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European Journal of Pharmacology 518 (2005) 140 – 144

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Short communication

Effects of selegiline alone or with donepezil on memory impairment in rats

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Received 18 April 2005; received in revised form 14 June 2005; accepted 20 June 2005

Abstract

Selegiline, a monoamine oxidase-B inhibitor, is reported to improve memory and learning in dementia of Alzheimer's type. However, only a few studies have reported its use in animal models. Here, we evaluated the effects of selegiline only or its combined use with donepezil, a selective acetylcholinesterase inhibitor on memory impairment, using a Morris water maze. Selegiline dose-dependently attenuated ethylcholine aziridinium ion-induced memory impairment. Co-administration of selegiline and donepezil, at doses that do not exert efficacy individually, significantly ameliorated scopolamine+*p*-chlorophenylalanine-induced memory deficits. These results suggest that selegiline improves memory impairment mediated by the cholinergic system, and provide evidence of the usefulness of co-treatment with selegiline and donepezil for treating spatial deficits in dementia.

Keywords: Selegiline; Donepezil; Water maze; Memory; Acetylcholine

1. Introduction

The central cholinergic system is known to play fundamental roles in cognitive function. Memory and learning deficits are induced by: 1) lesioning cholinergic structures such as the nuclei basales of Meynert, 2) pharmacologically intervening with cholinergic antagonists (Beatty et al., 1986), and 3) using a selective cholinotoxin ethylcholine aziridinium ion AF64A (Hortnagl, 1994; Jarrard et al., 1984). Cholinergic neurons degenerate in patients with Alzheimer's disease and senile dementia of Alzheimer's type, and the degree of degeneration correlates well with functional loss in these disorders (Davies and Maloney, 1976; Perry et al., 1978). Based on a cholinergic hypothesis, many attempts have been made to reverse cognitive deficits by increasing brain cholinergic activity through the cholinomimetic use of acetylcholinesterase inhibitors, acetylcholine precursors and cholinergic agonists. In fact, a selective acetylcholinesterase inhibitor, donepezil, has been used for the treatment of mild Alzheimer's disease (Doody, 1999).

The serotonergic system takes part in cognitive processes, partly through interactions with cholinergic mechanisms (Steckler and Sahgal, 1995). Co-treatment with a muscarinic receptor antagonist scopolamine and an inhibitor of serotonin biosynthesis *p*-chlorophenylalanine (PCPA) has been suggested to serve as potential model for cognitive consequences of loss of both cholinergic and serotonergic neurons in Alzheimer's disease (Vanderwolf, 1987). Moreover, AF64A-model has been reported to be of particular relevance for understanding the pathogenesis and progression of Alzheimer's disease (Hortnagl, 1994; Jarrard et al., 1984). Similar changes in non-cholinergic transmitters (e.g. noradrenaline and serotonin), monoamine oxidase-B activity, and markers of synaptic vesicles were found in Alzheimer's disease and the AF64A-model. AF64A (i.c.v. injection) produces long-term presynaptic cholinergic deficits specifically in the hippocampus (Jarrard et al., 1984; Walsh et al., 1984), whereas scopolamine causes cholinergic deficits by blocking muscarinic receptors.

Selegiline, a selective monoamine oxidase-B inhibitor, is used worldwide as a therapy for Parkinson's disease (Birkmayer et al., 1985). Several studies have shown that selegiline improves episodic memory and learning in patients with Alzheimer's disease (Filip and Kolibas, 1999; Mangoni

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doi:10.1016/j.ejphar.2005.06.024

et al., 1991; Piccinin et al., 1990; Tariot et al., 1987). However, according to recent data from meta-analysis, selegiline significantly improved cognition and activities of daily living at an earlier time point, but not at a later assessment time (Birks and Flicker, 2003; Wilcock et al., 2002). In the present study, we sought to ascertain whether selegiline has effects on memory impairment induced by AF64A, and to evaluate the effect of concurrent administration of selegiline and donepezil on scopolamine+PCPA-induced memory impairment, using a Morris water maze.

