Opinion TRENDS in Pharmacological Sciences Vol.27 No.12

Neuroprotective effects of huperzine A: new therapeutic targets for neurodegenerative disease

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In recent years, the most common pharmacological treatment for Alzheimer's disease (AD) has been acetylcholinesterase (AChE) inhibition. However, this single-target approach has limited effectiveness and there is evidence that a multitarget approach might be more effective. Huperzine A (HupA), a novel alkaloid isolated from a Chinese herb, has neuroprotective effects that go beyond the inhibition of AChE. Recent data have demonstrated that HupA can ameliorate the learning and memory deficiency in animal models and AD patients. Its potentially beneficial actions include modification of β-amyloid peptide processing, reduction of oxidative stress, neuronal protection against apoptosis, and regulation of the expression and secretion of nerve growth factor (NGF) and NGF signaling.

Introduction

The current mainstays of the symptomatic therapy of Alzheimer's disease (AD) are acetylcholinesterase (AChE) inhibitors, most of which are suitable only for mild to moderate disease. Drugs developed as NMDA-receptor antagonists, such as memantine, have been approved for the treatment of AD (Box 1). For the treatment of moderate to severe vascular dementia (VD) (see Glossary) or AD, memantine, in combination with donepezil, seems to be more promising than AChE inhibitors, and is well tolerated [1]. Moreover, several studies indicate that bifunctional or multifunctional compounds might provide greater symptomatic efficacy as potential neuroprotective drugs than single-target compounds [2]. Those data indicate that therapeutic strategies focusing on two or more targets might provide greater benefit in AD treatment. Drugs with multifunctional actions might be more proficient compared with multiple drug combinations because they simplify administration and obviate multiple single-target drugs with potentially different degrees of bioavailability and pharmacokinetics.

Huperzine A (HupA), a novel *Lycopodium* alkaloid isolated from the herb *Huperzia serrata* ('Qian Ceng Ta') and used in Chinese folk medicine, is a potent, reversible, selective and well-tolerated inhibitor of AChE. The potency of HupA in AChE inhibition is similar or superior to that of galanthamine, donepezil, rivastigmine and tacrine [3]. It has been widely shown to reverse or attenuate loss of cognition in several behavioral models and different animal species including non-human primates, in addition to ameliorating deficits in learning and memory in humans [3]. HupA is currently in a phase IV trial for the therapy of AD patients in China. It is also undergoing clinical trials in the USA for the treatment of age-related memory deficiency (http://www.clinicaltrials.gov/show/NCT00083590). Recent data indicate that the specific AChE inhibitory effect of HupA is only one aspect of its clinical and experimental efficacy compared with pure AChE inhibitors, which invariably have only limited success in AD therapy. In fact, HupA exerts multiple neuroprotective effects in several molecular sites that are not the result of its inhibition on AChE. This review will mainly focus on these novel effects and mechanisms of HupA on β-amyloid precursor protein (APP) processing, β-amyloid (Aβ)-associated neurotoxicity, nerve growth factor (NGF) and neurotransmission systems, highlighting its potential as a therapeutic agent targeting different pathologies.

Glossary

Catalase (CAT): an enzyme found in most plant and animal cells that functions as an oxidative catalyst. It decomposes hydrogen peroxide into hydrogen and water.
Tricarboxylic acid (TCA) cycle: a series of enzymatic reactions in aerobic organisms involving oxidative metabolism of acetyl units and producing high-energy phosphate compounds, which serve as the main source of cellular energy.
Dextrorotatory: optically rotating to the right. The rotation of interest in chemistry is induced by a chiral molecule. The direction of rotation cannot be predicted from the *R* or *S* designation of the configuration at the stereocenter: in some cases the *R* isomer is dextrorotatory and the *S* isomer is levorotatory, whereas in other cases it is the other way around.
Glutathione peroxidase (GPX): an enzyme in the body that is a powerful scavenger of free radicals; it helps to prevent peroxidation of cell membranes by consuming free peroxide in the cell.
Levorotatory: optically rotating to the left. The levorotatory isomer of optically active compounds is the form usually found in nature.
Malondialdehyde (MDA): a by-product of lipid metabolism in the body. It is also found in many foods and can be present in high amounts in rancid food.
Superoxide dismutase (SOD): an enzyme that catalyzes the decomposition of a superoxide into hydrogen peroxide and oxygen.
Tg2576 mice: a transgenic mouse strain that overexpresses a mutant form of human amyloid precursor protein with the Swedish mutation (APP_{Sw}), resulting in high Aβ levels in the brain.
Vascular dementia (VD): a dementia that is caused by disease of the blood vessels of the brain (cerebrovascular disease). It is the second most common form of dementia after AD.

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Available online 23 October 2006.

 0165-6147/$ – see front matter doi:10.1016/j.tips.2006.10.004

Effects on APP processing

Aβ is one of the main components of senile plaques and originates from APP, which processes through the α-secretase-mediated nonamyloidogenic pathway [4] and the β-secretase-mediated amyloidogenic pathway [5]. Previous studies suggested that secretory amyloid precursor protein α (sAPPα), which is produced by the nonamyloidogenic pathway, has potent neurotrophic and neuroprotective activities against excitotoxic and oxidative insults in various cellular models [6,7]. It also promotes neurite outgrowth [8], regulates synaptogenesis [9], exerts trophic effects on cerebral neurons in culture [10], and stabilizes neuronal calcium homeostasis [11].

