ISSN 0253-9756 Acta Pharmacol Sin 中国药理学报 2000 Dec; 21 (12): 1169-1173
http://www.chinaphar.com

Huperzine A reverses scopolamine- and muscimol-induced memory deficits in chick[1]

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KEY WORDS cholinesterases; huperzine A; scopolamine; muscimol; GABA; avoidance learning; memory; chickens

ABSTRACT

AIM: To study the effects of huperzine A on disruption of spatial memory induced by scopolamine (a muscarinic antagonist) and muscimol (a $GABA_A$ agonist) in passive avoidance task. **METHODS**: One-trial passive avoidance task was used to investigate the effects of huperzine A. The avoidance rate was used to evaluate memory retention. **RESULTS**: Both scopolamine (100 ng) and muscimol (50 ng), injected intracranially 5 min before training, resulted in a decreased avoidance rate. Huperzine A (25 ng), injected intracranially 15 min before training, reversed memory deficits induced by scopolamine and muscimol at 30 min after training, and this reversal persisted at least 1 h. The improving effects of huperzine A exhibited a bell-shaped dose-response curve. **CONCLUSION**: Huperzine A improved the process of memory formation not only by acting as a highly potent and selective inhibitor of AChE, but also by antagonizing effects mediated through the $GABA_A$ receptor.

INTRODUCTION

Huperzine A, a novel alkaloid isolated from the Chinese folk medicinal herb Qian Ceng Ta (*Huperzia serrata*), has proved to be a potent, selective, and reversible, inhibitor of acetylcholinesterase (AChE)[1,2]. This action, which leads to a rise in synaptic levels of the neurotransmitter, acetylcholine, at least partly explains the ability of huperzine A to improve performance in neurobehavioral tasks that involve learning and memory.

An inverse relationship was observed between acetylcholine levels and AChE activities in frontal cortex and whole brain following intramuscular injection of huperzine A[3]. The antiamnesic action of huperzine A was demonstrated in a number of different learning and memory models, such as the passive footshock avoidance, escape task of water maze, spatial discrimination of radial arm maze and delayed response performance. Beneficial effects were seen in intact adult rodents, in aged rodents and monkeys, and also in rodents and monkeys with cognitive impairment induced by treatment with scopolamine, electroshock, cycloheximide, $NaNO_2$, CO_2 or toxicants that induce selective cholinergic lesions in the brain[2,4-6]. Compared with other AChEIs, such as physostigmine, galanthamine, tacrine, and donepezil, huperzine A has better penetration through the blood-brain barrier, higher oral bioavailability and longer duration of AChE inhibitory action[1,2]. Thus, huperzine A appears to meet the criteria for an ideal AChE inhibitor for the symptomatic treatment of Alzheimer's disease and other memory disorder diseases.

The hyperstriatum ventrale (HV) of the chick telencephalon has been shown to play an important role in the learning and memory process of one-trial passive avoidance task and imprinting[7]. A variety of biochemical cascades have been shown to occur in this area, such as an up-regulation of *N*-methyl-*D*-aspartate (NMDA) glutamate receptors, an increase in *in vitro* phosphorylation of myristolyated, alanine-rich protein kinase C substrate protein and an increase in the expression of the immediate early gene product Fos[8,9]. Several measures of dendritic and synaptic morphology and density are specifically enhanced in the same region[10]. For these reasons, injection of agents into HV has been used widely to exam-

[1] Project supported by grant (G1998051110) from the Ministry of Science & Technology of China and grant (39900044) from National Natural Science Foundation of China.
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Received 2000-08-30 Accepted 2000-09-22

ISSN 0253-9756 Acta Pharmacol Sin 中国药理学报 2000 Dec; 21 (12)
E-mail aps@mail.shcnc.ac.cn Phn/Fax 86-21-6474-2629

ine the mechanisms of learning and memory[11].

The neurotransmitter, gamma-aminobutyric acid (GABA) and the $GABA_A$ receptors are rich in chick forebrain, especially in HV[12]. Muscimol, an agonist of $GABA_A$ receptor, has been found to impair memory retention in one-trial passive avoidance task following intracranial injection in the chick[13]. Since this memory-impairing effect of muscimol is reversed by the GABA antagonist, bicuculline[14], it is believed to be mediated through the $GABA_A$ receptor. Scopolamine, a muscarinic receptor antagonist, is also well known to impair learning and memory process in both animals and humans, presumably by blocking critical actions of the neurotransmitter, acetylcholine. In the present study we used muscimol and scopolamine impairment model in one-trial passive avoidance task to evaluate the ameliorating effects of huperzine A and the possibility of $GABA_A$-antagonism being involved.

