Neuroprotective Effects of Huperzine A

A Natural Cholinesterase Inhibitor for the Treatment of Alzheimer's Disease

Rui Wang Xi Can Tang

State Key Laboratory of Drug Research, Shanghai Institute of Materia Medica, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences, Zhangjiang Hi-Tech Park, Shanghai, China

Key Words

Huperzine A · Alzheimer's disease ·
Acetylcholinesterase · Cholinesterase inhibitor ·
Cognitive enhancer · Neuroprotection · Oxidative stress ·
Beta-amyloid · Amyloid precursor protein · Cerebral
ischemia · Apoptosis · Apoptotic-related gene ·
Mitochondria · Glutamate · NMDA receptor · Potassium
current

Abstract

Huperzine A (HupA), isolated from Chinese herb *Huperzia serrata*, is a potent, highly specific and reversible inhibitor of acetylcholinesterase. It has been found to reverse or attenuate cognitive deficits in a broad range of animal models. Clinical trials in China have demonstrated that HupA significantly relieves memory deficits in aged subjects, patients with benign senescent forgetfulness, Alzheimer's disease (AD) and vascular dementia (VD), with minimal peripheral cholinergic side effects compared with other AChEIs in use. HupA possesses the ability to protect cells against hydrogen peroxide, β -amyloid protein (or peptide), glutamate, ischemia and staurosporine-induced cytotoxicity and apoptosis. These protective effects are related to its ability to attenuate oxidative stress, regulate the expression of apoptotic proteins Bcl-2, Bax, P53 and caspase-3, protect mito-

chondria, and interfere with APP metabolism. Antagonizing effects on NMDA receptors and potassium currents may contribute to the neuroprotection as well. It is also possible that the non-catalytic function of AChE is involved in neuroprotective effects of HupA. The therapeutic effects of HupA on AD or VD are probably exerted via a multi-target mechanism.

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Alzheimer's disease (AD) is a progressive, neurodegenerative disorder associated with a global impairment of higher mental function, and presenting an impairment of memory as the cardinal symptom [1]. Histopathological hallmarks of the disease are the extracellular deposition of amyloid β -peptide ($A\beta$) in senile plaques, the appearance of intracellular neurofibrillary tangles (NFT), a loss of cholinergic neurons, and extensive synaptic changes in the cerebral cortex, hippocampus and other areas of brain essential for cognitive functions.

To date, the cause and the mechanism by which neurons die in AD remain unclear, but $A\beta$ has been established as a crucial factor in AD pathogenesis. $A\beta$ deposition may cause neuronal death via a number of possible mechanisms, including oxidative stress, excitotoxicity, energy depletion, inflammation and apoptosis. Despite this multifactorial etiology, genetics plays a key role in

disease progression. However, environmental factors (e.g. cytokines, neurotoxins) may be even more important in the development and progression of AD. Several lines of evidence support the involvement of oxidative stress [2, 40]. Oxidative damage, mediated by reactive oxygen species (ROS) generated following cell lysis, oxidative bursts, or an excess of free transition metals, has been hypothesized to play a pivotal role in AD neurodegeneration. On the other hand, postmortem studies provide direct morphological and biochemical evidence that some neurons in the AD brain degenerate via an apoptotic mechanism [30, 51], which may or may not be linked to ROS. The biological mechanism underlying the formation of AD is clearly complex, with many factors contributing to the neuropathology. Thus it is not surprising that a number of different intervention therapies are currently being researched to address distinct aspects of the disease.

