Effect of a Novel CNS-Selective Cholinesterase Inhibitor, SM-10888, on Habituation and Passive Avoidance Responses in Mice

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Abstract—The effects of the tacrine (THA) derivative SM-10888 (9-amino-8-fluoro-1,2,3,4-tetrahydro-2,4-methanoacridine citrate) on habituation and passive avoidance responses were studied in mice. We examined its effects on habituation of exploratory activity, measured by photo-cell beam interruptions in a small, simple cage and cycloheximide (CXM)- or electroconvulsive shock (ECS)-induced step-down type passive avoidance response (PAR) failures in comparison with those of THA, amiridin, HP-029 and physostigmine. SM-10888 (6 mg/kg, p.o.) administered post-acquisition session enhanced the retention of habituation. CXM- and ECS-induced PAR failures were improved by SM-10888 (6 mg/kg, p.o.) administered at pre-training or post-training, respectively. THA enhanced the retention of habituation and improved CXM-induced PAR failure at 30 mg/kg, p.o., but did not affect ECS-induced PAR failure at 1–15 mg/kg, p.o. Amiridin and HP-029 were also effective on habituation and CXM-induced PAR failure at 40–50 mg/kg, p.o., but did not affect ECS-induced PAR failure at the tested doses. Physostigmine showed a moderate improvement only in CXM-induced PAR failure. The results indicate that SM-10888 enhanced habituation and improved PAR failures at much lower doses than THA. This seems to depend on its high selectivity to the central nervous system.

The most striking symptoms of patients with senile dementia of the Alzheimer type (SDAT) are generally characterized by a memory loss of recent events and cognitive deficits. Among the abnormalities of several neurotransmitter systems in SDAT, the reduction of cholinergic markers is the most consistent and striking (1–3), and was found to correlate well with the cognitive deficits (4). Moreover, many reports have indicated that cholinergic function is involved in memory and cognitive functions (5, 6). Therefore, it is suggested that cholinergic dysfunction is related to the impaired memory observed in SDAT.

Recently, it was reported by Summers et al. that oral administration of the cholinesterase (ChE) inhibitor 9-amino-1,2,3,4-tetrahydroacridine (tacrine, THA) in combination with lecithin significantly ameliorated the symptoms of patients with SDAT (7). However, some patients had side effects (7), which were thought to be induced by the peripheral cholinergic action of THA. We have reported that the THA derivative SM-10888 (9-amino-8-fluoro-1,2,3,4-tetrahydro-2,4-methanoacridine citrate), which has a potent inhibitory effect on ChE, has higher selectivity to the central nervous system (CNS) than THA. According to our index of selectivity to the CNS, i.e., the ratio of ED50 values for hypothermia (CNS action) to that for salivation (peripheral action), the action of SM-10888 was about 3 times more selective to the CNS than THA, physostigmine and other THA derivatives (amiridin and HP-029) (8).

In the present paper, to investigate the effect of SM-10888 on the conditioned be-
haviors, we have studied the effect of SM-10888 on habituation and passive avoidance responses in mice. We have examined the effects of SM-10888, in comparison with that of THA, on the habituation of exploratory activity (9) and cycloheximide (CXM)- or electroconvulsive shock (ECS)-induced passive avoidance response (PAR) failures in mice. The other drugs we also examined in this study were ChE inhibitors, physostigmine and two THA derivatives (amiridin and HP-029), which are reported to be effective on cognitive behaviors or SDAT (9-15).

Materials and Methods

Animals
Male CD-1 mice (Charles River Japan, Hino) weighing 21–25 g and male ddY strain mice (Japan SLC Co., Ltd., Shizuoka) weighing 22–26 g were used. They were given food and water ad lib. in a temperature (23±2°C), humidity (55±10%)-controlled environment with a 12-hour dark (20:00–8:00) / light (8:00–20:00) cycle.

