

Improving Effects of Huperzine A on Spatial Working Memory in Aged Monkeys and Young Adult Monkeys with Experimental Cognitive Impairment

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ABSTRACT

Our previous studies demonstrated that huperzine A, a reversible and selective acetylcholinesterase inhibitor, exerts beneficial effects on memory deficits in various rodent models of amnesia. To extend the anti-amnesic action of huperzine A to nonhuman primates, huperzine A was evaluated for its ability to reverse the deficits in spatial memory produced by scopolamine in young adult monkeys or those that are naturally occurring in aged monkeys using a delayed-response task. Scopolamine, a muscarinic receptor antagonist, dose dependently impaired performance with the highest dose (0.03 mg/kg, i.m.) producing a significant reduction in choice accuracy in young adult monkeys. The delayed performance changed from an average of 26.8/30 trials correct on saline control to an average of 20.2/30 trials correct after scopolamine administration. Huperzine A (0.01–0.1 mg/kg, i.m.) significantly reversed deficits

induced by scopolamine in young adult monkeys on a delayed-response task; performance after an optimal dose (0.1 mg/kg) averaged 25.0/30 correct. In four aged monkeys, huperzine A (0.001–0.01 mg/kg, i.m.) significantly increased choice accuracy from 20.5/30 on saline control to 25.2/30 at the optimal dose (0.001 mg/kg for two monkeys and 0.01 mg/kg for the other two monkeys). The beneficial effects of huperzine A on delayed-response performance were long lasting; monkeys remained improved for about 24 h after a single injection of huperzine A. This study extended the findings that huperzine A improves the mnemonic performance requiring working memory in monkeys, and suggests that huperzine A may be a promising agent for clinical therapy of cognitive impairments in patients with Alzheimer's disease.

Alzheimer's disease (AD) is a slowly progressive neuropsychiatric illness, principally characterized by memory deficits. There is a substantial body of experimental work in animals and humans suggesting that the cholinergic mechanism plays an essential role in AD (Davies and Maloney, 1976; Bartus et al., 1982; Coyle et al., 1983). Dysfunction in cholinergic mechanisms may contribute to age-related memory impairments. The retrograde loss of the cholinergic system from the basal forebrain is the most common and the most severe neurochemical consequence of the disease (Susan, 1997). The cholinergic neuron clusters of the basal forebrain innervate the hippocampus and areas of association in the cortex involved in higher processes such as long-term memory, working memory, and attention. In these structures, the concentration of choline acetyltransferase (ChAT) decreased, accompanied by the impaired ability of high-affinity choline transport and synthesis of acetylcholine (ACh). The severity of memory impairments seen in AD is consistent with dys-

function of the cholinergic system (Coyle et al., 1983). Many attempts have been made to correct the cholinergic deficiency at various levels of cholinergic functioning to reduce, if not cure, some of the major cognitive disturbances of AD patients. Some cholinomimetic agents have been shown to improve age-related cognitive impairments. Among various cholinomimetic drugs, the acetylcholinesterase (AChE) inhibitor as a palliative agent in the treatment of AD has been the most promising so far (Parnetti et al., 1997). Physostigmine and tacrine have shown some clinical efficacy in AD patients (Mohs et al., 1985; Summers et al., 1986). They are not, however, ideal drugs for clinical use due to the short duration of action, the low bioavailability, and the frequent side effects with physostigmine (Winblad et al., 1991) and dose-dependent hepatotoxicity of tacrine (Watkins et al., 1994). Thus the search for a new cholinesterase inhibitor (ChEI) with properties that could overcome the limitations in the use of physostigmine and tacrine is still ongoing (Giacobini, 1997).

In addition to the central cholinergic system, other transmitter systems such as the monoaminergic system are

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ABBREVIATIONS: AD, Alzheimer's disease; ChEI, cholinesterase inhibitor; ChAT, choline acetyltransferase; NE, norepinephrine; DA, dopamine; PFC, prefrontal cortex; 1-ANOVA-R, one-way analysis of variance with repeated measures.

thought to participate in causing dementia in AD patients (Palmer and DeKosky, 1993). There is evidence of interaction between cholinergic and monoaminergic systems in the control of cognitive cortical function (Riekkinen et al., 1990). The positive clinical effect of ChEIs such as tacrine has been related to stimulation of both cholinergic and monoaminergic systems (Alhainen et al., 1993). Therefore, the nootropic effects of ChEIs may involve cholinergic mechanisms as well as monoaminergic mechanisms.

