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Combination of a novel antidementia drug FK960 with donepezil synergistically improves memory deficits in rats

Kenichi Tokita*, Shunji Yamazaki, Mayako Yamazaki, Nobuya Matsuoka, Seitaro Mutoh

Department of Neuroscience, Medicinal Biology Research Laboratories, Fujisawa Pharmaceutical Co. Ltd., 2-1-6 Kashima, Yodogawa-ku, Osaka 532-8514, Japan

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Abstract

FK960 [*N*-(4-acetyl-1-piperazinyl)-*p*-fluorobenzamide monohydrate] is a novel antidementia drug which has been demonstrated to have potential cognitive-improving actions through enhancement of somatostatin release. Since the mechanism of action is different from cholinesterase inhibitors (CEIs), FK960 might be more efficacious at alleviating cognitive deficiencies than CEIs alone, particularly when used in combination therapies with CEIs. We examined the ability of FK960 and donepezil, a CEI, to improve memory deficits in three rat models of dementia: scopolamine-treated rats, rats received with bilateral nucleus basalis magnocellularis (NBM) lesions, and aged rats using the passive avoidance task. FK960 (0.1–10 mg/kg ip) significantly ameliorated the memory deficits in all three models. Donepezil (0.032–3.2 mg/kg ip) significantly improved the deficits induced by both scopolamine or by NBM lesion, but no significant effect was observed in the aged rat model. To determine whether concomitant treatment would be more effective, we coadministered FK960 and donepezil in NBM-lesioned rats using the same task. Concurrent administration of FK960 and donepezil at dosages that were suboptimal when the compounds were administered alone (FK960, 0.1 mg/kg; donepezil, 0.1 mg/kg) significantly improved memory impairment in the animals. Furthermore, coadministration of FK960 and donepezil at optimal dosages for both (FK960, 1 mg/kg; donepezil, 0.32 mg/kg) produced marked amelioration of memory deficits that was more efficacious than when either compound was administered individually. These results demonstrate that FK960 is more efficacious than CEIs in improving memory deficits, and that FK960 has synergistic efficacy when combined with CEIs.

Keywords: FK960; Donepezil; Passive avoidance; Memory; Basal forebrain; Dementia; Somatostatin

1. Introduction

Brain cholinergic neurons degenerate in patients with Alzheimer's disease (AD) and senile dementia of the Alzheimer's type (SDAT), and the degree of degeneration parallels functional loss in these disorders (Davies and Maloney, 1976; Perry et al., 1978; Coyle et al., 1983). This clinical evidence, as well as experimental observations in animals, supports the hypothesis that cholinergic nerve activity plays a crucial role in various forms of cognitive function (Bartus et al., 1982; Hepler et al., 1985; Hagan and Morris, 1987; Matsuoka et al., 1991). Based on this "cholinergic hypothesis," many attempts have been made to reverse cognitive deficits by increasing brain cholinergic activity through the use of cholinomimetics such as acetylcholinesterase inhibitors (CEIs), acetylcholine (ACh) precursors and direct cholinergic agonists (Brinkman and Gershon, 1983).

Tacrine (9-amino-1,2,3,4-tetra-hydro-acridine hydrochloride) was the first drug approved by the FDA for the treatment of mild or moderate AD. Clinical trials with tacrine showed significant improvement in AD patients (Summers et al., 1981, 1986). However, the therapy was often accompanied by a variety of serious side effects, including hepatotoxicitiy. Recently, donepezil, a newly synthesized centrally acting CEI, became the second drug

Abbreviations: FK960, *N*-(4-acetyl-1-piperazinyl)-*p*-fluorobenzamide monohydrate; Donepezil (Aricept), 1-benzyl-4-[(5,6-dimethoxy-1-indanon)-2-yl] methylpiperidine hydrochloride; Tacrine, 9-amino-1,2,3,4-tetrahydro-acridine hydrochloride; CEI, cholinesterase inhibitor; NBM, nucleus basalis magnocellularis; ChAT, choline acetyltransferase; ACh, acetylcholine; AD, Alzheimer's disease; SDAT, senile dementia of the Alzheimer's type.

* Corresponding author. Tel.: +81-6-6390-1153; fax: +81-6-6304-5367.

E-mail address: kenichi_tokita@po.fujisawa.co.jp (K. Tokita).

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approved for AD. Donepezil has been reported to have 1000-fold higher selectivity for acetylcholinesterase than for butyrylcholinesterase, and to exert certain beneficial effects on learning and memory in animal studies (Ogura et al., 1988; Yamanishi et al., 1990; Rogers et al., 1991). Importantly, it has also been shown to improve cognitive decline in AD patients (Barner and Gray, 1998).

