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Neurotrophic Peptides: Potential Drugs for Treatment of Amyotrophic Lateral Sclerosis and Alzheimer's disease

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Abstract

Neurodegenerative diseases are characterized by the progressive loss of neurons and glial cells in the central nervous system correlated to their symptoms. Among these neurodegenerative diseases are Alzheimer's disease (AD) and amyotrophic lateral sclerosis (ALS). Neurodegeneration is mostly restricted to specific neuronal populations: cholinergic neurons in AD and motoneurons in ALS. The demonstration that the onset and progression of neurodegenerative diseases in models of transgenic mice, in particular, is delayed or improved by the application of neurotrophic factors and derived peptides from neurotrophic factors has emphasized their importance in neurorestoration. A range of neurotrophic factors and growth peptide factors derived from activity-dependent neurotrophic factor/activity-dependent neuroprotective protein has been suggested to restore neuronal function, improve behavioral deficits and prolong the survival in animal models. In this review article, we focus on the role of trophic peptides in the improvement of AD and ALS. An understanding of the molecular pathways involved with trophic peptides in these neurodegenerative diseases may shed light on potential therapies.

Keywords

Amyotrophic Lateral Sclerosis; Alzheimer's disease; ADNF-9; NAP; Colivelin

INTRODUCTION

Neurodegenerative diseases are difficult to treat and are frequently incurable due to the complicated nature of the underlying mechanisms of neuronal cell death. Neurodegenerative diseases including Alzheimer's disease (AD) and Amyotrophic lateral sclerosis (ALS) result in a debilitating loss of memory and motor function, respectively. Although the mechanisms of action in neurodegenerative diseases are unclear, the potential underlying mechanisms can be divided into two categories. The first, unique to each neurodegenerative disease, is a trigger that initiates activation of cell death machinery. The second, which is thought to be universal among neurodegenerative diseases, is a directorial process to complete death of neurons [For review, see ref. [1]].

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CONFLICT OF INTEREST
None

ALS is suggested to be related to a loss of motor neurons, progressively impairing the voluntary motor neuron system and resulting in motor paralysis. This is suggested to be caused by mutation of the superoxide dismutase-1 (SOD1) gene, causing motoneuronal death [2]. Alternatively, neuronal loss in AD results in memory deficit. A widely accepted theory for the mechanism of AD is that Aβ is the primary factor for its neurotoxic effect [3–7].

Although there are currently no effective drugs for the treatment of neurodegenerative diseases, potential therapeutic targets for symptomatic treatments of neurodegenerative diseases may include neuroprotective factors, encompassing neurotrophins and neuroprotective peptides [8–10]. A deficit in the presence of endogenous neurotrophic factors may play a key role in the attenuation of the progression of neurodegenerative diseases [For review, see ref. [11]]. The types of neuroprotection demonstrated by neurotrophic factors include the prevention of oxidative stress that induces apoptosis, promotion of cell survival, and possible cell growth. Neurotrophic factors may improve cellular function and increase neuronal metabolism, which can even lead to restoration of synaptic connections by growth of new axons. Neurotrophic factors are categorized by their activity in preventing neuronal cell death [For review, see ref. [11]].

We focus in this review on NAP (NAPVSIPQ) peptide derived from activity neuroprotective protein and ADNF-9 peptide derived from activity-dependent neurotrophic factor (ADNF). Both NAP and ADNF-9 display activity in the femtomolar range, enhancing cell survival and outgrowth of dendrites in the form of D-acid analogues such as D-NAP and D-ADNF-9 [12, 13]. ADNF-9 and NAP peptides share functional and structural similarities, originally intended for use against β-amyloid peptides for reduction of toxicity and increased protection of neurons [14]. A novel hybrid peptide called colivelin was synthesized by the addition of ADNF-9 to the N-terminus of a humanin derivative. Colivelin has potential neuroprotecrive effect in some neurodegenerative diseases such as AD [15]. We discuss in this review article the two most prevalent neurodegenerative diseases: ALS and AD, including the mechanisms, current treatments, and implications of trophic peptides in treating neurodegenerative diseases.

