Attenuation of Scopolamine-Induced and Age-Associated Memory Impairments by the Sigma and 5-Hydroxytryptamine_1A_ Receptor Agonist OPC-14523 (1-{3-[4-(3-chlorophenyl)-1-piperazinyl]propyl}-5-methoxy-3,4-dihydro-2[H]-quinolinone monomethanesulfonate)

KATSURA TOTTORI, MASAMI NAKAI, YASUFUMI UWAHODO, TAKASHI MIWA, SAKIKO YAMADA, YASUO OSHIRO, TETSURO KIKUCHI, AND C. ANTHONY ALTAR

Research Institute of Pharmacological and Therapeutical Development (K.T., Y.U., T.M., S.Y., T.K.), Second Institute of New Drug Discovery (M.N.), and Intellectual Property Department (Y.O.), Otsuka Pharmaceutical Co., Ltd., Tokushima, Japan; and Global Neuroscience Research (C.A.A.), Otsuka Maryland Research Institute, Rockville, Maryland

Received October 29, 2001; accepted December 20, 2001 This article is available online at http://jpet.aspetjournals.org

ABSTRACT

Sigma and 5-HT_1A receptor stimulation can increase acetylcholine (ACh) release in the brain. Because ACh release facilitates learning and memory, we evaluated the degree to which OPC-14523 (1-{3-[4-(3-chlorophenyl)-1-piperazinyl]propyl}-5-methoxy-3,4-dihydro-2[H]-quinolinone monomethanesulfonate), a novel sigma and 5-HT_1A receptor agonist, can augment ACh release and improve learning impairments in rats due to cholinergic- or age-related deficits. Single oral administration of OPC-14523 improved scopolamine-induced learning impairments in the passive-avoidance task and memory impairment in the Morris water maze. The chronic oral administration of OPC-14523 attenuated age-associated impairments of learning acquisition in the water maze and in the conditioned active-avoidance response test. OPC-14523 did not alter basal locomotion or inhibit acetylcholinesterase (AChE) activity at concentrations up to 100 μM and, unlike AChE inhibitors, did not cause peripheral cholinomimetic responses. ACh release in the dorsal hippocampus of freely moving rats increased after oral delivery of OPC-14523 and after local delivery of OPC-14523 into the hippocampus. The increases in hippocampal ACh release were blocked by the sigma receptor antagonist NE-100 (N,N-dipropyl-2-[4-methoxy-3-(2-phenylethoxy)-phenyl]-ethylamine). Thus, OPC-14523 improves scopolamine-induced and age-associated learning and memory impairments by enhancing ACh release, due to a stimulation of sigma and probably 5-HT_1A receptors. Combined sigma/5-HT_1A receptor agonism may be a novel approach to ameliorate cognitive disorders associated with age-associated cholinergic deficits.

Acetylcholine (ACh) is a crucial mediator of learning and memory (Blokland, 1995). Drugs that reduce cholinergic function, such as the muscarinic receptor antagonist scopolamine, cause profound memory impairments in animals and humans (Deutsch and Rocklin, 1967; Deutsch, 1971). The degeneration and dysfunction of cholinergic neurons is closely associated with the cognitive deficits of Alzheimer’s disease (Bartus et al., 1982; Coyle et al., 1983) and of aged rats and nonhuman primates (Gibson et al., 1981; Bartus et al., 1982). These findings provide a cholinomimetic rationale for treating Alzheimer’s disease (Becker, 1991) and support the use of animal models of learning impairments produced by age or muscarinic receptor antagonists (Yamazaki et al., 1995).

Although early trials with ACh biosynthetic precursors failed to improve cognitive impairments associated with Alzheimer’s disease (Growdon, 1997), acetylcholinesterase (AChE) inhibitors like tetrahydroaminoacridine (THA) and donepezil hydrochloride [(±)-2-[[1-benzylpiperidin-4-yl]methyl]-5,6-dimethoxy-indan-1-one monohydrochloride] (E–2020) were found to improve experimental and clinical memory impairments (Smith et al., 1996; Jann, 2000). The varying success of these and other AChE inhibitors indicates that the augmenta-