Huperzine A (HupA) is a novel *Lycopodium* alkaloid with recognized medicinal properties. In China the folk medicine *Huperzia serrata* (*Qian Ceng Ta*) (fig. 1), a source of HupA, has been used for centuries in the treatment of contusions, strains, swelling, schizophrenia, etc. The active principle, HupA, is a potent, reversible, and selective inhibitor of acetylcholinesterase (AChE) [56]. Its potency in AChE inhibition is similar or superior to that of physostigmine, galanthamine, donepezil and tacrine [60, 67]. AChE exists in multiple molecular forms that can be distinguished by their subunit associations and hydrodynamic properties [6, 41]. In mammalian brain, the bulk of AChE occurs as a tetrameric, G4 form (10S) together with much smaller amounts of a monomeric, G1 (4S) form [4, 22]. This phenomenon led us to investigate the possibility of HupA on differential inhibition of AChE forms [93]. We observed that HupA preferentially inhibited tetrameric AChE (G4 form), while tacrine and rivastigmine preferentially inhibited monomeric AChE (G1 form). Donepezil showed pronounced selectivity for G1 AChE in striatum and hippocampus, but not in cortex. Physostigmine showed no form-selectivity in any brain region [93].

HupA has been found to reverse or attenuate cognitive deficits in a broad range of animal models. Enhancement of learning and memory performance was documented in the passive footshock avoidance paradigm [37, 54, 98, 99], the classic water maze escape task [36, 79], and spatial discrimination in the radial arm maze [72]. Similarly, cognition enhancement was found in a delayed-response task in aged monkeys [78] and in reserpine- or yohimbine-treated monkeys [44]. Beneficial effects were seen not only in intact adult rodents, but also in rodents cognitive-

ly impaired by age [37, 79] or by treatment with scopolamine [16, 23, 36, 54, 59, 65, 98], AF64A [73, 9], electroshock [59, 99], cycloheximide [99], NaNO₂ [99], or CO₂ [37, 98]. In addition, HupA improved cognition in cholinergically lesioned rats [37, 99], and it reduced the spatial working memory deficit induced by lesion of the nucleus basalis magnocellularis [74]. These effects are attributable to increased synaptic ACh. Early on, HupA was found to cause a significant increase in ACh level in rat brain [55, 100]. More recently, a rise in cortical ACh levels has been demonstrated by microdialysis in awake, free-moving rats. In this study HupA was 8- and 2-fold more potent than donepezil and rivastigmine, respectively, and its effect was longer lasting [34].

Clinical trials of HupA in China have demonstrated a meaningful improvement in the memory of aged subjects, individuals with benign senescent forgetfulness and patients with AD. The results indicate minimal peripheral cholinergic side effects compared to other AChEIs in use; even more important, HupA lacks the dose-limiting hepatotoxicity induced by tacrine [75–77, 84, 90]. Adverse effects in the clinical trials were reported at a very low rate and are mainly cholinergic. Examples are dizziness, nausea, gastroenteric symptoms, headaches and depressed heart rate. Several clinical trials have addressed the effects of HupA on vascular dementia (VD). VD is currently considered to be the second most common form of dementia in Europe and the USA. However, in Asia and many developing countries including China, the incidence of VD exceeds that of AD. Multicenter, randomized, double-blind, placebo-controlled clinical trials in China proved that HupA markedly improved the cognitive function of VD [32, 38, 39, 90]. This article will summarize and discuss current research focused on elucidating the neuroprotective effect of HupA.

Protection of HupA against Hydrogen Peroxide and β -Amyloid Protein-Induced Injury by Attenuating Oxidative Stress

Several neurodegenerative disorders such as AD, cerebral ischemia-reperfusion and head injury are thought to be related to changes in oxidative metabolism. Increased oxidative stress, resulting from free radical damage to cellular function, can be involved in the events leading to AD, and is also connected with lesions called tangles and plaques. Plaques are caused by the deposition of A β and are observed in the brains of AD patients [7, 40, 45, 48]. Studies show that oxygen radicals initiate amyloid build-

Fig. 1. Chinese herb *Huperzia serrata* (Qian Ceng Ta).

Fig. 5. Anti-apoptotic mechanism of HupA. Black real line arrows = apoptotic-inducing pathway; red real line arrows = affecting sites of HupA; red dash arrows = speculative pathways; (-) = inhibiting; (+) = promoting.