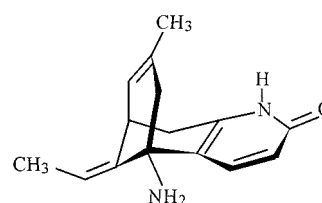
Huperzine A, a *Lycopodium* alkaloid isolated from the Chinese herb *Huperzia serrata* (Thunb) Trev, is a reversible and selective AChE inhibitor. The experiments showed that huperzine A can produce a long-term inhibition of AChE activity in rat brains and a sustained increase of ACh levels in the central nervous system (Tang et al., 1989). Compared with physostigmine, tacrine, and galanthamine, the AChE inhibitory effect of huperzine A is more potent, its selectivity for AChE other than butyrylcholinesterase is better, and its duration of inhibition is longer; its bioavailability is higher but the side effects are less (for review see Tang, 1996). It has been reported that huperzine A can produce a dose-dependent increase of other transmitters such as norepinephrine (NE) and dopamine (DA) in the rat cortex with either systemic or local intracerebral administration (Zhu and Giacobini, 1995). The previous studies in rodents showed that huperzine A improves performance in a variety of paradigms including spatial memory tasks (Tang, 1996) such as Y-maze (Tang et al., 1986; Lu et al., 1988) and the radial-arm maze (Xiong and Tang, 1995; Cheng et al., 1996). The duration of improving effects of huperzine A on learning and memory retention processes was longer than that of physostigmine or tacrine (Tang et al., 1994).

Scopolamine, a muscarinic receptor antagonist, has been shown in numerous studies to impair learning and memory under a variety of testing conditions, not only in small animals (Spencer and Lal, 1973), but also in monkeys (Ogura and Aigner, 1993; Rupniak et al., 1989) and in human (Ghoneim and Mewaldt, 1977; Rusted and Warburton, 1988); some of these impairments reflect neuropsychological similarities with the demented states in patients with AD (Molchan et al., 1992). The aged monkey is also a good candidate for studies of AD, because its behavioral impairments are similar to those that are characteristic of elderly human (Bartus, 1979). In particular, the similarities of neurochemical changes in aged monkeys with those in humans indicate that the aged monkeys may be a useful model for investigation of the age-associated transmitter abnormalities which are similar to those that occur in human (Wenk et al., 1989).

The aim of this study was to extend the findings as to whether huperzine A can improve the memory impairments in aged monkeys with a naturally occurring ACh decrease and in young monkeys with an experimental disruption of cholinergic system using scopolamine. The chemical structure of huperzine A is shown in Fig. 1.

Materials and Methods

Subjects. The subjects in this study consisted of eight rhesus monkeys (*Macaca mulatta*). Four young female monkeys (three were approximately 6–7 years old; one was approximately 4 years old) were used to evaluate the effect of huperzine A on scopolamine-induced memory impairments. The four aged monkeys (two females



(-)-Huperzine A

Fig. 1. Chemical structure of huperzine A.

and two males approximately 16–18 years old) were used to study the effect of huperzine A on age-related memory deficits. Because actual birth dates were unavailable, ages were estimated on the basis of prior breeding and behavioral testing records, dental records, and general appearance. Rhesus monkeys in captivity have been reported to live 20 to 25 years and longer. All young adult subjects were drug-naïve, whereas all aged subjects had prior behavioral testing experience, but none had been involved in drug tests in the 1 year preceding the present investigation. All animals were housed individually under standard laboratory conditions. Feeding occurred immediately after cognitive testing. Daily supplements of fruits and vitamins were also given. Water was available ad libitum.