1 Present address: Psychiatric Genomics, Inc. 19 Firstfield Road, Gaithersburg, MD 20878.

ABBREVIATIONS: ACh, acetylcholine; AChE, acetylcholinesterase; THA, tetrahydroaminoacridine; 8-OH-DPAT, 8-hydroxy-2-(di-n-propylamino)tetralin; CAR, conditioned-avoidance response; CSF, cerebrospinal fluid; E-2020, (±)-2-[[1-benzylpiperidin-4-yl]methyl]-5,6-dimethoxy-indan-1-one monohydrochloride; IC$_{50}$, 50% inhibitory concentration; NE-100, N,N-dipropyl-2-[4-methoxy-3-(2-phenylethoxy)-phenyl]-ethylamine; ANOVA, analysis of variance; NMDA, N-methyl-D-aspartate; OPC-14523, 1-{3-[4-(3-chlorophenyl)-1-piperazinyl]propyl}-5-methoxy-3,4-dihydro-2[H]-quinolinone monomethanesulfonate.
tion of endogenous ACh release can improve cognitive performance. Of particular interest are the antiamnesic effects of drugs that augment ACh release by different mechanisms. Selective sigma receptor agonists augment cognitive function of experimental animals (Matsuno et al., 1993; Senda et al., 1996) and the release of ACh in rat brain (Kobayashi et al., 1996). ACh release can also be increased by 5-HT_{1A} receptor agonists, including the specific, full agonist 8-hydroxy-2-(di-n-propylamino)tetralin (8-OH-DPAT) and the partial 5-HT_{1A} receptor agonists buspirone and ipsapirone (Wilkinson et al., 1994). On the other hand, presynaptic 5-HT_{1A} receptor stimulation and postsynaptic 5-HT_{1A} receptor blockade can improve scopolamine-induced deficits (Carl et al., 1998), indicating that a partial 5-HT_{1A} receptor agonist, which would be expected to preferentially stimulate presynaptic autoreceptors, may also reverse scopolamine-induced memory deficits. These findings suggest that a combined sigma and partial 5-HT_{1A} receptor agonist may increase ACh release and improve memory independently of effects on AChE.

OPC-14523, a novel antidepressant drug candidate currently in clinical trials, binds with highest affinity (IC_{50} = 2 nM) to 5-HT_{1A} receptors, as evaluated with [3H]-8-OH-DPAT binding in the rat cerebral cortex (Tottori et al., 2001). Although it is a potent ligand at the 5-HT_{1A} site, OPC-14523 is a partial agonist at both rat and recombinant human 5-HT_{1A} receptors as determined by [35S]GTP binding assays (Jordan et al., 2000). Its next highest affinity is for sigma-1 and sigma-2 receptors (IC_{50} = 40–60 nM), as determined with [3H]-pentazocine and [3H]-1,3-di(o-tolyl)guainidine binding in guinea pig brain, respectively (Tottori et al., 2001). OPC-14523 is an agonist at the sigma receptor (Oshiro et al., 2000). Single administration of OPC-14523 decreases immobility time in the forced swimming test in rats and mice, unlike the conventional antidepressants fluoxetine or imipramine. The acute antidepressant-like effects of OPC-14523 result from its combined sigma and 5-HT_{1A} receptor agonism and are independent of 5-hydroxytryptamine (5-HT) re-uptake-inhibitory activity or effects on locomotion (Tottori et al., 2001).

Also unlike some conventional antidepressants, OPC-14523 shows little affinity to muscarinic receptors (M1, M2, M3) at 10 μM. It does not produce peripheral anticholinergic effects in mice, even at very high doses (100 mg/kg, p.o.). Because of its sigma and 5-HT_{1A} receptor agonist properties and lack of anticholinergic effects, we examined the effects of OPC-14523 on ACh release in the rat hippocampus using in vivo microdialysis. We also evaluated the effect of OPC-14523 on learning and memory impairments induced in rats by scopolamine, as evaluated by the passive-avoidance test and the Morris water maze task. We evaluated the effects of OPC-14523 on age-associated learning impairments in rats using the Morris water maze and the conditioned-avoidance response test. The sigma receptor antagonist NE-100 was given at doses that do not affect ACh release by themselves to determine the contribution of sigma receptor stimulation to potential effects on ACh release. Furthermore, OPC-14523 was investigated for AChE inhibitory activity in comparison with the AChE inhibitor E–2020, and cholinimimetic effects were evaluated as side effects that could be associated with AChE-inhibitory activity.

### Experimental Procedures

#### Materials

Male Wistar rats (Japan SLC; Hamamatsu, Japan) and male Fisher 344 (F344) rats (Japan-Charles River, Yokohama, Japan) were housed in a room maintained at 23 ± 2°C and 60 ± 10% relative humidity, illuminated for 12 h per day (7:00 AM–7:00 PM). Food and water were freely available during the housing period.

Tetrahydroaminoacridine (THA) (Funakoshi Co., Tokyo, Japan), scopolamine HBr (Sigma-Aldrich, St. Louis, MO), OPC-14523, NE-100, and E-2020 (synthesized by Otsuka Pharmaceutical Co., Ltd., Tokushima, Japan) were used. OPC-14523 was suspended in 0.5% tragacanth gum-distilled water. NE-100, THA, and E-2020 were suspended in 5% gum arabic-0.9% saline solution. Scopolamine HBr was dissolved in 0.9% saline solution. The oral administration volume was 10 ml/kg and 5 ml/kg in mice and rats, respectively, and the subcutaneous and intraperitoneal injection volumes were 10 ml/kg.

#### Scopolamine-Induced Learning and Memory Impairments

##### Passive-Avoidance Response

The apparatus and experimental procedure were similar to those described previously (Wanibuchi et al., 1994). The passive-avoidance apparatus consisted of an illuminated compartment (15 × 20 × 20 cm) that contained a 100-W lamp placed 50 cm above the top of the compartment, and a dark compartment (20 × 20 × 20 cm) with a metal grid floor. The two compartments were separated by an electronically operated, vertically sliding door. In the training session, each male Wistar rat (200–310 g) was placed in the illuminated compartment, and 10 s later the door was opened. As soon as the rat moved into the dark compartment, the door was closed and a 1.5-mA foot shock was applied to the grid floor for 2 s. The rat was immediately removed from the apparatus and returned to its home cage. All test compounds were administered orally 60 min before the training session. The amnesic agent scopolamine (1.5 mg/kg) was administered intraperitoneally 30 min before training. At the retention test performed 24 h later, each animal was placed in the illuminated compartment, and the step-through latency was measured. If the animal did not enter the dark compartment within 180 s, the retention test was terminated and a maximum score of 180 s was assigned.