Fig. 2. Reduction of A β 25–35-induced DNA fragmentation by HupA. Neurons were treated with 20 μ M of A β 25–35 with or without 1 μ M of HupA. Fragmented DNA was isolated by NucleoBond DNA and RNA purification kit, electrophoresed with agarose gel, and finally stained with ethidium bromide. M = DNA size marker; Con = control.

up leading to neurodegeneration [19]. HupA has been found to protect against H₂O₂ and A β -induced cell lesion, to decrease the level of lipid peroxidation, and to increase antioxidant enzyme activities in rat PC12 and primary cultured cortical neurons [68–70]. Following a 6-hour exposure of the cells to H₂O₂ (200 μ M) or a 48-hour exposure of the cells to A β 25–35 (1 μ M), a marked reduction in cell survival and the activities of glutathione peroxidase (GSH-Px) and catalase (CAT) was observed, along with an increased production of malondialdehyde (MDA). Pretreatment of the cells with HupA (0.1–10 μ M) 2 h before H₂O₂ and A β exposure caused a significant increase in cell survival. HupA also partly reversed the H₂O₂- and A β -induced decrease of GSH-Px and CAT activity, as well as the increase in production of MDA and SOD. All these effects indicate a neuroprotective action.

The protective effect of HupA on A β -induced cell lesion was also observed in NG108–15 cells [86] and primary cultured cortical neurons [71]. In addition to elevating cell survival and GSH-Px and CAT activities while decreasing the level of MDA [70], HupA (0.1–10 μ M) significantly reduced A β 25–35-induced the formation of reactive oxygen species (ROS) in rat primary cultured cortical neurons [71].

In rat studies, an intracerebroventricular (i.c.v.) infusion of β -amyloid 1–40 (800 pmol $\times$ 3) induced a significant cognitive deficit, morphologic signs of injury and a decrease of cortical choline acetyltransferase activity [64]. Daily i.p. administration of HupA for 12 consecutive days produced partial reversal of the β -amyloid-induced deficit in learning a water maze task. This treatment ameliorated the loss of choline acetyltransferase activity in cerebral cortex and the neuronal degeneration induced by β -amyloid 1–40 [64]. HupA also reduced the level of lipid peroxidation and superoxide dismutase in the hippocampus, cerebral cortex and serum of aged rats [49]. In a rat model of chronic cerebral hypo-perfusion, HupA significantly reduced the increases in SOD and lipid peroxide while restoring lactate and glucose to their normal levels [61]. A clinical study also demonstrated a reduction of oxygen free radicals in plasma and erythrocytes from AD patients [77]. These findings indicate that HupA has protective effects against free radical and A β -induced cell toxicity, which might be beneficial in the treatment of patients with various kinds of dementia.

Experience with HupA enantiomers has shown that the neuroprotective properties have no relation to anticholinesterase potency. Thus, preincubation with (+)-HupA or (–)-HupA (0.1–10 μ M) protected cells with similar potency against A β toxicity and similar enhancement of survival [86]. This result contrasted with the stereoselectivity of cholinesterase inhibition in vitro and in vivo, in which (–)-HupA is approximately 50-fold more potent than (+)-HupA. In another study, we examined drug effects on the apoptosis induced by incubation with A β 25–35 and on the increase of AChE activity accompanying this reaction. We observed that inhibiting the hydrolyzing activity of AChE without decreasing AChE expression itself did not attenuate the A β 25–35 induced apoptosis [85]. In other words, the ability of HupA to block the catalytic activity of AChE did not parallel its neuroprotective effect. Therefore, the cytoprotective effect of HupA enantiomers may relate to some kind of non-catalytic actions on AChE, or to actions on other cellular targets.

Anti-Apoptotic Effect of HupA

Apoptosis is the process by which neurons die during normal development and is also a feature of chronic and acute neurodegenerative diseases and stroke [82]. The cellular commitment to apoptosis is regulated by the Bcl-2 family of proteins. High levels of Bcl-2 expression will

Fig. 3. Effects of HupA on H₂O₂-induced expression of bcl-2, bax and p53 in PC12 cells by RT-PCR. Cells were exposed to 100 μ M H₂O₂, and total RNA was extracted after the indicated recovery period and then subjected to RT-PCR. The PCR products were normalized by β -actin mRNA. Lane 1: non-treated intact control; lanes 2–5: 0, 2, 6 and 12 h after 30 min H₂O₂-treatment, respectively. Lanes 6–9 represent the same time points as lanes 2–5 but preincubated with 1 μ M HupA before H₂O₂ exposure.

