activation is a result of the binding of C1q to A β (Webster et al. 1995, Head et al. 2001, Tacnet-Delorme et al. 2001). It has been shown that C1q enhances A β aggregation at physiological concentrations, and that the kinetics of this enhancement are consistent with a nucleating interaction (Webster et al. 1995).

Acetylcholinesterase is an enzyme involved in the hydrolysis of the neurotransmitter acetylcholine and consistently co-localizes with amyloid deposits (Alvarez et al. 1997, Talesa 2001). *In vitro*, acetylcholinesterase promotes aggregation of A β by forming a complex with the growing fibrils (Alvarez et al. 1997). Recently, it has been shown that acetylcholinesterase interacts with A β via a hydrophobic domain close to the peripheral anionic binding site of the enzyme (De Ferrari et al. 2001). Basement membrane components, including entactin and laminin, also co-localize with senile plaques. Laminin inhibits A β fibril formation promoted by ApoE4 *in vitro* (Monji et al. 1998), and, more recently, entactin was also found to inhibit A β aggregation *in vitro* (Kiuchi et al. 2001).

Interactions between A β and phosphatidylinositol accelerate amyloid fibril formation, presumably through the structural conversion of β -amyloid from a random coil to β -sheet structure (Terzi et al. 1994, 1995, McLaurin and Chakrabarty 1996,

1997). By contrast, inositol stereoisomers (sugars involved in lipid biosynthesis, signal transduction and control of osmolarity) stabilize small A β aggregates, blocking the progress of fibril formation (McLaurin et al. 1998, 2000). Formation of a complex between A β and inositol significantly attenuates the toxicity of A β to neurons in culture (McLaurin et al. 2000). Inositol stereoisomers are physiological molecules that cross the blood-brain barrier, thus representing possible pharmacological tools in AD.

Gangliosides induce a distinct α -helical/ β -sheet conformation of A β at neutral pH (McLaurin and Chakrabarty 1996). Subsequent work by the same group showed that the interaction of A β with glycolipids, such as gangliosides of the GM1 type, prevents amyloid fibril formation and that the sialic acid moiety of gangliosides is necessary for the induction of α -helical structure (McLaurin et al. 1998, 2000).

Trimethylamine-N-oxide, a physiological osmolyte, and glycerol induce the conversion of A β from random coil to β -sheet structure, leading to the formation of tetrameric A β globular aggregates and early-stages protofibrils that are later transformed into mature fibrils (Yang et al. 1999).

Metals such as Zn²⁺, Fe³⁺ and Cu²⁺ are also associated with amyloid deposits found in AD patients (Lovell et al. 1998). The precise sources of these ions are not yet completely understood, but there is evidence indicating that they are released from metalloproteins under slightly acidic conditions during inflammatory responses (Brieland and Fantone 1991, Lamb and Leake 1994). A number of studies have shown that Zn²⁺, Ni²⁺ or Cu²⁺ induce fast amyloid aggregation *in vitro* and *in vivo* (Bush et al. 1994, Clements et al. 1996, Huang et al. 1997, Brown et al. 1997, Atwood et al. 1998). Aminoacid replacement studies have identified His13 as the metal ion ligand of A β (Liu et al. 1999). Replacement of His13 by Arg inhibits the structural transition from random coil to β -sheet and fibrillogenesis (Liu et al. 1999). Recent studies have shown that Cu²⁺ chelators, such as trien-

tine, penicilamine and bathophenanthroline can be used to solubilize $A\beta$ aggregates extracted in PBS buffer from AD brains (Cherny et al. 1999, 2000). These studies suggest that the combined properties of metal chelators and agents capable of dissolving $A\beta$ aggregates can be complementary in the treatment of Alzheimer's disease (Cherny et al. 1999, 2000, Cuajungco et al. 2000).

3. TARGETING β -AMYLOID PRODUCTION AND AGGREGATION AS POSSIBLE THERAPEUTIC APPROACHES IN AD

As noted above, the search for agents capable of blocking, decreasing or disrupting amyloid aggregation has become a focus of intense research interest. In this regard, a significant challenge consists in finding agents that interfere with amyloid aggregation and, at the same time, are non-toxic, capable of crossing the blood-brain barrier and stable against proteolytic degradation in plasma and cerebrospinal fluid. To date, several agents capable of interfering with β -amyloid aggregation have been characterized, including low molecular weight organic compounds, hormones, antibodies and peptides. Some of the properties, advantages and possible caveats of such agents are discussed below.