We have recently identified FK960, a putative cognitive enhancer of piperazine derivation, by a screening concept based on the hypothesis that drugs which specifically activate the hippocampal formation may ameliorate memory impairments. FK960 indeed enhances both short- and long-term memory in a variety of rodent and nonhuman primate models of dementia (Yamazaki et al., 1996; Matsuoka and Aigner, 1997). The memory-improving action is thought to be mediated by an activation of hippocampal somatostatinergic neurotransmission (Yamazaki et al., 1996; Matsuoka and Satoh, 1998). In our recent in vitro studies, FK960 enhanced the magnitude of long-term potentiation (LTP) in hippocampal slices (Matsuoka and Satoh, 1998) and also increased high K^+-evoked somatostatin release from hippocampal slices without altering basal release (Inoue et al., 2001). FK960 has been demonstrated to exert its selective facilitatory actions on somatostatin release from hippocampal nerve terminals by blocking the interplay between somatostatin autoreceptors, inhibitory G proteins and neuronal Ca^{2+} channels (Wang et al., 2000). Since the decline in cognitive function in AD patients is associated with a decrease in the brain somatostatin content (Davies et al., 1980; Alhainen et al., 1991), the activation of somatostatin by FK960 is expected to alleviate memory dysfunction in these patients. The primary mechanism of the pharmacological action of FK960 is different from that of the existing CEIs, tacrine or donepezil. It is, therefore, also expected to be more efficacious than CEIs not only when administered alone, but also when combined with CEIs.

To test this hypothesis, we compared the effects of FK960 and donepezil treatments on deficits in the learning of a passive avoidance task in scopolamine-treated rats, nucleus basalis magnocellularis (NBM)-lesioned rats, and aged rats. In addition, the effect of combined administration of FK960 with donepezil at suboptimal or optimal doses of each drug was investigated in NBM-lesioned rats in the same task.

2. Materials and methods

2.1. Animals

Male Wistar strain rats (7–8 weeks old) were used in the tests on scopolamine-induced amnesia. In the NBM-lesion model, 12–14-week-old male Fischer strain rats were used. For the study on memory deficit in aged rats, the aged and young groups consisted of 24–26-month-old Fischer rats and 10–11-week-old rats, respectively. Rats of all ages tested were used from the commencement of the studies. All animals were obtained from Charles River (Atsugi, Japan) at least 1 week before the experiments, and were given food and water ad libitum. They were housed in a stainless mesh cage in a temperature-controlled environment (22±2 °C) under a 12:12-h light/dark cycle with lights on at 8:00 a.m. They were handled 3 days prior to the start of the behavioral experiments. All animal procedures were carried out as approved by the Animal Care and Use Committee at Fujisawa Pharmaceutical Co. Ltd.

2.2. Behavioral procedures

The apparatus and experimental procedure used in the passive avoidance task were similar to those described previously (Matsuoka et al., 1992). Briefly, a two-compartment, step-through, passive avoidance apparatus consisting of illuminated and dark compartments attached to an electrified grid floor and separated by a guillotine door was used. During the habituation trial, the rat was placed in the illuminated compartment and the door was raised. After entering the dark compartment, the rat was returned to its home cage. In the scopolamine-treated model, rats were given an intraperitoneal injection of scopolamine (1 mg/kg) 30 min after the habituation trial. The rat was again placed in the illuminated compartment (acquisition trial), 60 min after the habituation trial. When the rat entered the dark compartment, the guillotine door was closed. In single-drug administration experiments, scrambled electrical foot shocks of 4–6.5 mA in the scopolamine-treated model, 4–4.3 mA in the NBM-lesioned model, and 3–3.8 mA in the aged rats were delivered for 3 s through the grid floor using a shock generator (Neuroscience, Tokyo, Japan; model SGS-001). In the coadministration study of FK960 and donepezil in the NBM-lesioned model, scrambled electrical foot shocks of 0.4 mA were delivered for 4 s through the grid floor using a shock generator (Neuroscience; model NS-SG01). The shock intensities used in the present study with two different models of shock generators were determined based on the level of shock intensity of the two machines and standardization of the animals aversive response. In our control experiments, electrical shocks with different intensities were given and then memory retention was measured 24 h after the acquisition trial. Experiments with model #SGS-001 revealed that the passive avoidance latencies were 17.1±6.0, 10.5±3.2, 13.6±5.9, 13.9±2.1, 100.2±31.7, 182.1±48.1, 252.1±28.4, 246.4±30.1, and 300±0 s in rats receiving a foot shock of 0, 0.5, 1, 1.5, 2, 2.5, 3, 3.5, and 4 mA, respectively, indicating that the foot shock strength of more than 3 mA produced sufficient memory retention. On the other hand, experiments with model #NS-SG01 showed that the passive avoidance latencies were 20.3±2.1, 34.2±8.2, 49.0±5.1, 131.5±21.6, 201.1±20.8, 289.2±43.2, 300.0±0, and 300±0 s in rats received with the foot shock of the strength of 0, 0.1, 0.15, 0.2, 0.25, 0.3, 0.4, and 0.5 mA, respectively, suggesting that foot shock strength of more

than 0.3 mA was sufficient for the model. FK960 or donepezil was administered intraperitoneally immediately after the acquisition trial (60 min after the habituation trial). In the retention trial, made 24 h after the acquisition trial, a rat was placed again in the illuminated compartment and the response latency to enter the dark compartment was measured up to a maximum of 300 s. The results were recorded as average latency for each treatment group. The percentage of rats reaching criterion (300 s) was also calculated.

