

REVIEW

Neuroprotective peptides as drug candidates against Alzheimer's disease

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Summary

First described by Alois Alzheimer in 1907, Alzheimer's disease (AD) is the most common dementia type, affecting approximately 20 million people worldwide. As the population is getting older, AD is a growing health problem. AD is characterized by the presence of neuritic plaques containing the amyloid-beta peptide (Abeta) and an intraneuronal accumulation of tubule-associated protein called tau. Currently available treatment used in AD is based on acetylcholinesterase inhibitors, since in the course of AD there is a substantial loss in cholinergic neurons. Another registered drug used in more severe AD is the NMDA antagonist-memantine. From a drug development point of view, some potential new AD therapeutics include neuroprotective peptides that may act in a variety of different ways, e.g. they help to break the amyloid plaque formation, modulate peptide processing enzymes (secretases) or are able to degrade Abeta toxic peptide. In this review, we present an overview of the new classes of compounds in use against AD.

Keywords: Alzheimer's disease – neuroprotective peptide – Abeta peptide

INTRODUCTION

Alzheimer's disease (AD) and related neurodegenerative disorders represent serious neurodegenerative disorders, are prevalent among

characterised by progressive dementia and the decline of intellectual functions. It is the most common cause of dementia among people aged 65 and older. A contemporary estimate of sufferers is approximately 20 million AD patients worldwide. In fact, nearly 30% of all people aged 85 and older are thought to have AD. It has been predicted that with our ageing population, by the year 2050 the AD patient population will almost double to become over 14 million people in the United States alone. It is thus of high importance to develop therapies to combat the disease and alleviate the devastating outcome. The drugs

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currently available treat mainly the decline in brain acetylcholine concentration, by means of inhibiting the acetylcholinesterase pathway leading to acetylcholine breakdown. However, in the best case this apparently contributes to improving the disease symptomatology for only a limited time period. It targets the cholinergic neurons – the most vulnerable population of neurons in the first stages of AD.

Four drugs, which all act in the same way to increase levels of acetylcholine, are currently used to improve symptomatic cognitive function in patients diagnosed with AD, i.e. Cognex (tacrine), Aricept (donepezil), Exelon (rivastigmine), and Reminyl (galantamine). They have not been shown, however, to alter the long-term progression of the disease. The majority of newly developing antidementia drugs are also cholinesterase inhibitors. Only some of them are based on other mechanisms of action, for example inhibitors of the NMDA ionotropic neurotransmitter receptors, such as memantine (Patočka 2001), although galantamine could act as a nicotinic receptor allosteric ligand potentiating the effect of acetylcholine at receptor level. From a drug development point of view, some potential new Alzheimer's disease therapeutics include peptides that may act in a variety of different ways, e.g. help to break the amyloid plaque formation, modulate peptide processing enzymes (secretases) or are able to degrade Abeta toxic peptides. In such a context, current research projects are specifically focused on reducing the formation of brain lesions resulting from the disease, especially those due to the amyloid peptide accumulation, and on reducing or even halting the clinical evolution of the disease and consequent neurodegenerative processes (Nieoullon 2004).

Peptides provide an attractive alternative but there are still some unanswered questions (Gozes 2001). For example, it is not quite clear if peptides are able to cross the blood-brain barrier. Nevertheless, peptides are important candidates for future drug development (Gozes and Spier 2002).

NEUROTOXIC PEPTIDES IN AD

The pathogenesis of AD is most probably connected with the deposition of beta-amyloid (Abeta) peptides in the cerebral cortex and hippocampus of affected individuals. Abeta peptides (the family of 40–43 amino acid long peptides) are derived from transmembrane protein, termed amyloid precursor protein (APP), by concerted action of the enzymes termed β - and γ -secretase(s). In contrast, α -secretase cleaves APP at a position that leads to nontoxic peptides. Recently, β -secretase was identified. However, the

identity of γ -secretase, which is responsible for the intramembranous processing of APP, is still enigmatic, although it was suggested that the membrane spanning presenilins (PS1 and PS2) function as γ -secretases (Sisodia et al. 2001). Though AD is largely a sporadic disease, mutations in APP and presenilins as well as the lipid carrier apolipoprotein E4 allele have been associated with hereditary AD (Sisodia and Tanzi 2001).

