ELSEVIER

European Journal of Pharmacology 453 (2002) 91–98

ejp

www.elsevier.com/locate/ejphar

Involvement of κ-opioid and σ receptors in short-term memory in mice

Masayuki Hiramatsu[a,b,*], Takashi Hoshino[b], Tsutomu Kameyama[b], Toshitaka Nabeshima[c]

[a] *Department of Neuropsychopharmacology, Graduate School of Environmental and Human Sciences, Meijo University, Nagoya 468-8502, Japan*
[b] *Department of Chemical Pharmacology, Faculty of Pharmaceutical Sciences, Meijo University, Nagoya 468-8503, Japan*
[c] *Department of Neuropsychopharmacology and Hospital Pharmacy, Nagoya University School of Medicine, Nagoya 466-8560, Japan*

Received 23 July 2002; received in revised form 4 September 2002; accepted 5 September 2002

Abstract

κ-Opioid receptor agonists, *trans*-(±)-3,4-dichloro-*N*-methyl-*N*-(2-[1-pyrrolidinyl] cyclohexyl) benzeneacetamide methanesulfonate (U-50,488H) and dynorphin A-(1–13), improve impairments of learning and memory in mice and rats. σ Receptor agonists, (+)-*N*-allylnormetazocine ((+)-SKF10,047) and 1-(3,4-dimethoxyphenethyl)-4-(3-phenylpropyl) piperazine dihydrochloride (SA4503), also reverse learning and memory impairment in various animal models. However, the mechanisms underlying these effects are not well understood. In the present study, the effect of coadministration of U-50,488H and (+)-SKF10,047 on scopolamine-induced memory impairment was investigated in mice using spontaneous alternation performance in a Y-maze. U-50,488H (0.21–2.15 μmol/kg, subcutaneously (s.c.)) and (+)-SKF10,047 (0.10–1.02 μmol/kg, s.c.) 25 min before the Y-maze test improved the impairment of spontaneous alternation induced by scopolamine (1.65 μmol/kg, s.c.). When U-50,488H and (+)-SKF10,047 were coadministered, no additive effect was observed. Furthermore, the ameliorating effects of U-50,488H and (+)-SKF10,047 were not antagonized by a selective σ receptor antagonist, *N*,*N*-dipropyl-2-[4-methoxy-3-(2-phenylenoxy)-phenyl]-ethylamine monohydrochloride (NE-100), and a selective κ-opioid receptor antagonist, nor-binaltorphimine, respectively. These results suggest that the mechanisms underlying the ameliorating effects on memory impairment are independent and no direct modulation exists in κ-opioid and σ receptors-mediated mechanisms.

Keywords: U-50,488H; (+)-SKF10,047; κ-Opioid receptor; σ Receptor; Y-maze; Short-term memory

1. Introduction

It is well known that cholinergic neuronal systems play an important role in the cognitive deficits associated with aging and neurodegenerative diseases (Bartus et al., 1982; Beninger et al., 1989; Coyle et al., 1983; Newhouse, 1990). Although investigation of learning and memory has focused primarily on cholinergic neurotransmission, reports of increased numbers of κ-opioid receptors in the limbic system (Hiller et al., 1987) and in the putamen and cerebellar cortex of postmortem brains of Alzheimer's patients (Mathieu-Kia et al., 2001) suggest that disruption of opioidergic neurotransmission may also be involved in the cognitive deficits associated with Alzheimer's disease and aging.

We previously reported that κ-opioid receptor agonists, dynorphin A-(1-13) and U-50,488H, improved the scopolamine-induced impairment of spontaneous alternation performance in mice (Itoh et al., 1993; Hiramatsu et al., 1996a,b, 1998b), carbon monoxide (CO)-induced delayed amnesia in mice (Hiramatsu et al., 1995, 1997) and also the β-amyloid peptide (25–35)- and carbachol-induced impairment of learning and memory in mice and rats, respectively (Hiramatsu et al., 1998a, 2000). Dynorphin A-(1–13) and U-50,488H reversed the decrease in acetylcholine release induced by carbachol and mecamylamine (Hiramatsu et al., 1996a,b, 1998a,b). Therefore, the κ-opioidergic system in the brain may play an important role in modulating learning and memory function, although the precise interaction between the κ-opioidergic and the cholinergic systems in the central nervous system has not yet been elucidated.

