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Reversal of scopolamine-induced deficits in radial maze performance by (−)-huperzine A: comparison with E2020 and tacrine

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Abstract

The effects of (−)-huperzine A ((5*R*,9*R*,11*E*)-5-amino-11-ethylidene- 5,6,9,10-tetrahydro-7-methyl-5,9-methanocycloocta[*b*]pyridin-2(1*H*)-one), and of the hydrochloride salt of E2020 ((*R*,*S*)-1-benzyl-4-[(5,6-dimethoxy-1-indanon)-2-yl]-methyl piperidine) and tacrine (9-amino-1,2,3,4-tetrahydroacridine), on the scopolamine-induced memory deficits in rats were compared in a radial maze, using a 4-out-of-8 baiting procedure. Scopolamine (0.15 mg/kg, i.p.) caused significant impairment in the rats' ability to fulfil the radial maze task. (−)-Huperzine A (0.2–0.4 mg/kg, p.o.; 0.1–0.4 mg/kg, i.p.) had greater efficacy than E2020 (0.6–0.9 mg/kg, p.o.; 0.3–0.6 mg/kg, i.p.) and tacrine (1.5–2.5 mg/kg, p.o.; 0.3–0.6 mg/kg, i.p.) on the improvement of scopolamine-induced working and reference memory errors, respectively. There appeared to be an inverse bell-shape dose-dependent effect for all three compounds tested. The compared data demonstrate that (−)-huperzine A is the most potent and orally active acetylcholinesterase inhibitor of the three, and fits more closely the established criterions for an ideal acetylcholinesterase inhibitor to be used in clinical studies.

Keywords: Acetylcholinesterase inhibitor; (−)-Huperzine A; E2020; Tacrine; Alzheimer's disease; Radial maze; Memory; (Rat)

1. Introduction

It is generally accepted that a cholinergic neural deficit is one of the main neurotransmitter-related deficits found in Alzheimer's disease which is characterised by a progressive loss of the memory function (Deutsch, 1971; Bartus et al., 1982; Coyle et al., 1983). In humans, changes in cognitive processes induced by the blockade of cholinergic activity have been associated with impaired attention and inability to store new information (Rusted, 1994). It has been reported that, using spatial tasks, cholinergic blockade results in specific impairments in both working and reference memory in rats (Watts et al., 1981). So far, cholinesterase inhibitors are the most popular strategies for increasing cholinergic activity in the brain, and show the most encouraging results as palliative therapy for Alzheimer's disease (Brufani and Filocamo, 1996). Among the inhibitors, physostigmine and tacrine have been evaluated on a large scale in Alzheimer's disease. They are not, however, the ideal drugs for clinical use because of their narrow therapeutic window, low bioavailability, and dose-dependent hepatotoxicity, especially of tacrine (Marx, 1987; Watkins et al., 1994). Therefore, it seems necessary to continue to seek for new cholinesterase inhibitors with markedly reduced side-effects and greater therapeutic window than those of the drugs tested.

Thus, there is now a more available new generation of acetylcholinesterase inhibitors, (−)-huperzine A and E2020, with minimal toxicity. (−)-Huperzine A, a novel natural *Lycopodium* alkaloid, was originally extracted from Chinese clubmoss, *Huperzia serrata*, with unique anti-acetylcholinesterase potency and pharmacokinetic properties (Tang, 1996). E2020, a specific piperidine-based acetylcholinesterase inhibitor, had a long duration of inhibitory action and was devoid of unexpected toxicity in the initial clinical studies (Rogers et al., 1991).

Previous studies with (−)-huperzine A and E2020 have suggested their improved abilities to overcome the problems associated with the use of physostigmine and tacrine. Thus, the present study aimed to provide comparative data on (−)-huperzine, E2020, and tacrine by testing their nootropic effects on scopolamine-induced amnesia—a widely cited model for human dementia in general and for

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Alzheimer's disease in particular (Drachman and Leavitt, 1974; Bartus and Johnson, 1976; Ober et al., 1985; Baddeley et al., 1991).