AD is characterized by overproduction of Abeta in the brain and with progressive loss of neuronal cells. The 42-amino acid form of the Abeta (Abeta42) is implied as a major causative factor, because it causes neuronal death through apoptosis and elicits inflammatory responses in the brain by activating microglial cells. Intracellular Abeta42 accumulates in the AD patients brain before plaque and tangle formation (Gouras et al. 2000) and is extremely toxic to human neuronal cells *in vitro* (Zhang et al. 2002).

In addition to the Abeta plaques, the neurofibrillary tangles composed of hyperphosphorylated microtubule-associated protein tau form inside the cells. Recent studies suggested that Abeta exposure may result in rapid tyrosine phosphorylation of neuronal proteins including tau and enhanced formation of neurofibrillary tangles (Gotz et al. 2001, Williamson et al. 2002). Glycogen synthase kinase 3 (GSK-3), a serine/threonine protein kinase that has been shown to be increased in AD, leads to tau hyperphosphorylation and apoptosis (Eldar-Finkelman 2002) and some GSK-3 inhibitors, such as lithium, can serve in the prevention of Alzheimer's disease (Strunecká and Patočka 2004).

NEUROPROTECTIVE PEPTIDES IN AD

Despite the overproduction of Abeta42, AD brain tissue also generates protective factor(s) that may antagonize the neurodestructive effect of Abeta. New pieces of evidence show that AD is associated with changes in the gene expression of many neuropeptides in the brain and their content in the cerebrospinal fluid (CSF). The significance of these changes resides in the fact that these neuropeptides may convey neuroprotection or induce changes in neuronal viability (Slaninová and Patočka 2003). In addition to the natural peptides, several of their synthetic analogues and fragments were studied for neuroprotectivity.

The neuroprotective potential of different peptides has become a matter of intensive investigation in many animal models. Many *in vitro* studies reveal that peptides protect neurons against apoptosis occurring naturally during CNS development and apoptosis induced by a series of neurotoxins, prion protein, Abeta, HIV envelope

glycoprotein (gp120), potassium ion deficit, and high glutamate concentrations (Sokolowska et al 2004). The neuroprotective potential of peptides has not been as thoroughly investigated in all peptides yet, but recent data have confirmed that numerous peptides are able to function as

neuroprotectants and may serve as a goal of modern therapeutic strategies in various neurodegenerative disorders. Minor and less explored peptides of this view are only briefly characterized in Table 1.

Table 1. Brief characterization of some neuroprotective peptides

<i>Peptide</i>	<i>Neuroprotective and other effects</i>	<i>Literature</i>
Somatostatin	Somatostatin levels in CSF are consistently decreased in AD. Somatostatin analogue infusion improved memory for patients with AD, perhaps through modulation of the insulin content	Craft et al. 1999
Neuropeptide Y (NPY)	NPY levels are decreased in AD. It may be involved in aluminium metabolism in animal models, and aluminium accumulation has been associated as a risk factor for AD, mainly in combination with fluorine.	Croom and Tailor 2001 Strunecká and Patočka 2003 Strunecká et al. 2004
Galanin	Galanin levels increase with the duration of AD. Galanin inhibits cholinergic transmission and long term potentiation in hippocampus. Galanin's excitatory action on cholinergic neurons may play a compensatory role by augmenting the release of acetylcholine from remaining cholinergic basal forebrain neurons.	Counts et al. 2001,2004 Jhamands et al. 2002
Insulin like growth factor I (IGF I)	IGF I protects in vitro primary neurons from cytotoxic mechanism of the London type Abeta PP mutant.	Niikura et al. 2004
Interleukin-6 and Interleukin-11	Both interleukins attenuate cytotoxicity of the London type Abeta PP mutant.	Niikura et al. 2004
Apoptosis-antagonizing transcription factor (AATF)	AATF protects neurons against Abeta-induced apoptosis in PC 12 cells	Xie and Guo 2004
SAL (SALLRSIPA)	SAL is active fragment of ADNF and prevents neuronal cell death produced by electrical blockade, N-methyl-D-aspartate, and Abeta	Brenneman et al. 2004
Activity-dependent neuroprotective protein (ADNP)	ADNP is glial cell mediator of VIP associated neuroprotection. The protein implicated in maintenance of cell survival through modulation of p53 expression. The ADNP was identified as a molecule that may mediate protection offered by lipophilic VIP analogues against ischemia cell death.	Bassan et al. 1999 Zamostiano et al. 2001 Sigalov et al. 2000
Bcl-w	Bcl-w is a member of the Bcl-2 anti-apoptotic protein family that promotes cell survival, significantly protects neurons against staurosporine and Abeta induced apoptosis.	Zhu et al. 2004 Weinreb et al. 2004
Gly-Pro-Arg	This tripeptide effectively protects and rescues cell death induced by Abeta.	Ioudina and Uemura 2003
RER peptides	Peptides containing the palindromic tripeptide RER sequence (Arg-Glu-Arg), present in the amyloid precursor protein, protects against memory loss cause by Abeta and acts as a cognitive enhancer.	Mileusnic et al. 2004
Autocamtide-related inhibitory peptides (AIP)	These peptides inhibit Ca/Calmodulin dependent protein kinase II, inhibit Abeta triggered activation of caspase 2 and 3, decrease tau phosphorylation and protect neuron against Abeta toxicity.	Lin et al. 2004
Substance P	This short peptide interacts with cholinergic ascending system of the nucleus basalis Meynert, resulting enhancement effects. Patients with AD show a marked loss of cholinergic neurons and diminished brain substance P expression.	Pompei et al. 2001 Patočka 2002