inhibit apoptosis. In contrast, an increased expression of P53 and Bax is associated with the initiation of apoptosis [29]. In accordance with previous reports, studies from our lab demonstrate typical apoptotic changes when neuron-like cells are exposed to stressors such as H₂O₂, A β peptide, oxygen-glucose deprivation (OGD), serum deprivation, or the PKC inhibitor, staurosporine. These changes include DNA laddering, cell shrinkage, the generation of nuclear apoptotic bodies, TUNEL positive staining, and other classic hallmarks of apoptosis (fig. 2) [63, 71, 87, 94]. Such abnormalities are markedly relieved by HupA. For example, in rats that received i.c.v. injections of β -amyloid1–40 (800 pmol $\times$ 3), administration of HupA (0.1, 0.2 mg/kg, i.p.) for 12 consecutive days gave substantial neuroprotection in the brain. This treatment greatly reduced the number of apoptotic-like neurons and partly reversed the down-regulation of Bcl-2 and up-regulation of Bax and P53. Anti-apoptotic effects of HupA were also found in primary cultured neurons. Preincubation with HupA at concentrations higher than 0.01 μ M led to a large, dose-dependent attenuation of cell toxicity induced by A β 25–35 (20 μ M). Moreover, HupA (1 μ M) caused large reductions in the amounts of subdiploid DNA detected in a flow cytometry assay and weakened the ladder pattern on agarose gel electrophoresis, typically seen after exposure to A β (fig. 2).

The anti-apoptotic actions of HupA may involve inhibition of the production or the effects of ROS [71]. We found that preincubation of PC12 cells with HupA before exposure to H₂O₂ substantially reduced apoptosis, by any of several measures. The same treatment also attenuated H₂O₂-induced overexpression of bax and p53, while restoring bcl-2 to normal levels (fig. 3) [63]. HupA was also effective in the OGD paradigm. Exposure to OGD for 3 h followed by reoxygenation for 24 h triggers apoptosis

characterized by chromatin condensation, nucleus fragmentation and DNA laddering, accompanied by altered levels of mRNA for c-jun, p53, bcl-2 and bax. In this model, HupA significantly attenuated apoptosis and reduced the up-regulation of c-jun and bax as well as the down-regulation of bcl-2 [94].

In the mitochondrial-mediated cell death pathway, a key step is transient opening of the mitochondrial permeability transition (MPT), involving a non-specific increase in the permeability of the inner mitochondrial membrane [25, 28, 53]. In this process, cytochrome c moves from the intermembrane space into the cytoplasm [5] where it binds to another factor (Apaf-1). In the presence of dATP, this complex polymerizes into an oligomer known as the apoptosome. The apoptosome activates the protease, caspase-9, which in turn activates caspase-3. The cascade of proteolytic reactions also activates DNAases, which leads to cell death [83].

When PC12 cells were pre-incubated with HupA at concentrations above 0.01 μ M, there was a marked neuroprotection against apoptosis induced by β -amyloid, with a significant reduction in mitochondrial swelling and an improvement in mitochondrial membrane potentials [unpubl. data of this lab]. Pretreatment of rat cortical neurons with HupA (0.01–10 μ M) significantly elevated cell survival and reduced all signs of apoptosis resulting from exposure to A β 25–35.