The σ receptor was initially considered a member of the opioid receptor family (Martin et al., 1976), but is

* Corresponding author. Department of Neuropsychopharmacology, Graduate School of Environmental and Human Sciences, and Department of Chemical Pharmacology, Faculty of Pharmaceutical Sciences, Meijo University, Tenpaku-ku, Nagoya 468-8503, Japan. Tel.: +81-52-832-1781 x342; fax: +81-52-834-8780.
E-mail address: mhiramt@ccmfs.meijo-u.ac.jp (M. Hiramatsu).

0014-2999/02/$ - see front matter © 2002 Elsevier Science B.V. All rights reserved.
PII: S0014-2999(02)02388-9

now considered to constitute a distinct group of receptors (Quirion et al., 1992). Interestingly, we demonstrated that a prototype of σ receptor agonist, (+)-*N*-allylnormetazocine ((+)-SKF-10,047), and (+)-pentazocine improved impairment of learning and memory in mice (Maurice et al., 1994). These findings were supported by Matsuno et al. (1996) using a novel σ_1 receptor agonist, 1-(3,4-dimethoxyphenethyl)-4-(3-phenylpropyl) piperazine dihydrochloride (SA-4503). Particularly, (±)-SKF-10,047 enhanced stimulation-evoked acetylcholine release in guinea pig cerebral slices (Siniscalchi et al., 1987), and other σ receptor agonists, (+)-SKF10,047, (±)-pentazocine, 1,3-di-*O*-tolylguanidin (DTG) and (+)-3-(3-hydroxyphenyl)-*N*-(1-propyl)-piperidine ((+)-3-PPP), increased extracellular acetylcholine levels in the rat frontal cortex (Matsuno et al., 1992, 1993) and hippocampus (Matsuno et al., 1995). The activating effect of (+)-SKF10,047 on the central cholinergic system was antagonized by haloperidol, a prototype of σ receptor antagonist (Matsuno et al., 1993, 1995). Therefore, σ receptor agonists may also be effective to improve memory impairment involving in the cholinergic system.

It was reported that a tonically active anti-opioid σ receptor system markedly influenced the sensitivity of mice to opioid analgesics, particularly κ-opioid drugs (Chien and Pasternak, 1993, 1994, 1995). σ_1 Receptor antisense enhanced the analgesic activity of both the κ_1- and κ_3-opioid receptor-mediated analgesics with U-50,488H and naloxone benzoylhydrazone, respectively (King et al., 1997), and haloperidol enhanced κ_1 and κ_3 analgesia more dramatically than did morphine through σ receptor-mediated mechanisms (Chien and Pasternak, 1994). These findings may indicate that an interaction between κ-opioid receptors and the σ receptor-mediated mechanism exists in the central nervous system that affects memory function.

In this study, we investigated whether κ-opioid receptors are involved in the σ receptor-mediated improvement of memory function or vice versa in mice, using spontaneous alternation behavior in a Y-maze.

2. Materials and methods

2.1. Animals

Seven-week-old male ddY mice (Japan SLC, Japan) were kept in a controlled environment, with controlled lighting (12-h light/dark cycle, lights on; 8 AM to 8 PM) and temperature (23 ± 2 °C) for at least 5 days before the experiments, and were given free access to food and water. Experimental protocols concerning the use of laboratory animals were approved by the committee of Meijo University and were performed in accordance with the guidelines of the Japanese Pharmacological Society (Folia Pharmacol. Japon, 1992, 99: 35A) and the interministerial decree of May 25th, 1987 (The Ministry of Education).

2.2. Drugs

The following drugs were used: *trans*-(±)-3,4-dichloro-*N*-methyl-*N*-(2-[1-pyrrolidinyl] cyclohexyl) benzeneacetamide methanesulfonate (U-50,488H, Sigma, St. Louis, MO); (+)-*N*-allylnormetazocine ((+)-SKF10,047, Research Biochemicals, Natrick, MA); *N*,*N*-dipropyl-2-[4-methoxy-3-(2-phenylenoxy)-phenyl]-ethylamine monohydrochloride (NE-100, Taisho Pharmaceutical, Tokyo, Japan); nor-binaltorphimine (Research Biochemicals); scopolamine hydrobromide (scopolamine, Tokyo Chemical Industry, Tokyo, Japan). All doses were calculated as those of the bases. Drugs were dissolved in isotonic saline solution (Otsuka Pharmaceuticals, Tokyo, Japan). Nor-binaltorphimine was administered, 25 min before the test session, into the lateral ventricular (i.c.v.) region of the mouse brain according to the method of Haley and McCormick (1957) in a volume of 5 μl/mouse under brief ether anesthesia. U-50,488H, (+)-SKF10,047 and scopolamine were administered subcutaneously (s.c.) 25, 25 and 30 min, and NE-100 was administered intraperitoneally (i.p.) 30 min before the Y-maze test, respectively.