Delayed-Response Testing. Monkeys were tested in the Wisconsin General Testing Apparatus. The test tray contained a left and a right food well spaced 15 cm apart. An opaque screen was lowered to separate the monkey from the test tray. For testing sessions, the test panel was attached to the home cage. While testing was in progress, the light in panel was on so that the monkey could see clearly what happened in the panel. Highly palatable food rewards (e.g., peanuts, raisins, or sugar chips) were used during testing to minimize the need for dietary regulation. The monkeys were tested daily at the same time of day in a quiet room by a trained observer. Using these conditions, no problems with motivation were evident.

The monkeys had previously been trained on the two-well, delayed-response task. During delayed response, the animal watched as the experimenter baited one of two food wells. The food wells were then covered with identical cardboard plaques, and an opaque screen was lowered between the animal and the test tray for a specified delay. At the end of this delay, the screen was raised and the animal was allowed to choose. Reward was quasi-randomly distributed between the left and right wells over the 30 trials that made up a daily test session. During the initial training phase, delays were held constant during a daily session and were gradually increased from 0 s according to a step-wise procedure over the 1000 trials.

Following the 1000 trials, the monkeys were prepared for drug testing. To observe the effects of drug on memory capacity, the animals were trained on a variable delayed-response task in which five different delay lengths were distributed over the 30 trials that made up the daily test session. For four aged monkeys, delays were adjusted until the animals exhibited stable baseline performance of approximately 67% correct. For example, the range of delays for aged monkey no. 35 was 0, 6, 12, 18, and 24 s. All aged subjects performed perfectly at 0-s delays and exhibited increasing difficulty with progressively longer delays, a pattern consistent with memory impairment. In young adult monkeys, delays were chosen to produce performance levels of about 90% correct out of 30 trials. For example, the range of delays was 0 to 20 s for monkey no. 19 and 0 to 8 s for monkey no. 36. The 0-s delay consisted of lowering the screen and immediately raising it again. Once performance was demonstrated to be stable at this baseline, drug treatment was initiated.

Drug Administration. Huperzine A (provided by the Department of Phytochemistry, Shanghai Institute of Materia Medica, Chinese Academy of Sciences) and scopolamine hydrobromide (Sigma Chemical Co., St. Louis, MO) were both dissolved in sterile 0.9% saline before injection. Scopolamine or saline and huperzine A or

saline were injected i.m. 30 min and 20 min, respectively, before delayed-response testing. The injection volume was kept constant at 0.1 ml/kg irrespective of dose.

The doses of scopolamine were 0.01, 0.02, and 0.03 mg/kg; huperzine A was coadministered with the highest dose of scopolamine (0.03 mg/kg) to young adult monkeys. At this dose of scopolamine, all the young subjects exhibited significant memory impairments so that there was enough room to test the effects of huperzine A. The doses of huperzine A were 0.001, 0.01, 0.1, and 0.2 mg/kg for young subjects and 0.0001, 0.001, 0.01, and 0.1 mg/kg for aged subjects. A wide range of doses was selected to ensure the optimal dose within it for each monkey.

Drugs were administered no more than twice per week (Monday-Saturday), and at least 3 days separated test sessions. Control injections of saline alone were given the day before each drug testing to assure that the performance was back to the baseline level. The experimenter testing the monkeys was unaware of the drug treatment conditions.

Data Statistics. Delayed-response performance on drug was compared with matched placebo (saline) control performance for the same week. Because the animals served as their own controls, statistical analyses employed repeated measures designs: one-way analysis of variance with repeated measures (1-ANOVA-R), and, if appropriate, followed by post hoc tests. The level of significance was $P < .05$.