Drugs
9-Amino-8-fluoro-1,2,3,4-tetrahydro-2,4-methanoacridine citrate (SM-10888), 9-amino-2,3,5,6,7,8-hexahydro-1H-cyclopenta(b)-quinoline monohydrate hydrochloride (amiridin) and (±)-9-amino-1,2,3,4-tetrahydroacridin-1-ol maleate (HP-029) were synthesized in our laboratory. Other drugs used were physostigmine sulfate (eserine sulfate, Wako), tacrine hydrochloride hydrate (THA, Aldrich) and cycloheximide (Calbiochem). Test compounds, SM-10888, amiridin, HP-029, physostigmine and THA, were dissolved in distilled water and were orally administered. CXM was dissolved 0.9% saline solution and was subcutaneously injected.

Habituation of exploratory activity

a) Apparatus: A photo-cell activity meter (9) was used. It consisted of six transparent acrylic cages (26×21 cm, 10 cm high) covered with a lid. Each cage was fitted with two photo-cell assemblies, and was connected to electromechanical counters that counted activity scores for each cage.

b) General procedure: The first session (session 1) consisted of placing CD-1 mice into the activity cages, one to a cage, and scoring photo-cell beam interruptions for 3 min. At the end of this session, mice were removed, immediately administered with test compounds or distilled water, and then replaced in their home cage. Animals within a cage received the same treatment. The second session (session 2), given at various intervals after session 1, was the same except that no injections were given. The experiments were conducted between 9:30–11:30 in a sound attenuated room.

c) Statistical analysis: The activity at the second session was compared with that at the first session using a paired Student's t-test. The difference between the scores of two sessions for each of the treated groups was compared with that of the control group using an unpaired Student's t-test.

Step down type passive avoidance failure

a) Apparatus: The test apparatus was a transparent acrylic box (30×40 cm, 30 cm high) with a grid floor (35 parallel steel rods, 4 mm in diameter, set 1 cm apart) and a white acrylic square platform (10×10×0.5 cm) in one corner. The foot shock (FS) was delivered by a shock generator (BRS/LVE, SGS-004, U.S.A.).

b) CXM-induced PAR failure: In the training session, each ddY strain mouse was placed on the platform facing the corner of the box. The FS (1 mA) was delivered during the session. We observed when the mouse touched the grid with its forepaws or stepped down on the grid floor and stepped back on to the platform, and counted the number of these behaviors. When the number of these behaviors reached three to four in 30–60 sec, then the mouse was removed. The animals that failed to show these behaviors three to four times within 60 sec and those that showed the behaviors more than four times within 30 sec were excluded from the experiments. Immediately after training, test compounds in combination with CXM (120 mg/kg, s.c.) or saline were administered. Twenty-four hours after the training session, the retention test was performed. In the test, each mouse was placed on the platform again, and the latency to step down from the platform and place all its paws on the grid floor was measured up to 120 sec. If the animals stayed longer than 120 sec on the platform,
they were assigned the maximum score of 120 sec.

c) ECS-induced PAR failure: The procedures for the training session and retention test were the same as those for CXM-induced PAR failure. In the case of the ECS-induced PAR failure, the test compounds were administered orally 30 min before the training session. Immediately after the training, the animals received either ECS or no shock. The ECS was applied transcorneally under the conditions of 30 mA, 0.2 sec by a EC stimulator (Muromachi-kikai Co., Ltd.).

d) Statistical analysis: The degree of PAR failure was evaluated by the step-down latency and the percentage of mice staying longer than 120 sec (retention %). The step-down latency was analyzed by the two-tailed Mann-Whitney U-test. The retention % was analyzed by the two-tailed z²-test.