MATERIALS AND METHODS

Animals Day-old Beijing White 939 chicks (male, weighing 40 g ± *s* 5 g) were commercially obtained from the Beijing Brood Chick Company.

Drugs Huperzine A (provided by Shanghai Institute of Materia Medica, Chinese Academy of Sciences), Scopolamine hydrobromide (Sigma Chemical Co) and muscimol (Sigma Chemical Co) were all dissolved in saline, and were bilaterally injected into HV in a volume of 10 μL per hemisphere. Huperzine A was injected in varying doses, 15 min before training. The other two drugs were injected in fixed doses (scopolamine, 100 ng in 20 μL; muscimol, 50 ng in 20 μL) 5 min before training. According to the chick brain atlas[15], the site of HV was 7 – 8 mm anterior ear bars, 2 mm lateral to the midline, and 3.5 mm ventral to the surface of the skull.

One-trial passive avoidance task Day-old chicks were placed in pairs into 20 cm × 20 cm × 25 cm wooden pens each illuminated with a 25 W light bulb and maintained at 25 – 28 ℃. After a 1-h equilibration, chicks were pre \ | trained by three 10-s presentations of a 2.5-mm diameter chrome bead coated with water. Each chick was then trained by a single 10-s presentation of a 5-mm diameter red bead coated with the bitter tasting methylanthranilate (MeA) either undiluted or dissolved in ethanol at 10 % concentration. Chicks which failed to show a clear disgust response (head shaking, bill wiping) on pecking the MeA coated red bead were discarded from further analysis. At 10, 30, 60, 90 or 120 min after training, each chick was tested by offering a dry red bead (identical to the one in training) and a novel blue bead (the same size as the red one), each for 10 s. Chicks that avoided the red bead on testing were regarded as remembering the task, those that pecked, as being amnesic.

Statistics Discrimination rate (DR) was calculated according to the formula: DR = Number of pecks at blue bead divided by total number of pecks at blue and red beads. The maximum value of DR is 1.0. A decline in DR means a reduction of the retention level for the discrimination task. One-way ANOVA followed by Duncan multiple-range test was used for comparison between the groups.

RESULTS

Effects of huperzine A on memory retention following weak training Chicks were trained on 10 % MeA and tested for retention at 60 min after training. The avoidance rate was used to evaluate memory retention. It was observed that retention levels were markedly improved in chicks treated with huperzine A (Fig 1). Analysis of the discrimination rate revealed significant overall group effects [$F_{(6,117)} = 9.76$, $P = < 0.001$]. A plot of the effects of huperzine A took a bell-shaped form with greatest positive effect on retention at 25 ng (100 ng, 50 ng, 25 ng, $P < 0.01$; compared with the saline control, respectively). Neither the lowest (6.25 ng, 12.5 ng, $P > 0.05$) nor the highest doses (200 ng, 400 ng, $P > 0.05$) had significant effects.

Effects of huperzine A on different memory

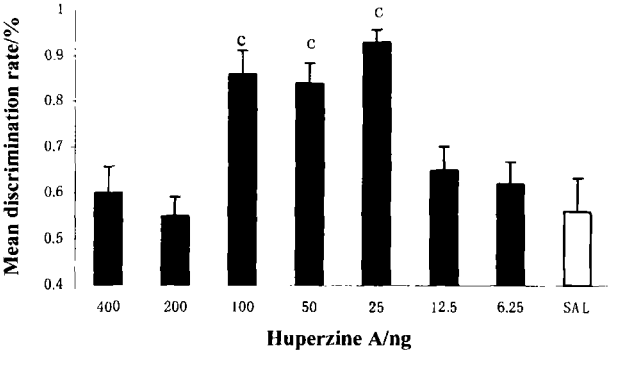

Fig 1. Effects of huperzine A on memory retention following weak training. $n = 16 – 19$. $\bar{x} \pm s$. Huperzine A or saline injected 15 min before training. $^cP < 0.01$ *vs* saline control.

stages Based on the results of the first experiment, the 25 ng dose of huperzine A was used for further studies on temporal effects. Chicks were trained on 10 % MeA and retention levels were tested at 10 to 120 min after training. Huperzine A treated chicks began to show improved avoidance rate 30 min post training and maintained this improvement at least to 120 min (30 min, 60 min, 90 min, 120 min, $P<0.01$ compared with the respective saline control groups). It was apparent that huperzine A had effects on improving intermediate-term and long-term memory but had no effects on short-term memory (Fig 2).

