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Huperzine A ameliorates the impaired memory of aged rat in the Morris water maze performance[1]

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KEY WORDS Huperzine A; cholinesterase inhibitors; aging; maze learning; scopolamine

ABSTRACT

AIM: To determine the memory-improving properties of huperzine A in aged rats with memory impairments naturally occurring or induced by scopolamine. METHODS: Morris water maze was used to investigate the effects of huperzine A on the acquisition and memory impairments. RESULTS: During 7-day acquisition trials, aged rats took longer latency to find the platform. Huperzine A (0.1－0.2 mg/kg, sc) could significantly reduce the latency. In the probe trials on the eighth day, huperzine A (0.1, 0.2 and 0.4 mg/kg, sc) significantly increased the time in the quadrant where plateform had disappeared in aged rats. In the acute experiment, scopolamine (0.1 mg/kg, ip) significantly impaired spatial memory in the trained aged rats. Huperzine A (0.4 mg/kg, sc) significantly reversed the memory deficits induced by scopolamine. CONCLUSION: Huperzine A ameliorates the impaired memory naturally occurring or induced by scopolamine in aged rats.

INTRODUCTION

Senescence in humans is accompanied by impairments in spatial learning and memory[1]. The spatial memory of aged rats is also impaired in various tests of spatial learning and memory such as the circular platform[2], the radial arm maze[3], and the Morris water maze[4]. There is a substantial body of experimental work suggesting that dysfunction in cholinergic mechanism may contribute to age-related memory impairments[5,6]. The retrograde loss of cholinergic system from the basal forebrain is the most common and the most severe neurochemical consequences of Alzheimer disease (AD), a slowly progressive neuropsychiatric illness[7]. The severity of memory impairments seen in AD is consistent with dysfunction of the cholinergic system[6]. Many attempts have been made to correct the cholinergic deficiency at various levels of cholinergic functioning to reduce some of major cognitive disturbance if AD patients are not cured.

Huperzine A (HupA), a novel *Lycopodium* alkaloid isolated from the Chinese herb *Huperzia serrata* (Thunb) Trev, is a reversible and selective acetylcholinesterase (AChE) inhibitor. It can amplify the physiological effect of released acetylcholine (ACh) by increasing its concentration in surviving cortical and subcortical cholinergic synapses of AD patients. Compared with the first generation of ChEIs such as physostigmine, tacrine, and galanthamine, the inhibitory effects of HupA on AChE are more potent, its selectivity for AChE other than butyrylcholinesterase is better, the duration of inhibition is longer, and its bioavailability is higher but the side effects are less[8].

The aim of this study was to investigate the effects of HupA on memory impairment naturally occurring or induced by scopolamine in aged rats by using Morris water maze.

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MATERIALS AND METHODS

Subjects All rats were naive male albino rats of Sprague-Dawley Strain (clean grade, Certification № 005) supplied by Shanghai Experimental Animal Centre, Chinese Academy of Sciences. Aged rats are 23 - 24 months old and young rats are about 2 months old. Rats were group-housed, five rats per cage, and were maintained in a climatically controlled room on a 12∶12 h light∶dark cycle (light phase 07∶00 - 19∶00 h) with free access to food and water.

Morris water maze All rats were trained in Morris water maze. The water maze apparatus consisted of a circular pool 150 cm in diameter, which was filled with water to depth of 30 cm and a circular platform, supported by a base resting on the bottom of the pool, placed 1.5 cm below the surface of the water. The water temperature was (23 ± 1) ℃ and made opaque by black ink in it. 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 the southwest quadrant except on the first and last day. On the first day, the rat was allowed to swim in the pool without the platform to be accustomed to swimming. From the second day, the rats were trained to find the platform. On the start of a trial, the rat was placed at one of the two cardinal starting locations facing the wall. When the rats found the platform, they were allowed to remain on it for 15 s. If the rats did not locate the platform within 60 s, they were removed from the water and placed on the platform for 15 s. The second daily trial began 1 min after the termination of the first. Starting locations were ordered in a semirandom manner. The first trial of each was started from one of the two location farthest from the platform (north or east) but never the same first location on 2 consecutive days. The starting location of the second trial was a random choice of one of the two remaining locations (south or west). The rats were trained in the water maze for 8 consecutive days. On the last day, a spatial probe trial was made by removing the platform and allowing the rat to swim for 60 s in search of it. The time swum in each of the four quadrants of the pool was calculated as a percentage over 60 s. If the rat showed a persistent preference during this trial to swim in the pool quadrant where the platform had been previously placed, this was taken to indicate that the rat had acquired the spatial task and remembered it.