In an intracerebroventricularly (i.c.v.) $A\beta_{1-40}$-infused rat 'AD' model, HupA administration significantly ameliorated an Aβ-induced decrement of sAPPα levels [12]. Similarly, HupA was also proved to increase sAPPα [12,13] and APP C-terminal fragment 83 [13] and reduce Aβ levels [13] in human embryonic kidney 293 Swedish mutant (HEK293sw) cells. HupA improves APP processing by increasing the levels of protein kinase C (PKC) isoform α. This finding agrees with previous evidence showing that the secretory pathway of APP is controlled by the activation of various neurotransmitter receptors coupled to PKC, especially by activating muscarinic acetylcholine (mACh) receptor subtypes M_1 and M_3, which might increase the release of sAPPα and decrease the levels of Aβ [14,15]. PKC might regulate diverse downstream signaling pathways including mitogen-activated protein kinase (MAPK) signaling, which was proved to be involved in the APP nonamyloidogenic pathway [16]. The precise mechanism of how PKCα reduces the amyloidogenic pathway still needs to be clarified. In addition, the effects of HupA on the elevation of ACh levels might contribute to the increase of sAPPα release because it might stimulate the mACh receptor M_1 and M_3-mediated PKC pathway.

Effects on Aβ-associated neurotoxicity

Abnormal processing of APP through the amyloidogenic pathway causes excessive accumulation of Aβ in the brain, which is one of the major characteristics of AD and is a possible cause of neurodegeneration [17]. Aβ was demonstrated to be harmful to PC12 (pheochromocytoma) cells, NG108 (neuroblastoma × glioma) cells and primary rat cortical cultures [18–21]. $A\beta_{1-40}$ i.c.v. injection in rat has been shown to cause severe spatial memory impairment evident in the Morris water maze [21], which can also be ameliorated or reversed by HupA administration [21]. However, the mechanism by which Aβ induces neurotoxicity and cognitive impairment and how HupA interferes with Aβ toxicity still need to be clarified.

Accumulating evidence indicates that the direct neurotoxic effects of Aβ involve activation of apoptosis pathways [22,23]. In our recent studies, HupA was shown to possess protective effects against various apoptosis models. Preincubation with HupA could markedly protect against apoptosis induced by $A\beta_{25-35}$ and $A\beta_{1-40}$ both *in vitro* and *in vivo* [21,24]. The antiapoptotic effect is not limited to the Aβ-mediated injury model. HupA can protect neurons from other apoptotic insults including hydrogen peroxide (H_2O_2) [25], oxygen–glucose deprivation [26], serum deprivation [27] and the PKC inhibitor staurosporine [28].

The main proteins involved in apoptosis are BCL-2, BAX and p53; abnormal regulation of these relates to the mitochondrial-mediated cell death pathway. The key step of this pathway is the transient opening of the mitochondrial permeability transition pore, involving a nonspecific increase in the permeability of the inner mitochondrial membrane [29]. In this process, cytochrome *c* moves from the intermembrane space into the cytoplasm, where it binds to apoptotic protease activating factor 1. The apoptosome then formed activates caspase-9, which in turn activates caspase-3 thereby inducing apoptosis [30] by cleaving proteins that contain DEVD consensus sequences. The antiapoptotic effect of HupA in Aβ-induced apoptosis in rats was mediated by upregulation of the antiapoptotic protein BCL-2 and downregulation of the proapoptotic proteins BAX and p53 [21]. HupA can also reduce the release of mitochondrial cytochrome *c* into cytosol [27] and decrease caspase-3 activities in apoptotic models [24,27,28]. In our further study, HupA was proved to protect against Aβ-induced mitochondrial ultrastructural abnormality in PC12 cells, and to maintain ATP concentration and membrane potential. These effects are achieved through preventing the reduction of key enzyme activities in the tricarboxylic acid (TCA) cycle and key protein levels in the electron transport chain, including complex I, complex II/III, complex IV, the pyruvate dehydrogenase complex and the α-ketoglutarate dehydrogenase complex, and increasing Na^+/K^+-ATPase activities to promote ion homeostasis [31].

Box 1. The pathology of AD and the sites of current drug action

One of the neuropathological hallmarks of AD is amyloid plaques primarily containing aggregates of β-amyloid (Aβ), which is generated from β-amyloid precursor protein (APP) [67]. The $A\beta_{1-42}$ form is predominant in amyloid plaques although the $A\beta_{1-40}$ form is more abundant in brain. $A\beta_{1-42}$ has a greater tendency to form the toxic small Aβ oligomer and aggregates more quickly than $A\beta_{1-40}$ [68]. $A\beta_{25-35}$ is the functional part that mediates the toxicity of whole-length Aβ; it displays toxicity with high reproducibility in various cellular systems [69]. Two pathways for APP processing have been described: the nonamyloidogenic pathway releases a soluble ectodomain – secretory amyloid precursor protein α (sAPPα) – and prevents Aβ formation [4], whereas the amyloidogenic pathway produces Aβ [5]. Targeting Aβ production and Aβ-mediated neurotoxicity could be a reasonable strategy for developing new drugs for AD therapy.

Another important characteristic feature of AD is acetylcholine-mediated hypofunction [70]. A beneficial therapeutic approach is to use AChE inhibitors to restore, at least partly, the lost acetylcholine neurotransmission in the central nervous system.

Most recently, it has been hypothesized that glutamate function is disrupted in AD patients [71]. Anomalous glutamate activity associated with AD might be due to defective postsynaptic receptors and downstream defects that produce glutamate activation of NMDA receptors, leading to neuronal injury and death and cognitive deficits associated with dementia [72]. Based on this theory, a low-affinity NMDA receptor antagonist, memantine, was developed to prevent excitatory neurotoxicity in AD. Memantine is approved in the USA and Europe for the treatment of patients, and currently is the only approved option for the treatment of moderate to severe AD.