OVERVIEW OF NEURODEGENERATIVE DISEASES: ALS AND AD

Alzheimer's disease

The most common neurodegenerative disease is AD, regularly known for its characteristic devastating memory and cognitive deficits. Neuronal cell death in the cortical area of the brain is believed to be related to the irreversible progression of dementia and cognitive disorders. The exact mechanism of action leading to AD is unknown, but there is a widely accepted theory regarding amyloid-β's (Aβs) as the primary factor for its neurotoxic effect in AD pathology. *In vivo* experiments have shown that cell death may result from extreme concentrations of toxic Aβs [15–20]. Studies in both mice and human AD patients demonstrated that aggregation of the β-amyloid peptide has been found to form oligomers along the microtubules of neuroprocesses in the AD brain [14]. There also have been studies indicating that toxic Aβ concentrations of 1–25 μM or higher are the cause of neuronal cell death *in vitro*, supporting the Aβ cascade theory [2]. An *in vitro* study suggested that Aβ-related cell death is mediated by Aβ receptors as well as severe potential death-mediating receptors for toxic Aβ [2].

Alternatively, amyloid precursor protein (APP) has been suggested to play a major role in activation of a neuronal cell-death signaling cascade when TGFbeta2 binds as a natural ligand for APP [21, 22]. Hashimoto and colleagues found TGFbeta2 to also be down-regulated by administration of toxic Aβ. Aβ binds to the extracellular domain of APP in

glial and neuronal cells, TGFbeta2 paracrinally and autonomically signaling the APP mediated cells. β-amyloid accumulation has been suggested to occur prior to Tau hyperphosphorylation, suggesting a possible cause and effect between accumulation and hyperphosphorylation [14]. At the present time, the FDA has approved acetylcholinesterase inhibitors and NMDA-type glutamate receptor antagonists for the treatment of moderate to severe AD [For review, see ref. [23]]. Currently there are no FDA approved treatments for behavioral and psychotic symptoms exclusive to AD, but many medications are used "off-label". Semagacestat, a γ-secretase inhibitor, is currently being studied under two Phase III clinical trials for the treatment of AD [24]. Semagacestat is thought to lower levels of Aβ in the brain by blocking cleavage of membrane-bound β-amyloid precursor proteins via γ-secretase, as seen in studies using transgenic mice [25, 26]. In addition, studies have been conducted to investigate the role of Aβ, tau proteins, and insulin on the onset and progression of AD [27–29]

Amyotrophic lateral sclerosis

Amyotrophic lateral sclerosis (ALS) is another neurodegenerative disease affecting the motor neurons, brainstem, and spinal cord. ALS is more commonly known as Lou Gehrig's disease. Degeneration of motor neurons leads to characterized progressive loss of motor control, eventually leading to muscular dystrophy, motor paralysis, and death due to respiratory failure. Most cases of ALS are sporadic in occurrence, but about 10% of cases are familial [30]. Both forms share similar characteristics, and onset occurs typically in adulthood [31], although juvenile onset ALS has been reported as an autosomal recessive mutation in ALS2.

The initial trigger for onset of this multifactorial disorder is still unknown. However, several factors may lead to motor neuron degeneration, including mitochondrial dysfunction, oxidative stress, protein aggregation, protein misfolding, neuro-inflammation, cytoskeleton abnormalities, defective axonal transport, dysfunctional growth factor signaling, and excitotoxicity [30–32]. Mitochondrial abnormalities occur early in ALS pathogenesis; mutant SOD1 was found to be associated with mitochondria in the intermembrane space, possibly triggering apoptosis [33]. SOD inclusion formation may recruit proapoptotic BAX to mitochondria. A possible non-cell autonomous process characterized in ALS is inflammation, which appears in microglial and astroglial cells, resulting in mitochondrial damage and apoptosis [34–36]. Protein misfolding and aggregation mechanisms are still unclear, but protein inclusions have been found in human ALS, including ubiquitinated skein-like inclusion, bunina bodies, and hyaline inclusions rich in neurofilament proteins [37]. Alternatively, patients with mutant SOD1 have shown decreased levels of excitatory amino acid transporters (EAAT2), causing a decreased removal of glutamate from the synapse as well as increased glutamate in the cerebrospinal fluid [38, 39].

Based on studies performed in transgenic mice, mutations on the SOD1 gene lead to the familial form of ALS [2, 15]. In SOD1 transgenic mice, evidence of dynein defects related to dynein-mediated axonal transport processes have been reported to be the earliest pathologies in ALS. Neurons are very sensitive to dynein dysfunction, as dynein is highly expressed in neurons; this may suggest the vulnerability of the motor neurons (For review, see ref.[30]). Mutations in the SOD1 gene are thought to lead to a toxic gain of function, as opposed to a loss of function found in other neurodegenerative diseases [40]. Other studies suggest that angiogenic activity plays a critical role in the development of ALS. Mutations in ALS patients may be associated with functional loss of angiogenic activity and null mutations in progranulin, an angiogenic protein [31].