Results

Habituation of exploratory activity

a) Effects of inter-session intervals on habituation: Each group of 18 animals was given session 1 and then tested again 1, 2, 3, 4, 5, 6, 7 or 10 days later (session 2). As shown in Fig. 1, the inter-session interval was 1–5 days, and there were significant decreases in activity. This decrement of activity became less marked as the inter-session interval increased.

b) Effects of the test compounds on habituation: From the above result, a 6-day inter-session interval, at which a decrease in activity was not observed, was chosen for testing drugs. The result obtained with SM-10888 is shown in Fig. 2. The control group showed no decrease in activity between the two sessions. In contrast, in the groups treated with SM-10888, the activity in session 2 was decreased dose-dependently, and the difference scores between the two sessions were significantly greater than in the control group at 6 and 10 mg/kg. As shown in Fig. 3, the groups treated with THA (10 or 30 mg/kg), physostigmine (2 mg/kg), amiridin (40 mg/kg) and HP-029 (25, 50 or 100 mg/kg) showed significant decreases in activity at session 2. The differences of the scores between the two sessions were significantly greater than in the control group for 30 mg/kg THA, 40 mg/kg amiridin and 50 mg/kg HP-029, but not for physostigmine.

Step-down type PAR failures

a) CXM-induced PAR failure: As shown in Fig. 4, the actions of CXM, indicated by the shortening of the step-down latency and the decrease of the retention %, were attenuated by SM-10888 in a dose-dependent manner, showing significant prolongation of the step-down latency and significant increase of the

![Fig. 1. Habituation of exploratory activity between session 1 (white columns) and session 2 (hatched columns) as a function of inter-session intervals. Eight groups of mice (n=18 per group) were given session 1 and tested for retention (session 2) 1, 2, 3, 4, 5, 6, 7 or 10 days later. Values are the means± S.E. * * * : Significantly different from session 1 (*P<0.05, ***P<0.001, paired t-test).](image-url)
Retention % at 6 and 10 mg/kg. Significant prolongation of the step-down latency or significant increase of the retention % were also obtained by 30 mg/kg THA, 1 mg/kg physostigmine, 50 mg/kg amiridin and 50 and 100 mg/kg HP-029 (Fig. 5).

b) ECS-induced PAR failure: Some animals that were treated with high doses of the tested drugs could not receive training because of drug-induced convulsion, tremor, sedation and depression of motor activity, etc. Accordingly, the dose range we used in this experiment was below the maximum dose at which the animals could receive the training session.

As shown in Fig. 6, the step-down latency and the retention % were shortened and decreased by ECS. SM-10888 attenuated the actions of ECS, showing significant prolongation of the step-down latency at 6 mg/kg. However, THA, physostigmine amiridin and HP-029 did not show any significant effects at the tested doses (Table 1).

Table 1. Effects of SM-10888 and other ChE inhibitors on the electroconvulsive shock (ECS)-induced passive avoidance response failures in mice

| Drugs          | Minimum effective dose (mg/kg, p.o.) |
|---------------|-------------------------------------|
| SM-10888      | 6                                   |
| THA           | Inactive (1–15)                     |
| Physostigmine | Inactive (0.1–0.4)                  |
| Amiridin      | Inactive (0.03–30)                  |
| HP-029        | Inactive (15–60)                    |

Drugs were given orally 30 min before the acquisition session. ECS (30 mA, 0.2 sec) was applied immediately after the acquisition session. Minimum effective dose was defined as the minimal dose among the doses administered p.o. that caused a significant (Mann-Whitney’s U-test, P<0.05) prolongation of the step-down latency or a significant (χ²-test, P<0.05) increase of retention %. The figures in parentheses after the word “inactive” are the dose ranges investigated.

Discussion

In the present study, we examined the effect of SM-10888 on habituation and PAR failures in mice. For comparison, THA, the ChE inhibitor physostigmine and THA derivatives (amiridin and HP-029) were also examined.

Habituation is the decrement of a response to a stimulus as a result of repeated or constant stimulus. Following the method of Platel...
and Porsolt (9), we exposed mice to a novel, previously unknown exploratory environment as a stimulus, measured the exploratory activity as a response to the stimulus and defined habituation as the decrease in activity observed on the second time. In our experiments, when the mice were placed for the second time in the same environment after the short intervals, the activities were significantly decreased. This phenomenon of habituation suggests that mice learned the novel environment at the first session, and due to familiarity with the environment (16), they showed the low level in exploratory activity.

