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# Effect of YM796, a Novel Muscarinic Agonist, on the Impairment of Passive Avoidance Response in Senescence-Accelerated Mice

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SUZUKI, M., T. YAMAGUCHI, Y. OZAWA, A. IWAI AND M. YAMAMOTO. *Effect of YM796, a novel muscarinic agonist, on the impairment of passive avoidance response in senescence-accelerated mice.* PHARMACOL BIOCHEM BEHAV **51**(4) 623–626, 1995.—We compared the effects of YM796 {(−)-*S*-2,8-dimethyl-3-methylene-1-oxa-8-azaspiro[4,5]-decane L-tartrate monohydrate}, a novel muscarinic agonist, on passive avoidance response with those of the cholinomimetics AF102B [(±)-*cis*-2-methylspiro-(1,3-oxathiolane-5,3′)-quinuclidine hydrochloride] and NIK247 [9-amino-2,3,5,6,7,8-hexahydro-1*H*-cyclopenta(*b*)-quinoline monohydrate hydrochloride] in senescence-accelerated mice. SAMP8//YAN (SAM-P/8, senescence-accelerated-prone substrain) showed an age-dependent shortening in the latency of step-through when compared with SAMR1/YAN (SAM-R/1, senescence-accelerated-resistant substrain). The shortened latency of step-through in SAMP8//YAN was prolonged by administration of YM796 (0.3 and 1 mg/kg, PO), AF102B (3 and 10 mg/kg PO), and NIK247 (30 mg/kg, PO) in a bell-shaped manner. In contrast, amitriptyline (10, 30, and 50 mg/kg, PO), with cholinolytic properties, had no effect on this shortened latency of step-through. These results suggest that YM796, AF102B, and NIK247 ameliorated the disturbance of learning behavior, presumably due to facilitation of the central cholinergic system in SAMP8//YAN mice and that SAMP8//YAN may be an appropriate age-dependent model of amnesia for evaluating pharmacological actions of drugs.

YM796 Passive avoidance response SAM AF102B NIK247 Amitriptyline

THE SENESCENCE-accelerated mouse (SAM), a murine model of accelerated senescence, was established by Takeda et al. (14). SAM consists of the senescence-accelerated-prone mouse (SAM-P) and senescence-accelerated-resistant mouse (SAM-R), which shows normal aging characteristics. It has been demonstrated that SAM-P/8 show earlier onset and irreversible progress of senescence as manifested by a grading score system designed to represent changes in behavior, appearance, eyes, and spine (3,14). In pathologic studies, these mice show spontaneous age-related systemic amyloidosis accompanying the presence of the amyloid protein (2,12,13,15). They also show a decrease in muscarinic receptor density in the hippocampus (6,7), and a disturbance in learning behaviors such as passive avoidance, active avoidance, and water maze tasks (4,9,20). The SAM-P/8 mice therefore are expected to be useful as an age-related model of amnesia for evaluating pharmacological actions of drugs. Yamamoto et al. reported that indeloxazine, dihydroergotoxine (cerebral activators), TRH, and azetireline (TRH analogue), which have facilitatory effects on the central cholinergic system, ameliorated the impairment of passive avoidance response in SAMP8//YAN (21–24).

YM796{(−)-*S*-2,8-dimethyl-3-methylene-1-oxa-8-azaspiro-[4,5]decane L-tartrate monohydrate} is a novel muscarinic agonist that possesses selective affinity for $M_1$ receptors (17,18): the drug stimulates phosphoinositide hydrolysis linked to $M_1$ receptors in both hippocampal slices (17,18) and transfected cells expressing the $M_1$ receptor gene (19). It has been pre-

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viously reported that YM796 improves the impairment of learning behavior in passive avoidance and Morris water maze tasks in rodents with cholinergic hypofunction (11,17,18). In the present study, the effects of YM796 on the impairment of passive avoidance response in SAMP8//YAN (SAM-P/8) were compared with those of AF102B (muscarinic agonist) (1) and NIK247 (acetylcholinesterase inhibitor) (8).

