



# Anti-amyloid $\beta$ hydrophobic peptides in Alzheimer's disease: biomarkers and therapeutic potential

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**Academic Editor:** Ryszard Pluta, Medical University of Lublin, Poland

**Received:** December 19, 2024 **Accepted:** February 10, 2025 **Published:** February 24, 2025

**Cite this article:** Gutierrez-Merino C. Anti-amyloid  $\beta$  hydrophobic peptides in Alzheimer's disease: biomarkers and therapeutic potential. *Explor Neurosci.* 2025;4:100672. <https://doi.org/10.37349/en.2025.100672>

## Abstract

Intracellular amyloid  $\beta$  oligomers (A $\beta$ O<sub>s</sub>) have been linked to Alzheimer's disease (AD) pathogenesis and to the neuronal damage in this neurodegenerative disease. Calmodulin, which binds A $\beta$ O with very high affinity, plays a pivotal role in A $\beta$ -induced neurotoxicity and has been used as a model template protein for the design of A $\beta$ O-antagonist peptides. The hydrophobic amino acid residues of the COOH-terminus domain of A $\beta$  play a leading role in its interaction with the intracellular proteins that bind A $\beta$ O with high affinity. This review focuses on A $\beta$ -antagonist hydrophobic peptides that bind to the COOH-terminus of A $\beta$  and their endogenous production in the brain, highlighting the role of the proteasome as a major source of this type of peptides. It is emphasized that the level of these hydrophobic endogenous neuropeptides undergoes significant changes in the brain of AD patients relative to age-matched healthy individuals. It is concluded that these neuropeptides may become helpful biomarkers for the evaluation of the risk of the onset of sporadic AD and/or for the prognosis of AD. In addition, A $\beta$ -antagonist hydrophobic peptides that bind to the COOH-terminus of A $\beta$  seem a priori good candidates for the development of novel AD therapies, which could be used in combination with other drug-based therapies. Future perspectives and limitations for their use in the clinical management of AD are briefly discussed.

## Keywords

Amyloid  $\beta$ , Alzheimer's disease, hydrophobic peptides, proteasome, endogenous neuropeptides, biomarkers, therapy

## Introduction

The 2022 world Alzheimer's report [1] pointed out the importance of early detection and diagnosis of Alzheimer's disease (AD), as nearly 75% of individuals with dementia are not diagnosed globally. The histopathological hallmarks of AD are the extracellular amyloid  $\beta$  (A $\beta$ ) plaques and intracellular neurofibrillary tangles [2]. A $\beta$  monomers aggregate to form low molecular weight oligomers (dimers, trimers, tetramers, and pentamers), mid-range molecular weight oligomers (hexamers, nonamers, and

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dodecamers), protofibrils, and fibrils [3]. Of special relevance for the main aims of this work is that the hydrophobic C-terminal of the A $\beta$  plays a critical role in triggering the transformation from  $\alpha$ -helical to  $\beta$ -sheet structure present in high-order aggregation states of A $\beta$  found in AD [4]. Guo et al. [5] reported that A $\beta$  and tau form soluble complexes that may promote self-aggregation of both into the insoluble forms observed in AD, and that middle and C-terminal A $\beta$  regions provide the A $\beta$  amino acid residues more strongly binding to tau, namely, peptide sequences A $\beta$ (11–16), A $\beta$ (27–32), and A $\beta$ (37–42). Therefore, A $\beta$ :tau complexes can be formed when intracellular free A $\beta$  monomers/oligomers reach concentrations in the saturation range for the formation of these complexes. Moreover, Guo et al. [5] hypothesized that in AD the intracellular binding of soluble A $\beta$  to soluble non-phosphorylated tau promotes tau phosphorylation and A $\beta$  nucleation, and proposed that blocking the sites where A $\beta$  initially binds to tau might arrest the simultaneous formation of plaques and neurofibrillary tangles in AD. Other molecular mechanisms linking A $\beta$  and tau pathology are reviewed in [6]. Also, it should be noted that the release of intracellular A $\beta$  to the extracellular medium caused by the neuronal damage elicited by the toxic intracellular A $\beta$  oligomers (A $\beta$ Os) and neurofibrillary tangles is likely to potentiate A $\beta$  plaque formation.