##### Morris Water Maze Task

The apparatus and experimental procedure were similar to those described previously (Morris, 1984). The apparatus was a white circular pool (130 cm in diameter, 40 cm in height) filled to a depth of 30 cm with 24°C water which was made opaque by the addition of nontoxic white dye. The pool was divided into four compass quadrants (north, south, east, and west). Diverse visual stimuli were permanently located on the walls beyond the tank in each of the four quadrants. A circular goal platform 10 cm in diameter was hidden 1 cm below the water level in the middle of one of the quadrants.

The continuous location of each swimming male F344 rat (150–245 g) from the start point to the goal was recorded by a video camera connected to a visual position sensor (3Z4SP-C12; Omron, Kyoto, Japan) placed 50 cm above the illuminated compartment, and the step-through latency was measured. If the animal did not enter the dark compartment within 180 s, the retention test was terminated and a maximum score of 180 s was assigned.
hidden platform between 5 and 10 s in time and between 100 and 200 cm in distance were used in subsequent experiments conducted in the afternoon of the same day. As in the scopolamine-induced amnesia experiments, a test compound was administered orally, and 30 min later, scopolamine (0.3 mg/kg) was injected subcutaneously. The rats underwent a block of four trials 30 min later.

Age-Associated Learning and Memory Impairments

Morris Water Maze Task. The normative swimming activity of young and older rats was evaluated on the day before the learning acquisition test. A straight course through the water was made with two parallel pieces of acrylic plastic spaced 10 cm apart, each 130 cm in length and 30 cm in depth. A visible platform was placed at the end of the water course. The swimming activity of the animals was calculated as the average time of three trials required to swim to the platform from the opposite end of the course. The water maze test was conducted as described above at 1 to 8 days after OPC-14523 (10 mg/kg, p.o.) daily administration to 5- and 15-month-old rats, and at 1 to 7 days after daily administration to 5- and 22-month-old rats (325–495 g).

Conditioned Active-Avoidance Response (CAR). The CAR test was conducted with 5- and 22-month-old rats (320–515 g) at 22 to 31 days after the start of daily single administration of OPC-14523 (10 mg/kg, p.o.). A two-chamber CAR (Nabata et al., 1973) system (Bio-Medica Ltd., Osaka, Japan) was contained in a sound-proof box room. Two 19 × 46 × 19 cm shuttle box compartments were separated by a 6-cm-high plastic hurdle. The position of the animal in the shuttle box was sensed by a microswitch attached to the floor. In 20 trials per day for 10 days, each rat was randomly placed in one of the compartments of the shuttle box for 1 min of free exploration. During the 20 trials, which lasted approximately 10 min per animal, a 10-s auditory stimulus (conditioned stimulus, CS) immediately preceded an electric shock (1 mA, unconditioned stimulus, US) delivered through the stainless steel bars in the floor. The US was terminated when the animal jumped over the hurdle into the other compartment, or in the case of no active escape, after 10 s. The variable interval between trials averaged 45 s and ranged from 10 to 75 s. An avoidance was recorded when the rat jumped the hurdle to enter the other compartment of the box during the CS interval. The mean number of avoidances for each group was used as an index for the acquisition of the CAR.

Fig. 2. Effect of OPC-14523 on the scopolamine-induced increase in the time (top) and the distance (bottom) required of rats to find the hidden platform in the water maze task. n = 16–17/group, ##, p < 0.05 versus scopolamine-treated control group (one-way ANOVA followed by two-tailed Dunnett’s test).
Microdialysis of Acetylcholine Release

**Surgery and Preparation.** Wistar rats (170–280 g) were anesthetized with pentobarbital (50 mg/kg, i.p.) and placed in a stereotaxic instrument (SR-5; Narishige Scientific Instrument, Tokyo, Japan). A small hole was drilled at the following coordinates relative to bregma: AP, −3.8 mm; L, 2.5 mm; DV, −2.2 mm, to permit implantation of a guide cannula that terminated just rostral to the dorsal hippocampus. The guide cannula was secured with dental cement and stainless steel screws threaded into the skull. After surgery, rats were housed individually for 24 h.

On the day of testing, a dialysis probe was inserted via the guide cannula (Izumi et al., 1994) and perfused at 2 μl/min with artificial CSF (125 mM NaCl, 2.5 mM KCl, 1.18 mM MgCl₂, 1.28 mM CaCl₂) containing 100 μM physostigmine sulfate. After 2 h from the start of the perfusion, the dialysate was collected every 15 min, mixed with 30 μl of 100 nM ethylhomocholine as an internal standard, and injected into a high pressure liquid chromatography system for quantification of ACh (Izumi et al., 1994).