Another group of naive aged rats were trained in Morris water maze like above until all subjects arrived at the platform within 20 s. Then these rats were used to investigate the effects of acute injection of HupA on memory impairments induced by scopolamine.

Data collection was automated by an on-line video tracking device designed to track the object in its field with the highest contrast, which was always the white rat on the black background. Tracking was achieved by a system consisting of a video camera mounted over the center of the pool. The tracker's digitized coordinate values were sampled in turn using a computer. Escape latency and swim path were recorded. The mean latencies of two trials in each day for finding the hidden platform are presented here.

Drug administration HupA (provided by the Department of Phytochemistry, this Institute) and scopolamine hydrobromide (Sigma Chemical Co) were dissolved in sterile 0.9 % saline prior to injection. Scopolamine (0.1 mg/kg) or saline was injected ip 30 min and HupA (0.1, 0.2 or 0.4 mg/kg) or saline sc 25 min prior to testing. The injection volume was kept constant at 1 mL/kg irrespective of dose.

Statistical analysis All results were expressed as $\bar{x} \pm s$. In the studies with the Morris water maze task, the one- or two- way analysis of variance (ANOVA) followed by Duncan's multiple-range test as a postdoc test was used for changes in mean latency in the drug dosing groups.

RESULTS

Effects of HupA on the memory impairmets in aged rats In naive rats, the mean latency in finding the platform declined progressively during the training period of 7 consecutive days. The typical swimming tracking paths are listed in Fig 1 A,B,C. The aged rats consistently took longer to find the platform than the young controls, and the effect was statistically significant [two-way ANOVA, $F(6, 54) = 8.977$, $P < 0.01$]. The prolonged latency in aged rats was shortened by HupA at the doses of 0.1 - 0.4 mg/kg, the main treatment effect of HupA being statistically significant [two-way ANOVA,

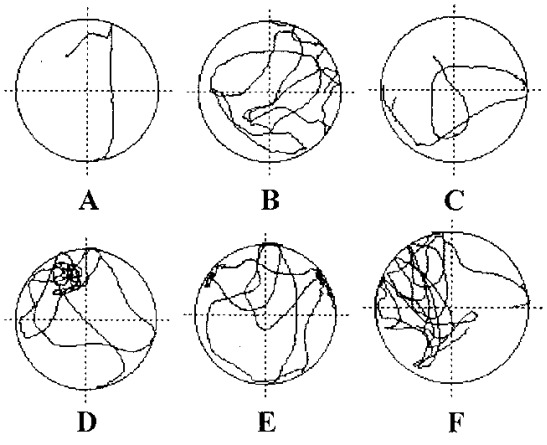

Fig 1. The typical swimming-tracking path in aged rats Morris water maze. A, B, and C are the performance on the seventh day. D, E, and F are the performance in the probe trial on the eighth day. A and D: Young control; B and E: Aged control; C and F: huperzine A-treated group.

$F(18, 162) = 2.9738$, $P < 0.01$]. The result in Fig 2B was parallel to Fig 2A and HupA significantly shortened the latency at the doses of 0.1 and 0.2 mg/kg.

In searching for the platform when this was removed after 7-day acquisition trials, the young control rats swam preferentially in the pool quadrant where the escaped platform had been placed during training. The aged rats, however, did not show any tendency to swim in the pool zone where the platform had been located during acquisition of the test compared with young control (Fig 1D, E, F). All doses of HupA (0.1, 0.2, and 0.4 mg/kg) significantly increased the swimming time in the training quadrant of aged rats (Fig 3), although the dose of 0.4 mg/kg did not improve even aggravated the memory impairments during the acquisition trials.