2.3. Neurosurgery

The surgical procedure used in the NBM-lesion model was similar to that described previously (Matsuoka et al., 1992). Briefly, bilateral neurotoxic lesions of the NBM were produced by injection of ibotenic acid. The animals were anesthetized with sodium pentobarbital (50 mg/kg ip) and were fixed on stereotaxic apparatus with the incisor bar set 2.3 mm below the intra-aural line. Positioning of the coordinates followed the stereotaxic atlas of König and Klippel (1963). A stainless injection needle (diameter 0.25 mm), connected via polyethylene tubing to a 10-μl microsyringe mounted on a microdrive injector, was inserted into the NBM. The coordinates were 1.6 mm posterior to the bregma, 2.8 mm lateral to the midline, and 7.8 mm ventral from the skull. Ibotenic acid (Sigma, St. Louis, MO) was dissolved in sterile physiological saline at a concentration of 8 μg/μl, and then infused directly into the area of one NBM in a volume of 1 μl over 6 min. After the injection, the cannula was left in place for 5 min to ensure that the drug had diffused away from the needle tip. One week later, when the weight of the rats had stabilized, an equivalent lesion was made in the contralateral NBM. Sham-operated rats were placed on stereotaxic apparatus under anesthesia and were infused with saline into the brain area for 6 min. Rats were subjected to behavioral tests 2–3 weeks after the last surgery.

2.4. Drugs

FK960 was synthesized at Fujisawa Pharmaceutical Co. Ltd., and was either suspended in 0.5% methylcellulose for the experiments using 10 mg/kg as the highest dose or dissolved in physiological saline for the lower-dose coadministration studies. Donepezil (Aricept, 1-benzyl-4-[(5,6-

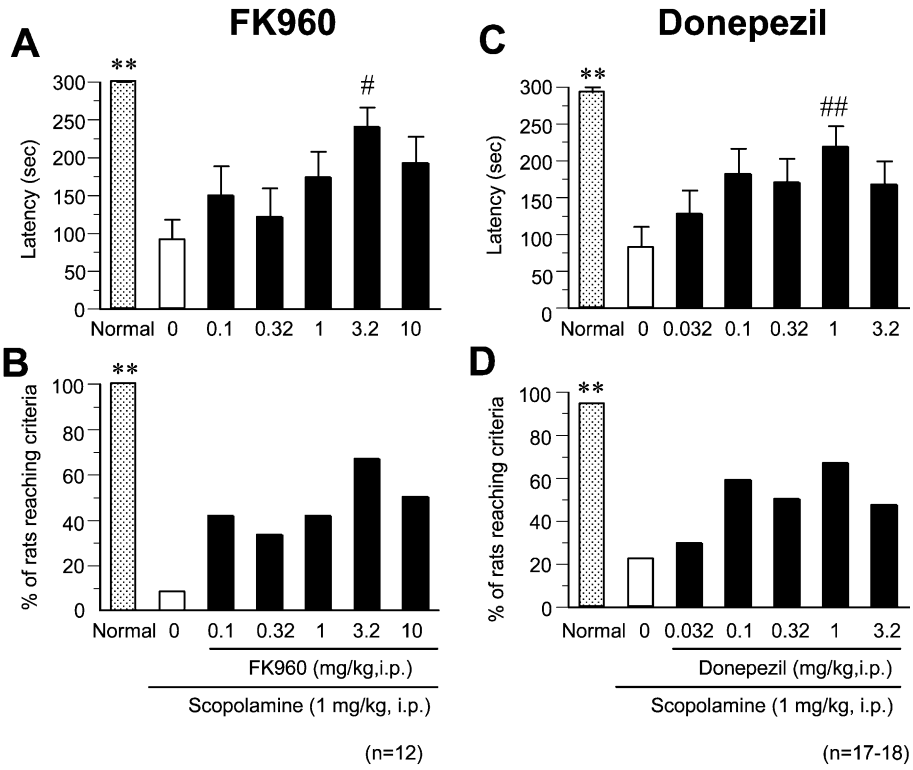

Fig. 1. Effect of FK960 (A, B) or donepezil (C, D) on memory deficits produced by scopolamine in rats in a passive avoidance task. (A, C) Retention latencies. Ordinate represents the median retention latencies in a 24-h retention test. $^{\#}P<.05$, $^{\#\#}P<.01$, statistically significant compared with scopolamine-alone controls (open columns) (by Kruskal–Wallis test followed by Dunnett's type test). $^{**}P<.01$, statistically significant compared with scopolamine-alone controls (open columns) (by Wilcoxon ranking test). Each column and bar represents the mean±S.E.M. (B, D) Percentage of rats reaching criteria. Ordinate represents the percentage of rats reaching criteria (300 s) in a 24-h retention test. $^{**}P<.01$, statistically significant compared with scopolamine-alone controls (open columns) (by Fisher's exact probability test). Numbers of rats are shown in parentheses. Scopolamine (1 mg/kg ip) was administered 30 min before the acquisition trial. FK960 or donepezil was administered intraperitoneally immediately after the acquisition trial.