The neurotoxic A $\beta$  peptide A $\beta$ (1–42), which is found in higher concentrations in the brain of AD patients and associated with A $\beta$  plaques [7, 8], is produced from the amyloid precursor protein (APP) by the so-called Amyloidogenic Pathway through the sequential activity of  $\beta$ -site APP cleaving enzyme 1 (BACE1) and  $\gamma$ -secretase [9]. Indeed, an enhanced activity of BACE1 and a shift towards the amyloidogenic pathway of APP processing has been reported to be linked to several factors known to foster the neurodegeneration in AD-affected brains, like iron dyshomeostasis [10–12], brain oxidative stress [13–15], hypercholesterolemia [16–18], and brain hypoxia [19]. Furthermore, A $\beta$  plaques and neurofibrillary tangles contain high concentrations of iron and Fe<sup>2+</sup> and Fe<sup>3+</sup> interactions with APP and A $\beta$  speed up A $\beta$  aggregation into fibrillar forms [12, 14, 20]. Nevertheless, nearly all BACE1 inhibitors used as candidate therapeutic agents in AD have failed in later phases of clinical trials, due to safety and/or efficacy issues, and others were discontinued early in favor of second-generation small-molecule candidates [21]. Thus, exploration of alternate approaches to reducing A $\beta$  toxicity seems a timely issue. Indeed, it has been noted recently that phytochemicals like indole-3 carbinol and diindolylmethane that inhibit A $\beta$ -induced neurotoxicity, A $\beta$  self-aggregation, and acetylcholinesterase enzyme activity show anti-AD effects [22]. Another novel potential therapeutic target for AD is the attenuation of signaling pathways leading to A $\beta$  overproduction, such as the WNT- $\beta$  catenin signaling [23]. As reviewed in [23], the dickkopf WNT signaling pathway inhibitor 1 (DKK1), which promotes A $\beta$  production and synapse degradation through downregulating WNT- $\beta$  catenin signaling, colocalizes in neurofibrillary tangles and dystrophic neurites in post-mortem AD brains.

The shortest A $\beta$ (1–42)-derived peptide that retains the toxicity of the full-length peptide is A $\beta$ (25–35) [24], and this experimental observation is of relevance for the identification of peptides that can antagonize the actions of neurotoxic A $\beta$  peptides. Cumulative experimental evidence shows that intracellular A $\beta$ Os are linked to AD pathogenesis and are the cause of neuronal damage [25–27]. Indeed, the metabolic and neurotoxic effects of A $\beta$ (1–42) have been linked with neuronal uptake of A $\beta$ O and the subsequent intracellular rise of their concentration [26–28]. Furthermore, it has been proposed that amyloid plaques can be considered reservoirs of A $\beta$  neurotoxic species [29]. As anti-A $\beta$  antibodies are expected to trap only extracellular A $\beta$ , this could, at least in part, account for the limited and partial protection reported for aducanumab treatment in AD [30]. It has been shown that binding of extracellular A $\beta$ (1–42) to lipid rafts of the plasma membrane elicits its oligomerization (to dimers or trimers) and uptake by the neuronal cells in culture, reviewed in [31]. It is to be recalled here that (i) the apolipoprotein E4 (apoE4) allele is a major genetic risk factor for late-onset AD [32], (ii) apoE binds with high affinity extracellular A $\beta$ (1–42) oligomers and the complex apoE:A $\beta$ (1–42) has been reported to strongly co-localize with lipid rafts [33], and (iii) apoE4 exacerbates the intraneuronal accumulation of A $\beta$  and plaque deposition in the brain parenchyma [34, 35]. Of note,  $\alpha$ 7 nicotinic cholinergic receptor and receptor for advanced glycation end products (RAGE) are other proteins that have been proposed to play a role in neuronal A $\beta$  uptake through binding with high affinity extracellular A $\beta$ O [31].

Briefly, in the brain undergoing degeneration in AD, A $\beta$ O is produced by neurons and, also, by reactive neurotoxic astrocytes [36, 37]. Thus, A $\beta$ O dynamics between extracellular and intracellular spaces, i.e., the balance between neuronal uptake and secretion, plays a relevant role in A $\beta$ O neurotoxicity. Only a few hours of incubation with 2 micromolar of A $\beta$ (1–42) oligomers is needed to allow for a significant internalization of A $\beta$ (1–42) in primary cultures of cerebellar granule neurons and in the HT-22 neuronal cell line [38, 39]. Internalized A $\beta$ (1–40) and A $\beta$ (1–42) peptides display a widespread intracellular distribution up to the perinuclear region in cerebellar granule neurons [38], in HT-22 cells [39], in differentiated PC12 cells and in rat primary hippocampal neurons [40], and in the neuroblastoma cell line SH-SY5Y [41].