Effects of acute administration of HupA on the memory impairment induced by scopolamine in the trained aged rats Scopolamine (0.1 mg/kg) impaired the Morris water maze performance of the trained aged rats. HupA ameliorated the impairment [one-way ANOVA, $F(3, 28) = 3.4021$, $P = 0.0313$]. The dose of 0.4 mg/kg showed statistical significance after post-doc test compare with scopolamine control (Fig 4).

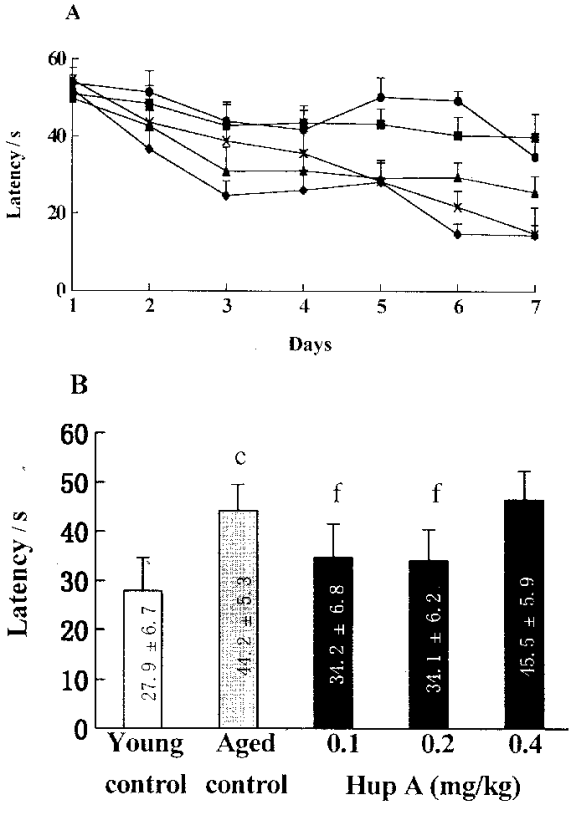

Fig 2. Effects of HupA (0.1, 0.2, and 0.4 mg/kg) on acquisition of Morris water maze in aged rats ($n = 10$). Saline or HupA was administered sc 30 min before testing. A: mean latencies to escape from the water onto the hidden platform are shown. Each rat was subjected to two trials per day for 7 consecutive days. ◆ Young rats + saline; ■ Aged + saline; × Aged + HupA 0.1 mg/kg; ▲ Aged + HupA 0.2 mg/kg; ● Aged + HupA 0.4 mg/kg. B: the mean latencies for 7 days. Each column and bar represents $\bar{x} \pm s$. $^cP < 0.01$ compared with young controls. $^fP < 0.01$ compared with aged controls.

DISCUSSION

The Morris water maze[9] is a popular test of age-related cognitive dysfunction in rodents. As a cognitive task which required the development of a spatial map, the Morris water maze seems analogous to nonverbal tests of cognitive function that are especially sensitive to senescence and dementing disorders in the clinical setting[10]. The validity of the Morris water maze as a measure of cognitive function in aged rats is supported by the fact that among aged rats the decline in noncognitive functions such as motor, sensorimotor, and visual abilities are unrelated to their performance in the Morris water maze[11].

In this study, we found that aged rats may be

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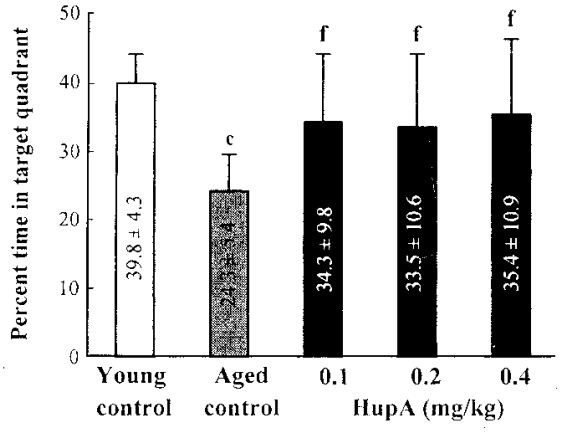

Fig 3. Effects of HupA (0.1, 0.2, and 0.4 mg/kg) on retention of Morris water maze at the eighth day in aged rats ($n = 10$). Saline or HupA was administered sc 30 min before testing. Each column and bar represents $\bar{x} \pm s$. $^cP < 0.01$ compared with young controls. $^fP < 0.01$ compared with aged controls.