Results

Effects of Scopolamine on Delayed-Response Performance in Young Adult Monkeys. Scopolamine at the doses of 0.01, 0.02, and 0.03 mg/kg produced a dose-related impairment in the performance of young monkeys [1-ANOVA-R: $F(3,9) = 6.66, P = .0116$] with the highest dose of scopolamine causing a significant disruption of choice accuracy in all of the young animals [$F(1,3) = 43.78, P = .0070$] (Fig. 2). After the 0.03-mg/kg dose, performance at all retention intervals was impaired but the magnitude of this effect increased as the retention interval lengthened (Fig. 3) [$9.7 \pm 4.7\%$, $10.3 \pm 7.2\%$, $25.7 \pm 8.7\%$, $30.5 \pm 14\%$, and $33.3 \pm 2.5\%$ decreases at A (0 sec), B, C, D, and E delays respectively]. 1-ANOVA-R suggested no significant effect of scopolamine at 0-s delays [$F(1,3) = 4.42, P = .1263$], but significant effect was found at the longest delays [$F(1,3) = 182.65, P = .0009$]. At the highest dose of scopolamine (0.03 mg/kg), some signs of the side effects of cholinergic antagonism, such as a slower

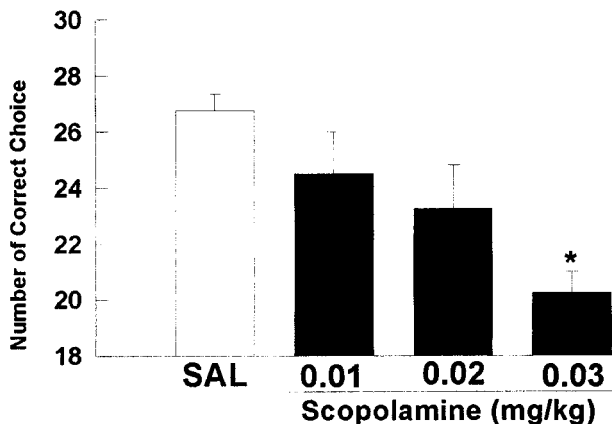


Fig. 2. Effects of scopolamine on delayed response task in young adult monkeys ($n = 4$). Saline or scopolamine administered i.m. 30 min before testing. Values represent mean \pm S.E.M. number of trials correct out of a possible 30 trials. * $p < .05$ versus saline control.

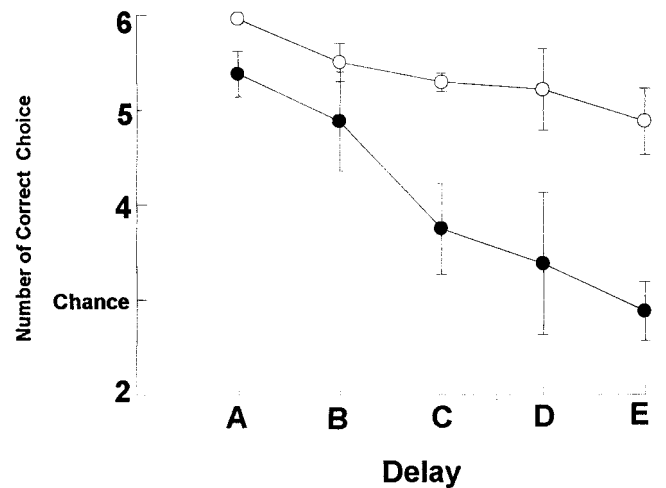


Fig. 3. Effects of scopolamine (0.03 mg/kg, ●) compared with saline (○) on delayed response performance for each of five delay intervals (A, B, C, D, and E) used in each testing session in young adult monkeys ($n = 4$). The A delay was always 0 s; the B, C, D, and E delays were incrementally increasing delays (e.g., 5, 10, 15, and 20 s) individually selected for each animal to produce an overall baseline performance of about 67% correct (see *Materials and Methods*). Values represent mean \pm S.E.M. number correct out of a possible six trials at each delay interval.

rate of chewing than usual and pupillary dilation, were observed.

Effects of Huperzine A on Scopolamine-Induced Deficit of Delayed Performance in Young Adult Monkeys. Huperzine A markedly improved the delayed-response performance of scopolamine-treated monkeys (Fig. 4A) [1-ANOVA-R: $F(4,12) = 14.3, P = .0002$]. The dose-response curve was bell-shaped with the maximum improvements at 0.1 mg/kg [$15 \pm 2.9\%$ increase, 1-ANOVA-R: $F(1,3) = 27.0, P = .0138$, compared with scopolamine control]. Neither the lowest nor the highest doses had effects [$F(1,3) = 2.45, P = .23$ for 0.001 mg/kg; $F(1,3) = 2.46, P = .21$ for 0.2 mg/kg] (e.g., young monkey no. 36, Fig. 4B). The beneficial effects of huperzine A were most evident at the longest delays [$27.1 \pm 2.5\%$ increases, $F(1,3) = 10.50, P = .048$].