dimethoxy-1-indanon)-2-yl] methylpiperidine hydrochloride) was synthesized at Fujisawa Pharmaceutical Co. Ltd., and was dissolved in physiological saline. Scopolamine hydrobromide (Nacalai Tesque, Kyoto, Japan) was also dissolved in physiological saline. For the coadministration studies of FK960 and donepezil, both drug solutions were mixed and this mixed solution was injected. All drugs were prepared immediately before use and were administered intraperitoneally in a volume of 1 ml/kg.

2.5. Statistical analysis

All results are expressed as the mean±S.E.M. Statistical analysis of retention latency and percentage of rats reaching criteria for drug treatment vs. control was conducted using a nonparametric Kruskal–Wallis test with Dunnett's posttest, or chi-square with Dunnett's posttest, respectively. For comparison of models with their control groups, statistical analysis of retention latency and percentage of rats reaching criteria was conducted using Wilcoxon ranking test or Fisher's exact probability test, respectively.

3. Results

3.1. Effect of FK960 or donepezil on scopolamine-induced memory deficits

We used a passive avoidance task to investigate the ability of FK960 or donepezil to ameliorate the memory deficits in scopolamine-treated rats. Scopolamine (1 mg/kg) given 30 min before the acquisition trial significantly ($P<.01$ by Wilcoxon ranking test) reduced latency to enter the dark chamber in a retention trial determined 24 h later (Fig. 1A,C). Administration of FK960 (0.1–10 mg/kg) immediately after the acquisition trial prolonged the latency in the scopolamine-treated rats, with a bell-shaped dose–response curve and a maximal efficacy at 3.2 mg/kg (Fig. 1A; $\chi^2=11.8$, $P<.05$ by Kruskal–Wallis test; $P<.05$ by Dunnett's type test). Donepezil (0.032–3.2 mg/kg) had a similar effect on latency, exhibiting a bell-shaped dose–response curve and a maximal effect at 1 mg/kg (Fig. 1C; $\chi^2=12.91$, $P<.05$ by Kruskal–Wallis test; $P<.01$ by Dunnett's type test). As shown in Fig. 1B,D, the percentage

