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Huperzine A attenuates cognitive dysfunction and neuronal degeneration caused by β-amyloid protein-(1–40) in rat

Rui Wang, Hai Yan Zhang, Xi Can Tang *

State Key Laboratory of Drug Research, Shanghai Institute of Materia Medica, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences, Shanghai 200031, People's Republic of China

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Abstract

Huperzine A, a promising therapeutic agent for Alzheimer's disease, was examined for its potential to antagonize the deleterious neurochemical, structural, and cognitive effects of infusing β-amyloid protein-(1–40) into the cerebral ventricles of rats. Daily intraperitoneal administration of huperzine A for 12 consecutive days produced significant reversals of the β-amyloid-induced deficit in learning a water maze task. This treatment also reduced the loss of choline acetyltransferase activity in cerebral cortex, and the neuronal degeneration induced by β-amyloid protein-(1–40). In addition, huperzine A partly reversed the down-regulation of anti-apoptotic Bcl-2 and the up-regulation of pro-apoptotic Bax and P53 proteins and reduced the apoptosis that normally followed β-amyloid injection. The present findings confirm that huperzine A can alleviate the cognitive dysfunction induced by intracerebroventricular infusion of β-amyloid protein-(1–40) in rats. The beneficial effects are not confined to the cholinergic system, but also include favorable changes in the expression of apoptosis-related proteins and in the extent of apoptosis in widespread regions of the brain.

Keywords: (−)-Huperzine A; β-Amyloid protein; Alzheimer's disease; Amnesia model; Apoptosis; Apoptosis-related protein

1. Introduction

Alzheimer's disease is associated with a marked hypofunction of the cholinergic system resulting from degeneration of cholinergic neurons in the forebrain (Bartus et al., 1982). The profound deficits in cortical cholinergic activity and the subsequent loss of subcortical cholinergic innervation in the nucleus basalis may be root causes of the memory impairment in this disorder (Coyle et al., 1983; Whitehouse et al., 1982). Histopathological hallmarks of Alzheimer's disease are extracellular senile plaques (Dayan, 1970), intracellular neurofibrillary tangles (Kowall et al., 1991), selective neuronal losses, and synaptic degeneration in many areas involved in cognitive function (Davies et al., 1987). The main component of senile plaques is a β-amyloid peptide of 39–43 amino acids, which is derived from a larger precursor protein (Golde et al., 1992; Kang et al., 1987) and tends to aggregate into a fibrillar and β-sheeted structure (Masters et al., 1985). Deposition of β-amyloid protein is considered a crucial event in initiating the neuritic and neuronal degeneration in Alzheimer's disease, which mainly affects the association cortices, some limbic structures, and the forebrain nuclei projecting to those areas. Neurotoxicity of β-amyloid protein has been demonstrated both in vitro (Pike et al., 1995; Yankner et al., 1990) and in vivo (Frautschy et al., 1996; Kowall et al., 1992). Of special interest is the observation that intracerebral infusion of this protein causes neurodegeneration in the brain, along with impairment of learning and memory (Nitta et al., 1997).

Mechanistic studies in tissue culture indicate that β-amyloid protein triggers neuronal degeneration by activating an apoptosis pathway (Backman et al., 1996). Several lines of evidence implicate apoptosis in the central neurodegeneration. Some neurons in Alzheimer's brains clearly die via an apoptotic mechanism (Lassmann et al., 1995; Smale et al., 1995). It has been reported that β-amyloid causes membrane blebbing and cell shrinkage followed by DNA damage, the generation of nuclear apoptotic bodies, DNA ladder, and other classic hallmarks of apoptosis (Loo et al., 1993; Gschwind and Huber, 1995; Paradis et al., 1996). Therefore, preventing the apoptosis induced by

* Corresponding author. Tel.: +86-21-6431-1833 ext. 405; fax: +86-21-6437-0269.

E-mail address: xctang@mail.shcnc.ac.cn (X.C. Tang).

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β-amyloid might be an optimal treatment for Alzheimer's disease. While awaiting a practical means of interfering with β-amyloid-induced apoptosis, however, much attention has been given to palliative therapies aimed at enhancing cholinergic function with pharmacological agents. Among the various agents investigated, cholinesterase inhibitors were the first to show potential for enhancing memory and learning. This enhancement may reflect the documented ability of such drugs to raise levels of synaptic acetylcholine. However, the precise mechanisms by which anticholinesterases induce cognitive improvement remain unclear, and other targets besides cholinesterases may contribute to the clinical efficacy of these drugs (Giacobini, 2000).