Effects of Huperzine A on Delayed-Response Performance in Aged Monkeys. Administration of huperzine A to aged monkeys produced a significant effect on delayed-response performance [1-ANOVA-R: $F(4,12) = 11.26, P = .0005$]. As can be seen in Fig. 5A, huperzine A produced a bell-shaped dose-response curve similar to the one described above in scopolamine-treated young monkeys. There were variances between the performance of four aged subjects. Of these four doses (0.0001–0.1 mg/kg), the best dose was 0.001 mg/kg for two monkeys, and for the remaining two animals the best dose was 0.01 mg/kg (e.g., monkey no. 34, Fig. 5B). The improvements following the best doses were most apparent at two longer delays (Fig. 6) [$F(1,3) = 16.94, P = .026$; $F(1,3) = 12.63, P = .0380$, respectively]. However, performance at 0-s delays did not change between monkeys on saline and on huperzine A [$F(1,3) = .36, P = .59$]. These results are consistent with changes in cognitive performance rather than a nonspecific performance variable, which would be expected to disrupt performance after the 0-s delay control trials.

During the sessions conducted 24 h after huperzine A injection at the dose of 0.01 mg/kg and 0.1 mg/kg, the improving performance remained evident (Fig. 7). Moreover,

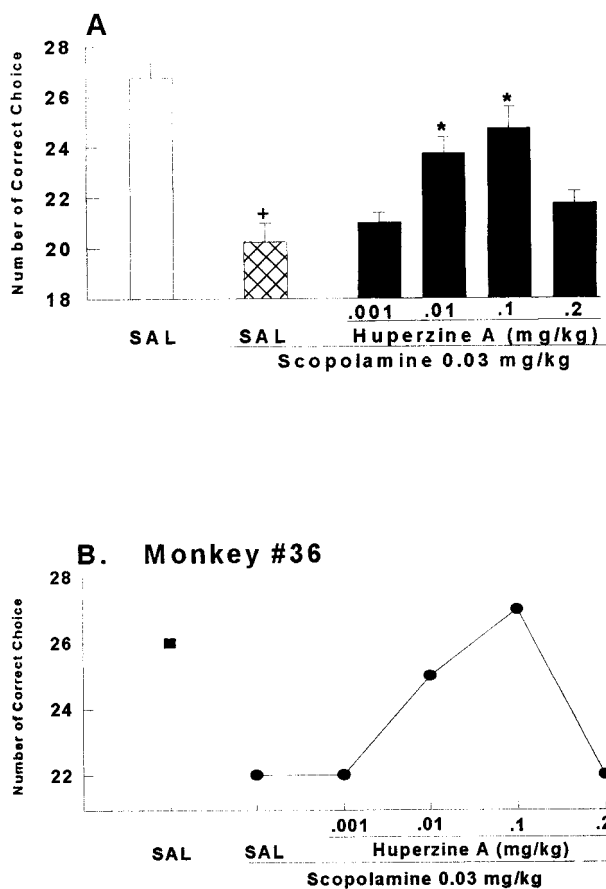


Fig. 4. Effects of huperzine A on the deficit of delayed-response performance induced by scopolamine in young adult monkeys. Saline or huperzine A was administered i.m. 20 min before testing. Scopolamine was administered 30 min before testing. A, huperzine A produced a dose-related improvement in the delayed-response performance of young monkeys ($n = 4$). Values represent mean \pm S.E.M. number of trials correct out of a possible 30 trials. $^+p < .05$ versus saline control; $^*p < .05$ versus scopolamine control. B, effects of monkey no. 36. Values represent mean number of trials correct out of a possible 30 trials.

these long-lasting beneficial effects were possibly dose dependent [24 h after 0.1 mg/kg, $10.8 \pm 0.82\%$ increases $F(1,3) = 172.166, P = .0010$; 24 h after 0.01 mg/kg, $5.5 \pm 2.23\%$ increases, $F(1,3) = 12.24, P = .0395$]. But performance had returned to baseline level by the sessions conducted 48 h after injection [$F(1,3) = 1.47, P = 1.000$; $F(1,3) = 3.000, P = .1817$, respectively]. Huperzine A was tolerated by all the monkeys even at the highest doses. No adverse signs were observed.