Local Delivery of OPC-14523 and Acetylcholine Release.

**OPC-14523 (10 μM) and the sigma receptor antagonist NE-100 (1 or 10 μM) were dissolved in artificial CSF containing 100 μM physostigmine sulfate. The dialysis probe was perfused with OPC-14523 or NE-100 alone. For the inhibition study, NE-100 perfusion was performed 1.5 h before perfusion of the mixed solution (10 μM OPC-14523 and 1 μM NE-100). Data were expressed as percentage of the mean baseline level (from fraction 1 to fraction 6).**

**ACh Analysis by High Pressure Liquid Chromatography.**

ACh was separated using a styrene polymer column (AC-GEL, 6.0 × 150 mm; EICOM, Kyoto, Japan) and converted to hydrogen peroxide by a post column enzyme reactor (AC-Enzympak; EICOM) with immobilized AChE and choline oxidase at 33°C. The mobile phase (0.1 M phosphate buffer, pH 8.5, 0.61 mM 1-decanesulfonate, 0.59 mM tetramethylammonium) was delivered at a rate of 1 ml/min using a pump (CCPS, TOSOH, Tokyo, Japan). The hydrogen peroxide was detected with an electrochemical detector (ECD-100; EICOM) equipped with a platinum electrode at an applied potential of +450 mV relative to an Ag/AgCl reference electrode.
versus vehicle-treated aged rats group (two-tailed t-test). Values are expressed as mean ± S.E.M. Results from 5-month-old (n = 6/group) or 22-month-old rats (n = 7/group) were analyzed by ANOVA for repeated measurements. Vehicle-treated young versus aged rats (group × days, p = 0.58; group, p = 0.023) and vehicle-treated versus OPC-14523-treated (10 mg/kg) aged rats (group × days, p = 0.020; group, p = 0.19). Statistical comparisons on each day were assessed by two-tailed t test. *, p < 0.05; **, p < 0.01 versus vehicle-treated young rats group (two-tailed t test). #, p < 0.05 versus vehicle-treated aged rats group (two-tailed t test).

Fig. 5. Effect of OPC-14523 on learning acquisition impairments in 22-month-old rats in the CAR test. OPC-14523 (10 mg/kg, p.o.) was administered 1 h before the CAR test conducted 22 to 31 days after the start of daily administration. Values are expressed as mean ± S.E.M. Results from 5-month-old (n = 6/group) or 22-month-old rats (n = 7/group) were analyzed by ANOVA for repeated measurements. Vehicle-treated young versus aged rats (group × days, p = 0.58; group, p = 0.023) and vehicle-treated versus OPC-14523-treated (10 mg/kg) aged rats (group × days, p = 0.020; group, p = 0.19). Statistical comparisons on each day were assessed by two-tailed t test. *, p < 0.05; **, p < 0.01 versus vehicle-treated young rats group (two-tailed t test).

Fig. 6. Effect of OPC-14523 on hippocampal ACh release in freely moving rats. OPC-14523 (10 mg/kg) or vehicle was administered immediately after the sixth fraction collection (n = 8/group). The efflux is expressed as percentage of basal efflux (100% = 151.4 ± 12.8, 110.0 ± 21.9, 113.7 ± 27.0, and 125.4 ± 21.8 fmol/min in vehicle, NE-100, OPC-14523, and NE-100 + OPC-14523 groups, respectively). The mean ACh level was increased by OPC-14523 compared with vehicle (group × time, p = 0.7048; group, p = 0.0001, two-way ANOVA). +, p < 0.05; **, p < 0.01 versus vehicle at each time point (two-tailed t test). In other animals (n = 8/group), NE-100 (10 mg/kg, i.p.) was injected immediately after the sixth fraction 15 min before injection of vehicle or OPC-14523. The interaction between the NE-100 group and the vehicle group was not significant on ACh levels (group × time, p = 0.9999; group, p = 0.4835, two-way ANOVA). The interaction between the OPC-14523 + NE-100 group, the NE-100 group, and the vehicle group was not significant (group × time, p = 0.7747; group, p = 1.0000, two-way ANOVA). ACh level of separate time points did not differ from that of the corresponding vehicle group (two-tailed Dunnett's test).