Further studies focused on caspase activation in primary cultures of rat cortical neurons subjected to a variety of stresses. Measurements of caspase-3-like fluorogenic cleavage demonstrated that HupA (1 μ M) attenuated an A β 25–35-induced increase in caspase-3 activity at 6, 12, 24, and 48 h [71]. Western blot analyses confirmed these results at the protein level. HupA also inhibited caspase-3 activation in models of apoptosis by serum deprivation

Fig. 4. Effect of HupA on caspase-3 activity (**a**) and Western blot detection of cytochrome c and COX4 (**b**) in primary cortical neurons. Neurons under serum deprivation for 24 h. HupA at a concentration of 1 μ M added to the culture 2 h in advance. **a** Data expressed as means $\pm$ SD. Statistical comparison was made using ANOVA followed by Duncan's test. There was a significant difference between the serum deprivation group and the untreated control group. $^{##}p < 0.01$ compared to control group. $^{*}p < 0.05$ compared to serum deprivation group.

b The mitochondrial and cytosolic fractions were isolated using an ApoAlert cell fractionation kit. They were then processed using the standard Western blot procedure on 12% SDS-PAGE and probe with COX4 antibody (~ 17 kDa) or cytochrome c antibody (~ 15 kDa). The presence of cytochrome c in the cytosolic fraction after induction indicates that apoptosis involves mitochondrial release of cytochrome c to the cytosol.

and staurosporine treatment. The apoptosis induced by 24 h of serum deprivation was accompanied by enhanced caspase-3 activity and a release of mitochondrial cytochrome c into the cytosol [97]. HupA (0.1–10 μ M) improved neuronal survival in this model, inhibiting the rise in caspase-3 activity and protein expression [97]. Likewise, cell survival was greatly enhanced when HupA (0.1–100 μ M) was introduced 2 h before a 24-hour exposure to 0.5 μ M staurosporine. Incubation with HupA at dose of 1 μ M also reduced staurosporine-induced DNA fragmentation, up-regulation of the pro-apoptotic gene, bax, down-regulation of the anti-apoptotic gene, bcl-2, and decrease in caspase-3 proenzyme protein level (fig. 4) [87].

A potassium channel with delayed rectifier characteristics may play an important role in A β -mediated toxicity. The up-regulation of an outward K $^{+}$ current known as I_k mediates several forms of neuronal apoptosis and could contribute to the pathogenesis of A β -induced neuronal death. Exposure to a 20- μ M concentration of A β 25–35 or A β 1–42 is known to enhance the apoptosis-related current, I_k [81]. Interestingly, HupA will reversibly inhibit the fast transient current, I_A , and the sustained potassium current, I_k , in CA1 pyramidal neurons acutely dissociated from rat hippocampus [33]. Such effects might contribute to this agent's anti-apoptotic effect.

In light of these findings and the effects of HupA on apoptosis-related genes, we propose that HupA blocks apoptosis by antagonizing the mitochondrial-dependent caspase pathway, directly or indirectly (fig. 5). The effects of HupA on the intrinsic caspase-3 pathway might be downstream consequences of altered expression of bcl-2 family genes. Functionally, bcl-2 is a potent cell death suppressor, whose over-expression can prevent cell death in response to a variety of stimuli. It is well known that Bcl-2 suppresses apoptosis by inhibiting cytochrome c release from the mitochondria. On the other hand, bax is a death-promoting factor, whose translocation to the mitochondrial membrane leads to a loss of mitochondrial membrane potential and increases mitochondrial permeability. Increased mitochondrial permeability results in the release of cytochrome c followed by activation of caspase-3 [21]. We consider it likely that HupA owes some of its anti-apoptotic effects to an effective antagonism of the up-regulation of bax and the down-regulation of bcl-2, which impairs mitochondria-dependent caspase pathway. At present, however, direct effects of HupA on cytochrome c and caspase-3 and other possible targets are not excluded.

Effects of HupA on Secretory Amyloid Precursor Protein and Protein Kinase C- α

A β is a self-aggregating 39–43-amino acid peptide that originates from a larger polypeptide termed Alzheimer's amyloid precursor protein (APP). Alternate pathways for APP processing have been described: the non-amyloidogenic secretory pathway, which releases a soluble ectodomain (APPs) and prevents A β formation [15], and the endosomal-lysosomal pathway, which produces amyloidogenic products [24]. The amyloid hypothesis of AD [17, 46] is focused on the potential toxic role of an excessive production of A β and suggests that the aberrant metabolism of APP is a central pathogenetic mechanism for the disease.