Discussion

Scopolamine has been used as a pharmacological tool for understanding pathological impairments such as AD, because it produces amnesiac effects similar to those identified in AD (Sakhakian et al., 1987). In this study, scopolamine dose dependently impaired spatial working memory of young adult monkeys in delayed-response tasks, consistent with the previous reports using other paradigms of delayed-response tasks in monkeys (Rupniak et al., 1989; Ogura and Aigner, 1993), indicating that spatial working memory processes are dependent upon the integrity of the brain cholinergic system. The capacity to perform these tasks requires the bilateral

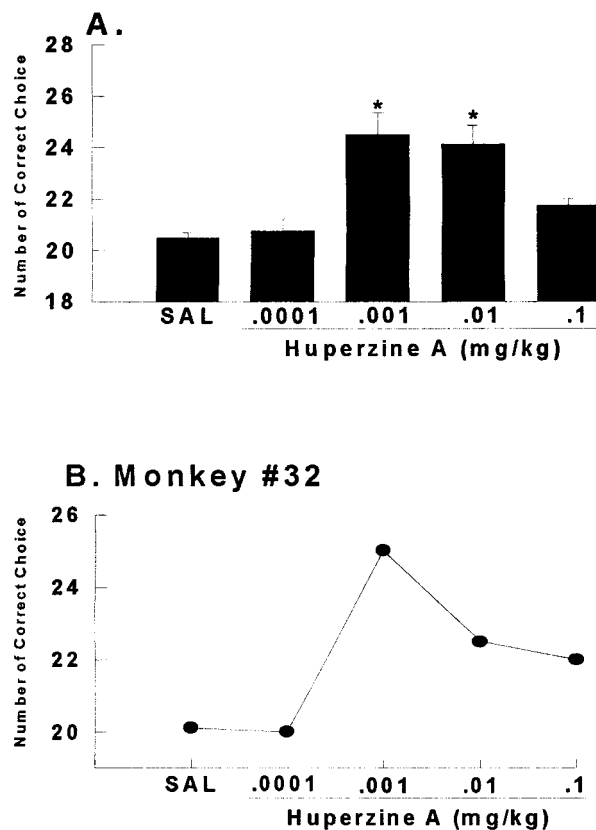


Fig. 5. Effects of huperzine A in aged monkeys. Saline or huperzine A was administered i.m. 20 min before testing. A, huperzine A produced a dose-related improvement in the delayed-response performance of aged monkeys ($n = 4$). Values represent mean \pm S.E.M. number of trials correct out of a possible 30 trials. B, effects of huperzine A in monkey no. 32. Values represent mean number of trials correct out of a possible 30 trials.

integrity of the dorsolateral prefrontal cortex (PFC) at both short and long delays and the hippocampus mainly at long delays (>15 sec) (Goldman-Rakic, 1987), which receive a massive projection of cholinergic axons originating in basal forebrain. So reduced-choice accuracy caused by scopolamine is due to disruption of the cholinergic system in PFC and the hippocampus through blockade of muscarinic postsynaptic receptors in synaptic clefts. The fact that there was a more significant decrease at longer delays than at shorter delays, although choice accuracy at all retention intervals decreased after scopolamine, showed that the major effects appear to be directly on memory processes.