Currently, riluzole is the only prescribed treatment for ALS, but it only provides moderate survival in patients. Riluzole was initially investigated as an antiseizure drug and was later

found to have neuroprotective properties [41]. Among other drugs, ceftriaxone a β-lactam antibiotic known to upregulate glutamate transporter 1 (GLT1), or EAAT2, and dexpramipexole are currently in Phase III clinical trials in ALS patients [42, 43].

ROLE OF NEUROTROPHIC DERIVED PEPTIDES IN ALS AND AD

NAP peptide derived from activity-dependent neuroprotective protein in ALS and AD

The parent protein, ADNP, is essential for brain development; it is found in the nuclei, cytoplasm, and occasionally along cytoplasmic microtubules [44, 45]. *In vitro* studies showed that recombinant ADNP was found to protect neurons against severe oxidative stress [46]. NAP mimics the neuroprotective activity of ADNP in its ability to cross the blood-brain barrier, interact with tubulin, enhance assembly of microtubules, and promote neuronal outgrowth [44, 47, 48]. The specific association of NAP with tubulin was detected by fluorescent NAD distribution at the cellular level in cells that express neuron-specific βIII-tubulin as well as astrocyte tubulin, which does not express βIII-tubulin [14, 44, 49]. It is demonstrated that NAP colocalizes with microtubules, which regulate Ca^{2+} signaling in neurons (Figure 1) [44, 50, 51+. NAP's association with Ca^{2+} mobilization may contribute to neuronal survival. Changes in mitochondrial Ca^{2+} homoestasis were found to be correlated with life-death pathways of cells in microtubule formation [49]. Other studies suggest that NAP is regulated by polyADP-ribosylation (Figure 1) [50] or that NAP stimulates the MAPK/ERK and PI3-K/Akt pathways [52]. Pascual and colleagues found that stimulation of MAPK/ERK and PI3-K/Akt leads to the phosphorylation of the transcription factor cAMP response element-binding protein (CREB), which produces neuronal outgrowth and differentiation (Figure 1). NAP protection and adaptation to enhance cognitive function can possibly be attributed to protection against apoptosis as a result of microtubule loss.

Studies regarding a mouse model for AD have shown deregulation of ADNP expression in the hippocampus [53]. Tau mutants have decreased affinity to microtubules, leading to less protection and aggregation of tau. NAP's neurotrophic effect lies in the activation of Fyn Kinase activated tyrosine phosphorylation and Crk-associated substrate (Cas) scaffold protein (Figure 1) [14]. NAP treatment is also associated with chromatin remodeling and neurite outgrowth due to enhanced poly-ADP ribosylation, connected with ADNP signaling (Figure 1) [44, 48–50]. While protecting against neurotoxins, NAP does not affect cell division [54]. In addition, studies have found that treatment with NAP in ADNP knockdown mice resulted in enhanced cognition and improved associated deficiencies [48]. Positive study results and clean toxicity reports have landed NAP in phase II clinical trials with a primary focus on AD-related cognitive impairment [14]. Studies are also being conducted to evaluate the effects of NAP in ALS models associated with cytoskeletal dysfunction. NAP extended life span in ALS mouse models when administered prior to disease onset, protecting against tauopathy [55].

ADNF-9 peptide derived from activity-dependent neurotrophic factor in AD and ALS

ADNF is a glial neurotrophic protein factor and, along with ADNP, it is released in response to vasoactive intestinal peptide (VIP) that protects neurons from tetrodoxin-induced cell death by electrical blockade as well as related insults from AD and ALS. ADNF is suggested to be essential for neuronal survival and embryonic growth regulated by VIP [56–61]. ADNF-9 derived from ADNF, also known as SAL (SALLRSIPA), is the active core of its parent compound and mimics its neuroprotective activity [56, 57]. In AD, ADNF-9 has been found to protect against Aβ, apolipoprotein E deficiencies, and oxidative insults, as well as enhancing synapse formation [56, 62–64]. It is noteworthy that ADNF-9 showed a greater prevention of cell death associated with stress than did other ADNF derived peptides