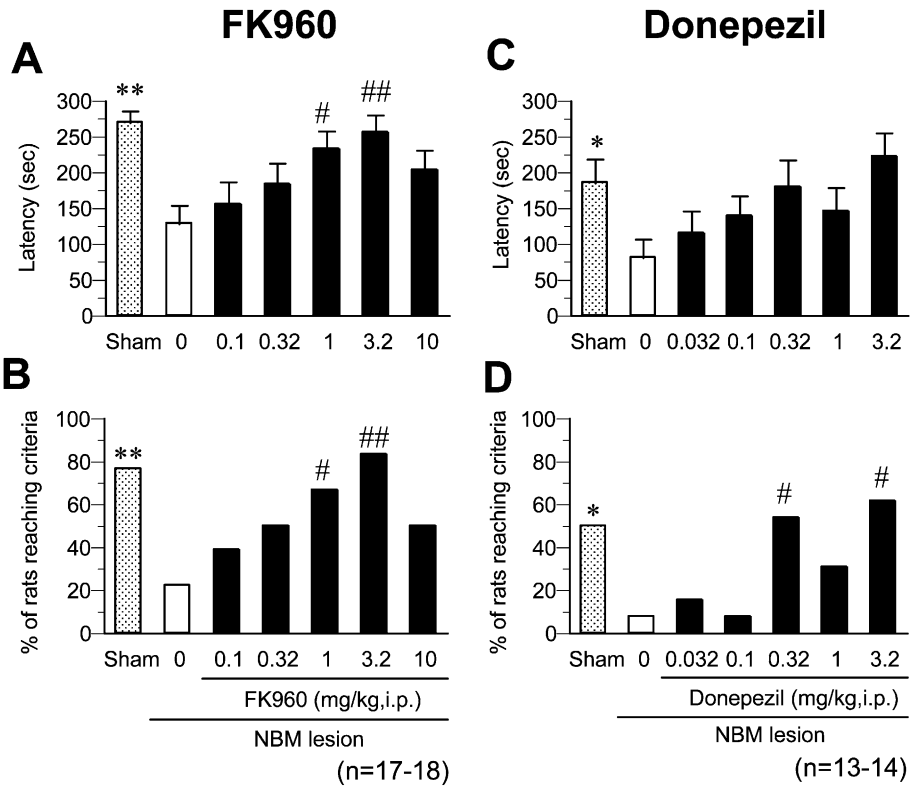

Fig. 2. Effect of FK960 (A, B) or donepezil (C, D) on the memory deficits in NBM-lesioned young rats in a passive avoidance task. (A, C) Retention latencies. Ordinate represents the median retention latencies in a 24-h retention test. $^{\#}P<.05$, $^{\#\#}P<.01$, statistically significant compared with vehicle controls (open columns) (by Kruskal–Wallis test followed by Dunnett's type test). $*P<.05$, $**P<.01$, statistically significant compared with vehicle controls (open columns) (by Wilcoxon ranking test). Each column and bar represents the mean±S.E.M. (B, D) Percentage of rats reaching criteria. Ordinate represents the percentage of rats reaching criteria (300 s) in a 24-h retention test. $^{\#}P<.05$, $^{\#\#}P<.01$, statistically significant compared with vehicle controls (open columns) (by chi-square test followed by Dunnett's type test). $*P<.05$, $**P<.01$, statistically significant compared with vehicle controls (open columns) (by Fisher's exact probability test). Numbers of rats are shown in parentheses. FK960 or donepezil was administered intraperitoneally immediately after the acquisition trial.

of rats reaching criteria, another index of memory, was significantly reduced by scopolamine ($P<.01$ by Fisher's exact probability test). Both FK960 and donepezil tended to ameliorate the deficits, although these were not statistically significant (Fig. 1B: $\chi^2=9.3$, $P=.098$ by chi-square test; Fig. 1D: $\chi^2=10.33$, $P=.066$ by chi-square test).

3.2. Effect of FK960 or donepezil on memory deficits in NBM-lesioned rats

Fig. 2 shows the effects of FK960 or donepezil on the memory deficits in NBM-lesioned young rats in a passive avoidance task. The latency in the retention trial was significantly shorter in the NBM-lesioned rats than in the control sham rats ($P<.01$, Fig. 2A; $P<.05$, Fig. 2C by Wilcoxon ranking test). FK960 (0.1–10 mg/kg) markedly diminished the retention deficit in NBM-lesioned rats. The dose–response curve was bell-shaped with the maximal and statistically significant change at 1 and 3.2 mg/kg ($\chi^2=14.44$, $P<.05$ by Kruskal–Wallis test; $P<.05$ by Dunnett's type test) (Fig. 2A). Although donepezil (0.032–3.2 mg/kg) showed a tendency to ameliorate the deficits, these changes were not statistically significant ($\chi^2=10.47$, $P=.063$ by Kruskal–Wallis test) (Fig. 2C). The percentage of rats reaching criteria paralleled the latency data. The percentage of rats reaching criteria was significantly reduced in the NBM-lesioned rats ($P<.01$, Fig. 2B; $P<.05$, Fig. 2D by Fisher's exact probability test). FK960 increased the percentage of rats reaching criteria with a bell-shaped dose–response curve with the maximal and statistically significant change at 1 and 3.2 mg/kg ($\chi^2=16.32$, $P<.01$ by chi-square test; $P<.05$ by Dunnett's type test) (Fig. 2B). Donepezil increased the percentage of rats reaching criteria and the statistically significant change ($\chi^2=17.33$, $P<.01$ by chi-square test; $P<.05$ by Dunnett's type test) was obtained at 0.32 and 3.2 mg/kg (Fig. 2D).

3.3. Effect of FK960 or donepezil on memory deficits in aged rats

Aged rats (24–26 months old) showed significantly ($P<.01$ by Wilcoxon ranking test) shorter latency compared with the young control (10–11 weeks old) animals (Fig. 3A,C). FK960 significantly prolonged the latency in the aged rats (Fig. 3A). The dose–response relationship was bell-shaped with the maximal and statistically significant effect at 1 mg/kg ($\chi^2=11.79$, $P<.05$ by Kruskal–Wallis test; $P<.01$ by Dunnett's type test). Administration of