3.1. MONOCLONAL ANTIBODIES

Early studies showed that immune complexes containing two monoclonal antibodies (6C6 and 10D5) raised against the N-terminal region of $A\beta$ disaggregated previously formed amyloid fibrils and protected neurons in culture from the toxic effects of $A\beta$ (Solomon et al. 1996, 1997). Further studies showed that the amino acid sequence EFRH, corresponding to residues 3-6 of the β -amyloid peptide, represents the epitope for monoclonal antibodies 6C6 and 10D5, acting as a regulatory site that controls the process of amyloid aggregation (Frenkel et al. 1998, 1999). More recently, it has been found that a new antibody, named 508F, directed at the same epitope prevents the neurotoxic effects of $A\beta$ and disrupts amyloid fibrils *in vitro* (Frenkel et al. 2000).

3.2. PEPTIDES

Based on their complementarities to specific $A\beta$ sequences, two peptides with sequences RDLPPFD-VPID and LPFFD have been designed (Soto et al. 1996). Due to the incorporation of proline residues in their sequences, these peptides exhibit low propensities to form β -sheet structures. Interestingly, such " β -sheet breaker peptides" were found to inhibit the fibrillogenesis of $A\beta$ and to dissolve pre-formed fibrils (Soto et al. 1996, 1998). The LPFFD peptide also prevents the neurotoxicity of $A\beta$ in primary neuronal cultures, reduces the *in vivo* deposition of $A\beta$ in a cerebral model of amyloidosis in rats (Soto et al. 1998), and reduces the extension of IL-1 positive microglial cells surrounding the amyloid deposits (Sigurdsson et al. 2000). Interestingly, β -sheet breaker peptides have also been shown to reverse conformational changes of the prion protein involved in transmissible spongiform encephalopathies (Soto et al. 2000). Possible problems associated with the use of peptides in the treatment of diseases of the central nervous system are related to their rapid proteolytic degradation in the plasma and/or cerebrospinal fluid, and low permeability across the blood-brain barrier. In this regard, a recent study has shown that covalent incorporation of polyamines to an 11-amino acid long β -sheet breaker peptide leads to an increase in both the permeability across the blood-brain barrier and resistance to proteolytic degradation (Poduslo et al. 1999).

3.3. HORMONES

The pineal hormone, melatonin, is involved in the regulation of circadian rhythms. Melatonin levels are known to decrease in normal aging, and are specially low in Alzheimer's disease (Skene et al. 1990). Recent studies have shown that melatonin interacts with $A\beta_{1-40}$ and $A\beta_{1-42}$, inhibiting fibrillogenesis (Pappolla et al. 1998) and preventing cellular death in culture, oxidative damage and the increase in intracellular Ca^{2+} induced by $A\beta$ (Pappolla et al. 1997, 2000). Additional studies have shown that

melatonin protects platelet membranes from lipid peroxidation induced by $A\beta$ (Daniels et al. 1998), and cells in culture from mitochondrial oxidative damage induced by $A\beta$ (Pappolla et al. 1999). The indol derivative, 3-indol-propionic acid, which is structurally related to melatonin, also exhibits neuroprotective action against $A\beta$ toxicity (Chyan et al. 1999) and a recent study has shown that melatonin reduces the secretion of interleukines 1 and 6 in mouse brain slices (Clapp-Lilly et al. 2001).