Huperzine A, a novel *Lycopodium* alkaloid isolated from the Chinese herb, *Huperzia serrata*, is a potent, reversible, and selective inhibitor of acetylcholinesterase that acts to enhance cognition in several animal species. Clinical trials have demonstrated that huperzine A relieves memory deficits in aged subjects and patients with Alzheimer's disease without any remarkable side effects (Tang and Han, 1999). We recently found that huperzine A protected PC12 cells and primary cultured neurons against β-amyloid-induced insult in vitro (Xiao et al., 2000). These unexpected findings prompted us to explore whether huperzine A could prevent the memory deficit and neurodegeneration induced by β-amyloid protein infusion in vivo. Here, we report that long-term treatment with hu-

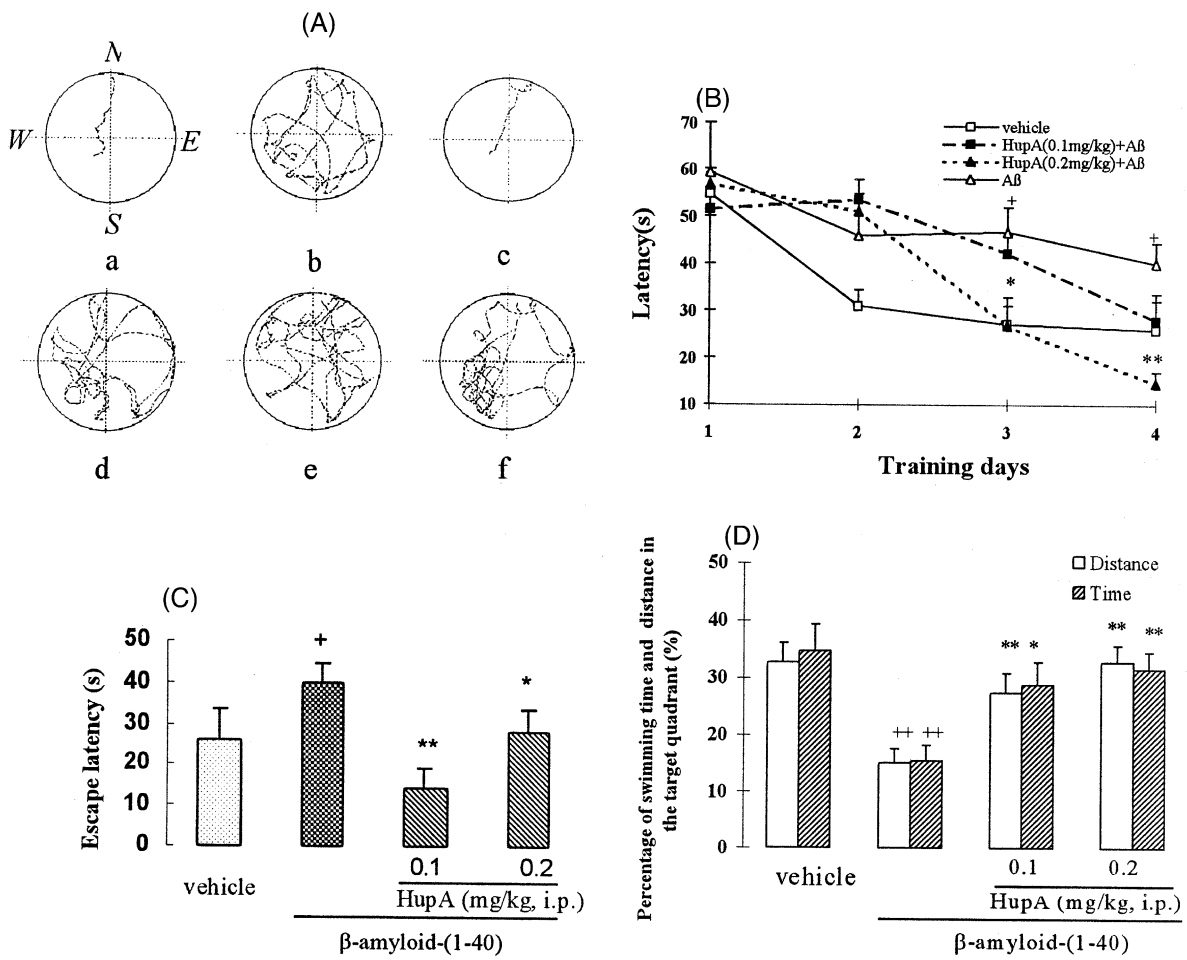

Fig. 1. Huperzine A improves the memory deficit induced by i.c.v. infusion of β-amyloid protein-(1–40) (800 pmol × 3) in rats ($n = 7$ for each group). (A) The typical swimming-tracking path in Morris water maze; a, b, and c are the performance on the fourth training day; d, e, f are the performance of probe trial on the fifth training day; a and d: vehicle control; b and e: β-amyloid protein-(1–40) (Aβ)-treated rat; c and f: rat treated with huperzine A (HupA) 0.2 mg/kg plus β-amyloid protein-(1–40) (Aβ). (B) Mean latencies to escape from the water onto the hidden platform. Each rat was subjected to two trials per day for 4 consecutive days. Data represent means ± S.E.M., $^{+}P < 0.05$ vs. vehicle-treated group. $^{*}P < 0.05$, $^{**}P < 0.01$ vs. β-amyloid protein-(1–40)-treated group. (C) Mean latencies to escape from the water onto the hidden platform in fourth training day. Data expressed as means ± S.E.M. indicated by vertical bar. $^{+}P < 0.05$ vs. vehicle-treated group. $^{*}P < 0.05$, $^{**}P < 0.01$ vs. β-amyloid protein-(1–40) (Aβ)-treated group. (D) The time spent and swum distance in the target quadrant (in which the platform had been placed during the training phase) in the probe trial (swimming 60 s without platform). Data expressed as means ± S.E.M. indicated by vertical bar. $^{++}P < 0.01$ vs. vehicle-treated group. $^{*}P < 0.05$, $^{**}P < 0.01$ vs. β-amyloid protein-(1–40) (Aβ)-treated group.

perzine A is able to reduce the deficits of learning and memory, and the damage of brain neurons in rats receiving intracerebroventricular β-amyloid protein-(1–40).

2. Materials and methods

2.1. Chemicals

Synthetic human β-amyloid protein-(1–40) was purchased from Sigma. (−)-Huperzine A (colorless crystals, purity > 98%) isolated from *H. serrata* was prepared by the Department of Phytochemistry in the Shanghai Institute of Materia Medica.

2.2. Animals

Male Sprague-Dawley rats, weighing 220–280 g at the beginning of the experiment, were housed individually in a room maintained at 23°C with a 12-h light–dark cycle for the duration of the experiment. Rats were allowed free access to food and water except during the learning test.

2.3. Surgery and drug administration