2.3. Spontaneous alternation behavior

Immediate working memory performance was assessed by recording spontaneous alternation behavior during a single session in a Y-maze (Hiramatsu et al., 1997). Each mouse, new to the maze, was placed at the end of one arm and allowed to move freely through the maze during an 8-min session. The series of arm entries was recorded visually. The observer usually did not know which drugs were administered because the drug treatments were randomly assigned. Alternation was defined as successive entries into the three arms, on overlapping triplet sets. The effect was calculated as percentage alternation according to the following formula:

$$\text{percentage alternation} = (\text{number of alternations} / \text{total number of arm entries} - 2) \times 100\%$$

2.4. Acetic acid-induced writhing test

The writhing test was conducted 25 min after s.c. injection of each drug. Mice were given 0.7% acetic acid solution (i.p.) 10 min before the writhing test, and the numbers of writhing responses were then counted for 10 min. Antinociception was quantified as % inhibition using the following formula:

$$\%\ \text{Inhibition} = [(\text{control responses} - \text{test responses}) / \text{control responses}] \times 100$$

% Inhibition was calculated for each dosage. Dose–response curves were generated using at least three doses

of test drugs. ED_{50} values were determined by log-probit analysis, and 95% confidence limits were determined using the method of Litchfield and Wilcoxon (1949).

2.5. Data analysis

The behavioral data are expressed in terms of means ± S.E.M for the writhing test, and median and interquartile ranges for the Y-maze test. The significance of differences was evaluated using the Mann–Whitney's *U*-test and the Kruskal–Wallis test followed by Bonferroni's test for multiple comparisons. The criterion for significance was $P<0.05$ in all statistical evaluations.

3. Results

3.1. Effects of U-50,488H and (+)-SKF10,047 on scopolamine-induced impairment of spontaneous alternation behavior and number of arm entries

Scopolamine (1.65 μmol/kg, s.c.) markedly impaired the spontaneous alternation behavior indicated as decreased percent alternation, and increased the total number of arm entries (Figs. 1 and 2). U-50,488H (0.21–2.15 μmol/kg, s.c.) dose-dependently attenuated the impairment of spontaneous alternation behavior induced by scopolamine, and its effect was significant at doses of 0.64 and 2.15 μmol/kg (Fig. 1A). The scopolamine-induced increase in the total number of arm entries was not attenuated significantly by the dose of U-50,488H used (Fig. 1B).

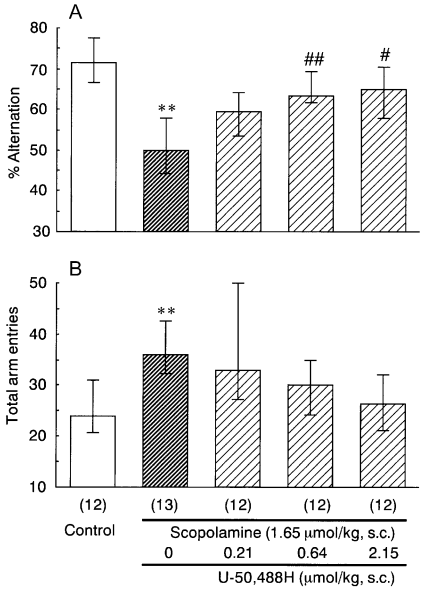

Fig. 1. Effects of U-50,488H on scopolamine-induced impairment of spontaneous alternation (A) and total arm entries (B) in the Y-maze test. Mice were treated with scopolamine (1.65 μmol/kg, s.c.) and U-50,488H (0.21, 0.64 and 2.15 μmol/kg, s.c.) 30 and 25 min before testing, respectively. Data are shown as median (vertical column) and first and third quartiles (vertical line). The number of mice used is shown in parentheses. Significance levels: $^{**}P<0.01$ vs. control (Mann–Whitney's *U*-test). $\#P<0.05$, $\#\#P<0.01$ vs. scopolamine alone (Bonferroni's test).