## METHOD

### *Animals*

SAMP8//YAN and SAMR1/YAN aged 2–6 months old were used. They were kept at a temperature of 23 ± 1°C under a 13L : 11D cycle (light on 0730–2030 h) with free access to food and water.

### *Passive Avoidance*

A one-trial step-through passive avoidance task was carried out as previously described (5). The apparatus (O'Hara & Co., Tokyo, Japan) consisted of two compartments, an illuminated box (14 × 10 × 10 cm, with a 60-W light at a height of 25 cm from the top the chamber) and a dark box (20 × 45 × 25 cm). These compartments were separated by a guillotine door. In the training session performed 45 min after oral administration of drugs, each mouse was placed in the illuminated compartment and allowed to enter the dark compartment through the door. Immediately after entry, a scrambled foot shock (30 V, 50 Hz for 2 s) was delivered through the grid floor. The mouse could escape from the shock only by stepping back into the "safe" illuminated side. In the test trial 24 h after the training, the mouse was again placed in the "safe" illuminated compartment and the response latency to enter the dark compartment was measured. The latency of mice not entering the dark compartment during the 300-s observation period was regarded to be 300 s.

### *Drugs*

YM796, AF102B, and NIK247 were synthesized in our laboratory. Amitriptyline hydrochloride (Yamanouchi Pharmaceutical Co. Ltd.) was purchased commercially. All drugs were dissolved in distilled water and administered orally compulsorily with a probe of 5 cm length at a volume of 0.1 ml/10 g body weight.

### *Data Analysis*

The results were analyzed by one-way ANOVA followed by Dunnett's multiple range test and Mann-Whitney's *U*-test.

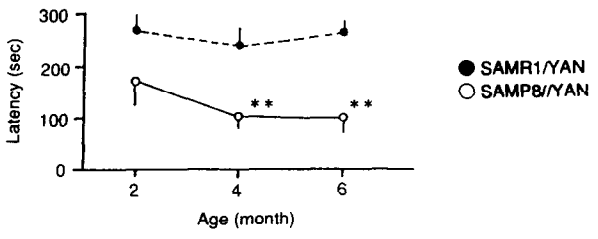


FIG. 1. Latency of step-through in passive avoidance of SAMP8//YAN and SAMR1/YAN aged 2, 4, and 6 months. Each value represents the mean ± SE of 7–9 mice. $^{**}p < 0.01$: significantly different from the value for SAMR1/YAN (Mann-Whitney *U*-test).

TABLE 1

EFFECTS OF YM796 AND OTHER COMPOUNDS ON THE STEP-THROUGH PASSIVE AVOIDANCE RESPONSE IN SAMP8//YAN

| SAM | Drug | Dose (mg/kg, PO) | Step-Through Latency (s) |
|---|---|---|---|
| R1/YAN | Solvent | – | 202 ± 34 |
| P8//YAN | Solvent | – | 64 ± 28* |
| | YM796 | 0.1 | 122 ± 29 |
| | | 0.3 | 204 ± 30† |
| | | 1 | 188 ± 30* |
| | AF102B | 1 | 128 ± 33 |
| | | 3 | 223 ± 34† |
| | | 10 | 178 ± 32‡ |
| | NIK247 | 10 | 79 ± 28 |
| | | 30 | 182 ± 29‡ |
| | | 50 | 148 ± 41 |
| R1/YAN | Solvent | – | 273 ± 74 |
| P8//YAN | Solvent | – | 75 ± 18§ |
| | Amitriptyline | 10 | 68 ± 31 |
| | | 30 | 75 ± 34 |
| | | 50 | 90 ± 40 |

SAMP8//YAN and SAMR1/YAN aged 6 months old were used. Each value represents the mean ± SE of 10 mice. The solvent and drugs were administered orally 45 min before training.