2. Materials and methods

2.1. Animals

Male Fisher 344 rats (8–9 weeks old, Nihon SLC, Shizuoka, Japan) were maintained in a humidity (55±10%) and temperature (23±2 °C)-controlled facility following a 12 h light/12 h dark cycle (light on at 7:00 a.m.) with free access to food (MF chow pellets, Oriental Yeast, Tokyo, Japan) and water. Rats were acclimated for 1 week before use in experiments. All animal procedures were in accordance with the guidelines of the Japanese Pharmacological Society.

2.2. AF64A-model

For surgical procedures, animals were anesthetized with pentobarbital sodium (50 mg/kg i.p.) and fixed on a stereotaxic apparatus. Stereotaxic procedures were used, and injections were done using a Hamilton syringe at the following coordinates: $A=-1.0$ mm, $L=\pm 1.6$ mm from bregma and $H=-4.3$ mm from the surface of the skull (Jarrard et al., 1984). Injection of 0.5 μl of either AF64A solution (12 mM; Research Biochemicals Inc., Natick, MA, USA) or vehicle was done into each ventricle over a 3 min period and the needle was left for two additional minutes to prevent spreading up in the tract. Thus, animals received a total of 12 nmol AF64A. Rats were subjected to a behavioral test 10 days after surgery.

2.3. Morris water maze (Morris, 1984)

A circular pool (150 cm in diameter and 45 cm deep) with walls and floor painted in black was filled with water (23±1 °C). A hidden circular platform (28 cm high, 12 cm diameter, 2 cm below the water surface, fixed position) was located in the pool away from the pool wall. The pool was conceptually divided into four quadrants of equal area: NE, NW, SE, and SW. Rats were given two trials per day at 1-min intervals for 5 consecutive days (total of 10 trials). A rat was placed in the water facing the pool wall at one of the 4 quadrants at a different place everyday, and allowed to swim for a maximum of 90 s to find the hidden platform where it was allowed to stay for 10 s. If the rat did not find the platform in 90 s, it was placed on the platform by hand and remained there for 10 s. The time to reach the platform (escape latency) was measured with a stopwatch.

2.4. Drugs and treatment

Selegiline (Fujimoto Pharmaceutical, Osaka, Japan), donepezil which was isolated from Aricept® (Eisai, Ibaraki, Japan) in our Department of Organic Synthesis, and scopolamine hydrobromide (Nacalai tesque, Kyoto, Japan) were dissolved in saline. PCPA (Nacalai tesque) was suspended in a solution of gum arabic (0.5%) in saline. For scopolamine+PCPA-induced memory impairment, rats were injected daily with PCPA at 300 mg/kg for 3 days prior to testing, and scopolamine (1 mg/kg) 30 min before testing. Thirty minutes before testing, scopolamine+PCPA-treated rats received the following treatments (i.p.): a single injection of either saline (1 ml/kg), donepezil (0.3 or 3 mg/kg) or selegiline (1 or 2.5 mg/kg); and combined administration of saline–saline, saline–donepezil (0.3 mg/kg), saline–selegiline (1 mg/kg) or donepezil–selegiline (0.3 and 1 mg/kg, respectively). The control group, which did not received scopolamine+PCPA, was given saline. AF64A-treated rats were injected with selegiline at 1–10 mg/kg 30 min before testing.

2.5. Statistical analyses

Values were expressed as means and S.E.M. for $n=8-34$. An SAS program (ver. 5.0, SAS Institute, Cary, NC, USA) was used to perform all analyses. Differences between the AF64A-treated and control or AF64A+selegiline-treated groups were assessed by one-way analysis of variance (ANOVA) followed by Steel's test. In the scopolamine model, the main effects (group) and the interactions (group × trials) were analyzed by two-way ANOVA followed by Dunnett's test. Differences were considered significant at $P<0.05$.