2. Materials and methods

2.1. Subjects

Fifty experimentally naive male albino rats of the Sprague–Dawley strain (from the Experimental Animal Central of Shanghai, Chinese Academy of Sciences) weighing 220–270 g were housed individually in a climate-controlled room under a reversed 12 h light–dark cycle (lights off: 0830). All treatments and behavioural testing were performed during the dark period of the light–dark cycle when the rats were normally most active. The rats were allowed access to water ad libitum and a standard diet to maintain 85% of the original body weight throughout the experiment.

2.2. Apparatus

The plastic radial maze was elevated 70.5 cm above the floor and had an octagonal centre platform (51.5 cm in diameter) with eight arms (61 cm long and 12 cm wide) radiating from the centre. The 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 lit with 40 W fluorescent tubes; several visually distinct extra-maze cues (e.g., wall picture, light, curtain) were present in the room and remained in the same position relative to the maze.

2.3. Methods

The experimental procedure was similar to those described elsewhere (Wirsching et al., 1984). Food deprivation was begun 1 week before training. On the seventh day, each rat was placed on the central platform and allowed to explore the paths and to consume the food (dustless pellet, 45 mg) scattered throughout the maze. Following the 3-day habituation, each rat received one training session daily for 6 days a week. At the beginning of the session, only four predetermined arms were used as baited arms. The baiting pattern remained the same throughout the experiments but was varied from rat to rat to limit the development of odour cues within the maze as well as to control for any directional preference concerning the extra maze cues. Each rat was placed on the central platform and left until all four baited arms were chosen or 14 choices were made or 10 min had elapsed. Training was considered completed when the rats attained the criterion of a maximum of one error over four consecutive trials. Once a rat reached the criterion, training for this rat was reduced to twice a week until all rats reached the criterion. A correct response was defined as the first entry to a baited arm. Two types of errors were recorded: re-entry to a baited arm was regarded as a working memory error; first entry to an unbaited arm was considered as a reference memory error.

2.4. Drug testing

Testing began once all rats reached the criterion. Trained rats were randomly assigned to subgroups and treatments were allocated according to a pseudo-latin square design. All the subjects thus received drugs in a counterbalanced order. (−)-Huperzine A ((5*R*,9*R*,11*E*)-5-amino-11-ethylidene-5,6,9,10-tetrahydro-7-methyl-5,9-methanocycloocta-[*b*]pyridin-2(1*H*)-one) (provided by Department of Phytochemistry, this Institute), E2020 ((*R*,*S*)-1-benzyl-4-[(5,6-dimethoxy-1-indanon)-2-yl]-methyl piperidine) (provided by Department of Synthetic Chemistry, this Institute) and tacrine (9-amino-1,2,3,4-tetrahydroacridine) (Sigma) were all dissolved in saline and administered p.o. or i.p. in a volume of 2 ml/kg or 1 ml/kg b.wt., respectively 30 min before the behavioural testing. Scopolamine hydrobromide

