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Effect of Huperzine A, a Novel Acetylcholinesterase Inhibitor, on Radial Maze Performance in Rats

ZHI QI XIONG AND XI CAN TANG[1]

Department of Pharmacology, Shanghai Institute of Materia Medica, Chinese Academy of Sciences, Shanghai 200031, P. R. China

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XIONG, Z. Q. AND X. C. TANG. *Effect of huperzine A, a novel acetylcholinesterase inhibitor, on radial maze performance in rats.* PHARMARCOL BIOCHEM BEHAV 51(2/3) 415–419, 1995.—Rats were trained to run in a spatial, radial arm maze using a procedure to determine two memory functions, working and reference memory. The muscarinic antagonist, not the nicotinic antagonist, impaired both working and reference memory of rats. Scopolamine (0.125, 0.15, and 0.2 mg/kg, IP, 30 min before a session) significantly impaired choice accuracy in the eight-arm maze. In contrast, mecamylamine (5, 10, and 15 mg/kg) did not affect the performance. Huperzine A (0.1, 0.2, and 0.3 mg/kg, IP, 30 min before testing) and physostigmine (0.3 mg/kg, IP, 20 min before testing) could reverse scopolamine-induced deficits in the task. Chronic treatment with huperzine A (0.25 mg/kg, PO, once a day) for 8 consecutive days was as potent as acute treatment on attenuating the scopolamine-induced amnesia.

Huperzine A Physostigmine Scopolamine Mecamylamine Working memory Reference memory Radial maze Cholinesterase inhibitor

THERE is considerable pharmacological evidence that the central cholinergic system plays an important role in learning and memory. Interest in the functions of the central cholinergic system has been stimulated by the proposal that their decline may underlie the part of the cognitive deterioration seen in normal and pathologic aging (1,3,4). Postmortem analysis of the brains of patients with senile dementia of the Alzheimer type has shown a decline in central cholinergic activity that correlates with their previous mental test scores (3,10,14). Analogous to the dopamine replacement treatment approach employed in Parkinson's disease, therapeutic trials of cholinomimetics, including cholinesterase inhibitor (ChEI), have aimed at augmenting cerebral cholinergic transmission. Thus far, only two cholinomimetic compounds, physostigmine (Phys) and tacrine, have been evaluated on a large scale in dementia. However, the therapeutic effect of Phys is limited by its short duration of action, narrow therapeutic window, and peripheral cholinergic effects (20). Tacrine at higher doses exhibited hepatotoxicity, which limits its clinical value (5). An ideal ChEI suitable for treatment of memory and cognition impairment should produce a long-term acetylcholinesterase (AChE) inhibition with minimal side effects at therapeutic dose. Such requirement has not been met so far. Huperzine A (Hup-A, Fig. 1) is a new *Lycopodium* alkaloid isolated from Chinese herb *Huperzia serrata* (Thunb.) Trev. (9). It is a potent and selective acetylcholinesterase inhibitor (19) with better therapeutic indices than that of Phys (16). Hup-A has been reported to improve the performance in the learning and memory task of both normal and memory-impaired animals (17,18). Therefore, Hup-A deserves further study as a promising candidate for therapy of cognitive impairment with aging and Alzheimer's disease. Both scopolamine, a muscarinic antagonist, and mecamylamine, a nicotinic antagonist, were reported to disrupt memory (2,8,11,12,21). The aim of the present study was to validate the effects of Hup-A on disruption of spatial memory induced by scopolamine and mecamylamine in a radial maze. Phys was selected for comparison with Hup-A.

METHOD

Subject

Thirty-five experimentally naive male albino rats of the Sprague-Dawley strain weighing 230–280 g were used. The

[1] To whom requests for reprints should be addressed.