3. Results

3.1. Effect of selegiline on AF64A-induced memory impairment

Escape latencies in AF64A-treated rats were significantly longer than those in vehicle-treated rats on day 3 [ANOVA: $F=8.37$, $df=4$, $P<0.0001$, Steel: $P=0.007$], day 4 [ANOVA: $F=4.41$, $df=4$, $P=0.0042$, Steel: $P=0.006$] and day 5 [ANOVA: $F=6.05$, $df=4$, $P<0.0005$, Steel: $P=0.001$] (Fig. 1). Selegiline dose-dependently attenuated the AF64A-induced increase of escape latencies on days 4 and 5. Selegiline (10 mg/kg)

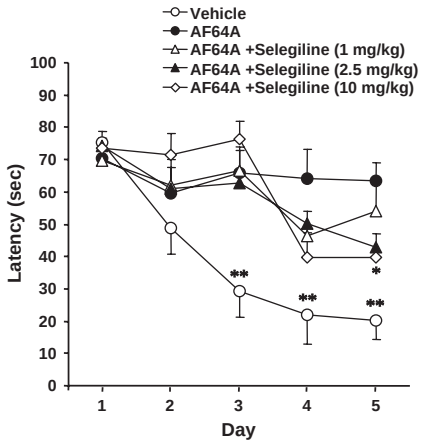

Fig. 1. Effects of selegiline on AF64A-induced memory impairment. Ten days after i.c.v. injection of AF64A, selegiline at 1–10 mg/kg or saline was i.p. administered 30 min before test. Values represent means±S.E.M. ($n=10-12$). *$P<0.05$, **$P<0.01$ vs. AF64A-treated group.

significantly reduced AF64A-induced memory impairment on day 5 [Steel: $P=0.044$].

3.2. Effects of co-treatment with selegiline and donepezil on scopolamine+PCPA-induced memory impairment

As shown in Fig. 2, scopolamine+PCPA caused increases in escape latencies, compared with the control group [two-way ANOVA: $F=27.26$, $df=3$, $P<0.0001$ (group), $F=13.67$, $df=4$, $P<0.0001$ (trial), $F=0.51$, $df=12$, $P=0.907$ (group × trial), Dunnett: $P<0.0001$]. In preliminary experiments, 1 mg/kg scopolamine alone increased escape latencies compared with the vehicle-treated group, and co-treatment with scopolamine+PCPA (150–500 mg/kg) caused dose-dependent increases in escape latencies compared with those in scopolamine alone-treated rats (data not shown). Selegiline at 1 and 2.5 mg/kg failed to reduce escape latencies in scopolamine+PCPA-treated rats [Dunnett: $P=0.214$ (1 mg/kg), $P=0.618$ (2.5 mg/kg)] (Fig. 2A). In contrast, donepezil at 3 mg/kg, but not at 0.3 mg/kg, significantly attenuated scopolamine+PCPA-induced increase of escape latencies [two-way ANOVA: $F=19.14$, $df=3$, $P<0.0001$ (group), $F=18.16$, $df=4$, $P<0.0001$ (trial), $F=0.63$, $df=12$, $P=0.815$ (group × trial), Dunnett: $P=0.260$ (0.3 mg/kg), $P=0.002$ (3 mg/kg)] (Fig. 2B). Although 1 mg/kg selegiline or 0.3 mg/kg donepezil alone failed to reduce escape latencies in scopolamine+PCPA-treated rats, concurrent administration with selegiline and donepezil at the same doses showed a significant reduction in escape latencies increased by scopolamine+PCPA [two-way ANOVA: $F=28.51$, $df=4$, $P<0.0001$ (group), $F=111.01$, $df=4$, $P<0.0001$ (trial), $F=0.75$, $df=16$, $P=0.745$ (group × trial), Dunnett: $P=0.026$] (Fig. 2C). In addition, co-administration of selegiline and donepezil significantly decreased escape latencies in scopolamine+PCPA+selegiline alone-treated rats [Dunnett: $P=0.014$], and tended to reduce escape latencies compared with those in donepezil alone-treated rats [Dunnett: $P=0.054$].