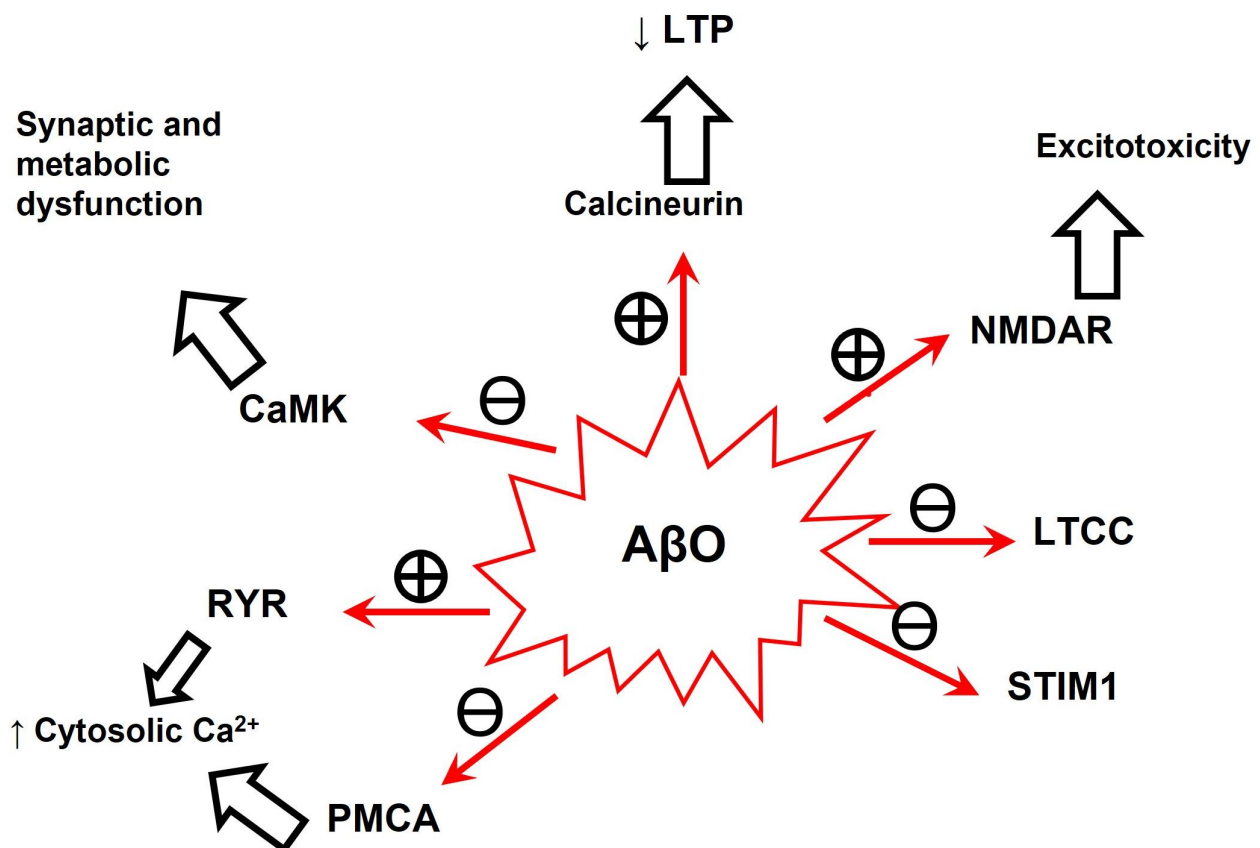
Thus, intracellular proteins that bind A $\beta$ O with high affinity play a major role in A $\beta$ -induced neurotoxicity, and antagonists of their interactions with A $\beta$ O are expected to provide neuroprotection against A $\beta$ -induced brain neurodegeneration. In the next section of this review, it is highlighted that the interaction of A $\beta$ O with calmodulin (CaM), one of the intracellular proteins displaying higher affinity to A $\beta$ O (if not the highest), can play a pivotal role in A $\beta$ -induced neurotoxicity. The following section points out that highly hydrophobic peptides binding to the COOH-terminus of A $\beta$ (1–42) inhibit the formation of A $\beta$ (1–42):CaM complexes, and, also, the aggregation and neurotoxicity of A $\beta$ . These are called A $\beta$ -antagonist hydrophobic neuropeptides. The brain endogenous neuropeptides of this type that have been reported to suffer significant variations in AD are analyzed next. Future perspectives on the AD therapeutic potential of A $\beta$ -antagonist hydrophobic neuropeptides are briefly dealt with in the final section of this review.