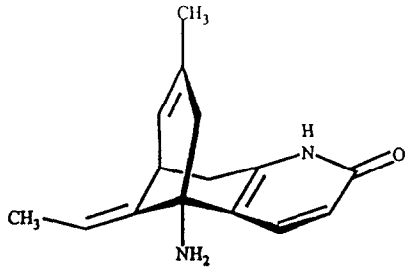

FIG. 1. Chemical structure of huperzine A.

rats were housed individually in a climatically controlled room on a 12L : 12D cycle, and were allowed access to water and a standard diet to maintain 80–85% (adjusted for growth) of original body weight throughout the experiment.

Apparatus

The plastic radial arm maze was elevated 70.5 cm above the floor, and had an octagonal center platform with eight arms radiating from the center. The platform was 51.5 cm in diameter, and each arm was 61 cm long and 12 cm wide. Plexiglas walls were 10 cm high extending along the length of each arm. Food wells, located 3 cm from the distal end of each arm, were 1 cm deep and 2 cm in diameter. The test room was well lit by 40-W fluorescent tubes; several visually distinct cues (e.g., wall, picture, light) were present in the room and remained in the same position with respect to the maze.

Procedure

The experimental procedure was similar to those described by Wirsching et al. (21). In brief, deprivation was begun 1 week prior to training. On the seventh day, each rat was placed on the central platform and allowed to explore the paths and to consume food (dustless pellet, 45 mg) scattered in the whole maze. This procedure was repeated for 3 consecutive days. Following the habituation period, rats received one training session daily, 7 days a week. At the start of each session, only four predetermined arms were used as the baited arms. The baiting pattern remained the same throughout the experiment but varied from rat to rat. The variation of the baited set for different rats limited the development of odor cues within the maze as well as controlling for any directional preference with respect to the extramaze cues. The rat was placed on the central platform and left until either all four baited arms were chosen or 10 min had elapsed.

A correct response was defined as the first entry to a baited arm. Three types of error were recorded. Reentry to a baited arm was regarded as a working memory (WM) deficit. First entry to an unbaited arm was considered as an error in reference memory (RM), and reentry to an unbaited arm was regarded as a deficit of both WM and RM (WRM). Rats were trained to a criterion of at most one error over four consecutive trials (range 26–38 days). Once a rat reached the criterion, training for this rat was reduced to twice a week until all rats reached the criterion.

Drug Testing

Testing began once all rats reached the criterion. Scopolamine hydrobromide (Sigma Chemical Co.), mecamylamine hydrochoride (Sigma Chemical Co.), physostigmine salicylate (Fluka Chemie), and huperzine A (provided by Phytochemistry Department, this Institute) were all dissolved in saline, and were administered in a volume of 1 ml/kg body weight. Phys was administered IP 20 min before behavioral testing because the drug has been shown to have a short half-life after systemic injection. All other drugs was administered IP 30 min prior to testing. Injection trials were made at 1-week intervals, and rats were retrained to the prior criterion before the next dose level of drug was given.

To test the chronic effect, Hup-A was orally administered at a dose of 0.25 mg/kg daily for 8 consecutive days at 0830–0930 h. Control subjects received a similar regime of saline administration. Rats were tested on the eighth day.

RESULTS

Effects of Scopolamine on Working and Reference Memory of Rats in the Radial Maze

All data plotted in the figures were expressed as mean ± SEM. The effects of the drugs on the number of errors were analyzed using a one-way analysis of variance (ANOVA) followed by Duncan's Multiple-Range test. Data from the rats that failed to learn the task ($n = 4$) were excluded from the statistical analysis.

The mean numbers for the three types of error (WM, RM, and WRM) under test conditions are shown in Fig. 2. The difference between conditions were significant for each type of error as indicated by ANOVA [$F(3, 40) = 10.55$ for RM errors, $F(3, 40) = 30.06$ for WM errors, and $F(3, 40) = 5.29$ for WRM errors, all $p < 0.01$]. Post hoc contrasts (Duncan's Multiple-Range test) indicated that the WM and RM errors were both significantly higher under scopolamine (0.125, 0.2 mg/kg, IP) than under control condition ($p < 0.01$). The mean number of WRM errors caused by scopolamine (0.125, 0.2 mg/kg) was significantly ($p < 0.05$) increased. However, there was no significant difference in errors between scopolamine (0.05 mg/kg) and saline ($p > 0.05$).