Fig. 7. Effect of NE-100 perfusion on hippocampal ACh release induced by OPC-14523 in freely moving rats. Top, n = 6/group. The hippocampus was perfused with artificial CSF solvent, then 1 and 10 μM OPC-14523 immediately were perfused after the sixth fraction collection. The efflux is expressed as percentage of basal efflux (100% = 142.2 ± 23.4, 131.2 ± 16.8, and 110.3 ± 16.2 fmol/min in solvent, and 1 and 10 μM OPC-14523 groups, respectively). The mean ACh level was increased by OPC-14523 compared with solvent (group × time, p = 0.2666; group, p = 0.0002, two-way ANOVA). Separate time point comparisons were significant (two-tailed Bonferroni test). Middle, perfusion with 1 μM NE-100 (n = 6/group) or 10 μM NE-100 (n = 4/group) was begun after the sixth fraction. The efflux is expressed as percentage of basal efflux (100% = 142.2 ± 23.4, 179.7 ± 35.4, and 141.2 ± 33.5 fmol/min in solvent, and 1 and 10 μM NE-100 groups, respectively). The mean ACh level was decreased by NE-100 compared with solvent (group × time, p = 0.8311; group, p = 0.0003, two-way ANOVA). +, p < 0.05 versus solvent at each time point (two-tailed Dunnett's test). Bottom, n = 6 to 7/group. NE-100 (1 μM) was perfused immediately after the sixth fraction was collected. OPC-14523 (10 μM) was perfused with NE-100 (1 μM) immediately after the twelfth fraction was collected. The efflux was expressed as percentage of basal efflux (100% = 110.3 ± 16.2, 179.7 ± 35.4, and 158.9 ± 29.9 fmol/min in 10 μM OPC-14523, 1 μM NE-100, and 1 μM NE-100 + 10 μM OPC-14523 groups, respectively). The mean ACh level did not differ between the OPC-14523 + NE-100 group and the NE-100 group (group × time, p = 0.6311; group, p = 0.3741, two-way ANOVA).
Acetylcholinesterase Activity

The procedure was similar to that described previously (Ellman et al., 1961). Rat striata were homogenized in 10 volumes of 0.1 M phosphate buffer (pH 8.0). Five minutes after the addition of 400 µl of homogenate, 30 µl of test compound solution at various concentrations, and 100 µl of dithiobisnitrobenzoic acid (10 mM) to 2.45 ml of 0.1 M phosphate buffer (pH 8.0), AChE activity was determined at 25°C over 1 min in a photocell after the addition of 20 µl of acetylcholine iodide (0.075 M) as substrate with a Shimadzu UV-2200 l of dithiobisnitrobenzoic acid (10 mM) to 2.45 ml of 0.1 M phosphate buffer (pH 8.0). AChE activity was converted to absolute concentration of tissue (mg/ml).

Cholinomimetic Side Effects

Cholinomimetic side effects were determined (Wanibuchi et al., 1994) in Wistar rats (160–310 g). Salivation: Secreted saliva was collected and weighed on blotting paper at 30 and 60 min after the oral administration of drugs. Total saliva secretion was then collected and weighed on blotting paper at 30 and 60 min after the administration of the test drugs. The change in pupil diameter (mm) was expressed as the difference between the predrug and postdrug values. Pupil diameter: The rats were allowed at least 10 min to adapt to the lighting conditions before the measurement. The right eye pupil diameter was measured using a microscope with a graduated eyepiece before and 60 min after the administration of the test drugs. The change in pupil diameter (mm) was expressed as the difference between the predrug and postdrug values.

Statistical Analysis

An SAS (Cary, NC) program (release 6.11) was used to perform all analyses. In the passive-avoidance response tests, the differences between the scopolamine-alone group were assessed by two-tailed Steel test, and the comparison with each vehicle group was assessed by the Wileoxon rank sum test. In the Morris water maze task, the differences between the scopolamine group and the scopolamine + drug group were assessed by one-way ANOVA followed by a two-tailed Dunnett’s test. In the Morris water maze task evaluating age-associated memory impairment, interaction (group × days) and main effects (group) were analyzed by ANOVA on repeated measurements. Statistical comparisons of escape latency or path distance, conditioned-avoidance response numbers on each day, and swimming activity were assessed by two-tailed t tests. In microdialysis studies, interactions between each group were assessed by two-way ANOVA. ACh levels of separate time points were assessed by a two-tailed Dunnett’s test. In the analysis of cholinomimetic side effects, salivation and hypothermia were assessed by one-way ANOVA followed by a two-tailed Dunnett’s test. Tremor was assessed by two-tailed Fisher’s exact test with Bonferroni correction. The pupil diameter was assessed by two-tailed Steel’s test.

Results

Scopolamine-Induced Learning and Memory Impairments

Passive-Avoidance Response. Scopolamine (1.5 mg/kg, i.p.) administered 30 min before training to the rat reduced by 90% the step-through latency in the retention test (Fig. 1). Pretreatment with OPC-14523 reversed the scopolamine-induced inhibition of the passive-avoidance response with a bell-shaped dose-response curve, and the maximum effect was observed at 3 and 10 mg/kg as well as that observed at 1 mg/kg E-2020. THA altered the amnesic effect of scopolamine in a pattern similar to that of E-2020, although unlike E-2020, the reversal of amnesia by THA was not statistically significant.

Morris Water Maze Task. Scopolamine (0.3 mg/kg, s.c.) increased the time (latency) and distance required of rats to find the hidden platform in the water maze task compared with their predrug, trained performance values. A pupil diameter: The rats were allowed at least 10 min to adapt to the lighting conditions before the measurement. The right eye pupil diameter was measured using a microscope with a graduated eyepiece before and 60 min after the administration of the test drugs. The change in pupil diameter (mm) was expressed as the difference between the predrug and postdrug values.