such as ADNF-14, which also protects against cell death [56] and provides neuroprotection against AD-related toxicity [57, 65]. ADNF-9 protects against Aβ peptide and oxidative stress through regulation of mitochondrial function, reduction of accumulating reactive oxygen species, and regulation of the Nf-kb transcription factor (Figure 2) [62, 66]. Moreover, ADNF-9 also promotes axonal elongation through cAMP-dependent mechanisms and increases chaperonin expression of heat shock protein 60, which leads to neuroprotection against Aβ insult (Figure 2) [66–68]. *In vitro* studies using both hippocampal and cortical neurons demonstrated that ADNF-9 stimulates synapse formation [69]. ADNF-9 was also shown to have an effect on memory and learning in association with polyADP ribosylation catalyzed by poly(ADP-ribose)polymerase-1 (Figure 2) [50]. Other possible mechanisms of neuroprotection include the Bcl2 mitochondrial intrinsic signaling pathway, which regulates cell survival and apoptosis [70] and the extrinsic JNK signaling pathway [71]. The all D-amino acid analogue of ADNF-9 was found to protect against Aβ tau hyperphosphorylation, in early events, *in vitro* and *in vivo*, with regards to neuroprotection and maintenance of neuronal organization [72]. ADNF-9 was tested in *in vitro* and *in vivo* ALS models [58]. This study demonstrated that ADNF-9 suppressed SOD-1-mediated cell death. The neuroprotective effect of ADNF-9 involves CaMKIV and tyrosine kinase signaling pathways. Neuroprotection is thought to occur as a result of CaMKIV and tyrosine kinase involvement when ADNF is administered intracerebroventricularly. Although prolonged survival of the ALS mouse model was marginal, it did provide insight into a possible treatment for ALS.

Colivelin, hydrbrid synthetic peptide of ANDF-9 and Humanin, in AD and ALS

Neuroprotective peptide colivelin was created by adding ADNF-9 to the N-terminus of the humanin peptide [2, 73]. Humanin was identified as an endogenous neuroprotective peptide, which is suggested to protect against AD-related toxicity and cytotoxicity as a result of various stimuli [1, 74]. Moreover, studies have shown that humanin suppresses neurotoxicity through extracellular cell surface receptors, which induce cytoprotective signals [74, 75]. It is suggested that humanin interacts with the Bcl-2 family of proapoptotic proteins, blocking their action in the cytosol (Figure 3) [74, 76, 77]. Although the mechanisms of action of colivelin in neuroprotection are unclear, two independent prosurvival signals have been suggested: a humanin mediated STAT3 pathway and ADNF-9 mediated Ca^{2+}/calmodulin-dependent protein kinase IV pathway (Figure 3) [73]. It is noteworthy that ADNF-9 is active in the femtomolar concentration range, but activity is lost by about 10 nM, whereas humanin analogue AGA(C8R)HNG17 is active starting at 10 pM [56–58, 73].

Colivelin is active in the femtomolar range and does not lose activity at higher concentrations [73]. Humanin was found to provide neuroprotection against AD-related insults such as Aβ neurotoxicity [75]. The JAK2/STAT3 signaling pathway showed importance in colivelin neuroprotection against AD-related memory loss [78–80]. An *in vivo* study found that colivelin has more potent neuroprotective effects than humanin and ADNF-9 have when tested against Aβ neurotoxicity [73].

Although, ADNF-9 was found to suppress the FSOD1 ALS-related gene [58], colivelin was found to be more neuroprotective than ADNF-9 in this model. Colivelin neuroprotection was associated with motor performance improvement but not increased lifespan in the ALS mouse model of SOD1 [58]. Moreover, another study showed that colivelin improved motor performance and increased lifespan when compared to FSOD1, in addition to suppressing motoneuronal death [15].

CONCLUSION

The roles of neurotrophic factors and peptides are coming into focus for the treatment of neurodegenerative diseases such as AD and ALS. We discussed the potential application of neurotrophic peptides derived from ADNF and ADNP on the attenuation of the progression of ALS and AD. It is noteworthy that NAP is in Phase II clinical trials for the treatment of AD. ADNF-9 shows potential therapeutic effects in animal models of ALS. In addition, the hybrid peptide, colivelin, has been shown to be effective in animal models of ALS. In contrast to neurotrophic factors, these trophic peptides have the ability to cross the blood-brain barrier for efficacy. Ample evidence suggests that these trophic peptides have potential for the treatment of ALS and AD.