## CaM and CaM binding proteins are major intracellular targets for intracellular A $\beta$ O

Patients with symptoms ranging from mild cognitive impairment to early mild AD suffer a progressive loss of functional synapses in hippocampal and cortical brain regions [42–44]. Neurotransmitter secretion, synaptic plasticity, neurite growth and sprouting, and signaling pathways that mediate the metabolic neuronal responses to many relevant extracellular stimuli are strongly dependent on cytosolic calcium concentration [45, 46]. In addition, mitochondrial Ca<sup>2+</sup> signaling is altered in familial AD due to mutations in the presenilins [47], and this has been proposed to cause mitochondria dysfunction [48]. Furthermore, it has been shown that mutations in presenilins and APP can produce a substantial increase in the endoplasmic reticulum-mitochondria connectivity through upregulation of the mitochondria-associated endoplasmic reticulum membrane functions, a common feature in both familial and sporadic AD [49, 50]. Indeed, a sustained increase in mitochondrial Ca<sup>2+</sup> concentration impairs ATP production, increases reactive oxygen species production, and the opening of the mitochondrial permeability transition pore [51].

Many studies have shown that the activity of systems playing key roles in the control of neuronal intracellular calcium homeostasis and signaling are altered by A $\beta$ O, reviewed in [52]. It is worth noting here that CaM binding proteins (CaMBPs) are major targets for intracellular A $\beta$ O, as schematically shown in Figure 1, see also [53]. It is to be highlighted that at normal neuronal resting cytosolic calcium concentrations, i.e.,  $\leq 100$  nM, CaM is mainly in the apo-CaM conformation [46]. Therefore, a dysregulation of intracellular calcium homeostasis that raises the cytosolic calcium concentration shifts the apo-CaM/Ca<sup>2+</sup>-CaM equilibrium towards the Ca<sup>2+</sup>-CaM conformation, which is the CaM conformation that binds to most CaMBPs. This bears a special relevance because, due to the high reactivity and short lifetime of reactive oxygen species produced by iron-A $\beta$  redox cycling [14, 20], its relative proximity to the iron-A $\beta$  source will determine the extent of oxidative modifications of these calcium transport systems.

In addition, as briefly explained below, A $\beta$ O induces a harmful feedback loop that fosters A $\beta$  production. In vitro experiments have shown that BACE1 is stimulated around 2.5-fold by Ca<sup>2+</sup>-CaM [53, 54]. Moreover, Giliberto et al. [55] showed that the treatment of neuronal and neuroblastoma cells with 1  $\mu$ M soluble A $\beta$ (1–42) increased BACE1 transcription and that this was reverted by an anti-A $\beta$ (1–42) antibody. It has been suggested that this could be due to A $\beta$ -induced oxidative stress because this increase



**Figure 1. Neuronal intracellular calcium signaling systems whose activity has been shown to be modulated by AβO.** See [39, 52] for detailed references of each calcium signaling system modulation by AβO. The encircled + and – mean stimulation and inhibition, respectively. The ↑ and ↓ mean increase and decrease, respectively. AβO: amyloid β oligomer; CaMK: calmodulin-dependent kinase; LTCC: L-type calcium channels; LTP: long-term potentiation; NMDAR: *N*-methyl *D*-aspartate receptor; PMCA: plasma membrane Ca<sup>2+</sup>-ATPases; RYR: ryanodine receptor; STIM1: stromal interaction molecule-1

in BACE1 transcription was shown to be mediated by the activity of nuclear factor kappa light-chain enhancer of activated B cells (NFκB) [56].