Age-Associated Learning and Memory Impairment

Morris Water Maze. No statistically significant differences were noted between 5- and 15-month-old rats in normative swimming activity on the day prior to the learning acquisition test (Fig. 3, top). Vehicle-treated 5-month-old rats progressively learned to locate the hidden platform (Fig. 4). Vehicle-treated 15-month-old rats exhibited a longer escape latency (group × days: p = 0.93, group: p = 0.012) and path distance (group × days: p = 0.78, group: p = 0.0042) when compared with the vehicle-treated young rats (Fig. 4, A and B). The prolonged escape latency and path distance in vehicle-treated 15-month-old rats were partly but not significantly shortened by daily administrations of OPC-14523 (10 mg/kg, p.o.).

The normative swimming time of 5-month-old rats was significantly shorter than that of 22-month-old rats in tests.
performed on the day before the learning acquisition test (Fig. 3, bottom). Nevertheless, the initial escape latency and path distance did not differ between 5- and 22-month-old rats or those treated with OPC-14523 (Fig. 4, C and D). The learning acquisition was independent of the rate of basal swimming activities. Vehicle-treated 22-month-old rats exhibited longer escape latencies (group \( \times \) days: \( p = 0.026, \) group: \( p = 0.0001 \)) and path distances (group \( \times \) days: \( p = 0.0021, \) group: \( p = 0.0001 \)) compared with the young rats. OPC-14523 (10 mg/kg, p.o.) decreased the escape latency (group \( \times \) days: \( p = 0.81, \) group: \( p = 0.050 \)) and, in particular, path distance (group \( \times \) days: \( p = 0.66, \) group: \( p = 0.011 \)) in 22-month-old rats (Fig. 4, C, and D).

**CAR.** Young control rats showed steadily increasing CAR performance, reaching a success level of about 85% after 10 days (Fig. 5). Vehicle-treated 22-month-old rats showed an initial CAR deficit, and learned to produce the CAR with levels of acquisition that were delayed compared with the young rats (group \( \times \) days: \( p = 0.58, \) group: \( p = 0.023 \)). The magnitude of the CAR in the OPC-14523 (10 mg/kg, p.o.)-treated aged group exceeded that of the vehicle-treated aged group (group \( \times \) days: \( p = 0.020, \) group: \( p = 0.19 \)), and did not differ from that of the young vehicle-treated controls.

**Microdialysis Studies.**

**Oral Delivery of OPC-14523 and Acetylcholine Release.** OPC-14523 (10 mg/kg, p.o.) increased the release of ACh in the hippocampus compared with the vehicle group (group \( \times \) time, \( p = 0.7048, \) group: \( p = 0.0001 \); Fig. 6). The maximum increase appeared at 15 and 30 min after OPC-14523. The ability of OPC-14523 to increase hippocampal ACh release was completely blocked by pretreatment with a 10 mg/kg i.p. dose of the sigma receptor antagonist NE-100 (group \( \times \) time, \( p = 0.9999, \) group: \( p = 0.4834 \)). NE-100 alone had no effect on hippocampal ACh release (group \( \times \) time, \( p = 0.7747, \) group: \( p = 1.0000 \)).

**Local Delivery of OPC-14523 and ACh Release.** The release of ACh from the hippocampus was increased when 10 \( \mu \)M OPC-14523 was delivered directly to the hippocampus via the dialysis probe (group \( \times \) time, \( p = 0.2666, \) group: \( p = 0.0002 \); Fig. 7, top). Unlike 1 \( \mu \)M NE-100, 10 \( \mu \)M NE-100 alone decreased ACh levels (group \( \times \) time, \( p = 0.8311, \) group: \( p = 0.0003 \); Fig. 7, middle). The greatest decreases of ACh release by 10 \( \mu \)M NE-100 appeared at 1, 1.25, and 1.5 h after perfusion. Therefore, 1 \( \mu \)M NE-100 was continuously administered starting 1.5 h before OPC-14523 to investigate its ability to block the ACh-releasing effect of locally delivered OPC-14523. The ability of 10 \( \mu \)M OPC-14523 to increase hippocampal ACh release was completely blocked by co-perfusion with 1 \( \mu \)M NE-100 (group \( \times \) time, \( p = 0.6311, \) group: \( p = 0.3741 \), whereas 1 \( \mu \)M NE-100 alone again failed to affect basal ACh release (group \( \times \) time, \( p = 0.7797, \) group: \( p = 0.6311 \); Fig. 7, bottom).

**Effect on Rat Striatal AChE Activity.** Basal rat striatal AChE activity was 36 ± 0.9 \( \mu \)mol substrate hydrolyzed/min/g tissue. The IC\(_{50}\) value of E-2020 for inhibiting striatal AChE activity was 20 nM with 95% confidence intervals ranging from 18 to 22 nM. OPC-14523 showed no inhibition of AChE activity even at 100 \( \mu \)M.

**Cholinomimetic Side Effects.**

OPC-14523 increased salivation only at a very high dose of 300 mg/kg, p.o., and did not induce tremor or alter pupil diameter even at that dose. OPC-14523 reduced rectal temperature at doses of 100 and 300 mg/kg. In contrast, the AChE inhibitors THA and E-2020 were far more potent at increasing saliva secretion and tremor, and lessening pupil diameter, compared with OPC-14523 (Table 1).