Possible biological effects of estrogen on neurotransmitter activity and neuronal development have been proposed (for a review, see Alonso-Solis et al. 1996). These studies suggest that estrogen (specially its prevalent form in human ovaries, estradiol) exhibits antioxidant properties and may affect relevant events in Alzheimer's disease (Behl et al. 1995, Kawas et al. 1997). In addition, estrogen has been shown to regulate APP processing, causing increased secretion of the non-amyloidogenic fragment sAPP α and a concomitant decrease in β -amyloid peptide formation (Jaffe et al. 1994, Xu et al. 1998, Manthey et al. 2001). This effect appears to be mediated via the extracellular-regulated kinase 1 and 2 (ERK1/2) pathways (Manthey et al. 2001). Estrogen protects neurons in culture from the toxic effects of exogenously added $A\beta$ (Gridley et al. 1997, Mook-Jung et al. 1997, Zhang et al. 2001) and protects neuronal cells from $A\beta$ -induced apoptotic cell death (Hosoda et al. 2001). A recent study has shown that estrogen enhances $A\beta$ uptake in human cortical microglial cultures, suggesting an important role of estrogen in $A\beta$ peptide clearance (Li et al. 2000). In line with this idea, another study has shown that testosterone also interferes with APP processing, leading to an enhancement of sAPP α production and a reduction in $A\beta$ levels in neurons in culture (Gouras et al. 2000). These results are in accordance with epidemiological studies that suggest that women that received estrogen replacement therapy in the post-menopausal phase are less susceptible to the development of Alzheimer's disease than women not receiving estrogen (Kawas et al. 1997, Seshadri et al. 2001). However, the protective role

of estrogen in β -amyloid toxicity is still controversial, as other epidemiological studies have failed to identify a lower risk of developing Alzheimer's disease in women that received hormonal replacement therapy (Seshadri et al. 2001).

3.4. INHIBITION OF $A\beta$ PEPTIDE FORMATION

Current strategies to decrease or prevent β -amyloid peptide formation are based on the inhibition of γ - and β -secretase activities or enhancement of α -secretase activity. Since $A\beta$ formation precedes amyloid plaque formation and neuronal death, blocking its production may constitute an effective therapeutic approach in AD.

Activation of α -secretase, which forms the sAPP α fragment, may be used as a strategy to prevent APP processing by β - and γ -secretases. In this regard, *in vitro* studies have shown that phosphatase inhibitors, protein kinase C activators and acetylcholinesterase inhibitors enhance APP cleavage by α -secretase, increasing the release of sAPP α (Gandy and Greengard 1994, da Cruz e Silva et al. 1995, Giacobini 1997). Nevertheless, it is believed that different proteases (including desintegrin, metalloproteases, TNF- α , ADAM-17 and ADAM-10) contribute to the α -secretase activity (Nunan and Small 2000). Thus, it seems difficult to specifically regulate, from the pharmacological point of view, this pathway of APP proteolysis.

β -secretase (BACE) has only recently been identified (Sinha et al. 1999, Vassar et al. 1999, Yan et al. 1999) and it remains unclear whether presenilins and nicastrin are required components for γ -secretase activity. Therefore, studies involving pharmacological inhibition of these enzymes have mainly been carried out in an indirect way, through the observation of the decrease in $A\beta$ production in the presence of certain inhibitors (e.g., gelatinase A, bafilomicin A, calpain inhibitor, brefeldin and NH₄Cl) (Asami-Odaka et al. 1995, Knops et al. 1995, Higaki et al. 1995, Citron et al. 1996).

Recent studies have identified new inhibitors of BACE, including the protease inhibitor MG132 and calcium ionophore A23187 (Steinhilb et al. 2000,

Sennvik et al. 2001). In addition, the proteolytic domain structure of β -secretase complexed to an octapeptide inhibitor was recently solved (Hong et al. 2000). BACE has structural homology to the HIV protease, and, thus, many inhibitors of the latter can modulate BACE activity (Nunan and Small 2000). Transgenic mice over-expressing APP and deficient in BACE expression are viable, present normal phenotype and a decrease in $A\beta$ peptide production (Luo et al. 2001), suggesting that *in vivo* inhibition of BACE may be therapeutically valuable. However, it is important to note that, in humans, BACE can have other physiological substrates in addition to APP, and may be involved in important biological functions that could be affected by the use of inhibitors (Nunan and Small 2000).

Recent studies have also identified inhibitors of γ -secretase activity, including difluoroketone, difluoro alcohol peptideomimetics, a bromoacetamide derivative, and a benzofenone analog (Seiffert et al. 2000, Moore et al. 2000). Of considerable interest, a very recent study has shown, for the first time, the *in vivo* inhibition of γ -secretase activity through the oral administration of the compound N-[N-[3,5-difluorophenacetyl]-L-alanyl]-S-phenylglycine t-butyl ester to transgenic mice over-expressing human APP, resulting in the reduction of $A\beta$ levels in the brain (Dovey et al. 2001). However, as in the case of BACE, it is not clear whether the inhibition of γ -secretase may become a realistic therapy for Alzheimer's disease, as these enzymes can play other important physiological roles in addition to APP processing and β -amyloid peptide production, and, thus, the inhibition of secretases could lead to important cell disfunctions.