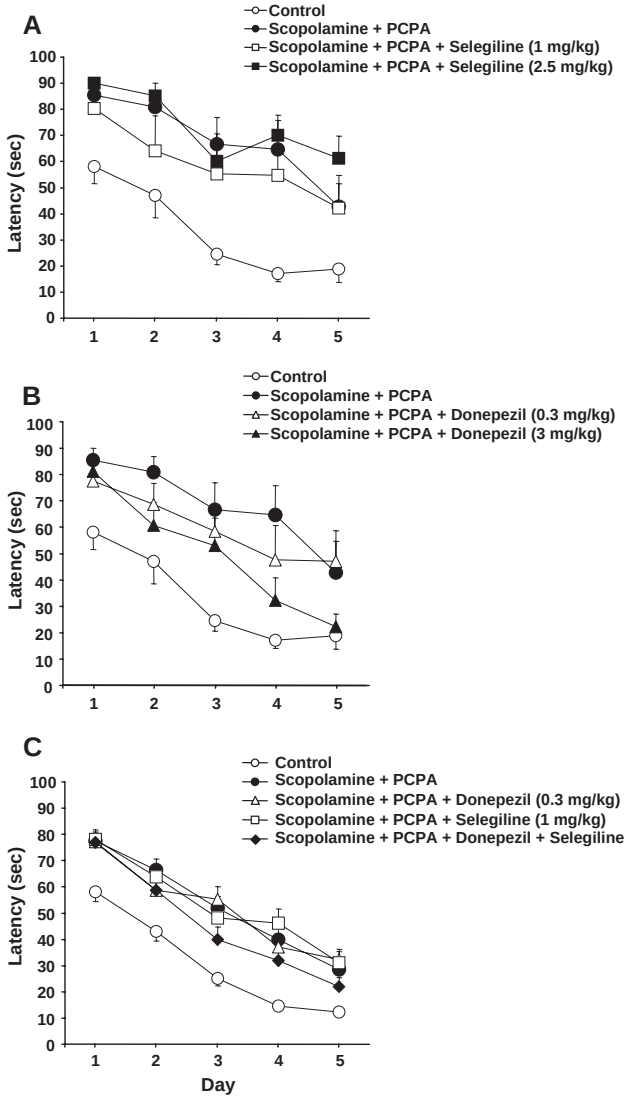

Fig. 2. Effects of a combined treatment with selegiline and donepezil on scopolamine+PCPA-induced memory impairment. Test compounds or saline were i.p. administered 30 min before test. Values represent means±S.E.M. ($n=8–34$). Panel A, B and C show the effects of selegiline alone, donepezil alone, and combined treatment on scopolamine+PCPA, respectively. A; scopolamine+PCPA vs. control ($P<0.0001$), B; scopolamine+PCPA vs. control ($P<0.0001$) or scopolamine+PCPA+donepezil: (0.3 mg/kg; $P=0.260$, 3 mg/kg; $P=0.002$), C; scopolamine+PCPA vs. control ($P<0.0001$) or scopolamine+PCPA+selegiline+donepezil ($P=0.026$), scopolamine+PCPA+selegiline+donepezil vs. scopolamine+PCPA+selegiline ($P=0.014$) or +donepezil ($P=0.054$).

4. Discussion

Cholinergic hypofunction is currently considered one of the main causes of dementia and cognitive deficits in Alzheimer's disease. In animal models, cholinergic deficits have been induced by a muscarinic antagonist scopolamine or a selective cholinotoxin AF64A. Scopolamine interferes with memory and cognitive function in humans by blocking muscarinic receptors in several brain regions (Beatty et al., 1986). AF64A causes damage to the septo-hippocampal pathway without altering muscarinic M1 receptor density (Thorne and Potter, 1995). In the present study, we evaluated the effect of selegiline on AF64A-induced memory impairment, and the effect of co-treatment with selegiline and donepezil on scopolamine+PCPA-induced memory impairment, using a Morris water maze. Selegiline significantly improved memory impairment induced by AF64A. Moreover, combined treatment with selegiline and donepezil at doses which individually failed to reduce the escape latencies in scopolamine+PCPA-treated rats, significantly ameliorated memory impairment induced by scopolamine+PCPA, which was in agreement with the results of previous studies showing that co-treatment with selegiline and an acetylcholinesterase inhibitor tacrine improved performance in scopolamine+PCPA-treated rats (Dringenberg et al., 2000). In several clinical trials, selegiline was shown to improve episodic memory and learning in patients with Alzheimer's disease (Filip and Kolibas, 1999; Mangoni et al., 1991; Piccinin et al., 1990; Tariot et al., 1987). However, recent meta-analysis data has shown that the magnitude of selegiline's effect was considered unlikely to reach clinical importance, although it improved cognition