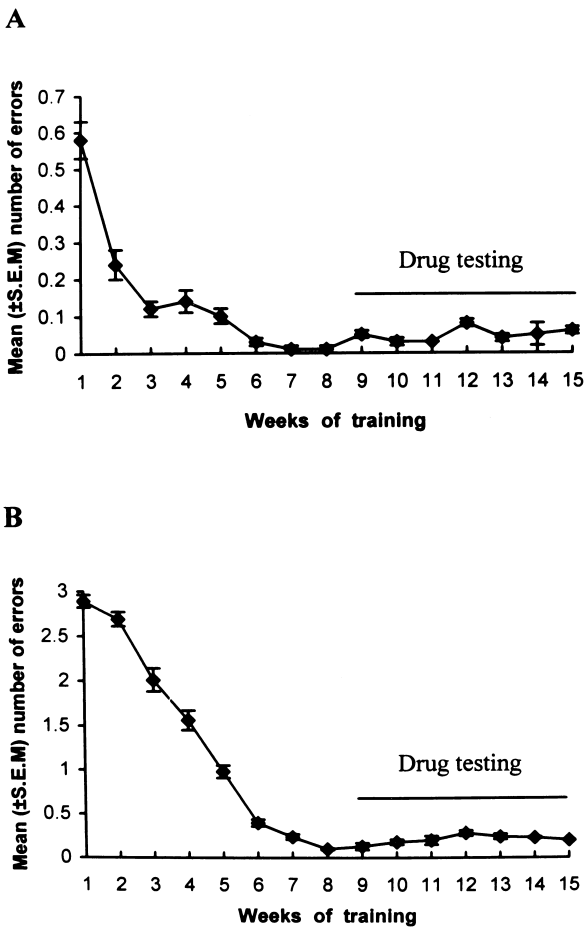

Fig. 1. The working (A) and reference (B) memory components of the rats trained in the partially baited radial maze, represented by mean number of working and reference memory errors ± S.E.M. ($n = 48$).

(Sigma) dissolved in saline was administered i.p. 30 min before testing in a volume of 1 ml/kg b.wt. Administration trials were all made at 1-week intervals for washout and the rats were retrained to the prior criterion before the next dose level of drug was given.

2.5. Statistics

Data were expressed as means ± S.E.M. Analysis of variance (ANOVA) followed by Duncan's multiple range test was used for comparison between the treated groups.

3. Results

3.1. Acquisition

The acquisition of the working and reference memory components of the radial arm maze task on Weeks 1–8 is presented in Fig. 1. It took fewer weeks for the acquisition of the working memory component (3 to 4 weeks) to reach a relatively stable level of about 0.1 errors per rat each session than for acquisition of the reference memory component (7 to 8 weeks) of about 0.3 errors per rat each session. We trained rats to this criterion to decrease baseline error rates, which is permitting the deficit in the reference memory to be manifest.

3.2. Effects of (−)-huperzine A, E2020 and tacrine on the working memory errors induced by scopolamine

The number of working memory errors ($F(1,45) = 76.73$, $P < 0.0001$; $F(1,49) = 139.22$, $P < 0.0001$) (Fig. 2A and Fig. 3A) was significantly increased by scopolamine (0.15 mg/kg i.p. 30 min before the testing), compared with both saline p.o. + i.p. and saline i.p. alone, respectively. ANOVA showed that (−)-huperzine A at