Rats anesthetized with chloral hydrate (350 mg/kg, i.p.) were positioned in a Narishige stereotaxic instrument, and a cannula was implanted into the left cerebral ventricle (A 1.4, L 0.9, V 4.0). At least 2 days were allowed for recovery from the surgery. For administration in vivo, β-amyloid protein-(1–40) was dissolved in 35% acetonitrile/0.1% trifluoacetic acid, and 800 pmol was infused on day 5, day 8 and day 11 after surgery (15 μl, 2 μl/min), with the aid of a mini-pump. Sham control rats were infused with the vehicle (35% acetonitrile/0.1% trifluoacetic acid). Administration of huperzine A (0.1, 0.2 mg/kg, i.p., once per day for 12 consecutive days) or its vehicle (saline) began on the day of the first infusion of β-amyloid. Upon completion of behavioral testing, the rats were sacrificed by decapitation. The brains were rapidly removed on ice. Half of the brain tissue was fixed for histology involving hematoxylin and eosin, Congo red, terminal deoxynucleotidyl transferase-mediated dUTP nick-end labeling (TUNEL) staining, immunohistochemistry and electron microscopy. The other tissue sample was used for assay of choline acetyltransferase activity.

2.4. Morris water maze task

The water maze apparatus consisted of a circular pool 150 cm in diameter, 60 cm deep, filled to height of 30 cm with water at 23 ± 1°C to cover a black platform (diameter 10 cm). The platform was submerged approximately 1.5 cm below the surface of the water, which was darkened with 50 ml Chinese ink. For descriptive data collection, the pool was subdivided into four equal quadrants formed by imaging lines, which intersected in the center of the pool at right angles called north, south, east and west. The platform always resided in the center of southwest quadrant except on the last day. Each rat's swimming was monitored by a video camera linked to a computer-based image analyzer. Learning performance was tested for 5 consecutive days beginning 1 day after the last infusion of β-amyloid protein ($n = 7$ for each group). Each rat was trained to find the platform, with two trials a day. To begin a trial, the rat was placed facing the wall at one of the two cardinal starting locations (N and E shown in Fig. 1Aa). The time taken to find the platform (escape latency) was measured and averaged over two trials. If a rat failed to find the platform within 60 s, it was placed on the platform. Regardless of whether the rat found the platform or not, it was kept there for 10 s. There was a 30 s recovery period between trials. On day 5 of training, a probe trial was made by removing the platform and allowing the rat to swim for 60 s in search of it. Time and distance swum in each of the four quadrants in the pool were calculated as percentages of the totals. A persistent preference for the quadrant previously occupied by the platform was taken to indicate that the rat had acquired and remembered the spatial task.

2.5. Measurement of choline acetyltransferase activity

The brain cortex was separated on ice and rapidly stored in liquid nitrogen until assay. Each cortex was weighed and homogenized in 9 vol ice-cold phosphate buffered saline. Choline acetyltransferase activity was determined by measuring the rate of formation of acetylcholine from acetyl-CoA, using a radiochemical method (Fonnum, 1975). Protein content was determined by the Coomassie blue protein binding method (Bradford, 1976), using bovine serum albumin as standard.