Only a few intracellular proteins expressed in neurons are known to bind Aβ peptides with nanomolar dissociation constants. These are cellular prion protein [57], glycogen synthase kinase 3α [58], tau [5], stromal interaction molecule-1 (STIM1) [39], and the EF-hand calcium binding proteins CaM [59], and calbindin-D28k [60]. The nanomolar dissociation constant of the CaM:Aβ(1–42) complex [in Aβ(1–42) monomer concentration] reported by Corbacho et al. [59] from fluorescence studies, has been confirmed by Kim et al. [61] using titration microcalorimetry. As these dissociation constants were obtained using solutions of AβO (dimers/trimers), the CaM:Aβ(1–42) oligomers dissociation constant is < 1 nM AβO [59], implying that CaM is the intracellular protein with the highest affinity for AβO reported until now. This bears a special relevance, because the expression level of CaM in neurons (in the micromolar range) is several orders of magnitude higher than that of the other protein targets displaying a high affinity for Aβ(1–42), with the exception of calbindin-D28k in brain regions more prone to neurodegeneration in AD, namely, pyramidal neurons of hippocampus and in cortical neurons of the central nervous system. Moreover, extensive co-localization of internalized Aβ(1–42) and CaM in cerebellar granule neurons, and, also, co-immunoprecipitation of CaM with Aβ(1–42) in cell lysates strongly suggested that CaM is a major intracellular sink for Aβ(1–42) [38]. On these grounds, we have proposed that CaM and calbindin-D28k help to maintain the intracellular free neurotoxic Aβ concentrations in the low nanomolar range, i.e., serve as Aβ-trap or Aβ-buffer system [38, 52, 60].

## Highly hydrophobic peptides that bind to the COOH-terminus of A $\beta$ (1–42) inhibit the formation of A $\beta$ (1–42):CaM and of A $\beta$ (1–42):calbindin-D28k complexes, and, also, the aggregation and neurotoxicity of A $\beta$

The rational design of peptides to antagonize the interaction of A $\beta$ 0 with target proteins in the neurons requires the knowledge of the three-dimensional structure of the target proteins with resolution at the atomic level, and, also, the amino acid residues of A $\beta$ 0 more relevant for the formation of the complex between A $\beta$ 0 and the target protein. Due to this, soluble target proteins become the first choice for this task, since the three-dimensional structures of only a few membrane-inserted proteins have been resolved at an atomic level, and their conformations can be significantly affected by their interaction with vicinal lipids in the native cell membrane. In addition, the smaller the protein size the better to minimize the possibility of the presence of alternate binding sites with different affinity for A $\beta$ 0. CaM, a small size soluble target protein with high affinity for A $\beta$ 0, fulfills these basic criteria.

In addition, CaM seems a good model protein template for the search for peptides that can antagonize A $\beta$ (1–42) neurotoxicity because the two most recognized neuropathological hallmarks of AD, i.e., A $\beta$  and tau, bind CaM with high affinity. Moreover, the activities of CaM-dependent kinase II (CaMKII), cyclin-dependent kinase 5, and glycogen synthase kinase 3 $\alpha$  (protein kinases that contribute to tau hyperphosphorylation in AD) are modulated by CaM [52].

Salazar et al. [60] concluded that hydrophobic interactions drive the formation of the A $\beta$ (1–42):CaM complex. Extensive docking analysis was performed in Salazar et al. [60] using the three-dimensional structure of CaM saturated by Ca<sup>2+</sup> [Protein Data Bank file identification code (PDB ID): 1CLL] because Corbacho et al. [59] previously showed that the Ca<sup>2+</sup>-CaM conformation displays more than 10-fold higher affinity for A $\beta$ 0 than apo-CaM. Since hydrophobic domains of CaM are more exposed in the Ca<sup>2+</sup>-CaM conformation than in the apo-CaM conformation, the latter result gives experimental support to their critical role in the complexation between CaM and A $\beta$ (1–42). Hydrophobic amino acids of the COOH-terminus domain of A $\beta$ , i.e., 24–42 amino acid residues of A $\beta$  were identified as those more strongly interacting with CaM using interface analysis of the more probable structures generated by docking for the A $\beta$ (1–42):CaM complex [60]. Using multi-tilt nanoparticle-aided cryo-electron microscopy sampling, Kim et al. [61] have reported that the complexation with A $\beta$ (1–42) elicits structural changes in Ca<sup>2+</sup>-CaM, which is shifted to a structure intermediate between the classical Ca<sup>2+</sup>-CaM and apo-CaM conformations. This provides a rational basis to explain, at least in part, the modulation of CaMBPs by A $\beta$ (1–42), a point that needs further experimental studies to be demonstrated. Another point that merits a brief comment is the presence of several methionines of CaM among the more strongly interacting amino acid residues with A $\beta$ (1–42) in the interface domain of the A $\beta$ (1–42):CaM complex, for example, Met71, Met51, Met36, and Met72 [60]. This location makes these methionines highly prone to oxidation, as the redox cycling of iron bound to A $\beta$  is a source of reactive oxygen species [14, 20]. Oxidation of CaM's methionines has been shown to impair the activity of CaMBPs [62]. However, to the best of my knowledge, the extent of the oxidation of CaM's methionines in the A $\beta$ 0-induced dysregulation of CaMBPs remains to be experimentally studied.