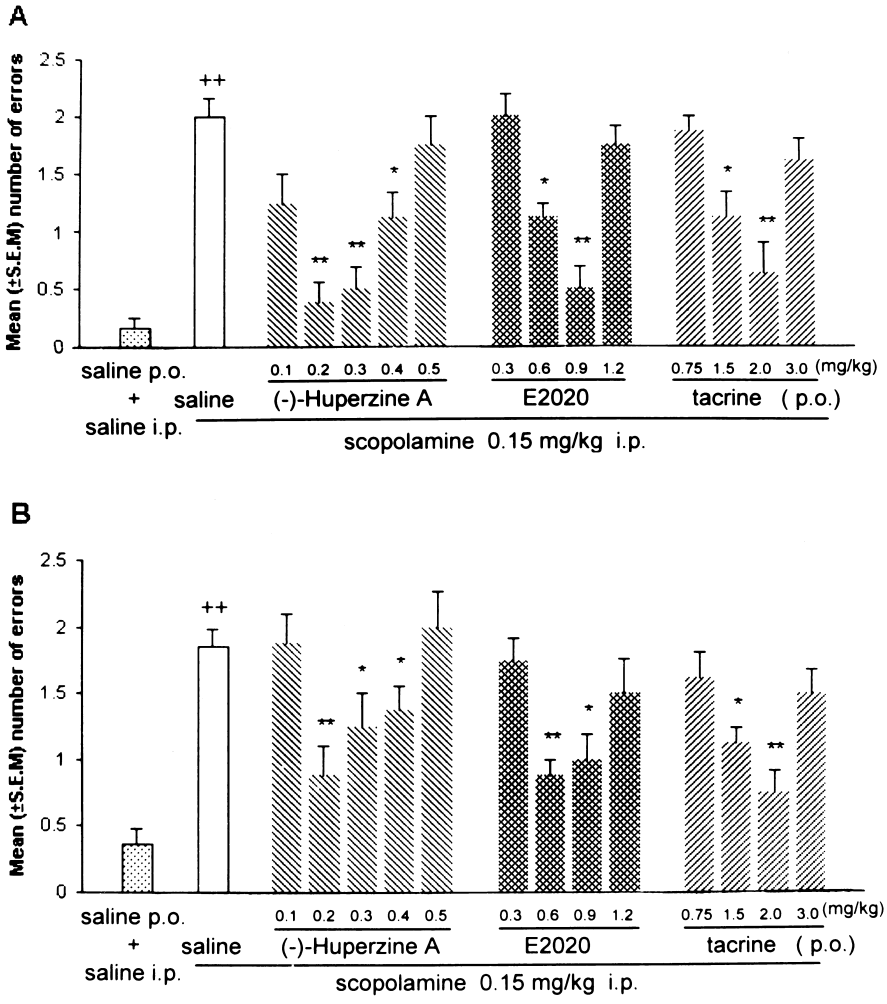

Fig. 2. Effects of oral (−)-huperzine A, E2020, and tacrine on the scopolamine-induced (A) working and (B) reference memory disruption of the partially baited radial maze performance in rats. (−)-Huperzine A, E2020, and tacrine were administered orally 30 min before the behavioural testing. Data represent means ± S.E.M. ($n = 8$). $^{++}P < 0.01$ vs. saline p.o. + saline i.p. ($n = 21$). $*P < 0.05$ and $**P < 0.01$ vs. saline p.o. + scopolamine i.p. ($n = 29$).