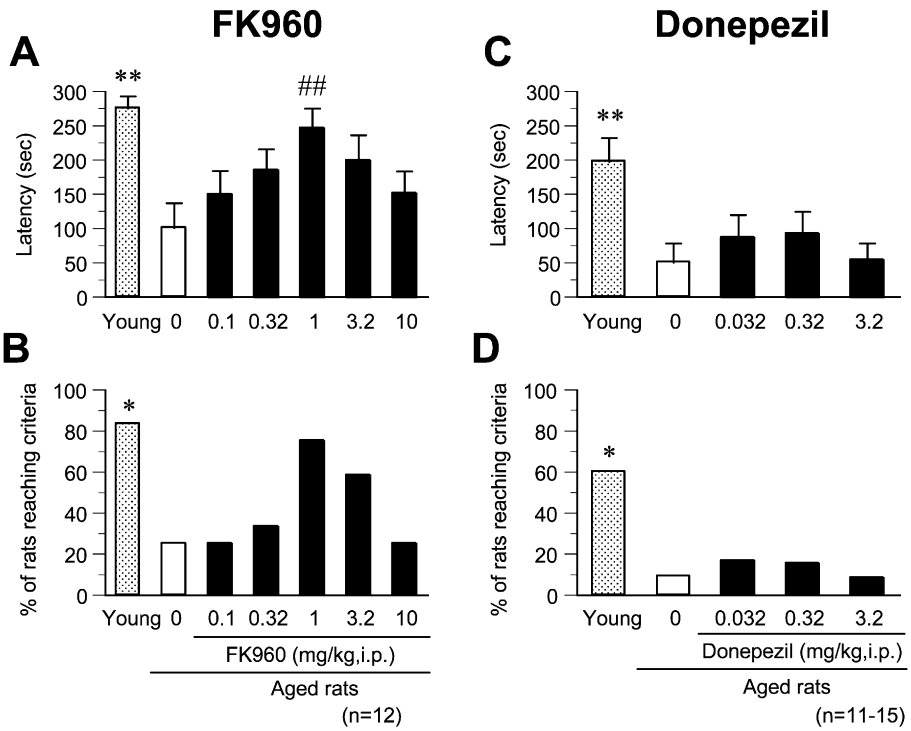

Fig. 3. Effect of FK960 (A, B) or donepezil (C, D) on memory deficits in aged rats in a passive avoidance task. (A, C) Retention latencies. Ordinate represents the median retention latencies in a 24-h retention test. $^{\#\#}P<.01$, statistically significant compared with vehicle controls (open columns) (by Kruskal–Wallis test followed by Dunnett's type test). $^{**}P<.01$, statistically significant compared with vehicle controls (open columns) (by Wilcoxon ranking test). Each column and bar represents the mean±S.E.M. (B, D) Percentage of rats reaching criteria. Ordinate represents the percentage of rats reaching criteria (300 s) in a 24-h retention test. $^{*}P<.05$, statistically significant compared with vehicle controls (open columns) (by Fisher's exact probability test). Number of rats are shown in parentheses. FK960 or donepezil was administered intraperitoneally immediately after the acquisition trial.

donepezil (0.032–3.2 mg/kg), however, only marginally ameliorated amnesia in the aged rats (χ^2=1.87, P=.599 by Kruskal–Wallis test) (Fig. 3C). The percentage of rats reaching criteria paralleled the latency. The percentage of rats reaching criteria was significantly reduced in the aged rats (P<.05 by Fisher's exact probability test) (Fig. 3B,D). FK960 increased the percentage of rats reaching criteria exhibiting a bell-shaped dose–response curve with the maximal change at 1 mg/kg, although the change was not statistically significant (χ^2=11.37, P<.05 by chi-square test; P>.05 by Dunnett's type test). Donepezil did not significantly alter the percentage of rats reaching criteria in aged animals at all doses tested (χ^2=0.6, P=.897 by chi-square test) (Fig. 3D).

3.4. Effect of concomitant administration of FK960 with donepezil on memory deficits in NBM-lesioned rats

The effects of concomitant administration of FK960 and donepezil at doses previously established as suboptimal or optimal (Fig. 2) were examined using the NBM-lesioned rats model in the passive avoidance task. Suboptimal and optimal doses of FK960 were determined to be 0.1 and 1 mg/kg, respectively. Similarly, suboptimal and optimal doses of donepezil were determined to be 0.1 and 0.32 mg/kg.

As shown in Fig. 4, the latency in the retention trial was significantly (P<.01 by Wilcoxon ranking test) shorter in the NBM-lesioned rats than in the control sham rats (Fig. 4A). Suboptimal doses of FK960 (0.1 mg/kg) or donepezil (0.1 mg/kg) treated alone tended to prolong the latency, although, it did not reach statistical significance. Concomitant administration of FK960 and donepezil at their suboptimal doses significantly ameliorated memory deficits (χ^2=22.73, P<.01 by Kruskal–Wallis test; P<.05 by Dunnett's type test). Optimal doses of FK960 (1 mg/kg) or donepezil (0.32 mg/kg) provided a significant restoration (P<.05 by Kruskal–Wallis test followed by Dunnett's type test) on the retention deficits. Concomitant administration of FK960 at optimal dose and donepezil at suboptimal dose significantly (P<.01 by Kruskal–Wallis test followed by Dunnett's type test) prolonged the latencies. Similarly, concomitant administration of FK960 at suboptimal dose and donepezil at optimal dose significantly (P<.05 by Kruskal–Wallis test followed by Dunnett's type test) improved the deficits. However, the latencies in those combinations were almost completely consistent with those in FK960 alone at optimal dose and in donepezil alone at optimal dose. Concomitant administration of FK960 and donepezil at optimal doses significantly (P<.01 by Kruskal–Wallis test followed by Dunnett's type test) improved the impaired performance, and the latency in this combination was increased slightly compared to that in each drug alone. The percentage of rats reaching criteria paralleled the latency (Fig. 4B). Concomitant administration of FK960 and donepezil tended to ameliorate the deficits, although, it was not statistically significant (χ^2=14.16, P=.078 by chi-square test).