Fradinger et al. [63] have shown that COOH-terminus peptides of A $\beta$ (1–42) assemble into A $\beta$ (1–42) oligomers, disrupt oligomer formation, and protect neurons against A $\beta$ (1–42)-induced neurotoxicity. These and other investigators have concluded that the COOH-terminus plays a major role in the formation of A $\beta$ (1–42) oligomers [63, 64]. Also, Fradinger et al. [63] showed that A $\beta$ (31–42) is the most potent inhibitor of A $\beta$ (1–42)-induced neurotoxicity. Other results that highlight the relevance of the hydrophobic amino acid residues close to the COOH-terminus domain in the neurotoxicity of A $\beta$  peptides are that 1  $\mu$ M of A $\beta$ (25–35) has the same early neurotrophic and late neurotoxic activities as 1  $\mu$ M of A $\beta$ (1–40), while up to 20  $\mu$ M A $\beta$ (1–16) and A $\beta$ (17–28) did not show trophic or toxic activity [65]. Moreover, Andreetto et al. [66] concluded from the results obtained using membrane-bound peptide arrays and fluorescence titration assays that the amino acid residues 27–32 and 35–40 of A $\beta$ (1–40) are part of the interacting domain leading to A $\beta$ (1–40):A $\beta$ (1–40) self-association. As the neurofibrillary tangles are a well-established

histopathological hallmark of AD, it is to be recalled here that the A $\beta$  COOH-terminus binds to multiple tau domains and subsequently forms soluble A $\beta$ -tau complexes in vitro [5].

Noteworthy, the hydrophobic peptide VFAFAMAFML (amidated-C-terminus amino acid), designed to bind to the A $\beta$ (1–42) amino acid residues of the COOH-terminus domain strongly interacting with CaM [60], is a potent inhibitor of the formation of A $\beta$ (1–42):CaM and of A $\beta$ (1–42):calbindin-D28k complexes. Of value for its potential therapeutic use is that the incubation of HT-22 cells in culture for 24 h with up to 1 micromolar concentration of VFAFAMAFML does not have any significant effect on cell viability, while sub-micromolar concentrations of this peptide efficiently antagonizes A $\beta$ (1–42) complexation with CaM and calbindin-D28k [60]. Many alternate sequences of highly hydrophobic amino acids share similar hydrophobicity plots and, also, fit the geometrical constraints of the 3D structure of the 29–42 segment of A $\beta$ (1–42) [67]. However, it must be noted that highly hydrophobic sequences of 8–10 amino acids are buried within the inner core of proteins. Therefore, these will be accessible to intracellular A $\beta$  only after an extensive intracellular protein degradation up to peptides of 8–10 amino acid residues. Only the ubiquitin-proteasome system, an intracellular system that functions to maintain intracellular proteostasis, fulfills these requirements. Indeed, most of the antigenic peptides of 8–10 amino acids presented by the major histocompatibility complex (MHC) class I molecules are produced by the proteasome [68, 69], and many of them display a hydrophobicity quite like the above mentioned A $\beta$ (1–42) antagonist peptide.

Finally, it is worth noting here that peptides able to antagonize the binding of extracellular A $\beta$ (1–42) and A $\beta$ (1–40) to proteins involved in their uptake by neurons, like apoE and RAGE, have been shown to ameliorate A $\beta$  peptide-mediated neuronal disorder [70–73].

## A $\beta$ -antagonist hydrophobic neuropeptides

The endogenous neuropeptides that can afford neuroprotection against the toxic actions of A $\beta$  on neurons through binding to A $\beta$  hydrophobic domains are called here A $\beta$ -antagonist hydrophobic neuropeptides, as in [67]. As will be briefly analyzed in this section, many studies have reported the occurrence of significant alterations in the levels of these types of neuropeptides and/or of their receptors in the brain of AD patients in relation to healthy brains of age-matched individuals.