Table 1
Huperzine A attenuates the reduction of cortical choline acetyltransferase activity induced by i.c.v. β-amyloid in rats
Rats were killed 2 h after the fifth performance of water maze. Huperzine A was administered i.p. once per day for 12 consecutive days. Data are expressed as a percent of vehicle control ± S.E.M; $n = 5–6$. Basal vehicle control values of choline acetyltransferase in the cortex were $(5.967 \pm 1.353) \times 10^{-3}$ nmol/h/mg protein.

Group	Dose	Choline acetyltransferase activity (% of control)
Vehicle	–	100.00 ± 13.69
β-amyloid-(1–40) + saline	800 pmol × 3	71.22 ± 5.77[a]
β-amyloid-(1–40) + Huperzine A	800 pmol × 3	
	0.1 mg/kg	95.89 ± 7.91
	0.2 mg/kg	96.61 ± 2.32[b]

[a] $P < 0.05$ vs. vehicle-treated group.
[b] $P < 0.05$ vs. β-amyloid-treated group.

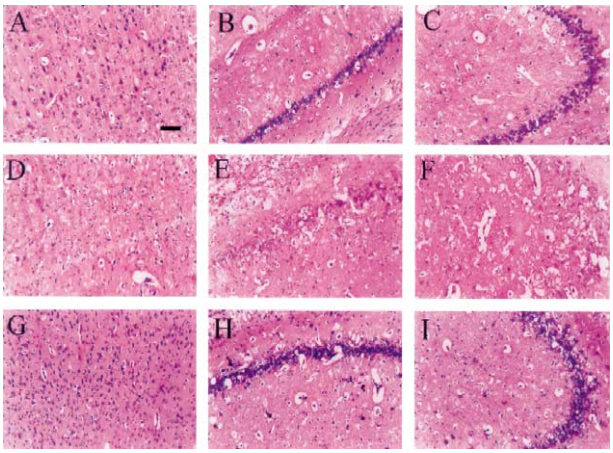

Fig. 2. Effects of huperzine A on morphologic changes induced by i.c.v. infusion of β-amyloid protein-(1–40) (800 pmol × 3) in rats (haematocylin and eosin staining). Rats were killed after fifth performance of water maze. Daily i.p. administration of huperzine A 0.2 mg/kg for 12 consecutive days. Sections from four rats in each group ($n = 7$) were examined. A, D, G: cortex; B, E, H: CA1 region in hippocampus; C, F, I: CA3 region in hippocampus. A, B, C: vehicle-treated group; D, E, F: β-amyloid protein-(1–40)-treated group. G, H, I: β-amyloid protein-(1–40)-treated plus i.p. administration of huperzine A 0.2 mg/kg. Scale bar = 20 μm.

2.6. Morphology

Brains were rapidly removed on ice, fixed in 10% buffered formalin, embedded with paraffin, cut into consecutive 6 μm sections with a microtome and placed on poly-D-lysine-coated glass slides. Alternate sections were stained with haematoxylin and eosin to observe general morphologic alteration, or with Congo red to detect amyloid deposits. Neighboring sections were used for immunohistochemistry and TUNEL staining.

TUNEL staining was performed on brain sections treated with 2% hydrogen peroxide in phosphate buffered saline for 20 min at room temperature. After washing with phosphate buffered saline, the sections were stained with In situ Cell Detection Kit, AP (Boehringer Mannheim) for apoptosis detection according to the manufacturer's protocol. In other sections, immunohistochemistry was used to assess the levels of Bcl-2, Bax and P53 protein. Sections were pretreated with microwave in citrate buffer for 10 min. Following treatment with 3% hydrogen peroxide for 10 min at room temperature to eliminate endogenous peroxidase, sections were incubated with goat serum for 10 min, followed by primary antibody (rabbit anti-rat SANTA CRUZ, USA) for 60 min at 37°C, then treated with biotinylated secondary antibodies (goat anti-rabbit) and the ABC kit. Peroxidase labeling was visualized by 3,3′-diaminobenzidine. For analysis and photography, an Olympus microscope type BH2 with a video camera system was linked to a computer to obtain digitized images. The images were analyzed with color image analysis system to determine the staining index (color intensity value multiplied by area immunostained). Sections from four rats in each group ($n = 7$) were analyzed, and values from each of three random images for each compartment of a given rat were averaged.

For electron microscopy detection, epoxy-embedded blocks of cortex and hippocampus from two rats in each group were sectioned with ultramicrotome, placed on 200-mesh copper grids, and stained in saturated ethanol/uranyl acetate and bismuth subnitrate, as described previously (Masliah et al., 1993), and examined with a Zeiss EM 10/C transmission electron microscope at 80 kV.