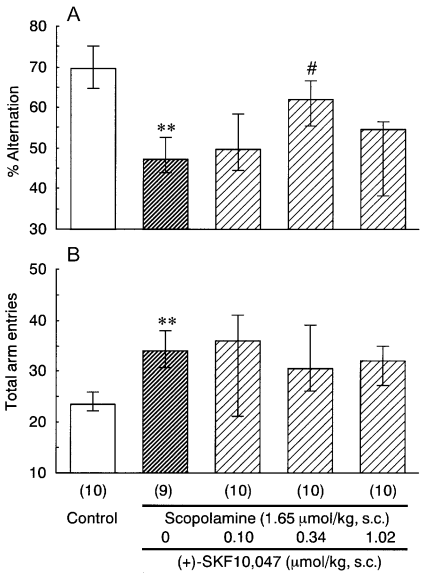

Fig. 2. Effects of (+)-SKF10,047 on scopolamine-induced impairment of spontaneous alternation (A) and total arm entries (B) in the Y-maze test. Mice were treated with scopolamine (1.65 μmol/kg, s.c.) and (+)-SKF10,047 (0.10, 0.34 and 1.02 μmol/kg, s.c.) 30 and 25 min before testing, respectively. Data are shown as median (vertical column) and first and third quartiles (vertical line). The number of mice used is shown in parentheses. Significance levels: $^{**}P<0.01$ vs. control (Mann–Whitney's *U*-test). $\#P<0.05$ vs. scopolamine alone (Bonferroni's test).

(+)-SKF10,047 (0.10–1.02 μmol/kg, s.c.) also attenuated the impairment of spontaneous alternation behavior induced by scopolamine, but the dose–response curve was bell-shaped and only 0.34 μmol/kg of (+)-SKF10,047 showed a significant effect (Fig. 2B). The scopolamine-induced increase in the total number of arm entries was not attenuated by doses of (+)-SKF10,047 (Fig. 2B). The injection of (+)-SKF10,047 alone had no effect on the spontaneous alternation behavior and total number of arm entries (data not shown).

3.2. Effects of coadministration of U-50,488H and (+)-SKF10,047 on scopolamine-induced impairment of spontaneous alternation behavior and number of arm entries

In the next experiment, we examined whether an additive effect was observed when subactive doses of U-50,488H (0.21 μmol/kg) and (+)-SKF10,047 (0.10 μmol/kg) were coadministered. Coadministration of U-50,488H (0.21–2.15 μmol/kg, s.c.) with a subactive dose of (+)-SKF 10,047 (0.10 μmol/kg) did not show any additive effect (Fig. 3A) compared with (+)-U-50,488H alone (Fig. 1A). Furthermore, coadministration of (+)-SKF10,047 (0.10–1.02 μmol/kg, s.c.) with subactive doses of U-50,488H (0.21 μmol/kg) also did not show an additive effect (Fig. 4A) compared with (+)-SKF10,047 (Fig. 2A). Moreover, coadministration of active doses of U-50,488H (0.64 μmol/kg, s.c.)

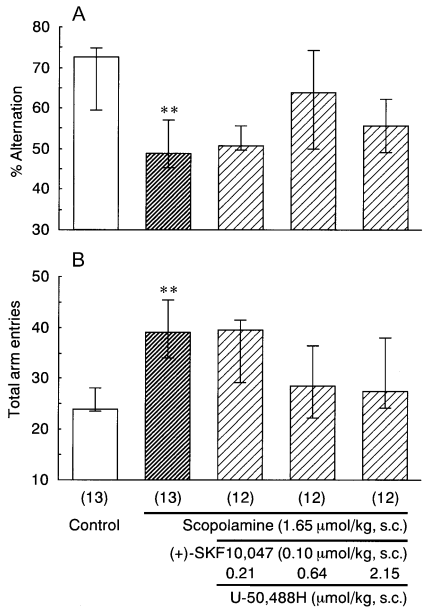

Fig. 3. Effects of (+)-SKF10,047 and its combination with U-50,488H on scopolamine-induced impairment of spontaneous alternation (A) and total arm entries (B) in the Y-maze test. Mice were treated with (+)-SKF10,047 (0.10 μmol/kg, s.c.) and U-50,488H (0.21, 0.64 and 2.15 μmol/kg, s.c.) 25 and 25 min before testing, respectively. Scopolamine (1.65 μmol/kg, s.c.) was injected 30 min before testing. Data are shown as medians (vertical column) and first and third quartiles (vertical line). The number of mice used is shown in parentheses. Significance levels: $^{**}P<0.01$ vs. control (Mann–Whitney's *U*-test).