Effects of Mecamylamine on Spatial Memory of Rats in the Radial Maze

The mean number of two types of error (RM, WM) did not changed significantly under mecamylamine (5, 10, and 15 mg/

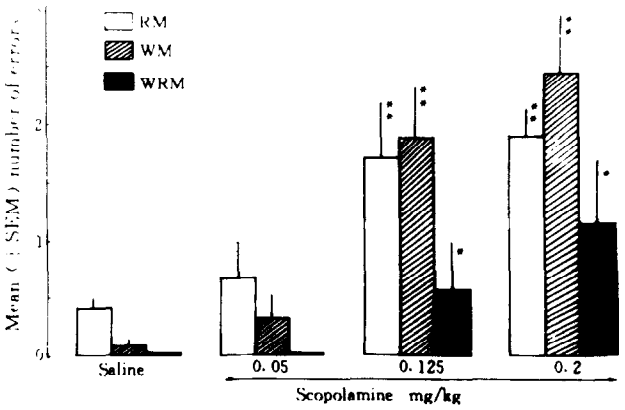

FIG. 2. Effects of scopolamine on working and reference memory of rats in the radial maze. Each bar represents the mean ± SE of 24 animals for the saline and 7–8 animals for the scopolamine. Saline or scopolamine hydrobromide was administered IP 30 min before testing. $^{*}p < 0.05$, $^{**}p < 0.01$ compared with the saline group.

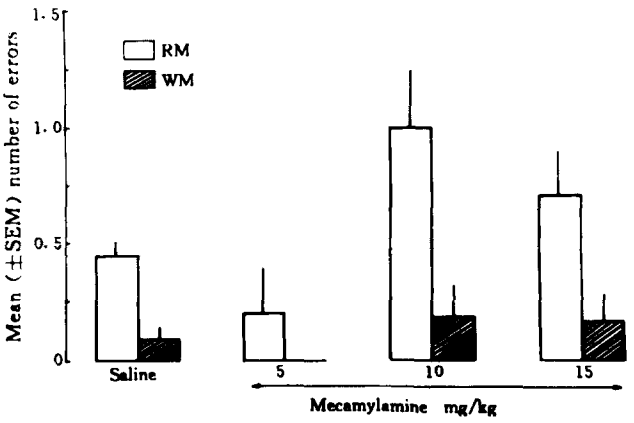

FIG. 3. Effects of mecamylamine on radial maze performance in rats. Saline or mecamylamine was given IP 30 min before testing. Data represent mean ± SEM of 7-8 animals in each group.

kg, IP) treatment [$F(3, 38) = 2.04$ for RM, $F(3, 38) = 0.38$ for WM]. Additional analysis with Duncan's Multiple-Range test indicated that the types of error were not significantly affected by the different dose levels ($p > 0.05$) (Fig. 3).

Effects of Hup-A and Phys on Working and Reference Memory Deficits Induced by Scopolamine

Scopolamine (0.2 mg/kg, IP, 30 min before testing) significantly increased the number of three types of error [$F(1, 33) = 55.45$ for RM errors, $F(1, 33) = 51.25$ for WM errors, and $F(1, 33) = 38.50$ for WRM errors, all $p < 0.01$] (Fig. 4). The difference in the number of errors between scopolamine-treated rats with and without Hup-A was significant at doses ranging from 0.1 to 0.3 mg/kg [$F(4, 36) = 2.64$, $p < 0.05$ for RM errors, $F(4, 36) = 7.61$, $p < 0.01$ for WM errors, and $F(4, 36) = 4.84$, $p < 0.01$ for WRM errors]. The scopolamine-induced memory deficit was reversed by Phys (0.3 mg/kg, IP) [$F(1, 16) = 20.86$, $p < 0.01$ for RM errors, $F(1,16) = 11.68$, $p < 0.01$ for WM errors, and $F(1, 16) = 6,37$, $p < 0.05$ for WRM errors]. Hup-A at 0.05 mg/kg did not affect the radial maze performance disrupted by scopolamine. There was no significant difference between 0.3 mg/kg Hup-A and 0. 3 mg/kg Phys [$F(1, 13) = 0.73$ for RM errors, $F(1, 13) = 0.54$ for WM errors].