Fig. 3. Effects of THA, physostigmine, amiridin and HP-029 on the habituation of exploratory activity between session 1 (white columns) and session 2 (hatched columns). Drugs were given orally to mice immediately after session 1. The inter-session interval was 6 days. Activity count is the difference between sessions 1 and 2. Values are the means±S.E. *: Significantly different from session 1 (P<0.05, paired t-test); **: Significantly different from the 0 group (P<0.001, Student’s t-test).
In addition, these decrements in activity were less marked as the interval increased. This time-dependency suggests that forgetting occurred over time. Accordingly, it is possible to consider that the decrement in activity at the second session indicates that the mice keep the memory of having been there before.

The poor habituation with a 6-days inter-session interval was improved by SM-10888, which was administered immediately after the acquisition session. THA, amiridin and HP-029, which showed significantly greater decreases in activity than that in the control group, also showed habituation. Their minimum effective doses that showed the significant decreases in activity compared with each control group were 5–8 times as much as that of SM-10888.

We administered drugs immediately after the first session, but we suspected that it was possible for the influences of drugs on motor activity or sensomotor function to appear at the retention session. To investigate this point, we administered drugs without the acquisition session and measured activity counts for 3 min 6 days later. We found that there was no significant change in activity compared to the control group (data not shown). Platel and Porsolt reported that aversive or rewarding treatment after the first session had no effect on habituation (9). From our results and those of Platel and Porsolt, it is suggested that the decreases in activity observed in the drug treated mice reflect habituation, and they are not due to the alternations of non-specific factors such as motor activity, sensomotor function, and aversive or rewarding properties of the drugs. Therefore the effective drugs in this study may act on the retention of habituation, that is, the memory of a novel environment.

The treatments by CXM or ECS are widely used to disrupt the performance of passive avoidance tasks in animal models. It has been reported that administration of protein synthesis inhibitors shortly before or immediately after the training impairs memory in a variety
of learning tasks in rodents and other species (17), and the treatment by ECS induces retrograde memory loss (18, 19). In the present study, to avoid the effects of CXM or ECS on behaviors at the training session, mice were treated by CXM or ECS immediately after the training session. SM-10888, THA and other drugs administered at pre or post training did not alter motor activity, sensomotor function or shock sensitivity at the doses used in these studies, on the training session or the retention test (data not shown). Therefore, the changes in latency in the retention test were supposed to depend mainly on the memory of the FS delivered on the grid floor during the training.

In the CXM-induced PAR failure, SM-10888 and other drugs were administered at the same time with CXM. The drugs tested in the present study may act on a process after the training such as consolidation, maintenance and retrieval of the memory. In the ECS-induced PAR failure, post training administration of SM-10888 had no effect (data not shown). Longini et al. (20) have reported that the sharp and short lasting decrease in brain ACh level following ECS treatment may be related to ECS-induced amnesia. SM-10888, THA and other drugs increase brain ACh level with the peak at
about 30 min after the p.o. administration (8). From these observations, we administered SM-10888 and other drugs 30 min before the training and ECS-application. Therefore, there is a possibility that the tested drugs can act on the learning process in addition to the consolidation, maintenance and retrieval of the memory in ECS-induced PAR failure.

The CXM- and ECS-induced PAR failures were both attenuated by the treatment with SM-10888 at 6–10 mg/kg. THA, amiridin and HP-029 improved CXM-induced PAR failure at 30–50 mg/kg, but did not affect ECS-induced PAR failure at the dose range we tested. The potency of the ChE inhibitory action of SM-10888 is similar to that of THA (8), but the minimum effective dose in the present study was about 1/5 that of THA, and THA was not effective on ECS-induced PAR failure. This may be due to the higher selectivity to the CNS of SM-10888 than THA, which was shown by the difference of the ratio of ED50 values for hypothermia (CNS action) to that for salivation (peripheral action) (8). Amiridin has been reported to improve CXM- and ECS-induced PAR failure in mice at low doses (12). According to the report, the dose-response curves for amiridin were bell-shaped curves. So we also examined the effect of amiridin on CXM- and ECS-induced PAR failures at low doses (1–10, 0.03–1 mg/kg respectively), but we could not detect any effectiveness (data not shown). This may be explained by the difference of the experimental conditions such as the dose of CXM, the size of the apparatus, the FS-level and the training procedure.