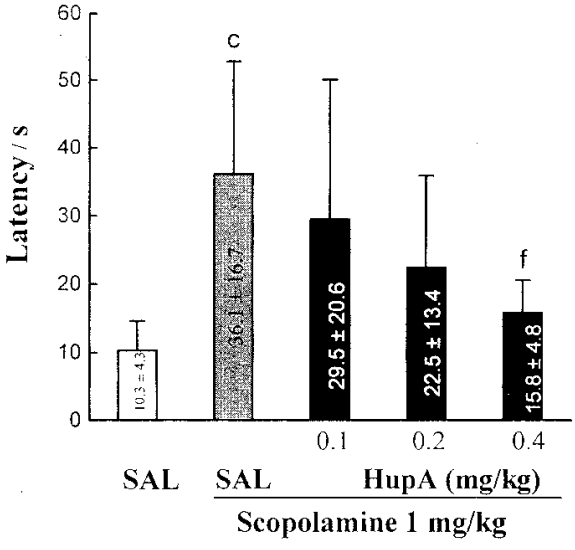

Fig 4. Effects of HupA (0.1, 0.2, and 0.4 mg·kg^{-1}) on performance in scopolamine-treated aged rats ($n = 8$). Every aged rats was pre-trained and the escape latency onto the platform was within 20 s. Saline (SAL) or HupA was administered sc 25 min and SAL or scopolamine was administered ip 30 min before testing. Each column and bar represents $\bar{x} \pm s$. $^cP < 0.01$ compared with SAL control; $^fP < 0.01$ compared with scopolamine control.

more sensitive to scopolamine compared with young subjects (unpublished data). The dose of scopolamine which can significantly impair the spatial memory of aged rats can not produce the same significant effects in young rats. It may be due to the decreased ACh level and muscarinic receptor in aged animals.

HupA significantly improved the performance of aged rats including acquisition and retention. The improving effect of HupA was bell shaped, probably owing to the dose-dependent increase in ACh via direct cholinesterase inhibition. Since HupA shows no significant affinity for muscarinic receptors[12], no evident pre- or post- synaptic effects[13], and no effect on ChAT[14], its effects on spatial memory in task are due primarily to the dose-dependent increase in ACh resulting from direct AChE inhibition. In addition, after administration of HupA, norepinephrine (NE) and dopamine (DA) levels were significantly increased over baseline for several hours, while ACh levels reached a maximum. These increases in NE and DA levels may be related to the increase of extracellular ACh levels through subcortical mechanism[15]. Since the brain levels of NE and DA decreased significantly with aging[16], and there is evidence of interaction between cholinergic and monoaminergic system in the control of cognition[17], it suggested that the increase of NA and DA may also be involved in memory improvement of HupA.

There was no marked side-effect after 7 consecutive days of administration of HupA. It suggests that HupA has no tolerance. The present results, coupled with the improving effects observed in cholinergically lesioned rats[18] and aged monkeys[19], suggest that HupA may have potential value in the treatment of aged-related memory disorders such as Alzheimer' disease.

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石杉碱甲改善老年大鼠水迷宫操作记忆障碍[1]

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关键词 石杉碱甲；胆碱酯酶抑制剂；衰老；迷宫学习；东莨菪碱

目的：测试石杉碱甲对自然衰老及东莨菪碱导致的空间记忆缺损的作用. **方法**：采用大鼠的水迷宫操作，检测石杉碱甲对获得及记忆的作用. **结果**：连续 7 天获得试验期间，皮下注射石杉碱甲 0.1－0.2 mg/kg 能明显缩短老年大鼠找到平台的潜伏期. 在第 8 天撤去平台的记忆测试，石杉碱甲 0.1，0.2 与 0.4 mg/kg 明显延长老年大鼠在该平台区的游泳时间. 单次腹腔注射东莨菪碱 0.1 mg/kg 明显损害已训练达标老年大鼠的空间记忆. 皮下注射石杉碱甲 0.4 mg/kg 明显翻转东莨菪碱产生的记忆损害作用. **结论**：石杉碱甲能改善老年大鼠自然衰老或东莨菪碱产生的记忆障碍.

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