VIP (vasoactive intestinal peptide), originally discovered in the intestine, was later found to be a major brain peptide having neuroprotective activities (Gozes and Brenneman 1989).

Some lipophilic derivatives of *VIP* cross the blood brain barrier and provide nerve-cell protection and accelerate learning and memory in animal models (Gozes et al. 1999, Sokolowska et al. 2004). Also *PACAP* (pituitary adenylate cyclase-activating polypeptide), *PHI* (peptide histidine-isoleucine), *PHM* (peptide histidine-methionine), and *PHV* (peptide histidine-valine) belong to the same superfamily of peptides as *VIP*, share a high level of structural and functional similarity, and have been shown to be neuroprotective (Dejda et al. 2004).

ADNF (activity-dependent neurotrophic factor) is a recently isolated factor secreted by glial cells under the action of *VIP* (Brenneman and Gozes 1996). This protein was named 'activity-dependent neurotrophic factor' as it protected neurons from death associated with blockade of electrical activity. Subsequent structure-activity studies identified the novel *ADNF* peptide, *ADNF-9*, more potent than the parent protein and with a broader range of effective concentration (Brenneman et al. 1998). *ADNF-9* exhibits protective activity in *Abeta* toxicity (Brenneman et al. 1998), presenilin 1 mutation (Guo et al. 1999), and apolipoprotein E deficiencies (Bassan et al. 1999). *ADNF-9* provides protection against oxidative stress (Steingart et al. 2000) through maintenance of mitochondrial function and a reduction in the accumulation of intracellular reactive oxygen species (Glazner et al. 1999). *ADNF-9* regulates transcriptional activation associated with neuroprotection and increases heat shock protein 60 expression, thus providing cellular protection against *Abeta* toxicity (Zamostiano et al. 1999).

In synaptosomes, *ADNF-9* enhanced basal glucose and glutamate transport, and it attenuates oxidative impairment of glucose and glutamate transport induced by *Abeta* (Guo and Mattson 2000) and in hippocampal neurons, *ADNF-9* stimulated synapse formation (Blondel et al. 2000). In hippocampal culture system, *ADNF-9* caused the secretion of neurotrophin 3 (*NT-3*), and both *NT-3* and *ADNF-9* regulated *NMDA* the receptor subunit 2A (*NR2A*) and *NR2B*, suggesting in vivo effects on learning and behavior in the adult nervous system. In a rat model of choline deficiency, *ADNF-9* enhanced performance in a water maze, indicative of spatial learning and memory (Gozes et al. 2000). Longer peptides including the *ADNF-9* sequence, such as *ADNF-14*, have been shown to promote axonal elongation through transcriptionally regulated cAMP-dependent mechanisms (White et al., 2000) and to activate protein kinase C and mitogen-associated protein kinase kinase, protecting the

developing mouse brain against excitotoxicity (Gressens et al. 1999).

NAP (*NAPVSIPQ*), an eight-amino-acid peptide, the smallest active element of *ADHP*, provides neuroprotection at very low doses in a variety of animal models (Alcalay et al. 2004). It was identified as a most potent neuroprotective peptide in an animal model of apolipoprotein E deficiency (knockout mice) (Bassan et al., 1999) and in an animal model of cholinotoxicity (Gozes et al. 2000). Because apolipoprotein E has been implicated as a risk factor in *AD*, this peptide holds promise for future treatment against *AD*-associated short-term memory deficits (Gozes et al. 2004). *AD* is associated with the death of cholinergic neurons and rats treated with the cholinotoxin ethylcholine aziridium (*AF64A*) provide a model of cholinotoxicity (Fisher and Hanin 1986). In this model a significant improvement in short-term spatial memory in *NAP*-treated animals was observed (Gozes et al. 2000). Cognition enhancement was also found in the Morris water maze in rats treated daily with *NAP* (Gozes et al. 2002). *NAP*-treated animals also exhibit faster recovery of motor ability, balancing, and alertness and a single *NAP* subcutaneous injection after closed head injury dramatically reduced mortality and facilitated clinical recovery in mice (Beni-Adani et al. 2000).