Several factors can affect the secretory non-amyloidogenic pathway of APP. For example, the stimulation of phospholipase C (PLC)-coupled receptors, such as muscarinic m1 and m3, has been shown to potentiate the secretion of APP in cell cultures. These effects are probably mediated mainly by protein kinase C (PKC) [43]. It has also been reported that several anticholinesterases affect APP processing in addition to the catalytic function of AChE [18, 42].

Our own studies showed that HupA could alter APP processing in the brains of rats given i.c.v. infusions of A β 1–40, and in otherwise untreated human embryonic kidney 293 (HEK293sw) cells [88]. In the A β treated rats, levels of APPs and PKC α were significantly decreased by treatment with A β 1–40. These decreases were much reduced by 12 consecutive days of HupA treatment (0.2 mg/kg, i.p.), but HupA in normal rats caused no change in either APPs or PKC α . In normal HEK293sw cells, on the other hand, the levels of APPs and PKC α rose progressively during an 18-hour exposure to HupA (1 μ M). However, no significant alternations in the levels of PKC δ and PKC ϵ were found after HupA treatment. Taken together these findings suggest that HupA may affect the processing of APP by up-regulating PKC, especially PKC α .

In an attempt to clarify the receptor mechanisms involved in such effects, we treated HEK293wt cells with cholinergic receptor antagonists [unpubl. data]. The non-selective muscarinic antagonist, scopolamine, partly blocked the HupA-induced rise in levels of APPs and PKC α . By contrast the nicotinic antagonist, mecamylamine, had little effect. These results suggest that muscarinic ACh receptors may mediate, at least in part, the effects of HupA on the regulation of APPs and PKC α in HEK293sw cells.

Our recent results provide the first demonstration that HupA can reduce the disturbance of PKC and APPs both in rats and in an isolated cell line. The effect of HupA to enhance non-amyloidogenic processing of APP and elevate APPs levels likely depends on the activation of muscarinic receptors and the PLC/PKC cascade. A number of biological activities such as cell proliferation, promotion of cell-substratum adhesion, neurite outgrowth and the prevention of intracellular calcium accumulation and cell death have been attributed to APPs [47]. Since PKC is a key enzyme in signal transduction, and since APPs itself has neuroprotective effects, modulating the levels of these two proteins by HupA may well be beneficial in AD therapy (fig. 6).

Protection of HupA against Hypoxic-Ischemic and Glutamate Induced Brain Injury and Cytotoxicity

Apart from AD, the most common dementia in the elderly is VD. This disorder, like AD, presents a clinical syndrome of intellectual decline produced by ischemia, hypoxia, or hemorrhagic brain lesion. Cerebral ischemia in rats with permanent bilateral ligation of the common carotid arteries (CCA) provides a useful model of VD, in which to investigate the effects of HupA. These animals experience a significant reduction of cerebral blood flow and exhibit learning and memory impairments and neuronal damage resembling those in VD. Daily oral administration of HupA (0.1 mg/kg) to such rats for 14 days produced significant improvement in the learning of a water maze task. Simultaneously there was marked recovery from the decrease in choline acetyltransferase activity in hippocampus and a restoration of SOD, lipid peroxide, lactate and glucose to normal levels [61]. Similar protection was also observed in gerbils given subchronic oral doses of HupA (0.1 mg/kg, twice daily for 14 days) following 5 min of global ischemia [96].

An in vitro model of neuronal ischemia is the rat pheochromocytoma PC12 cell treated with OGD for 30 min. In our hands, this treatment causes death in more than 50% of the cells in culture, along with major changes in morphology and biochemistry, including elevated levels of lipid peroxide, SOD activity and lactate. Cells pre-treated for 2 h with HupA (0.1, 1 and 10 μ M), however, showed increased survival and reduced biochemical and morphologic signs of toxicity. HupA protected PC12 cells against OGD-induced toxicity, most likely by alleviating disturbances of oxidative and energy metabolism [95].