2.7. Statistical analysis

The data were expressed as means ± S.E.M. Group differences in the escape latency in the Morris water maze training task were analyzed using two-way analysis of variance (ANOVA) with repeated measures. One-way ANOVA followed by the Duncan multiple group comparison was used to analyze group differences of the data collected during fourth training day, probe trials, biochemical assay and image analysis.

3. Results

3.1. Huperzine A reduces memory impairment after infusion of β-amyloid protein-(1–40)

Learning and retention of a Morris water maze was used to evaluate spatial memory in rats given β-amyloid protein-(1–40) (800 pmol × 3) by intracerebroventricular

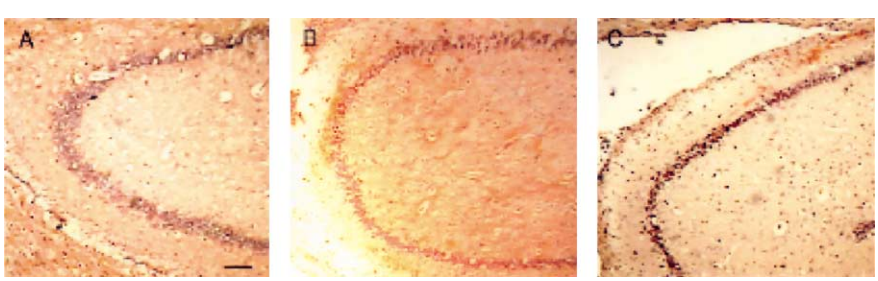

Fig. 3. β-amyloid protein deposits stained by Congo red in the hippocampus in rats. Rats were killed after fifth performance of water maze. Sections from four rats in each group ($n = 7$) were examined. (A) The vehicle-treated rat; (B) the β-amyloid protein-(1–40)-treated rats. (C) Rats treated with β-amyloid protein-(1–40) and daily administration of huperzine A (0.2 mg/kg, i.p.) for 12 consecutive days. Scale bar = 40 μm.

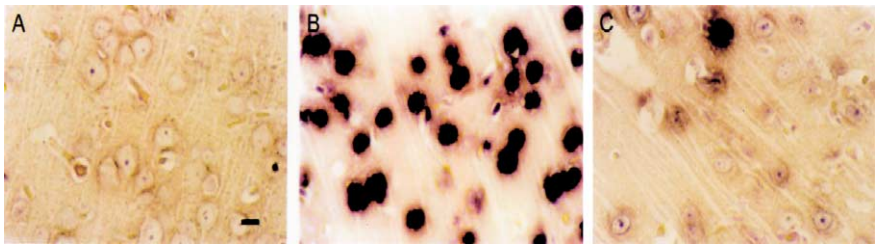

Fig. 4. Apoptotic cell death induced by i.c.v. infusion of β-amyloid protein-(1–40) (800 pmol × 3) in the cortex in rats. DNA fragmentation was observed using the TUNEL method. Four rats in each group ($n = 7$) were examined. (A) Vehicle-treated rat; (B) β-amyloid protein-(1–40)-treated rats. (C) rats treated with β-amyloid protein-(1–40) and daily administration of huperzine A (0.2 mg/kg, i.p.) for 12 consecutive days. Scale bar = 5 μm.

(i.c.v.) infusion. In the acquisition trials, typical swimming paths on the fourth training day (Fig. 1Aa,b,c), and quantitative escape latencies (Fig. 1B), indicate that rats treated with β-amyloid took longer to find the platform than did vehicle-treated rats [$F(3,18) = 4.8799$, $P < 0.05$]. This prolongation of latency was shortened by huperzine A at the doses of 0.1 and 0.2 mg/kg, and the main treatment effect was statistically significant [two-way ANOVA, $F(9,54) = 3.1030$, $P < 0.01$]. The result in Fig. 1C also showed that the prolonged latency was shortened significantly by huperzine A at doses of 0.1 and 0.2 mg/kg in the fourth training day (one-way ANOVA, $P < 0.05$).

In the probe trials, the time spent and swimming distance in the quadrant that had held the hidden platform was used to estimate performance. The data (Fig. 1Ad,e,f and D) indicate that the rats treated with vehicle alone or β-amyloid plus huperzine A swam longer in the probe quadrant than did the rats treated with β-amyloid alone. According to one-way ANOVA, huperzine A at doses of 0.1, 0.2 mg/kg significantly increased the time spent and the swimming distance in the probe quadrant.

3.2. Effects of huperzine A on cortical choline acetyltransferase activity

As shown in Table 1, the infusion of β-amyloid protein-(1–40) induced a decrease of about 30% in the choline acetyltransferase activity of cerebral cortex ($P < 0.05$). Huperzine A at 0.2 mg/kg significantly attenuated the decrease in choline acetyltransferase activity. The dose of 0.1 mg/kg exhibited a similar tendency, but the statistical significance was borderline ($P = 0.05$).