and activities of daily living at an earlier time point (Birks and Flicker, 2003; Wilcock et al., 2002). Selegiline might have limited effects in Alzheimer's disease owing to its lack of dose-dependence, because it is reported to provide an improvement in memory with a bell-shaped dose response curve in patients with Alzheimer's disease (Tariot et al., 1987) and rats (Molinengo and Ghi, 1997). Although the efficacy of selegiline alone may be not sufficient to use in patients with Alzheimer's disease, our and previous studies have proposed that the therapeutic effect of cholinergic drugs in cognitive disorders with lowered cholinergic–monoaminergic neurotransmission can be enhanced by monoaminergic stimulation (Dringenberg et al., 2000; Schneider et al., 1993).

In the forebrain of Alzheimer's patients, pathological abnormalities in serotonergic and noradrenergic innervations are known to exist in addition to cholinergic innervation abnormality. This also indicates the rationality of a combination therapy of cholinergic and monoaminergic drugs in Alzheimer's disease. Selegiline can enhance dopaminergic neurotransmission due to its monoamine oxidase-B inhibitory action, but not serotonergic. The dopaminergic stimulation by selegiline may contribute to its adjuvant effect to cholinergic drugs. Actually, forebrain dopaminergic system is related to cognitive function (Marie and Defer, 2003; Remy and Samson, 2003). Furthermore, it has been reported that increases in dopamine levels enhanced a compensatory release of acetylcholine (Nilsson et al., 1992). Shimazu et al. (1996) showed that selegiline increased acetylcholine release in the frontal cortex, and that its effect was mimicked by dopamine D1 receptor agonist and blocked by dopamine D1 receptor antagonist. As well, the synergistic effects of co-treatment with selegiline and acetylcholinesterase inhibitors such as donepezil or tacrine on memory impairment may not be due to pharmacokinetic mechanism related to metabolism by cytochrome P450 (CYP), because donepezil, tacrine, and selegiline are mainly metabolized through CYP2D6 and 3A4, CYP1A2, and CYP2B6, respectively (Jann et al., 2002; Salonen et al., 2003).

Monoamine oxidase-B activity in Alzheimer's disease patients is significantly increased in the cortex and hippocampus (Barber et al., 1993; Reinikainen et al., 1988). In this context, monoamine oxidase-B inhibitory action of selegiline may be useful in the treatment of Alzheimer's disease.

It is also suggested that oxidative stress plays an important part in the disease and is induced by several processes related to β-amyloid, energy failure and toxic inflammatory responses. Selegiline has been shown to reduce neuronal death in the frontal cortex and hippocampus (Amenta et al., 1994; Barber et al., 1993), and to exert neuroprotective activities through several mechanisms, including upregulation of antioxidant enzymes (Carrillo et al., 1992). Moreover, donepezil was recently reported to protect cortical neurons against glutamate neurotoxicity via α4β2- and α7-nicotinic acetylcholine receptors (Takada et al., 2003). Combined treatment with selegiline and donepezil might show neuroprotective actions in addition to amelioration of cognitive deficits.

Although we did not evaluate swimming speed in the present study, selegiline or donepezil have already been reported to have no effects on swimming speed in a water maze (Abe et al., 2003; Yavich et al., 1996). Further investigations will be required to confirm whether or not these improvements by selegiline or donepezil individually, or combined treatment are due to the facilitated motor function, and to clarify whether or not combined treatment reverses AF64A-induced memory impairment. In conclusion, selegiline improved memory impairment in AF64A-treated rats. The combination of selegiline and donepezil, at doses that do not exert efficacy individually, was found to ameliorate memory impairment in scopolamine+PCPA-treated rats. These results support our view of the therapeutic value of co-treatment with selegiline and donepezil for treating cognitive disturbances related to dysfunctions of the cholinergic system.

Acknowledgements

We thank Ms. S. Egawa and Ms. M. Watanabe for technical support; Ms. H. Urabe and Ms. R. Eguchi for technical assistance.

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