3.5. CLEARANCE OF $A\beta$

The correct balance between production and catabolism of $A\beta$ appears directly related to Alzheimer's disease, as the over-production of β -amyloid peptide is not followed by a parallel increase in its clearance in both familiar and sporadic forms of AD (Scheuner et al. 1996, Hardy 1997, Selkoe 1998, Price et al. 1998). Furthermore, recent observations suggest

that reduction of $A\beta$ catabolism leads to brain accumulation of this peptide, triggering initial processes of the disease (Iwata et al. 2000). In this regard, an interesting therapeutic approach to AD might consist of increasing the degradation and clearance of $A\beta$. However, knowledge of the mechanisms involved in $A\beta$ degradation and clearance is still limited. Early studies showed that microglial cells uptake $A\beta$ *in vitro* through receptor-mediated mechanisms (Shaffer et al. 1995, Paresce et al. 1996), and that this process can be slowed down in the presence of proteoglycans (Shaffer et al. 1995). Recently, it has been shown that the uptake of $A\beta$ by microglia is induced by a chaperonin, BiP/GRP-78 (Kakimura et al. 2001). In addition, it has been shown that inhibition of $A\beta$ fibrillogenesis by 4'-iodo-4'-deoxydoxorubicin (IDOX) facilitates clearance of the peptide (Merlini et al. 1995).

The insulin degrading enzyme (insulysin) appears to play an important role in regulating extracellular β -amyloid peptide levels (Vekrellis et al. 2000), by hydrolyzing $A\beta$ into various fragments that are not neurotoxic (Vekrellis et al. 2000, Mukherjee et al. 2000, Chesneau et al. 2000). α_2 -macroglobulin is also known to enhance $A\beta$ clearance via interaction with the LDL receptor related protein (Qiu et al. 1999, Lauer et al. 2001). On the other hand, serine protease inhibitors, such as α_1 -antichymotrypsin, inhibit the clearance of $A\beta$ both *in vitro* and *in vivo* (Abraham et al. 2000), increasing plaque formation in transgenic mice (Abraham et al. 2000, Mucke et al. 2000).

Nepriylisin, a neutral endopeptidase, has recently been identified as responsible for the major $A\beta_{1-42}$ catabolic pathway in brain parenchyma (Iwata et al. 2000). Infusion of thiorphan, a nepriylisin inhibitor, in rat brains causes extracellular amyloid deposition of endogenous $A\beta$ (Iwata et al. 2000, Shirotani et al. 2001). Nepriylisin deficiency resulted in defects both in the degradation of exogenously administered $A\beta$ and in the metabolic suppression of endogenous $A\beta$ in a gene dose-dependent manner (Iwata et al. 2000) and an inverse association between vulnerability to $A\beta$ de-

position and immunohistochemical localization of neprilysin in human cerebral cortex has been reported (Akiyama et al. 2000).

3.6. IMMUNIZATION WITH A β PEPTIDE

Studies using transgenic mice over-expressing human APP have shown that immunization with A β peptide leads to a significant reduction in brain amyloid plaques (Schenk et al. 1999). In a subsequent study, antibodies raised against the β -amyloid peptide were peripherally administered (i.e., in a passive immunization transfer protocol), and were found to gain access to the central nervous system and to reduce amyloid plaque burden in transgenic mice (Games et al. 2000). Passive immunization also decreased astrogliosis and brain inflammatory response induced by A β peptide (Bard et al. 2000). Further studies by other groups confirmed that immunization of transgenic mice with A β reduced the fibrillar deposition of β -amyloid peptide (Sigurdsson et al. 2001) and protected against cognitive dysfunction (Janus et al. 2000, Morgan et al. 2000). In accordance with these studies, direct *in vivo* observations in transgenic mice brains through the use of multi-photon fluorescence microscopy have recently shown that immunization with A β leads to the clearance of amyloid plaques (Bacskai et al. 2001, DeMattos et al. 2001).