Huperzine A significantly improved the performance of scopolamine-treated monkeys, producing a bell-shaped dose-response curve, similar to previous findings in rodents (Xiong and Tang, 1995; Cheng et al., 1996). The bell-shaped dose-response curve is common with most drugs that have been reported to exert cognitive enhancing actions; the precise mechanisms of this effect remain to be established. Smaller doses of huperzine A stimulate cognitive function through increasing ACh levels, whereas larger doses mask it via an unknown mechanism. Huperzine A produced AChE inhibition in whole brain or brain regions in a dose-dependent manner following peripheral administration (Tang et al., 1994; Wang and Tang, 1998). Because huperzine A shows no significant affinity for muscarinic receptors (Tang et al., 1989), no evident pre- and postsynaptic effects (Lin et al.,

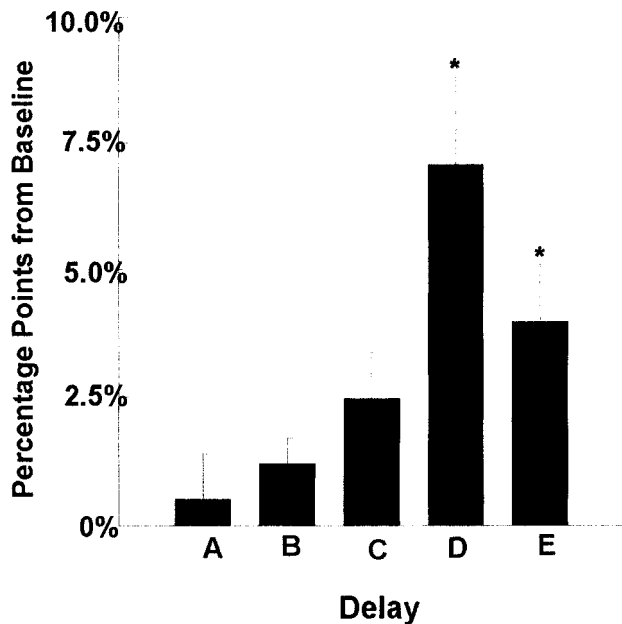


Fig. 6. Effects of huperzine A at the best doses compared with saline control on delayed-response performance for each of the five delayed intervals (A, B, C, D, and E) used in each testing session in aged monkeys ($n = 4$). The number of trials correct on saline was subtracted from the number of trials correct on huperzine A; this difference score was then multiplied by 3.3% because each trials constituted 3.3% of the total number of trials: [(number correct huperzine A – number correct saline) \times 3.3%]. Values in the figure represent mean \pm S.E.M. * $p < .05$ versus saline control.

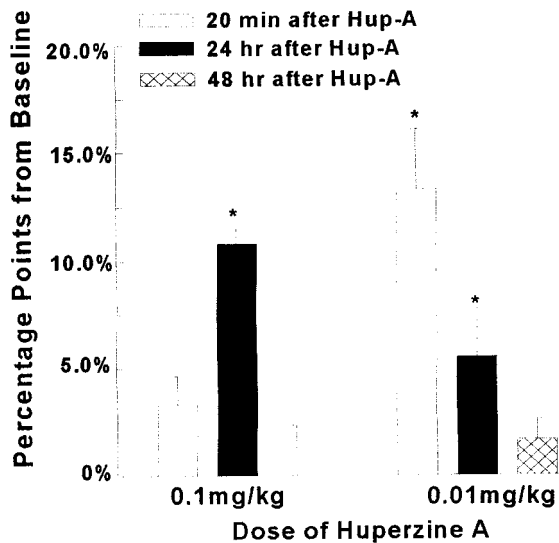


Fig. 7. Effects of huperzine A [0.1–0.01 mg/kg] on delayed-response tasks in aged monkeys ($n = 4$). Performance was measured 20 min and 24 and 48 h after i.m. injection of huperzine A, respectively. The number of trials correct on saline was subtracted from the number of trials correct on huperzine A; this difference score was then multiplied by 3.3% because each trials constituted 3.3% of the total number of trials: [(number correct huperzine A – number correct saline) \times 3.3%]. Values in the figure represent mean \pm SEM. * $p < .05$ versus saline control.

1997), and no effect on ChAT (Tang et al., 1994), its effects on spatial memory in tasks are due primarily to the dose-dependent increase in ACh resulting from direct AChE inhibition. The degree of ACh elevation after huperzine A is selectively highest in frontal and parietal cortex and there are smaller increases in other brain regions (Tang et al., 1989). Consid-

ering that ACh is particularly low in the cerebral cortex of AD patients (Bowen et al., 1983), this particularly regional specificity may constitute a therapeutic advantage.