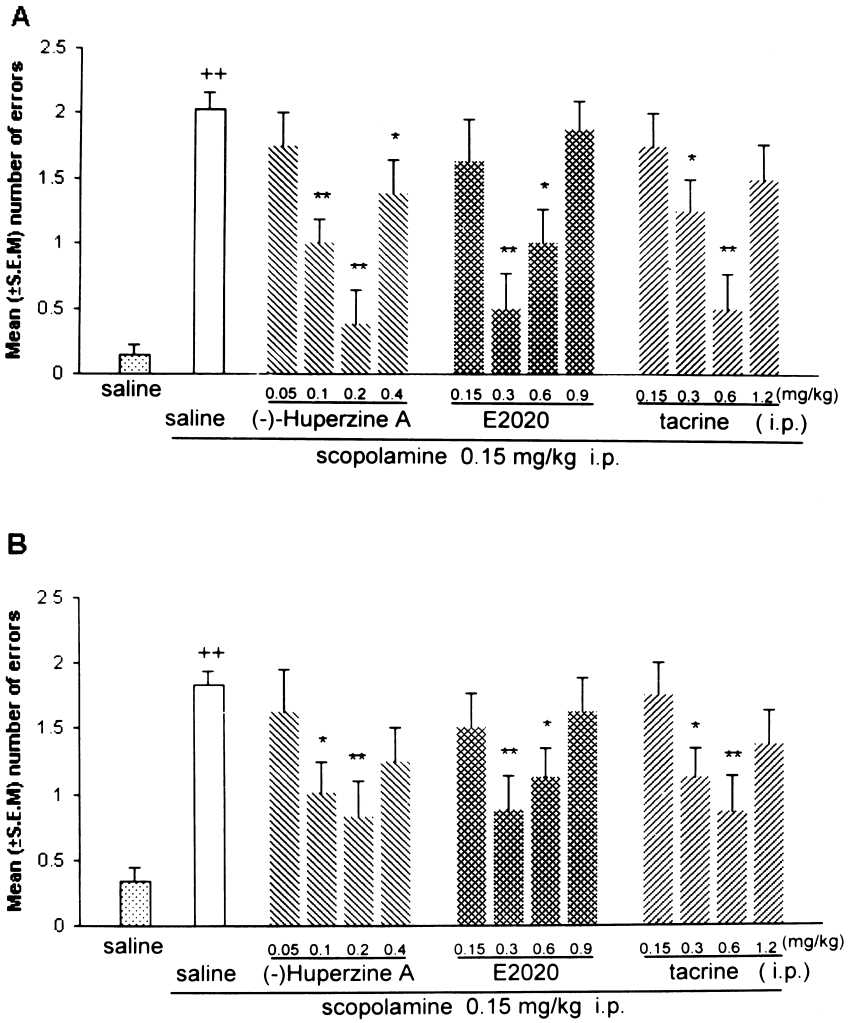

Fig. 3. Effects of (−)-huperzine A, E2020, and tacrine on the scopolamine-induced (A) working and (B) reference memory disruption of the partially baited radial maze performance in rats. (−)-Huperzine A, E2020, and tacrine were administered intraperitoneally 30 min before the behavioural testing. Data represent mean ± S.E.M. ($n = 8$). $^{++}P < 0.01$ vs. saline i.p. ($n = 21$). $*\ P < 0.05$ and $**\ P < 0.01$ vs. scopolamine i.p. ($n = 30$).

p.o. doses from 0.2 to 0.4 mg/kg ($F(5,60) = 55.76$, $P < 0.001$) and at i.p. doses from 0.1 to 0.4 mg/kg ($F(4,57) = 11.43$, $P < 0.001$) significantly improved the working memory errors produced by scopolamine. The scopolamine-induced working memory deficits were also reversed by E2020 at doses of 0.6–0.9 mg/kg p.o. ($F(4,53) = 39.40$, $P < 0.001$) or of 0.3–0.6 mg/kg i.p. ($F(4,57) = 8.99$, $P < 0.001$) and by tacrine 1.5–2.5 mg/kg p.o. ($F(4,53) = 7.36$, $P < 0.001$) or 0.3–0.6 mg/kg i.p. ($F(4,57) = 8.41$, $P < 0.001$), respectively. It was apparent that (−)-huperzine A whether given p.o. or i.p. had almost the same improving effects on the working memory errors produced by scopolamine, whereas E2020 and tacrine required higher doses when administered p.o. than administered i.p. to reverse the scopolamine-induced working memory errors. The dose–response curves were bell-shaped with the maximum improvements at 0.2 mg/kg p.o. and i.p. for (−)-huperzine A, at 0.9 mg/kg p.o. and 0.3 mg/kg i.p. for E2020, and at 2.5 mg/kg p.o. and 0.6 mg/kg i.p. for tacrine, respectively.

3.3. Effects of (−)-huperzine A, E2020 and tacrine on the reference memory errors induced by scopolamine

It is showed in Fig. 2BFig. 3B that the number of reference memory errors ($F(1,45) = 71.23$, $P < 0.0001$; $F(1,49) = 84.18$, $P < 0.0001$) were significantly increased by scopolamine (0.15 mg/kg i.p. 30 min before testing), compared with both saline p.o. plus i.p. and saline i.p. alone, respectively, and that the number of reference memory errors was significantly reduced by the treatment of with oral (to) − huperzine A 0.2–0.4 mg/kg ($F(5,60) = 4.81$, $P < 0.001$) or (−)-huperzine A 0.1–0.2 mg/kg i.p. ($F(4,57) = 7.40$, $P < 0.001$), oral E2020 0.6–0.9 mg/kg ($F(4,53) = 7.27$, $P < 0.001$) or E2020 0.3–0.6 mg/kg i.p. ($F(4,57) = 8.24$, $P < 0.001$), and oral tacrine 1.5–2.5 mg/kg ($F(4,53) = 8.44$, $P < 0.001$) or tacrine 0.3–0.6 mg/kg i.p. ($F(4,57) = 6.05$, $P < 0.001$), compared with administration of scopolamine, respectively.