*$p > 0.01$: significantly different from values for SAMR1/YAN (Mann-Whitney *U*-test).†$p > 0.01$: significantly different from values of the SAMP8//YAN solvent control group (one-way ANOVA followed by Dunnett's multiple range test).

*‡$p > 0.05$: Significantly different from values of the SAMP8//YAN solvent control group (one-way ANOVA followed by Dunnett's multiple range test.

*§$p > 0.05$: Significantly different from values for SAMR1/YAN (Mann-Whitney *U*-test).

## RESULTS

Latency of step-through in passive avoidance of SAMP8//YAN was shortened in an age-dependent manner when compared with that of SAMR1/YAN (Fig. 1), in agreement with the report of Miyamoto et al. (9). SAMP8//YAN aged 4 and 6 months had significantly shorter latency compared with SAMR1/YAN at the same respective ages. In SAMP8//YAN aged 6 months, the shortened latency of step-through was significantly prolonged by administration of YM796 (0.3 and 1 mg/kg, PO) in a bell-shaped manner (Table 1). AF102B (3 and 10 mg/kg, PO) and NIK247 (30 mg/kg, PO) also significantly prolonged the shortened latency of step-through in a bell-shaped manner. On the other hand, amitriptyline at doses of 10, 30, and 50 mg/kg had no influence on the shortened latency. No significant difference in latency to enter the dark compartment in the training session was found among the groups (data not shown). All mice escaped from the dark compartment immediately after the scrambled foot shock.

## DISCUSSION

The present study demonstrated that latency of step-through in passive avoidance of SAMP8//YAN was shortened in an age-dependent manner, in agreement with previous observations (9,20). Latency in SAMP8//YAN aged 4 and 6 months was significantly shorter than that in SAMR1/YAN at the same respective ages. SAMP8//YAN and SAMR1/YAN aged 6 months were therefore used to evaluate pharma-

cological actions of drugs. YM796 prolonged this shortened latency of step-through in SAMP8//YAN. It is known that changes in spontaneous movement and sensitivity to pain may affect the passive avoidance response; in the present study, however, latency from the illuminated compartment to the dark compartment and escape behavior from the electric shock in the training session remained unchanged after administration of YM796. These findings suggest that the ameliorating effects of YM796 on the impairment of passive avoidance response in SAMP8//YAN is attributable not to any effect on spontaneous movement and sensitivity to pain but to the amelioration of cognitive function per se. Both AF102B and NIK247 showed a similar action to YM796, but with less potency than YM796.

Kitamura et al. reported that the $B_{max}$ of [$^3$H]quinuclidinyl benzilate (QNB) binding in the hippocampus of SAM-P/8 was decreased when compared with that of SAM-R/1 (6,7). The impairment of learning behavior in SAMP8//YAN may therefore be partly attributable to the decrease in muscarinic receptors in the central nervous system. It has already been reported that the muscarinic agonists YM796 (11,17,18) and AF102B (1,17) and the cholineacetylesterase inhibitor NIK247 (10,16) ameliorate the impairment of passive avoidance response in rodents with cholinergic hypofunction induced by treatment with scopolamine and aridinium ion (AF64A) or lesion of the nucleus basalis magnocellularis (NBM). We showed that amitriptyline, with monoaminergic and cholinolytic properties, had no effect on the impairment of passive avoidance response in SAMP8//YAN. These suggest that the ameliorating effects of the present cholinomimetics on the impairment of learning behavior in SAMP8//YAN may therefore be due to their facilitatory effects on the central cholinergic system.