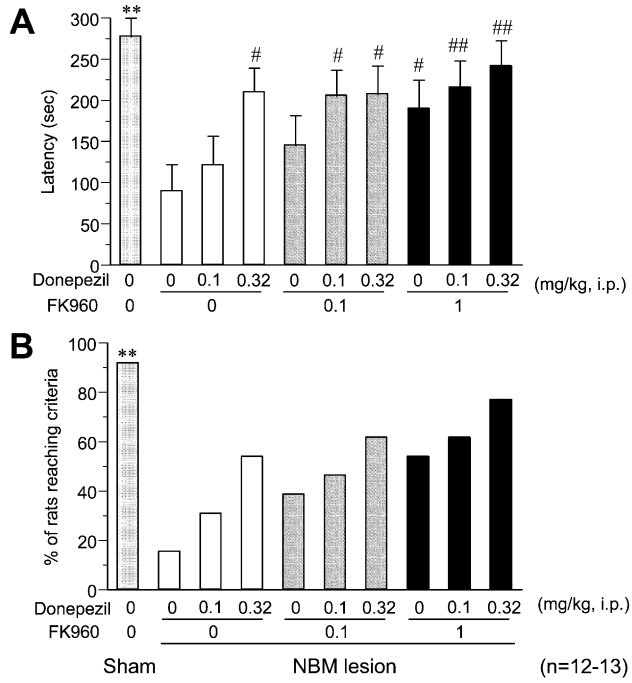

Fig. 4. Effect of FK960 and donepezil on memory deficits in NBM-lesioned young rats in a passive avoidance task. (A) Retention latencies. Ordinate represents the median retention latencies in a 24-h retention test. $^{\#}P$<.05, $^{\#\#}P$<.01, statistically significant compared with vehicle controls (open columns) (by Kruskal–Wallis test followed by Dunnett's type test). $^{**}P$<.01, statistically significant compared with vehicle controls (open columns) (by Wilcoxon ranking test). Each column and bar represents the mean±S.E.M. (B) Percentage of rats reaching criteria. Ordinate represents the percentage of rats reaching criteria (300 s) in a 24-h retention test. $^{**}P$<0.01, statistically significant compared with vehicle controls (open columns) (by Fisher's exact probability test). Number of rats are shown in parentheses. FK960 or donepezil was administered intraperitoneally immediately after the acquisition trial.

4. Discussion

In the present study, we evaluated the antidementia effects of FK960 and donepezil in three different rat models of dementia using a passive avoidance task. In addition, the efficacy of concomitant administration of FK960 and donepezil was investigated for the same task. FK960 significantly improved impaired memory not only in scopolamine-treated rats, but also in NBM-lesioned rats in the passive avoidance task. These results suggest that FK960 ameliorates the memory deficits produced by brain cholinergic hypofunction. The findings agree with our previous data on memory-improving actions of FK960 in other behavioral models like the Morris water maze and the eight-arm radial maze in rats (Yamazaki et al., 1996), as well as the delayed nonmatching-to-sample task in monkeys (Matsuoka and Aigner, 1997). Previous mechanistic studies on FK960 in vitro have demonstrated that FK960 selectively activates

somatostatinergic neurotransmission in the hippocampus (Matsuoka and Satoh, 1998; Wang et al., 2000; Inoue et al., 2001). Somatostatin is known to play a vital role in modulating mnemonic functions. Intracerebral or intrahippocampal injections of somatostatin improve memory deficits produced by cholinergic hypofunction in rats (Matsuoka et al., 1994; Ohno et al., 1994), and somatostatin depletion by cysteamine impairs memory formation (Matsuoka et al., 1995). Therefore, it is reasonable to assume that the memory-improving action of FK960 is mediated by a potentiation of hippocampal somatostatinergic neurotransmission.