It must be noted that the proteasome activity in the AD brain is lower than in age-matched healthy brains [74, 75]. Therefore, the production of hydrophobic peptides of 8–10 amino acid residues declines in the AD brain. As these peptides are endogenous A $\beta$ -antagonists, it follows that the AD brain is more prone than a healthy brain to A $\beta$ -induced neurodegeneration. In addition, the proteasome is the major source of hydrophobic antigenic peptides needed to maintain the normal activity of MHC class I molecules [76], and since A $\beta$ (1–42) is not a good substrate for cytosolic endopeptidases it may serve as a “chaperone” for these antigenic peptides [68]. Thus, this “chaperone-like” role of A $\beta$ (1–42) may impair the functional activity of MHC class I molecules in the AD brain, a hypothesis that, due to its potential relevance, deserves to be experimentally assessed.

Substance P [77, 78], islet amyloid polypeptide (IAPP) [79, 80], somatostatin [81, 82], chromogranin A and B-derived peptides [83, 84] and cocaine- and amphetamine-regulated transcript encoded peptides [85] are other A $\beta$ -antagonist hydrophobic neuropeptides that have been reported to co-localize with A $\beta$  plaques in brain areas of AD patients.

Substance P was one of the firstly identified A $\beta$ -antagonist hydrophobic neuropeptides. Yankner et al. [65] noticed that there is a high homology between the amino acid sequences of tachykinin peptides and that of A $\beta$ (25–35), and reported that sub-micromolar concentrations of substance P, a tachykinin neuropeptide, inhibit the neurotoxic effects of A $\beta$ (1–40) in hippocampal neurons. The findings that substance P level decreases in cortex, hippocampus, and dentate gyrus of AD patients [86, 87], lend further support to a protective role of substance P against A $\beta$  neurotoxic effects in vivo.

The interaction of intrinsically disordered IAPP with A $\beta$ (1–42) and A $\beta$ (1–40) has been extensively studied, and the amino acid residues of A $\beta$  involved in the formation of the complex A $\beta$ :IAPP have been identified, see [66, 88, 89]. Andreetto et al. [66] concluded that A $\beta$ (29–40) and A $\beta$ (25–35) are the amino

acid residues of A $\beta$  peptides acting as the stronger ligands for IAPP. As IAPP is associated with type 2 diabetes, this interaction bears a special biomedical relevance, because epidemiological and pathophysiological evidence point out that the AD and type 2 diabetes are linked diseases [79, 80].

Somatostatin, a small cyclic neuropeptide, is an A $\beta$ -antagonist hydrophobic peptide selectively enriched in human frontal lobes [81, 82]. The levels of somatostatin in the cerebral cortex decline during aging and this decline is more pronounced in AD [90]. In addition, it has been proposed that this decline in somatostatin levels could produce a reduced clearance of A $\beta$ , because somatostatin has been shown to induce the release of A $\beta$  degrading enzymes [91]. Regarding chromogranin A and B-derived peptides, it should be noted that their levels in the cerebrospinal fluid of AD patients have been reported to be altered relative to those found in age-matched controls [92].

Finally, the mitochondrial-derived peptide humanin merits a brief comment, because mitochondrial dysfunction has been associated with AD-induced brain neurodegeneration, see, e.g., [93]. Humanin seems to be a good candidate for A $\beta$ -antagonist hydrophobic neuropeptide, because it has been reported that humanin interacts with A $\beta$  oligomers and counteracts A $\beta$  in vivo toxicity [94].

It must be noted that the relative efficiency of the hydrophobic neuropeptide listed above as A $\beta$ -antagonists in early or late stages of AD depends on their dissociation constants from A $\beta$  monomers or oligomers, which is not known at present for all of them. Also, the identification of the amino acid residues of A $\beta$  in the interacting domain with many of these neuropeptides is still poorly defined.

## **Future perspectives on the AD therapeutic potential of A $\beta$ -antagonist hydrophobic neuropeptides**

During last few years, global neuropeptidome analyses have pointed out that there are significant variations in the levels of brain neuropeptides between AD patients and age-matched individuals. Interindividual variation in the level of hydrophobic endogenous neuropeptides that bind to the COOH-terminus of A $\beta$  monomers/oligomers is likely to affect the onset of sporadic AD, and, also, the rate of brain damage progression in this disease. Therefore, these endogenous brain neuropeptides may become helpful biomarkers in the evaluation of the risk of the onset of sporadic AD and/or for the prognosis of AD.