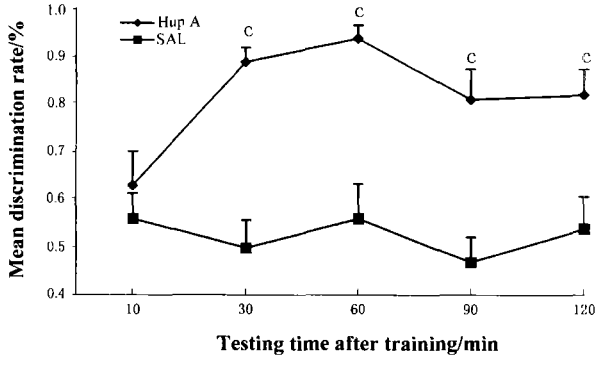

Fig 2. Effects of huperzine A (Hup A) on different memory stages. $n=17-19$. $\bar{x}\pm s$. Hup A or saline injected 15 min before training. $^cP<0.01$ *vs* saline control.

Effects of huperzine A on scopolamine-induced memory deficits Chicks were trained on 100 % MeA, and retention levels were tested at 10 to 90 min after training (Fig 3).

Scopolamine-treated groups showed low avoidance rates at all the testing time points. Huperzine A (25 ng) produced a significant effect on retention levels at 30, 60, and 90 min after training as compared to the corresponding time points of the groups treated with scopolamine alone (30 min, 60 min, 90 min, $P<0.01$). On the other hand, huperzine A had no effect at 10 min ($P>0.05$).

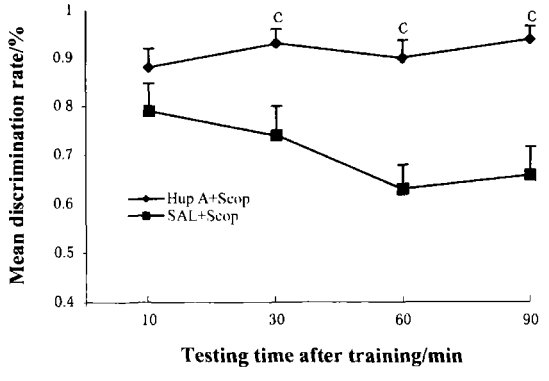

Fig 3. Effects of huperzine A (Hup A) on scopolamine (Scop)-induced memory deficits. $n=18-20$. $\bar{x}\pm s$. Hup A injected 15 min before training, Scop injected 5 min before training. $^cP<0.01$ *vs* saline + Scop.

Effects of huperzine A on muscimol-induced memory deficits Chicks were trained on 100 % MeA and tested at 10 to 90 min after training (Fig 4). Muscimol produced memory amnesia, with a lack of retention after 10 min. The retention levels were better in the huperzine A treated groups at 30, 60, and 90 min after training ($P<0.01$).

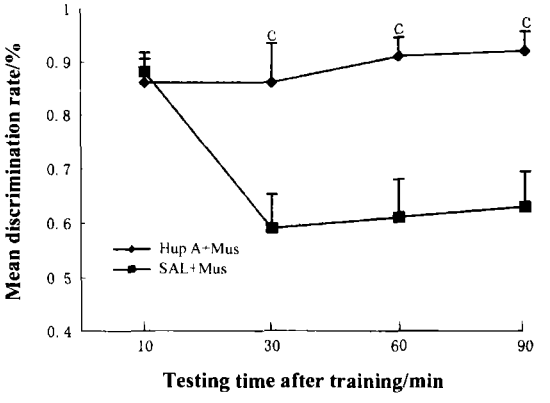

Fig 4. Effects of huperzine A (Hup A) on muscimol (Mus)-induced memory deficits. $n=19-20$. $\bar{x}\pm s$. Hup A injected 15 min before training, muscimol injected 5 min before training. $^cP<0.01$ *vs* saline + Mus.