Yamamoto et al. have reported that drugs with facilitatory effects on central cholinergic and monoaminergic systems such as indeloxazine, dihydroergotoxine (cerebral activators), TRH, and azetireline (TRH analogue) ameliorate the impairment of passive avoidance response not only in cycloheximide-treated mice and scopolamine-treated rats currently used for evaluating pharmacological actions of drugs but also in SAMP8//YAN at similar doses (21-24). Amitriptyline, with monoaminergic property, had no effect on the impairment of passive avoidance response, presumably due to having cholinolytic property in SAMP8//YAN. From these results, it is suggested that SAM-P/8 may be an appropriate age-dependent model of amnesia for evaluating the effects of drugs on brain dysfunction.

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## REFERENCES

1. Fisher, A.; Brandeis, R.; Karton, I.; Pittel, Z.; Gurwitz, D.; Haring, R.; Sapir, M.; Levy,A.; Heldman, E. (±)-cis-2-methylspiro(1,3-oxathiolane-5,3′)quinuclidine, an $M_1$ selective cholinergic agonist, attenuates cognitive dysfunctions in an animal model of Alzheimer's disease. J. Pharmacol. Exp. Ther. 257:392-403; 1991.
2. Higuchi, K.; Matsumura, A.; Honma, A.; Takeshita, S.; Hashimoto, K.; Hosokawa, M.; Yasuhira, K.; Takeda, T. Systemic senile amyloid in senescence-accelerated mouse. A unique fibril protein demonstrated in tissues from various organs by unlabeled immunoperoxidase method. Lab. Invest. 48:231-240; 1983.
3. Hosokawa, M.; Kasai, R.; Higuchi, K.; Takeshita, S.; Shimizu, K.; Hamamoto, H.; Honma, A.; Irino, M.; Toda, K.; Matsumura, A.; Matsushita, M.; Takeda, T. Grading score system: A method for evaluation of the degree of senescence in senescence accelerated mouse (SAM). Mech. Ageing Dev. 26:91-102; 1984.
4. Ikegami, S.; Shumiya, S.; Kawamura, H. Age-related changes in radial-arm maze learning and basal forebrain cholinergic systems in senescence accelerated mice (SAM). Behav. Brain Res. 51:15-22; 1992.
5. Jarvik, M. E.; Kopp, A. An improved one-trial passive avoidance learning situation. Psychol. Rep. 21:221-224; 1967.
6. Kitamura, Y.; Zhao, X.; Ohnuki, T.; Nomura, Y. Ligand-binding characteristics of [$^3$H]QNB, [$^3$H]prazosin, [$^3$H]rauwolscine, [$^3$H]TCP and [$^3$H]nitrendipine to cerebral cortical and hippocampal membranes of senescence accelerated mouse. Neurosci. Lett. 106:334-338; 1989.
7. Kitamura, Y.; Ohmuki, T.; Nomura, Y. Effects of bifemelane hydrochloride, a brain function improver, on muscarinic receptors in the CNS of senescence-accelerated mouse. Jpn. J. Pharmacol. 56:231-235; 1991.
8. Kojima, J.; Yoshida, S.; Ishii, Y.; Fujii, K.; Okumura, M. Effects of NIK-247 and 9-amino-1,2,3,4-tetrahydroacridine on scopolamine-induced amnesia, long-term potentiation and acetylcholinesterase. Eur. J. Pharmacol. 183:929; 1990.
9. Miyamoto, M.; Kiyota, Y.; Yamazaki, N.; Nagaoka, A.; Matsuo, T.; Nagawa, Y.; Takeda,T. Age-related changes in learning and memory in the senescence-accelerated mouse (SAM). Physiol. Behav. 38:399-406; 1986.