Fig. 7. Photomicrographs of coronal brain sections stained with cresyl violet at the levels of the striatum (**a, b**) and the dorsal hippocampus (**c, d**) for representative saline-treated and huperzine A 0.1 mg/kg-treated rats. Intraperitoneal administration of huperzine A or saline for 5 weeks after hypoxic-ischemic (HI) brain injury in neonatal rats. Note the gross infarction and atrophy in left hemisphere of saline-treated HI rats (**a, c**) ($n = 11$) and the subtle reduction in the left hemisphere in huperzine A-treated HI rats (**b, d**) ($n = 12$).

to saline-treated HI group. These results raise the possibility that HupA have potential utility in treating HI encephalopathy in neonates.

Glutamate is the main excitatory neurotransmitter in the CNS, with important roles in neurotransmission and functional plasticity. Excitatory amino acid neurotransmitters are also involved in CNS pathology. The deleterious effects of overstimulation with excitatory amino acids have been implicated in a variety of acute and chronic neurodegenerative disorders such as ischemic brain damage, AD and neuronal cell death [11] [for reviews, see 13, 14, 26, 27, 35]. Glutamate-mediated overactivation of receptors induces excessive Ca^{2+} influx, which results in elevated intracellular Ca^{2+} concentrations [10, 12] with serious consequences such as necrosis and apoptosis [31]. Blockade of glutamate receptors prevents most of the Ca^{2+} influx and neuronal cell death induced by glutamate exposure [50, 57].

It has been reported that HupA protects against glutamate-induced toxicity. HupA ($100 \mu\text{M}$) decreased neuronal cell death caused by a toxic level of glutamate (also

$100 \mu\text{M}$). In those experiments, HupA reduced glutamate-induced calcium mobilization but did not affect the increase in intracellular free calcium induced by exposure to high KCl or a calcium activator Bay-K-8644 [58]. HupA dose-dependently inhibited the NMDA-induced toxicity in primary neuronal cells, most likely by blocking NMDA ion channels and the subsequent Ca^{2+} mobilization at or near the PCP and MK-801 ligand sites [20]. Wang et al. [66] reported that HupA reversibly inhibited NMDA-induced current in acutely dissociated rat hippocampal pyramidal neurons and blocked specific [^3H]MK-801 binding in synaptic membranes from rat cerebral cortex. Of all the AChE inhibitors tested, HupA is the most powerful both in protecting mature neurons and in blocking the binding of [^3H]MK-801. Studies on the mechanism of receptor inhibition showed that HupA reversibly inhibited NMDA-induced currents. The effect was non-competitive, and showed neither 'voltage-dependency', nor 'use-dependency' [89]. Studies of [^3H]MK-801 binding in cortex membranes suggest that HupA acts as a non-competitive antagonist of the NMDA receptors, via a

competitive interaction with one of the polyamine binding sites [92]. Of interest, natural (–)-HupA and synthetic (+)-HupA reduced the binding of [³H]MK-801 with similar potencies [91] indicating that HupA inhibits NMDA receptors in rat cerebral cortex without stereoselectivity. This result is in dramatic contrast with the stereoselective inhibition of acetylcholinesterase.

NMDA-receptor activation also mediates the generation of long-term potentiation (LTP) – a cellular process that underlies learning and memory [3, 52]. There is evidence that the suppressive action of A β on LTP in both CA1 and dentate gyrus operates via a NMDA receptor-independent pathway that involves cholinergic terminals in the hippocampus. Of some interest, HupA (1.0 μ M) was found to enhance LTP, while a much lower dose (0.1 μ M) largely blocked the suppressive effects of A β on LTP induction [8, 80].

Neuronal cell death caused by overstimulation of glutamate receptors has been proposed as the final common pathway for a variety of neurodegenerative diseases including AD. The ability of HupA to attenuate glutamate-mediated neurotoxicity may be one additional reason for considering this agent as a potential therapeutic for dementia and as a means of slowing or halting the pathogenesis of AD at an early stage [20].

Acknowledgements

This work was supported in part by the grants from Ministry of Science and Technology of China (G199805110, G199805115) and National Natural Science Foundation of China (39170860, 39770846, 3001161954, 30123005 and 30271494). The authors are grateful to Professor Stephen W. Brimijoin (Mayo Clinic, USA) for English revision on the manuscript.

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