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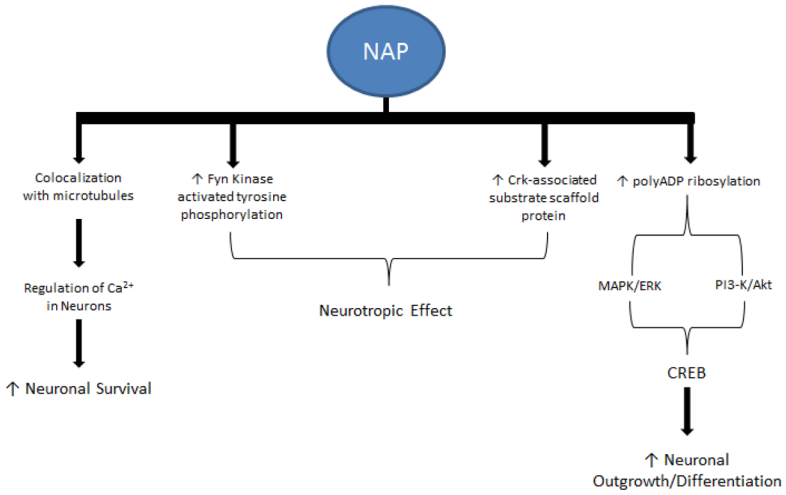

Figure 1.

Diagram shows several pathways involving NAP neuroprotection. Among these pathways, NAP can co-localize with microtubules to regulate neuronal Ca^{2+} homeostasis to increase neuronal survival. The neuroprotective effect of NAP, mediated by increases in Fyn kinase, activated tyrosine phosphorylation and the level of Crk-associated substrate scaffold protein. Alternatively, increases in neuronal outgrowth and differentiation might be mediated through CREB involving MAPK/ERK and PI3-K/Akt pathways, which are activated by increased polyADP ribosylation.

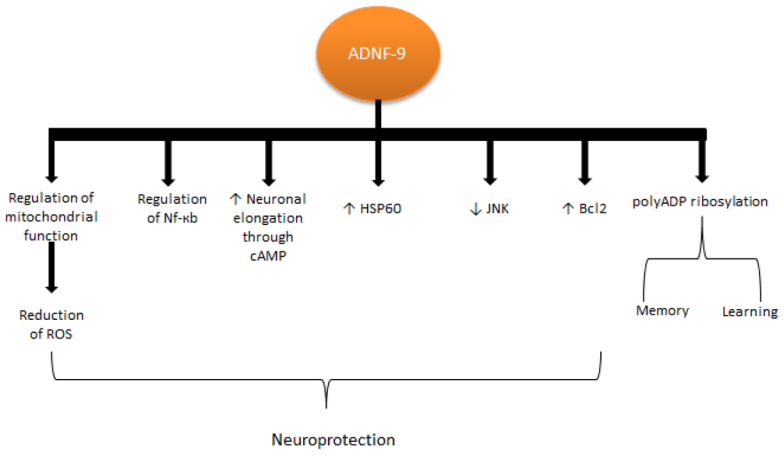

Figure 2.

Diagram shows several pathways involving ADNF-9 neuroprotection. Among these pathways, ADNF-9 regulates mitochondrial function and NF-kB, increases neuronal elongation, decreases HSP60, decreases JNK, and increases Bcl2. ADNF-9 ameliorates learning and memory through polyADP ribosylation.

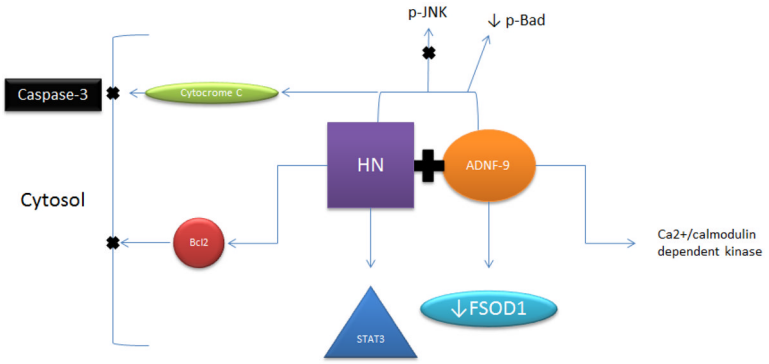

Figure 3.

Diagram shows pathways involved in colivelin neuroprotection. Colivelin neuroprotection is mediated through Bcl2, cytochrome c and caspase-3 pathways. In addition, colivelin neuroprotection may involve STAT3, JNK and Bad pathways. It is suggested that ADNF-9 fragment of colivelin involves Ca^{2+}/calmodulin dependent kinase.