3.3. Effects of huperzine A on morphologic alterations induced by β-amyloid protein-(1–40)

No remarkable neuronal abnormalities were observed in brains from rats infused with vehicle (Fig. 2A,B,C), but after i.c.v. β-amyloid protein-(1–40), all tested brains showed neuronal degeneration in cerebral cortex and hippocampus in different degree (Fig. 2D,E,F). Neuronal death and loss were especially obvious in cortical regions near the lateral ventricle and in the CA3, CA1 and CA4 regions of hippocampus. Co-treatment with huperzine A (0.1, 0.2 mg/kg) markedly reduced these pathological changes (Fig. 2G,H,I).

Extracellular deposits of amyloid as evaluated by Congo red staining were absent from the brain sections of vehicle-treated rats but were obvious in sections from rats treated with β-amyloid protein-(1–40). In fact, distinct deposits of amyloid were widely disseminated throughout the frontoparietal cortex and also present in the hippocampus. Huperzine A (0.2 mg/kg) attenuated deposits of amyloid in the cortex and hippocampus (Fig. 3).

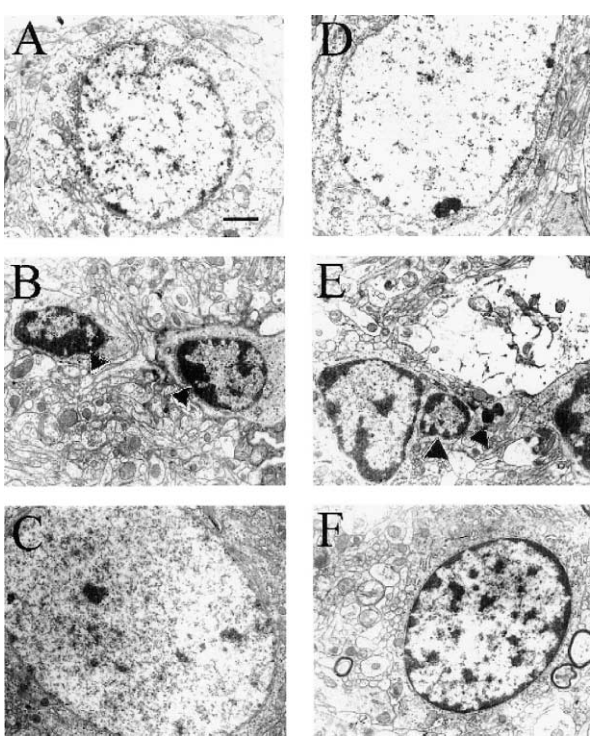

Fig. 5. Changes of electron micrographs of neurons in the cortex and hippocampus induced by i.c.v. infusion of β-amyloid protein-(1–40) (800 pmol × 3) in rats. Rats were killed after fifth performance of water maze. The cortex and hippocampus from two rats in each group ($n = 7$) were examined. A, B and C: neuron in cortex; D, E and F: neuron in hippocampus. A and D: vehicle-treated group; B and F: β-amyloid protein-(1–40)-treated group, arrows show the irregular nucleus and formation of dense intranuclear. C and F: huperzine A (0.2 mg/kg, i.p. once per day for 12 consecutive days) attenuates the pathologic changes induced by β-amyloid. Scale bar = 1 μm.

3.4. Effects of huperzine A on β-amyloid protein-(1–40) induced apoptosis

Rats treated with β-amyloid protein-(1–40) exhibited increased TUNEL labeling in patches and neuronal layers in the cortex and hippocampus. Fig. 4 depicts examples of the labeling in the cortex and also illustrates TUNEL-labeled nuclei. The nuclei were irregularly shaped and appeared to be degenerating. Strikingly, the TUNEL-positive cell population appeared to decrease after treatment with huperzine A at a dose of 0.2 mg/kg, suggesting a neuroprotective effect. To evaluate this possibility more rigorously, the pathologic changes were also examined by electron microscopy. With this method, we observed apoptotic-like changes in both cortical and hippocampal neurons of β-amyloid protein-(1–40)-treated rats (Fig. 5). The neurodegenerative changes included segmentation of nuclear chromatin and formation of dense intranuclear and intracytoplasmic bodies. Huperzine A prominently attenuated these neurodegenerative changes induced by β-amyloid infusion.

For further insight into the effects of huperzine A on β-amyloid-induced apoptosis, we used immunohistochemistry to evaluate the expression of two pro-apoptotic proteins, Bax and P53, along with the anti-apoptotic protein, Bcl-2 (Fig. 6). Brain samples were collected on the fifth day after the last infusion of β-amyloid protein-(1–40). At this time, vehicle-treated controls exhibited moderate basal expression of Bcl-2 and weak expression of Bax and P53 in the cortex and all regions of hippocampus. In contrast, rats given i.c.v. β-amyloid protein-(1–40) exhibited strongly increased Bax and P53 reactivity and decreased Bcl-2 reactivity, predominantly in cortical and hippocampal regions bordering the lateral ventricle. Quantitative image analysis confirmed this qualitative picture (Fig. 6). Thus, β-amyloid treatment significantly reduced the index of Bcl-2 intensity and raised that of p53 in cortex and CA1, CA3, CA4, as well as DG regions of hippocampus.