**Discussion.**

OPC-14523 is an antidepressant candidate that exhibits sigma and 5-HT\(_{1A}\) receptor agonism as determined in vivo (Tottori et al., 2001). The inability of OPC-14523 to alter locomotor activity after single administration (Tottori et al., 2001) and after 7 consecutive days of chronic administration (unpublished observation) rules out general effects on locomotion on the diverse effects on learning and memory observed here.

**Scopolamine-Induced Learning and Memory Impairments.** Scopolamine is a muscarinic cholinergic receptor antagonist and, when used at a low dose as in the present study, is well known to cause memory impairments in rodents. OPC-14523 lessened scopolamine-induced deficits in the passive-avoidance and the Morris water maze tasks. OPC-14523 produced less of a cognition-enhancing effect at the highest dose, resulting in a bell-shaped dose-dependent effect in both tasks. Cholinergic stimulators frequently produce bell-shaped dose-response curves in studies of learning and memory performance (Senda et al., 1998). It has been proposed that the decreasing effects at higher doses are caused by an agonistic action of ACh on muscarinic autoreceptors that dampen endogenous ACh release (Baghdoyan et al., 1998). The ability of sigma receptor agonists to potentiate hippocampal responses to glutamate also occurs with a bell-shaped dose-response (Debonnel et al., 1996). Because hippocampal glutamate activation of the N-methyl-d-aspartate (NMDA) receptor is crucial for learning and memory (Cammarota et al., 2000), combined biphasic effects of OPC-14523 on glutamate and ACh release might explain the decreased learning effects of higher-dose OPC-14523. Microdialysis studies of both glutamate and ACh release in the hippocampus at doses of 3, 10, and 30 mg/kg OPC-14523 would be ideal to evaluate this hypothesis. 5-HT\(_{1A}\) receptor agonism at presynaptic receptors can attenuate scopolamine-induced acquisition impairments, whereas postsynaptic agonism potentiates such impairments (Cole et al., 1994). Thus, it is speculative but consistent with the action of partial monoamine receptor agonists that the partial 5-HT\(_{1A}\) receptor agonism of OPC-14523 (Jordan et al., 2000) is first manifested at somatodendritic autoreceptors and only at higher doses at postsynaptic receptors. This pharmacological sequence could also contribute to its bell-shaped effect on learning and memory.

**Age-Associated Learning and Memory Impairment.**

In the present study, aged rats exhibited deficits in the Morris water maze test of spatial learning and the CAR test of preservative learning. The administration of OPC-14523 (10 mg/kg, p.o.) did not improve either form of spatial learning impairment in 15-month-old rats but did so in 22-month-old rats. This difference may reflect the greater acquisition deficits for 22-month-old rats compared with 15-month-old rats.
rats, which increases the statistical power to observe improvements. These findings suggest that OPC-14523 can improve spatial memory deficits of aged rats but does not affect cognitive performance in younger, or uncompromised, rats. The repeated administration of OPC-14523 also improved the age-associated deficits of learning acquisition determined with the CAR test in rats.

Age-associated impairments of spatial learning can result from central cholinergic and glutamatergic dysfunction. Thus, the ameliorating effect of OPC-14523 in the age-associated cognitive impairments studied here could have been through activation of central cholinergic or glutamatergic receptors. Sigma receptor agonists can improve learning impairments induced by scopolamine (Maurice et al., 1998) and by carbon monoxide or ethanol intoxication (Maurice et al., 1999). Sigma receptor agonists such as SA4503 and (+)-SKF-10,047 also lessen amnesia induced by muscarinic cholinergic receptor antagonists or bilateral cholinergic lesions of the basal forebrain (Senda et al., 1998). These events are mediated by sigma receptor activation of central cholinergic systems (Matsuno et al., 1993; Kobayashi et al., 1996; Senda et al., 1996). Ionotropic glutamate receptors can also promote learning and memory (Nayak et al., 1998). However, OPC-14523, even at 10 μM, does not bind to α-amino-3-hydroxy-5-methoxy-4-isoxazole propionic acid, kainate, or NMDA receptors (unpublished observations). Interestingly, however, sigma receptor agonists can dramatically potentiate the depolarization of hippocampal neurons by glutamate via the NMDA receptor (Debonnel et al., 1996). Thus, the sigma receptor agonist property of OPC-14523 may increase the stimulation of cholinergic receptors and of hippocampal NMDA receptor by glutamate to promote learning and memory.

Whether the stimulation of 5-HT_{1A} receptors by OPC-14523 or any drugs that enhance memory remains controversial. Stimulation of presynaptic 5-HT_{1A} receptors can reverse memory impairments induced by scopolamine (Carli et al., 1998, 1999). In contrast, however, Carli and Samanin (1992) showed that the full 5-HT_{1A} receptor agonist, 8-OH-DPAT, impairs performance in a dose-dependent manner in two different versions of the Morris water maze test. They also showed that intraventricular 5-hydroxytryptamine depletions induced by 5,7-dihydroxytryptamine increases the amnesic effects of 8-OH-DPAT in the Morris water maze test, indicating that full agonism of postsynaptic 5-HT_{1A} receptors can impair spatial navigation. Thus, the improvement of scopolamine-induced and age-associated memory impairments by OPC-14523 may also be related to 5-HT_{1A} receptor partial agonistic activity as well as sigma receptor agonism.