DISCUSSION

Our results add to accumulating evidence that huperzine A is effective in alleviating memory deficits induced by a variety of means. Thus, when huperzine A was concurrently injected with scopolamine or muscimol, marked attenuation of the scopolamine-induced or muscimol-induced impairment were observed at 30 min to 90 min, but not at 10 min after training. These data indicated that huperzine A participated in the modulation of intermediate-term memory and long-term memory formation in passive avoidance task of chick.

The effects on muscimol treated chicks are especially interesting because of their implications that huperzine A may interact with GABAergic systems in the chick brain. Intracranial administration of the inhibitory neurotransmitter, GABA, produces a well-documented amnesic effect in humans and laboratory animals across a wide range of

ISSN 0253-9756 Acta Pharmacol Sin 中国药理学报 2000 Dec; 21 (12)
E-mail aps@mail.shcnc.ac.cn Phn/Fax 86-21-6474-2629

cognitive paradigms. The impaired working and reference memory in a double Y-maze paradigm, the impaired choice accuracy in discrimination task and the impaired retention of inhibitory avoidance all serve to confirm that GABA plays an important role in the performance of learning and memory[16-18]. In our study of chicks in a passive avoidance task, the dose-dependent impairment of memory formation by the $GABA_A$ agonist, muscimol, is consistent with the findings from other animal models.

Previous studies in different models of learning and memory suggested that huperzine A exerts its beneficial effects primarily by increasing synaptic acetylcholine levels through inhibition of the degradative enzyme, AChE[4-6]. However, our new finding that huperzine A significantly reversed the muscimol-induced memory deficits at 30, 60, and 90 min post training, suggests an additional action on GABAergic systems. It may be considered that huperzine A, besides inhibiting AChE, may also be acting as a competitive antagonist of the $GABA_A$ receptor. An alternative explanation for our results is that huperzine A indirectly reduces GABA-mediated memory attenuation by enhancing a physiological antagonism between acetylcholine and GABA. To distinguish these possibilities, direct studies of huperzine A binding at GABA receptors are needed.

Our data may have some relevance to the treatment of Alzheimer's disease (AD), a neurodegenerative disorder characterized by progressive deterioration of memory and cognition. Deficiencies in acetylcholine and GABA content have been observed in the cortical regions of Alzheimer brains[19]. Anatomical evidence also suggests an important interaction between GABA and cholinergic neurons in the septum and hippocampus[20], two additional centers of critical importance for learning and memory. Post training intraseptal injection of the $GABA_A$ agonist muscimol decreases hippocampal high-affinity choline transport along with acetylcholine turnover and releases[21]. Thus, it might be possible to reduce the major cognitive disturbances in Alzheimer patients by treatments with drugs that counteract abnormalities of GABA and acetylcholine levels. A drug which is able to enhance synaptic acetylcholine and also antagonizes the $GABA_A$ receptor could be ideal for that purpose. Huperzine A appears to be an especially promising candidate, since it reverses not only scopolamine-induced but also muscimol – induced memory deficits. These findings indicate that, in addition to its highly potent, selective inhibition of AChE, huperzine A improves memory amnesia through direct or indirect interactions with GABAergic systems. This evidence for a new mediative function of huperzine A confirms that huperzine A is a promising clinical drug for therapy of cognitive impairment observed with aging and Alzheimer's disease.

ACKNOWLEDGEMENTS The authors thank Prof ZHU Da-Yuan for providing huperzine A.

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石杉碱甲改善东莨菪碱和蝇蕈醇所致小鸡记忆障碍[1]

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关键词 胆碱脂酶类；石杉碱甲；东莨菪碱；蝇蕈醇；GABA；回避学习；记忆；小鸡

目的：研究石杉碱甲对东莨菪碱和蝇蕈醇所致记忆障碍的改善作用. **方法**：采用小鸡的一次性被动回避学习模型研究记忆形成过程，辨别率作为评价记忆保持水平的指标. **结果**：学习前 5 分钟，双侧上纹体腹核(hyperstriatum ventral HV)内注射东莨菪碱 100 ng 或蝇蕈醇 50 ng 显著降低小鸡的逃避率. 学习前 15 分钟，双侧 HV 内注射石杉碱甲 25 ng 能明显逆转东莨菪碱和蝇蕈醇所致记忆损害. **结论**：石杉碱甲在改善记忆形成的过程中，除了通过高选择性抑制乙酰胆碱脂酶外，还与调节 $GABA_A$ 受体有密切关系.

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