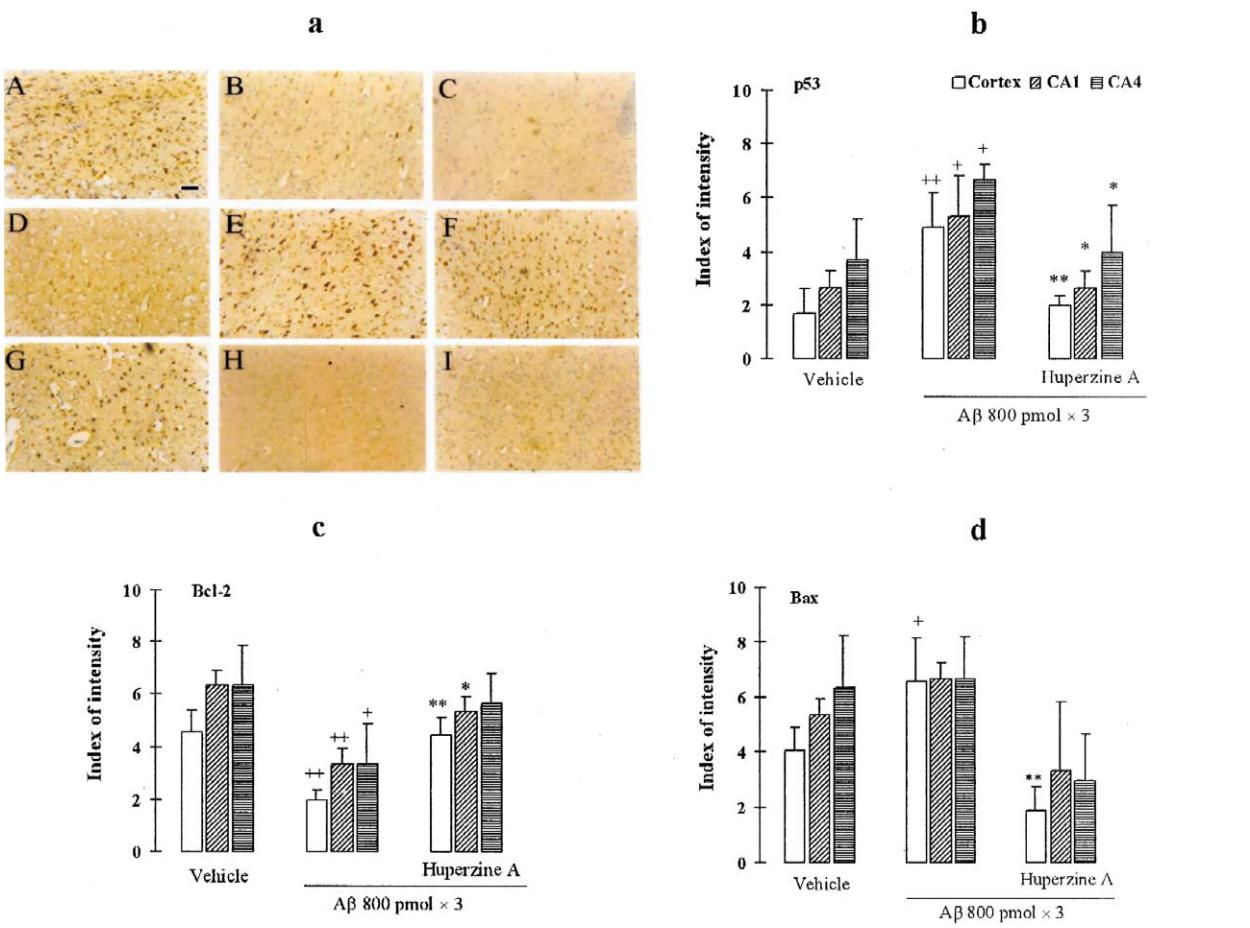

Fig. 6. Effects of huperzine A on apoptotic related protein expression in rats. Rats were killed after fifth performance of water maze. Huperzine A 0.2 mg/kg i.p. was administered once per day for 12 consecutive days. Sections from four rats in each group ($n = 7$) were analyzed, and values from each of three random images for each compartment of a given rat were averaged for the image analysis. a: photographs of Bcl-2, Bax and P53 immunostaining in the cortex. A, B, C: vehicle treated group; D, E, F: β-amyloid protein-(1–40) [Aβ-(1–40)]-treated rats; G, H, I: rats infusion of Aβ-(1–40) and administration of huperzine A. A, D, G: the Bcl-2 expression in cortical region; B, E, H: Bax; and C, F, I: P53 in the same region. Scale bar = 20 μm. b, c, d: quantitative image analysis of Bcl-2, Bax and P53 proteins in the cortex and hippocampal CA1, CA3 and CA4 regions, respectively. The levels of protein expression were indicated by the index of intensity. Data expressed as means ± S.E.M., $^{+}P < 0.05$, $^{++}P < 0.01$ vs. vehicle treated group; $^{*}P < 0.05$, $^{**}P < 0.01$ vs. Aβ-(1–40)-treated group.