Humanin is a 24-amino acid peptide, which protects neuronal cells from damage by *Abeta*₄₂ (Hashimoto et al. 2001; Patočka et al. 2003) and is a new peptide in the etiology of Alzheimer's disease (Patočka and Slaninová 2004). *Humanin* induced chemotaxis of mononuclear phagocytes by using a human G protein-coupled formylpeptide receptor-like-1 (*FPRL1*) and its murine counterpart *FPR2*. Coincidentally, *FPRL1* and *FPR2* are also functional receptors used by *Abeta*₄₂ to chemoattract and activate phagocytic cells. *Humanin* reduced the aggregation and fibrillary formation by suppressing the effect of *Abeta*₄₂ on mononuclear phagocytes. In neuroblast cells, *humanin* and *Abeta*₄₂ both activated *FPRL1*; however, only *Abeta*₄₂ caused apoptotic death of the cells, and its cytopathic effect was blocked by *humanin*. It was concluded that *humanin* shares human *FPRL1* and mouse *FPR2* with *Abeta*₄₂ and suggested that *humanin* may exert its neuroprotective effects by competitively inhibiting the access of *FPRL1* to *Abeta*₄₂ (Ying et al. 2004). Also some peptides derived from *humanin* have a neuroprotective effect and represent a beneficial drug for the impairment of learning and memory (Mamiya and Ukai 2001, Krejčová et al. 2004).

Beta sheet breaker peptides represent an interesting group of new compounds that are able to reverse the effects of pathogenic prion proteins (Reily 2000). Since fibril formation *Abeta*, considered to be responsible for the pathology of

AD, is formed by a protein misfolding process in which intermolecular beta-sheet interactions become stabilized abnormally, compounds which have a profile as a beta-sheet breaker can probably be important as an anti-AD candidate. These peptides are able to bind soluble amyloid peptide and prevent and reverse its conversion to the beta-sheet rich aggregated structure, precursor of the amyloid plaques (Chacon et al. 2004). Results in vitro, in cell culture and in vivo suggest that beta-sheet breaker peptides might be candidates for an AD-therapy focused on reducing amyloid deposition (Permanne et al. 2002).

NAA (N-acetylaspartate) and *NAAG* (N-acetylaspartylglutamate), short endogenous peptides, were significantly reduced in the hippocampus (by 38 and 24%) and the amygdala (by 28 and 22%), but not in the olfactory bulb and the cerebellar cortex of patients with AD. These results indicate that the concentrations of *NAA* and *NAAG* are selectively decreased in brain areas affected by pathology in AD and can be important in the development of new antidementia drugs (Jaarsma et al. 1994). Short peptides have a better chance to overcome the blood-brain barrier. Orlando et al. (1997) found that *NAAG* co-injected with quinolinic acid significantly reduced lesion volumes due to this neurotoxic compound. *NAAG*'s protective effect may be mediated through actions on N-methyl-D-aspartate receptors or metabotropic glutamate receptors (Thomas et al. 2001).

CONCLUSION

As the population is getting older, AD is a growing health problem. AD is currently treated by symptomatic drugs, the acetylcholinesterase inhibitors, based on the cholinergic hypothesis. During the past decade, advances in neurobiology have led to the identification of new targets. Although some of these innovative approaches tend to delay the onset of AD, others are still symptomatic. Acetylcholinesterase inhibitors have beneficial effects in improving the cognitive impairment in patients with mild to moderate AD. In addition, a channel blocker of N-methyl-D-aspartate receptor, memantine hydrochloride, was approved as a therapeutic agent for patients with moderate to severe AD. In contrast, the pharmacotherapy for a prime cure against AD is not available in the market, although there has been a worldwide search for novel compounds. The fact that so many peptides were found in vitro neuroprotective is giving hope that soon some of them will be found active also in animal models in vivo and that the most active compounds will proceed to clinical trials (Gozes et al. 2004, Horouchi 2004).

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