Many reports have indicated the effectiveness of physostigmine on memory in various animal models. The ChE inhibitory action of physostigmine is about 10 times as potent as SM-10888 (8). However, physostigmine showed only a moderate improvement in CXM-induced PAR failure. The reason is not clear, but the difference of the administration route between our experiments and other reports may be involved. In our ex-

Fig. 6. Effect of SM-10888 on the electroconvulsive shock (ECS)-induced passive avoidance response failures in mice. White columns: step-down latency, hatched columns: retention %. SM-10888 was orally given 30 min before the training session. ECS (30 mA, 0.2 sec) was applied immediately after the training session. The retention test was given 24 hours after the training session. In the retention test, the latency to step down from the platform was measured up to 120 sec. Retention % shows the percent of mice staying on the platform for at least 120 sec. Values are the means±S.E. N=22–25. **: Significantly different from the O+CXM group (P<0.01, Mann-Whitney’s U-test); #: Significantly different from the O+CXM group (P<0.01, z²-test).
Experiments, physostigmine was p.o.-administered to be compared with the action of SM-10888. In many of the reports that show the effectiveness of physostigmine on memory, physostigmine is not p.o.-administered, but often i.p.- or s.c.-injected at low doses (9, 21–23). Physostigmine, when p.o.-administered, may show a weaker action on the behaviors than by i.p. or s.c. injection due to the difference of pharmacodynamic responses, such as the duration or the potency of action.

The role played by brain cholinergic mechanisms in the memory process has been well-documented (5, 6), and facilitation of central cholinergic transmission has been shown to enhance memory retention. The mechanisms of CXM- and ECS-induced PAR failures are not known, but some data suggest that CXM and ECS induce PAR failures through a functional reduction of cholinergic neurotransmission (24, 25). Comparing the present data with the increasing effects of the tested drugs on brain ACh (8), there is a good correlation ($r=0.95$, $P<0.001$) between the minimum effective doses of SM-10888, THA, amiridin and HP-029 on retention of habituation and PAR failures and those of increasing brain ACh level in mice. From these results, SM-10888 is suggested to influence memory processes via activation of cholinergic mechanisms. Actions on habituation and PAR failures of SM-10888 at lower doses than THA and other THA derivatives seem to depend on its high selectivity towards the CNS. Therefore, it may be suggested that SM-10888 is expected to improve memory processes with much less peripheral cholinergic side effects than THA, amiridin, HP-029 and physostigmine.

References

1 Davies, P. and Maloney, A.J.F.: Selective loss of central cholinergic neurons in Alzheimer’s disease. Lancet 2, 1403 (1976)

2 Whitehouse, P.J., Price, D.L., Clark, A.W., Coyle, J.T. and DeLong, M.R.: Alzheimer disease: Evidence for selective loss of cholinergic neurons in the nucleus basalis. Ann. Neurol. 10, 122–126 (1981)

3 Rossor, M. and Iversen, L.L.: Non-cholinergic neurotransmitter abnormalities in Alzheimer’s disease. Br. Med. Bull. 42, 70–74 (1986)

4 Pery, E.K., Tomlinson, B.E., Blessed, G., Bergmann, K., Gibson, P.H. and Perry, R.H.: Correlation of cholinergic abnormalities with senile plaques and mental test scores in senile dementia. Br. Med. J. 2, 1457–1459 (1978)

5 Drachman, D.A. and Leavitt, J.: Human memory and the cholinergic system. Arch. Neurol. 30, 113–121 (1974)

6 Drachman, D.A.: Memory and cognitive function in man; does the cholinergic system have a specific role? Neurology 27, 783–790 (1977)