This result indicates an up- and down-regulation of pro-apoptotic and anti-apoptotic proteins, respectively. Most importantly, huperzine A at a dose of 0.2 mg/kg caused an increase in the index of Bcl-2 intensity and decreases in the indices of Bax and P53 intensity, all of which were statistically significant. These findings lead us to propose that the anti-apoptotic effects of huperzine A are connected with an ability to influence the expression of apoptosis-related proteins.

4. Discussion

Animal models are playing a critical role in ongoing attempts to understand the pathology and therapeutics of Alzheimer's disease (Barry et al., 1996). Although no current model develops the full pathologic spectrum of the disease, injection of β-amyloid into brain has been shown to impair memory and elicit a degree of Alzheimer-type neurodegeneration (Nitta et al., 1997). The present studies showed that repeated i.c.v. infusion of β-amyloid protein-(1–40) induced marked amnesic effects along with signs of neurodegeneration, including (1) decrease of cortical choline acetyltransferase activity and (2) significant neuronal loss in the cortex and hippocampus especially in CA3 region. In addition, this treatment generated extracellular amyloid deposits throughout the frontoparietal cortex and hippocampus as detected by Congo red staining. These results are in accordance with previous reports (Nitta et al., 1994, 1997) indicating that β-amyloid protein deposition in the brain is related to cognitive impairment, hypofunction of cholinergic neurons, and neuronal death.

Our finding that huperzine A was able to attenuate β-amyloid-induced neuropathology has major therapeutic implications. One implication is that the beneficial effects on memory deficits may reflect more than a simple enhancement of cholinergic function by inhibition of acetylcholinesterase. It now appears likely that huperzine A also has an ability to protect brain neurons from β-amyloid-induced damage. Since excessive exposure to β-amyloid has been proposed as a cause of Alzheimer's disease, such a protective effect might prove useful in slowing disease progression as opposed to mere symptomatic palliation.

We present initial evidence that the neuronal degeneration induced by infusing β-amyloid i.c.v. may proceed through an apoptotic pathway. TUNEL staining and electron microscopy showed that the cortical and hippocampal neurons treated with β-amyloid protein-(1–40) undergo morphologic changes that resemble apoptosis. The data are compatible with the idea that neurodegeneration is directly triggered by the accumulation of β-amyloid, which leads to the localized activation of a series of apoptotic events. Much evidence has accumulated dealing with the roles of certain proto-oncogenes, such as bcl-2, bax, and p53, in the induction of apoptosis (Allsopp et al., 1993; Oltval et al., 1993; Sakhi et al., 1994). Most relevant to our work is a previous report of down-regulation in the expression of bcl-2 and up-regulation of bax and p53 during the onset of apoptosis following exposure to β-amyloid fragment 31–35 in tissue culture (Yan et al., 1999). These changes are similar to those that we observed in the expression of the same proteins after i.c.v. infusion of β-amyloid protein-(1–40) in live rats. Hence, it appears that different fragments of β-amyloid induce an apoptosis that proceeds through similar alterations in the expression of similar sets of genes.

Huperzine A attenuates apoptotic-like changes and neuron losses and it reverses the shift in the expression pattern of apoptosis-related proteins induced by β-amyloid protein-(1–40) in the cortex and hippocampus. These effects are neuroprotective, by definition, and might be specifically anti-apoptotic. Perhaps neuroprotection is involved to some extent in the favorable effect of huperzine A on β-amyloid-induced memory deficits. Acetylcholinesterase is increasingly seen as having actions in addition to the hydrolysis of synaptic acetylcholine (Broide et al., 1999). There is also evidence that acetylcholinesterase activation might be involved in apoptosis (Soreq et al., 1994; Calderon et al., 1998; Robitzki et al., 1998). These non-cholinergic roles of acetylcholinesterase could be important in Alzheimer's disease, and they might be influenced in a beneficial manner by huperzine A. On the other hand, the ability of huperzine A to reduce cognitive deficits and neurodegeneration in β-amyloid-treated rats may involve direct actions on targets other than acetylcholinesterase. In either case, the therapeutic effects are probably based on a multi-target mechanism, and may be helpful in the treatment of Alzheimer's disease.

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