Effect of Hup-A After Chronic Treatment on Scopolamine-Induced Memory Deficit

RM errors, WM errors, and WRM errors were significantly increased by scopolamine (0.15 mg/kg, IP, 30 min before testing) compared to saline group [$F(1, 19) = 102.60$, 119.36, and 16.89, respectively, all $p < 0.01$] (Fig. 5). The decrease was significant for RM errors, WM errors, and WRM errors by single dose and eight repeated doses of oral Hup-A (0. 25 mg/kg) on scopolamine-induced deficit [$F(2, 21) = 15.00$, 16.03, and 6,67, respectively, all $p < 0.01$]. There was no significant difference between chronic and acute Hup-A treatment in RM errors, WM errors, and WRM errors [$F(1, 15) = 1.99$, 0.22, and 1.54, respectively].

DISCUSSION

The radial maze is a valuable apparatus in the study of spatial memory. It offers an alternative that closely resembles the natural food-seeking behavior of species such as rats. Scopolamine has been used in many studies to assess the possible role of cholinergic mechanisms in maintaining accurate spatial memory (11). There has been inconsistency in the studies that examine both types of memory in a radial maze. Wirsching et al. reported that scopolamine impaired working memory selectively (21). We did not find a dissociation between the working and reference memory. Lower doses of scopolamine (0.125, 0.15, and 0.2 mg/kg) impaired both working and reference memory in the radial maze. Our results were consistent with those of Okaichi et al. (11). Difference in the amount of training may account for some of the discrepancy in studying the effects of antimuscarinic drug on the two types of memory. We trained rats to a criterion to decrease baseline error rates, which permitted the deficit in reference memory to be manifest.

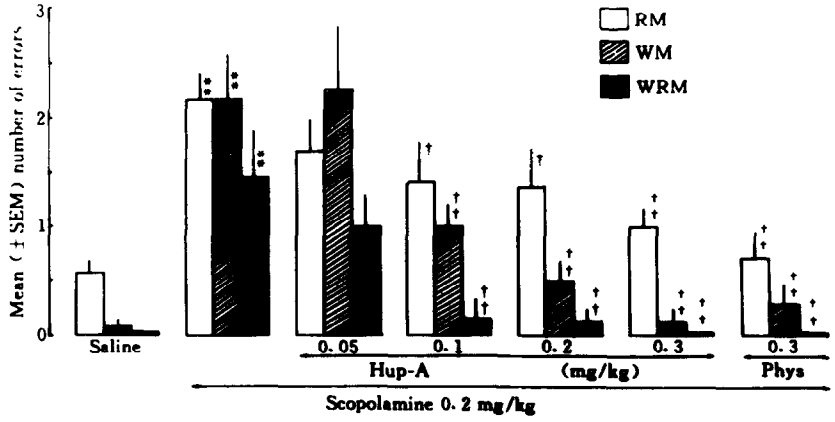

FIG. 4. Effects of Hup-A and Phys on scopolamine-induced disruption of radial maze performance in rats. Saline, scopolamine, or Hup-A was administered IP 30 min before testing. Phys was administered 20 min before testing. Data represent mean ± SEM of 7-8 animals in each group. $^{**}p < 0.01$ compared with the saline group. $\dagger p < 0.05$, $\dagger\dagger p < 0.01$ compared with scopolamine alone.