Peripheral effects were negligible for all three compounds tested.

4. Discussion

The partially baited radial maze, due to its close resemblance to the natural food-seeking conditions for species such as rats, is widely used in the study of drug effects on spatial memory. A muscarinic acetylcholine receptor antagonist, scopolamine, that is widely accepted as providing a model of cognitive deficits in experimental animals, has been extensively used as a preclinical tool to test the efficacy of new drugs which may have cognition-enhancing potential (Bymaster et al., 1993; Preda et al., 1993). In the present studies scopolamine impaired both working and reference memory in the partially baited radial maze, confirming again that the spatial memory processes were dependent on the integrity of the central cholinergic system. Our results were consistent with those of Okaichi et al. (1989), but not with the reports of Wirsching et al. (1984) who concluded that scopolamine does not impair reference memory. Differences in the amount and extent of training may account for some of the discrepancies encountered when the effects of the anti-muscarinic drug in the two types of memory are studied (Lydon and Nakajima, 1992).

The present experiments indicated that a lower oral dose of (−)-huperzine A than of E2020 and tacrine was required to ameliorate the working and reference memory deficits. The relative potency of oral (−)-huperzine A on the improvement of working memory errors was 3.5 and 9 times that of oral E2020 and tacrine, respectively. While E2020 and tacrine had greater efficacy when given i.p. than p.o., the advantage of (−)-huperzine A was its near equal activity whether given orally or intraperitoneally for reversal of the working and reference memory errors produced by scopolamine. Parallel results have also been obtained for the inhibition of acetylcholinesterase (Wang and Tang, 1998). It has been reported that the relative potency of E2020 to inhibit acetylcholinesterase in vitro (Cheng et al., 1996) or i.c.v. injected (Cheng and Tang, 1997) is greater than that of (−)-huperzine A. These results indicated that (−)-huperzine A had higher bioavailability and stronger potency to penetrate the blood–brain barrier than E2020 and tacrine did. The higher dose of tacrine required p.o. might be explained by its low bioavailability and/or rapid metabolism (Hartvig et al., 1990). Moreover, tacrine's affinity for both acetyl- and butyryl-cholinesterase (Wang and Tang, 1998) and for muscarinic and nicotinic receptors (Perry et al., 1988) contributes to its poor tolerability. The highly selective acelytcholinesterase inhibitors, (−)-huperzine and E2020, may retain beneficial effects on cognition and have a marked tolerability compared to that of tacrine.

The use of an acetylcholinesterase inhibitor as a palliative agent in the treatment of Alzheimer's disease has been the most promising approach so far. The reliable ability of (−)-huperzine A, E2020, and tacrine to antagonise most of the cognition impairments induced by scopolamine further supports the ideas of drug development for potential cognitive enhancers based on attempts to increase cholinergic neurotransmission by exerting effects on the surviving cholinergic system. An inhibitor without higher affinity for the central nervous system (CNS) might, however, inhibits general acetylcholinesterase activity, resulting in significant peripheral side-effects. The basic requirements for an acetylcholinesterase inhibitor to be useful therapeutically in Alzheimer's disease are higher bioavailability, good selectivity for acetylcholinesterase, ability of the compound to penetrate to the CNS, and long duration of action (Becker et al., 1991). Previous findings (Tang, 1996) and the present results indicate that (−)-huperzine A possesses these properties and therefore approaches the established criterions for an ideal acetylcholinesterase inhibitor to be used in clinical studies.

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