**Effects on ACh Release.** The enhancement of ACh release in the hippocampus has been closely associated with the improvement of age- and drug-related memory impairments including those induced by low-dose scopolamine (Blokland, 1995). This is consistent with the reversal of age- and scopolamine-induced memory impairments by OPC-14523. The systemic administration of OPC-14523 enhanced ACh release within the hippocampus of freely moving rats more rapidly than after probe-delivered OPC-14523. This may have been caused by differences in brain concentrations of OPC-14523 achieved with the two methods of delivery. Orally administered OPC-14523 is rapidly absorbed and distributed to brain, where it attains a peak concentration within 0.5 h (Altar et al., 2000; unpublished observation). The peak increases of ACh release at 15 and 30 min after orally administered OPC-14523 parallels its appearance in brain (Altar et al., 2000). The less rapid effects of probe-delivered OPC-14523 on ACh release may be due to slower diffusion throughout hippocampal parenchyma or to more distal sites, e.g., the medial septum or the dorsal raphe nucleus, after it exudes from the probe.

The increases in ACh release after OPC-14523 were blocked by coadministration of a low, 1 μM concentration or a 10 mg/kg i.p. dose of the sigma receptor antagonist NE-100, which by itself did not affect ACh release. This demonstrates that the sigma receptor agonism of OPC-14523 mediates its ACh-releasing effects, consistent with the increase in vivo ACh release by sigma receptor agonists (Kobayashi et al., 1996). It is interesting to note that a higher concentration of NE-100 (10 μM) alone decreased hippocampal ACh release. This reveals an ACh-releasing role of constitutive sigma receptor activation. These results suggest that OPC-14523 increases ACh release by its sigma receptor agonist activity, and that hippocampal ACh release is promoted by constitutive sigma activity.

The systemic administration of full (Fuji et al., 1997) or partial (Wilkinson et al., 1994) 5-HT_{1A} receptor agonists like 8-OH-DPAT or buspirone and ipsapirone, respectively, can increase ACh release in brain. Pretreatment with the selective 5-HT_{1A} receptor antagonist WAY-100635 (Nakai et al., 1998) can completely eliminate the enhancement of hippocampal ACh release induced by 8-OH-DPAT. OPC-14523 is a partial 5-HT_{1A} receptor agonist to the same degree as buspirone (Jordan et al., 2000). Therefore, together with the sigma receptor agonism, the enhancement of ACh release in the hippocampus by OPC-14523 may be attributed to partial 5-HT_{1A} receptor agonism.

**Acetylcholinesterase Activity and Cholinomimetic Side Effects.** AChE inhibitors can improve experimental and clinical memory impairments (Smith et al., 1996; Jann, 2000). In low-dose scopolamine models, these actions demonstrate that an elevation in synaptic ACh can reverse partial muscarinic receptor blockade and improve impaired learning and memory. However, OPC-14523 did not inhibit central AChE activity, nor did it cause cholinomimetic side effects such as tremor or the reduction of pupil diameter even at an oral dose of 300 mg/kg. OPC-14523 produced a slight increase in saliva secretion at the high dose of 300 mg/kg and produced hyperthermia, but only at doses of 100 and 300 mg/kg. This hyperthermia is consistent with 5-HT_{1A} receptor agonistic activity, exemplified by full agonists such as 8-OH-DPAT. Thus, OPC-14523 appears to increase ACh levels in brain but not in the periphery, and this action is independent of effects on AChE or interactions with muscarinic receptors, with which OPC-14523 does not bind (Tottori et al., 2001).

**Conclusion**

The present results suggest that OPC-14523 can enhance memory without cholinomimetic side effects, unlike AChE inhibitors such as THA or E-2020. The ACh-releasing and, probably, cognition-enhancing effects of OPC-14523 may be attributed to the sigma receptor and partial 5-HT_{1A} receptor agonism. OPC-14523, which is active in rodent models predictive of antidepressant effects (Tottori et al., 2001), may prove to be an antidepressant drug that does not produce...
memory impairments, unlike tricyclic antidepressants and selective serotonin reuptake inhibitors, which can impair human memory (Lane and Baldwin, 1997). Furthermore, because decreased cholinergic dysfunction is involved in the neurobiological mechanism that links stress with depression (Fratranska et al., 1987), the ACh-releasing effects of OPC-14523 may contribute not only to antiamnesic effects but also to antidepressant activity. Depressive symptoms frequently coexist with dementia, and its incidence increases with age. OPC-14523 may help the increasing number of elderly persons who suffer from both depression and cognitive impairments.

References


Address correspondence to: Dr. Katsura Tottori, Research Institute of Pharmaceutical and Therapeutic Development, Otsuka Pharmaceutical Co., Ltd., 463-10 Kagasuno Kawachi-cho Tokushima 771-0192, Japan. E-mail address: k_tottori@research.otsuka.co.jp