It has been observed that in aged monkeys the number of nicotinic and muscarinic type-1 receptors decreased only slightly with aging, which suggests that postsynaptic indicators of cholinergic function are only mildly impaired with aging. However, the level of ChAT decreased significantly with aging (Wenk et al., 1989). It is mainly because of the loss of cholinergic neurons in forebrain basal nucleus, which projected into the PFC and hippocampus. These age-related changes may underlie a decline in cognitive abilities. Similar to the results found in scopolamine-treated monkeys, the improving efficacy of huperzine A in aged monkeys also exhibited a bell-shaped response. However, the optimal dose is smaller than that for scopolamine-treated monkeys. High-choice accuracy at 0-s delays (about 95%) and unaffected visual discrimination (Bartus and Dean, 1979) shows that the deficits in aged monkeys did not appear to be due to problems with perceptual sensory processing but with memory. The increased ACh concentration induced by huperzine A supplemented the impaired ability of ACh synthesis caused by the decreased level of ChAT, so that the cholinergic transmission is restored close to normal in a certain period. On the other hand, biochemical, electrophysiological, and behavioral studies have indicated an interaction between the cholinergic and noradrenergic systems, as well as the dopaminergic system (Decker and McGaugh, 1991), which influences learning and memory (Kruglikov, 1982). After administration of huperzine A, NE and DA levels were significantly increased over baseline for several hours, whereas ACh levels reach a maximum. These increases in NE and DA levels may be related to the increase of extracellular ACh levels through subcortical mechanism (Zhu and Giacobini, 1995). Therefore, the effects of huperzine A on memory may be associated with the increased levels of NE and DA, which decreased significantly with aging in monkeys (Wenk et al., 1989).

In this study, the performance of all aged subjects after administration of huperzine A was improved. But wide variations in the most-effective dose of huperzine A in aged subjects were observed. This finding is consistent with various levels of loss in the cholinergic system in aged monkeys, suggesting that in clinical therapy the optimal doses of huperzine A must be selected according to pathological situation of the patients with AD. Their overall response as a group, however, was less variable than that of physostigmine-treated aged monkeys in earlier studies (Bartus, 1979), in which some subjects did not benefit from physostigmine at all. The performance of all aged subjects after administration of huperzine A was improved. In the present study, a consistent finding was the long-lasting effect of huperzine A on delayed-response tasks. The performance was significantly different as compared with saline control even 24 h after a single injection of huperzine A (0.1, 0.01 mg/kg) and showed a possible dose-dependent manner. The facts that the terminal half-life of huperzine A was 288 min in humans (Qia et al., 1995), and that huperzine A could produce a long-term inhibition of AChE activity in brain (Wang and Tang, 1998), suggested that the long-lasting effects of huperzine A on cognitive function might simply result from its AChE inhibition.

Compared with rodents, rhesus monkeys may be more

sensitive to pharmacological manipulation of central cholinergic systems (Matsuoka and Aigner, 1997). In addition, primates are able to perform many complex behavioral tasks identical to those impaired in human amnesiac states, including dementia (Freedman and Oscar-Berman, 1986; Sakhakian et al., 1988), in which the delayed-response performance is commonly used to test the mental status of nonhuman primates. Memory-impaired humans show significant performance deficits when tested by this kind of task (Rice, 1987), and the fact that these tasks are sensitive to the impairments associated with human memory loss, supports the validity of using nonhuman primates performing delayed-response tasks as models for development of drugs designed to improve human memory. Huperzine A could improve memory deficits either induced by scopolamine in young adult monkeys or occurring naturally in aged monkeys. These findings extend our previous studies in rodents, in which huperzine A markedly reversed the memory impairments induced by central cholinergic blockade with scopolamine treatment, lesions of the nucleus basalis magnocellularis, or aging (Lu et al., 1988; Cheng et al., 1996; Xiong et al., 1998). Taken together, these results can confirm that huperzine A is a promising candidate for clinical evaluation as treatment for AD.

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