7 Summers, W.K., Majovski, L.V., Marsh, G.M., Tachiki, K. and Kling, A.: Oral tetrahydroaminoacridine in long-term treatment of senile dementia. N. Engl. J. Med. 315, 1241–1245 (1986)

8 Natori, K., Okazaki, Y., Irie, T. and Katsube, J.: Pharmacological and biochemical assessment of SM-10888, a novel cholinesterase inhibitor. Japan. J. Pharmacol. 53, 145–155 (1990)

9 Platel, A. and Porsolt, R.D.: Habituation of exploratory activity in mice; a screening test for memory enhancing drugs. Psychopharmacology (Berlin) 78, 346–352 (1982)

10 Thal, L.J., Masur, D.M., Blau, A.D., Fulld, P.A. and Klauber, M.R.: Chronic oral physostigmine without lecithin improves memory in Alzheimer’s disease. J. Am. Geriatr. Soc. 37, 42–48 (1989)

11 Stern, Y., Sano, M. and Mayeux, R.: Long-term administration of oral physostigmine in Alzheimer’s disease. Neurology 38, 1837–1841 (1988)

12 Nabeshima, T., Yoshida, S. and Kameyama, T.: Effects of the novel compound NIK-247 on impairment of passive avoidance response in mice. Eur. J. Pharmacol. 154, 263–269 (1988)

13 Kuribara, H.: Effects of amiridin on ambulatory activity and discrete shuttle avoidance response in mice. Folia Pharmacol. Japon. 88, 299–307 (1986) (Abs. in English)

14 Shutstke, G.M., Pierrat, F.A., Cornfeldt, M.L., Szewezak, M.R., Huger, F.P. and Bores, G.M.: (+)-9-Amino-1,2,3,4-tetrahydroacridin-1-ol. A potential Alzheimer’s disease therapeutic of low toxicity. J. Med. Chem. 31, 1278–1279 (1988)

15 Ueki, A. and Miyoshi, K.: Effects of cholinergic drugs on learning impairment in ventral globus pallidus-lesioned rats. J. Neurol. Sci. 90, 1–22 (1989)

16 File, S.E.: Effect of chlorpromazine on exploration and habituation in the rat. Br. J. Pharmacol. 49, 303–310 (1973)

17 Davis, H.P.: Protein synthesis and memory: A Review. Psychological Bull. 96, 518–559 (1984)

18 McGaugh, J.L.: Time-dependent processes in
memory storage. Science 153, 1351–1358 (1966)

19 Squire, L.R.: ECT and memory loss. Am. J. Psychiatry 134, 997–1001 (1977)

20 Longoni, R., Mulas, A., Novak, B.O., Pepeu, I.M. and Pepeu, G.: Effect of single and repeated electroshock applications on brain acetylcholine levels and choline acetyltransferase activity in the rat. Neuropharmacology 15, 283–286 (1976)

21 Boggan, W.O. and Schlesinger, K.: Pharmacological correlates of ECS induced disruption of a passive avoidance task in mice. Behav. Biol. 12, 127–134 (1974)

22 Kameyama, T., Nabeshima, T. and Noda, Y.: Cholinergic modulation of memory for step-down type passive avoidance task in mice. Res. Commun. Psychol. Psychiat. Behav. 11, 193–205 (1986)

23 Nabeshima, T., Noda, Y., Itoh, K. and Kameyama, T.: Role of cholinergic and GABAergic neuronal systems in cycloheximide-induced amnesia in mice. Pharmacol. Biochem. Behav. 31, 405–409 (1988)

24 Spignoli, G. and Pepeu, G.: Oxiracetam prevents electroshock-induced decrease in brain acetylcholine and amnesia. Eur. J. Pharmacol. 126, 253–257 (1986)

25 Rose, S.P.R., Gibbs, M.E. and Hambley, J.: Transient increase in forebrain muscarinic cholinergic receptor binding following passive avoidance learning in the young chick. Neuroscience 5, 169–172 (1980)