Donepezil was demonstrated to be effective on memory deficits both in scopolamine-treated rats and in NBM-lesioned rats in the present study. The memory-improving action of donepezil in the scopolamine-induced amnesia model is in line with the data of Eisai's group showing that donepezil reverses scopolamine-induced memory deficits in an eight-arm radial maze (Ogura et al., 1988). The improvement of scopolamine amnesia by donepezil in our study and others could be simply explained by neurochemical changes in the brain where available synaptic ACh was increased via acetylcholinesterase inhibition (Giacobini et al., 1996). The result in NBM-lesioned model was also consistent with previous results by others who demonstrated the beneficial effects of CEIs (Dokla et al., 1989; Mandel et al., 1989; Ogura et al., 2000). Nevertheless, it was an unexpected observation to us, because we previously observed that physostigmine, another CEI, failed to ameliorate memory deficits in NBM-lesioned rats in a passive avoidance task (Matsuoka et al., 1992). Based on the finding with physostigmine, we thus speculated that intact NBM–cortical cholinergic pathways are necessary for CEIs to ameliorate memory disturbance. Therefore, other pharmacological actions of donepezil besides its cholinesterase inhibiting action could be involved in the improvement of NBM-lesion-induced amnesia. For instance, donepezil is also known to have noncholinergic activities; it has binding affinity to adrenergic-α receptors (Miyamoto and Goto, 1997) and σ-receptors (Kato et al., 1999). Rogers et al. (1991) have observed significant changes in the contents of catecholamines and their metabolites in the brain of rats following donepezil treatment. These results taken together prompted us to speculate that donepezil but not physostigmine ameliorated the memory deficits produced by NBM-lesioning in rats through its noncholinergic activity, although this hypothesis requires further investigation.

An important finding of the present studies was that while in aged rats FK960 ameliorated impaired memory, donepezil minimally ameliorated memory deficits. We have previously demonstrated that physostigmine failed to ameliorate memory deficits in aged rats in a passive avoidance task (Matsuoka et al., 1992). The lack of efficacy of CEIs on memory deficits associated with aging suggests that brain cholinergic hypofunction might not be involved in the memory deficits seen in aged rats. This view is supported by our previous neurochemical finding that there were no changes in brain ChAT activity in aged rats compared to control young rats (Matsuoka et al., 1995). A number of studies on aged rats have demonstrated age-related changes in the brain aminergic system as well as neuropeptides that may play a role in memory disturbance in aged animals (Kubanis and Zornetzer, 1981; Ponzio et al., 1982; Luine et al., 1990; Zhang et al., 1991; Wang et al., 1993). Another implication of the present studies is that our findings in aged rats clearly show a difference between the mechanisms of action of FK960 and CEIs such as donepezil or physostigmine, and furthermore, support our hypothesis that cholinergic activation is not the primary mechanism of action of FK960. In fact, FK960 has little effect on acetylcholinesterase activity, the binding to ACh muscarinic receptors, or on ACh release from hippocampal slices induced by high K^+ stimulation at concentrations up to 10^{-5} M (unpublished observations and Inoue et al., 2001). Therefore, taken together, it was speculated that an activation of hippocampal somatostatin could play a role in functional improvement by FK960 on senescence-associated memory disturbance.

The most important finding of the present study was that concomitant administration of FK960 and donepezil at suboptimal doses for each drug synergistically improved memory impairment in NBM-lesioned rats in a passive avoidance task. These facilitatory actions are likely to be attributable to the synergistic interaction of different neuronal mechanisms of the two drugs. The primary mechanism of the pharmacological action of FK960 is the activation of the somatostatinergic nervous system in the hippocampus (Yamazaki et al., 1996; Matsuoka and Satoh, 1998, Inoue et al., 2001), whereas donepezil primarily activates cholinergic transmission, although noncholinergic activities may be partly involved as previously mentioned. Bidirectional interactions between somatostatin and ACh in the cortex or the hippocampus have been documented (Lamour and Epelbaum, 1988). Therefore, it would be conceivable that activation of these two important intermediates modulate cognitive dysfunction via parallel pathways. Further investigations are needed to delineate the mechanisms underlying this interaction.

The combination of a suboptimal dose and an optimal dose or the combination of optimal doses for both agents improved memory deficits with an equivalent effect to that observed for each drug given individually at an optimal dose. The results may indicate that the effects of the optimal dose of FK960 or donepezil are almost saturated and that, at least in this model, there is a ceiling effect. Our studies demonstrated that the dose–response curve of FK960 is bell-shaped not only in the NBM-lesion model but also in other models. Donepezil also showed a bell-shaped dose–response relationship both in the NBM-lesion model and in scopolamine-treated rats. Concomitant administration of FK960 with donepezil at each compound's optimal dose did not cause a decrease below the effectiveness seen for each drug alone. Thus, coadministration in the clinical

setting may ameliorate the symptoms in a more efficacious manner than that of either drug alone.

In conclusion, FK960 improved the memory impairment in three different animal models of dementia, while donepezil ameliorated the memory deficits in only two: in scopolamine-treated and in NBM-lesioned rats but not in aged rats. Furthermore, combination of FK960 and donepezil at suboptimal dosages, which do not exert efficacy individually, significantly improved memory impairment. The findings suggest that FK960 could be superior to CEIs like donepezil in terms of efficacy against different degrees of cognitive disturbance, and also that FK960 may improve memory impairment not only when administered alone but also when combined with CEIs like donepezil. These results taken together continue to support our view that FK960 might be of therapeutic value against dementing disorders, such as AD or SDAT.

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