In addition, several A $\beta$ -antagonist hydrophobic peptides that bind to the COOH-terminus of A $\beta$  seem, a priori, candidates to become targets for the development of novel therapies against A $\beta$ -induced neurodegenerative diseases. It is important to recall here that the amino acid residues 24–42 of the COOH-terminus domain of A $\beta$ (1–42) play the leading role in the interaction of A $\beta$ (1–42) with the intracellular proteins displaying high affinity for A $\beta$ O analyzed in this review. As briefly commented in the Introduction, extracellular A $\beta$ O internalization plays a critical role in A $\beta$  neurotoxicity. Three proteins that bind extracellular A $\beta$ O with high affinity have been proposed to play an active role in the internalization of extracellular A $\beta$ , namely, apoE4,  $\alpha$ 7 nicotinic acetylcholine receptor, and RAGE [31]. Interestingly, the interacting domain of A $\beta$ (1–42) with these proteins comprises amino acid residues 12–28 for apoE4 [95, 96] and  $\alpha$ 7 nicotinic acetylcholine receptor [97], and 17–23 for RAGE [98]. Therefore, it can be foreseen that peptides binding to amino acid residues 12–28 may act synergically with those binding to the 24–42 COOH-terminus of A $\beta$ (1–42) to antagonize A $\beta$  neurotoxicity interfering with A $\beta$  internalization and intracellular A $\beta$ -induced toxic actions, respectively.

Although the efficient delivery of peptides into the brain is still a cumbersome technical issue under active research, recently developed nanocarrier particles show promising advances for the transport across the blood-brain barrier of short peptides directed to brain structures, reviewed in [99]. In addition, considering that AD is a multifactorial disease, a priori, A $\beta$ -antagonist hydrophobic peptide-based therapy could be used in combination with other currently used therapies.

## Conclusions

This review is focused on hydrophobic peptides that have been shown to be antagonists of neurotoxic A $\beta$  forms based on studies performed *in vitro*, *ex-vivo* with cellular cultures, and *in vivo* with animal models of AD. Two major conclusions can be reached from these studies: (1) measurements of the brain levels of hydrophobic endogenous neuropeptides that bind to the COOH-terminus domain of A $\beta$  monomers/oligomers may serve as a complementary tool for the prognosis of the onset and course of AD; (2) A $\beta$ -antagonist hydrophobic peptides that bind to the COOH-terminus domain of A $\beta$  provide insights for the development of novel drugs of use in therapeutic treatments against A $\beta$ -induced neurodegenerative diseases. Although these conclusions open new perspectives for the clinical management of AD, further studies are needed to overcome the current limitations for the translational application of these findings. The protection against the loss of the proteasome activity in AD brain, which plays a major role in the production of this type of hydrophobic peptides in the brain, is yet a challenging scientific issue and pharmacological treatments to stimulate the brain proteasome activity are yet to be established. In addition, despite that studies performed with brains of AD patients show that several endogenous hydrophobic neuropeptides co-localize with A $\beta$  plaques, it must be noted that specific radiochemical tracers will be needed for the use of neuroimaging tools to monitor regional brain changes of these endogenous neuropeptides during AD progression at the early stages of the disease. Finally, for synthetic peptides, further experimental studies with synthetic peptides in animal models of AD are needed before their use in clinical trials, like development of nanoparticles for their efficient transport across the blood-brain barrier, pharmacological, toxicological, and pharmacokinetic studies.

## Abbreviations

AD: Alzheimer's disease

apoE4: apolipoprotein E4

APP: amyloid precursor protein

A $\beta$ : amyloid  $\beta$

A $\beta$ Os: amyloid  $\beta$  oligomers

BACE1:  $\beta$ -site amyloid precursor protein cleaving enzyme 1

CaM: calmodulin

CaMBPs: calmodulin binding proteins

IAPP: islet amyloid polypeptide

MHC: major histocompatibility complex

RAGE: receptor and receptor for advanced glycation end products

## Declarations

### Author contributions

CGM: Conceptualization, Data curation, Formal analysis, Investigation, Writing—original draft, Writing—review & editing.

### Conflicts of interest

The author declares that he has no conflicts of interest.

### Ethical approval

Not applicable.



### Consent to participate

Not applicable.

### Consent to publication

Not applicable.

### Availability of data and materials

Not applicable.

### Funding

Not applicable.

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