It is believed that immunization can modulate the metabolism of A β through distinct mechanisms, including its destruction by microglial phagocytosis (Bard et al. 2000) and redistribution of A β from neuritic plaques to diffuse plaques (Janus et al. 2000). These distinct effects can reflect differences in antigen presentation, or "lineage-specific" immune response (Schenk et al. 1999, Bard et al. 2000, Janus et al. 2000). If indeed this is found to be the case, such differences may complicate the use of active immunization in humans (St. George-Hyslop and Westaway 1999, Janus et al. 2000). Furthermore, it is important to note that despite the important reduction of plaque formation and prevention of cognitive decline, no study so far has been able to demonstrate the complete reversion of amyloid

plaque formation by immunization with A β (Janus et al. 2000).

3.7. SMALL MOLECULE INHIBITORS OF A β AGGREGATION: NITROPHENOLS

Another approach that has been pursued consists in the search for anti-amyloidogenic compounds capable of preventing the neurotoxicity of A β . In the lack of detailed molecular structures of either soluble or fibrillar A β (which precludes a structure-based drug design approach), one strategy to identify potential anti-amyloidogenic compounds has relied on an investigation of the stability of amyloid fibrils (De Felice et al. 2001, Ferreira and De Felice 2001). These studies have indicated that a significant contribution to the stability of A β fibrils comes from entropy-driven hydrophobic interactions, leading to the hypothesis that low molecular weight hydrophobic compounds could be effective in destabilizing and disaggregating amyloid fibrils.

After examining a number of moderately hydrophobic compounds, we found that 2,4-dinitrophenol (DNP) and 3-nitrophenol (NP) prevent amyloid aggregation *in vitro* and cause the disassembly of pre-aggregated fibrils (De Felice et al. 2001). Of greater interest, nitrophenols block the neurotoxicity of A β to rat hippocampal neurons in primary culture, and cause a marked reduction in the area occupied by amyloid deposits in a rat model system of amyloidosis (De Felice et al. 2001). DNP is known for its toxic effects related to mitochondrial uncoupling, which raises concerns about the possible therapeutic applications of this compound. Interestingly, however, at the low concentrations employed to destabilize amyloid aggregates, no toxic effects of DNP were detected in either neuronal cultures or in brains slices of animals that received intra-cerebral injections of DNP (De Felice et al. 2001). Thus, since at present there is no effective treatment available for amyloidoses, including AD, Type II diabetes and prion-related spongiform encephalopathies, we have proposed that nitrophenols and their derivatives should be explored as possible drug candidates or lead compounds for the develop-

ment of drugs to prevent amyloid aggregation and neurotoxicity in Alzheimer's disease.

4. CONCLUSIONS

Despite intense research efforts into elucidating the molecular and cellular basis of Alzheimer's disease, no effective treatments are yet available to stop it or to prevent its development. As reviewed above, several different approaches addressing distinct aspects of the disease are being pursued in an attempt to develop effective therapies. Given the multifactorial nature of this disease (Selkoe 1999), it seems unlikely that a single therapeutic target may lead to an effective treatment for AD. Instead, the simultaneous employment of distinct strategies aiming at decreasing A β production (through manipulation of the activities of secretases), stimulation of the physiological mechanisms of clearance of the peptide and inhibition of amyloid aggregation may eventually constitute an effective approach to the prevention and treatment of AD.

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RESUMO

Os mecanismos biológicos envolvidos na neuropatologia da doença de Alzheimer (DA) são complexos, já que vários fatores contribuem para o desenvolvimento da doença. Portanto, não é surpreendente que diferentes abordagens terapêuticas possíveis envolvendo aspectos distintos da doença estejam sendo investigados atualmente. Estas abordagens incluem a prevenção da agregação e/ou deposição amilóide, a prevenção da degeneração neuronal

e o aumento do nível de neurotransmissores. Nesta revisão, nós discutimos possíveis papéis de moduladores endógenos da agregação de peptídeo A β na fisiopatologia da DA e algumas estratégias atualmente sob consideração para interferir com os níveis do peptídeo A β , sua agregação e neurotoxicidade.

Palavras-chave: doença de Alzheimer, peptídeo A β , agregação, neurotoxicidade, moduladores fisiopatológicos.

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