10. Nabeshima, T.; Yoshida, S.; Kameyama, T. Effects of the novel compound NIK-247 onimpairment of passive avoidance response in mice. Eur. J. Pharmacol. 154:263-269; 1988.
11. Ohyama, M.; Ozawa, Y.; Yamaguchi, T.; Takahashi, K.; Yamamoto, M. Effect of a new $M_1$ muscarinic agonist, YM796, on impairment of passive avoidance task in mice with acetylcholine hypofunction. Jpn. J. Pharmacol. 61(Suppl. 1):93; 1993.
12. Shimizu, K.; Kasai, R.; Yamamuro, T.; Hosokawa, M.; Takeshita, S.; Takeda, T. Amyloid deposition in the articular structures of AKR senescent mice. Arthritis Rheum. 24:1540-1543; 1981.
13. Shimizu, K.; Ishii, M.; Yamamuro, T.; Takeshita, S.; Hosokawa, M.; Takeda, T. Amyloid deposition in intervertebral discs of senescence-accelerated mouse. Arthritis Rheum. 25:710-712; 1982.
14. Takeda, T.; Hosokawa, M.; Takeshita, S.; Irino, M.; Higuchi, K.; Matsushita, T.; Tomita, Y.; Yasuhira, K.; Hamamoto, H.; Shimizu, K.; Ishii, M.; Yamamuro, T. A new murine model of accelerated senescence. Mech. Ageing Dev. 17:183-194; 1981.
15. Takeshita, S.; Hosokawa, M.; Irino, M.; Higuchi, K.; Shimizu, K.; Yasuhira, K.; Takeda, T. Spontaneous age-associated amyloidosis in senescence-accelerated mouse (SAM). Mech. Ageing Dev. 20:13-23; 1982.
16. Ueki, A.; Miyoshi, K. Effects of cholinergic drugs on learning impairment in ventral globus pallidus-lesioned rats. J. Neurol. Sci. 90:1-21; 1989.
17. Wanibuchi, F.; Konishi, T.; Harada, M.; Terai, M.; Hidaka, K.; Tamura, T.; Tsukamoto,S.; Usuda, S. Pharmacological studies on novel muscarinic agonists, 1-oxa-8-azaspiro[4,5]decane derivatives, YM796 and YM954. Eur. J. Pharmacol. 187:479-486; 1990.
18. Wanibuchi, F.; Nishida, T,; Hidaka, K.; Akiho, H.; Nakamura, Y.; Takahashi, K.; Tsukamoto, S.; Usuda, S. Muscarinic activity of a novel $M_1$ agonist, YM796, and itsoptical isomer. Soc. Neurosci. Abstr. 17:1234; 1991.
19. Wei, H.; Roeske, W. R.; Lai, J.; Wanibuchi, F.; Hidaka, K.; Usuda, S.; Yamamura, H. I. Pharmacological characterization of a novel muscarinic partial agonist, YM796, intransfected cells expressing the $M_1$ or $M_2$ muscarinic receptor gene. Life Sci. 50: 355-363; 1992.
20. Yagi, H.; Katoh, S.; Akiguchi, I.; Takeda, T. Age-related deterioration of ability of acquisition in memory and learning in senes-

cence accelerated mouse: SAM-P/8 as an animal model of disturbances in recent memory. Brain Res. 474:86-93; 1988.
21. Yamamoto, M.; Shimizu, M. Cerebral activating properties of indeloxazine hydrochloride. Neuropharmacology 26(7A):761-770; 1987.
22. Yamamoto, M.; Shimizu, M. Effects of a new TRH analogue, YM-14673 on a passive avoidance test as a possible criterion of improvement in cognitive disturbance in rodents. Naunyn Schmiedebergs Arch. Pharmacol. 338:262-267; 1988.
23. Yamamoto, M.; Iwai, A.; Kawabata, S.; Ozawa, Y.; Shimizu, M. Effects of indeloxazine hydrochloride on passive avoidance behavior of senescence-accelerated mice. Eur. J.Pharmacol. 166: 345-348; 1989.
24. Yamamoto. M.; Iwai, A.; Ozawa, Y. Effects of a new TRH analogue, YM-14673, on disturbance of passive avoidance learning in senescence-accelerated mice. Pharmacol. Biochem. Behav. 35:727-729; 1990.