Mecamylamine, the principal nicotinic antagonist, has been found to impair performance of rodents in several tasks including passive avoidance and the working memory of the radial-arm maze (8,12). Levin et al. found mecamylamine (10 mg/kg) impaired performance in the radial maze study (8). But Clarke and Fibiger did not find significant memory deficits in a spatial alternation task with mecamylamine (up to 5 mg/kg) (2). Our finding was in agreement with that of Clarke and Fibiger. Mecamylamine 5–15 mg/kg did not cause any deficits in working and reference memory. Motor activity of rats was not affected by mecamylamine during the test. Rats could reach the food quite quickly but spent a relatively long time consuming pellets. When the pellet was moistened by water, rat could swallow the pellet easily and complete the choice accurately. Our failure to observe effects of mecamylamine on the performance in the radial maze is unlikely due to the inadequate blockade of nicotinic receptors. The lack of effects of mecamylamine on radial maze performance suggests that the central nicotinic receptor may not be a necessary substrate for the formation of working and reference memory.

Hup-A completely reversed the scopolamine-induced deficit of maze performance. The memory improvement achieved with Hup-A was comparable to that produced by Phys. Besides, Hup-A exhibited a wide dose range for attenuating scopolamine-induced memory impairment. Chronic treatment with Hup-A was as potent as acute treatment. It indicated that no significant tolerance to the memory-improving effect of Hup-A was seen. This finding was consistent with the study of Laganiere et al., who reported that Hup-A-induced inhibition of AChE activity was as potent after chronic as it was after acute treatment (7).

Compared to Phys, Hup-A is more potent at inhibiting acetylcholinesterase than Phys in vitro (19). Hup-A produces a long-term inhibition of AChE activity in brain (up to 360 min) and increases the ACh levels up to 40% at 60 min (16). Phys produces a rapid (15 min) peak of 55% inhibition but this effect is over within 120 min (16) . After attaining peak plasma concentration in humans at approximately 30–60 min, Phys is cleared from plasma with a half-life of about 30 min (15). The terminal half-life of Hup-A is 5.8 h. The preliminary clinical trials showed that Hup-A did improved short-term and long-term memory in patients of multi-infarct dementia or senile dementia (23,24). At therapeutic dose, parasympathomimetic side effects of Hup-A were minimal when compared with those caused by Phys and tacrine (6,22). These findings and present results suggest that Hup-A may be an interesting candidate for cholinomimetic therapy of cognitive impairments with aging and Alzheimer's disease.

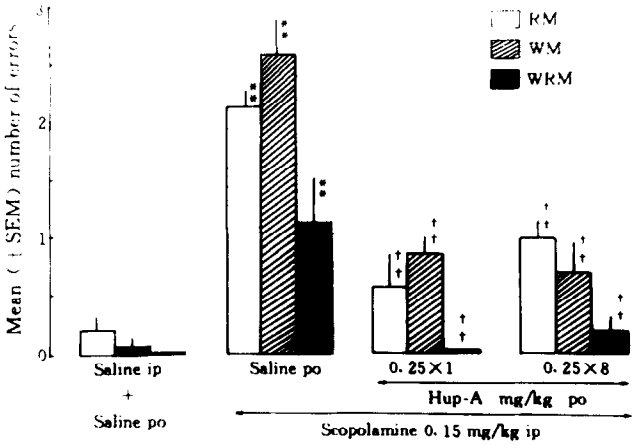

FIG. 5. Effects of chronic administration of Hup-A on scopolamine-induced amnesia on radial maze performance in rats. Hup-A was orally administered at a dose of 0.25 mg/kg daily for 8 consecutive days. Control subject received a similar regime of saline administration. Subjects were tested on the eighth day. Data represent mean ± SEM of 8–10 animals in each group. $^{**}p < 0.01$ compared with the saline + saline group. $\dagger\dagger p < 0.01$ compared with scopolamine + saline.

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