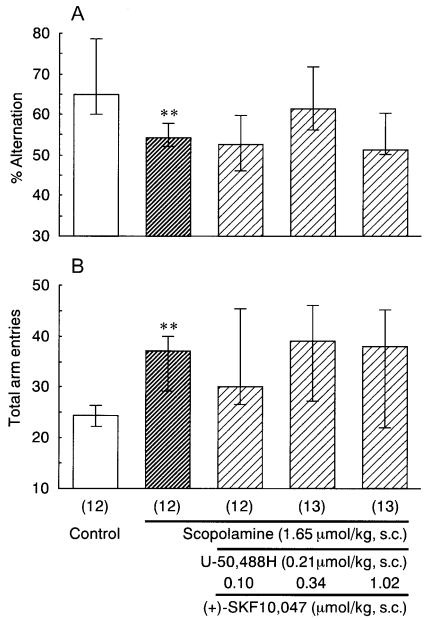

Fig. 4. Effects of U-50,488H and its combination with (+)-SKF10,047 on scopolamine-induced impairment of spontaneous alternation (A) and total arm entries (B) in the Y-maze test. Mice were treated with U-50,488H (0.21 μmol/kg, s.c.) and (+)-SKF10,047 (0.10, 0.34 and 1.02 μmol/kg, s.c.) 25 and 25 min before testing, respectively. Scopolamine (1.65 μmol/kg, s.c.) was injected 30 min before testing. Data are shown as median (vertical column) and first and third quartiles (vertical line). The number of mice used is shown in parentheses. Significance level: $^{**}P<0.01$ vs. control (Mann–Whitney's *U*-test).

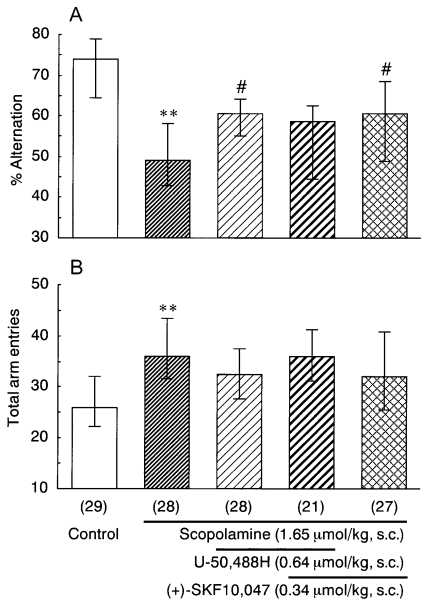

Fig. 5. Effects of active doses of U-50,488H and its combination with (+)-SKF10,047 on scopolamine-induced impairment of spontaneous alternation (A) and total arm entries (B) in the Y-maze test. Mice were treated with U-50,488H (0.64 μmol/kg, s.c.) and (+)-SKF10,047 (0.34 μmol/kg, s.c.) 25 and 25 min before testing, respectively. Scopolamine (1.65 μmol/kg, s.c.) was injected 30 min before testing. Data are shown as median (vertical column) and first and third quartiles (vertical line). The number of mice used is shown in parentheses. Significance levels: $^{**}P<0.01$ vs. control (Mann–Whitney's *U*-test). $^{\#}P<0.05$ vs. scopolamine alone (Bonferroni's test).

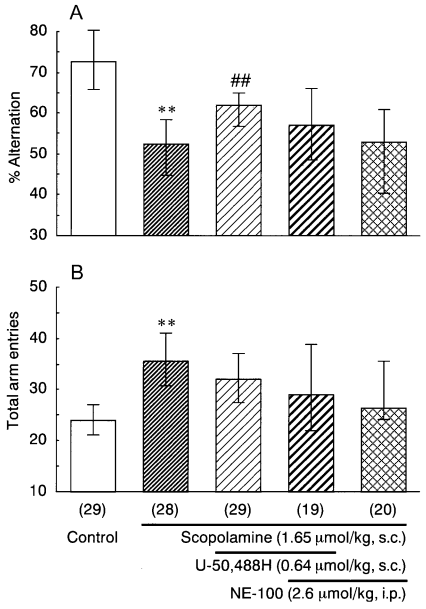

Fig. 6. Effects of U-50,488H and its combination with NE-100 on scopolamine-induced impairment of spontaneous alternation (A) and total arm entries (B) in the Y-maze test. Mice were treated with NE-100 (2.6 μmol/kg, i.p.) and U-50,488H (0.64 μmol/kg, s.c.) 30 and 25 min before testing, respectively. Scopolamine (1.65 μmol/kg, s.c.) was injected 30 min before testing. Data are shown as median (vertical column) and first and third quartiles (vertical line). The number of mice used is shown in parentheses. Significance levels: $^{**}P<0.01$ vs. control (Mann–Whitney's *U*-test). $^{\#\#}P<0.01$ vs. scopolamine alone (Bonferroni's test).

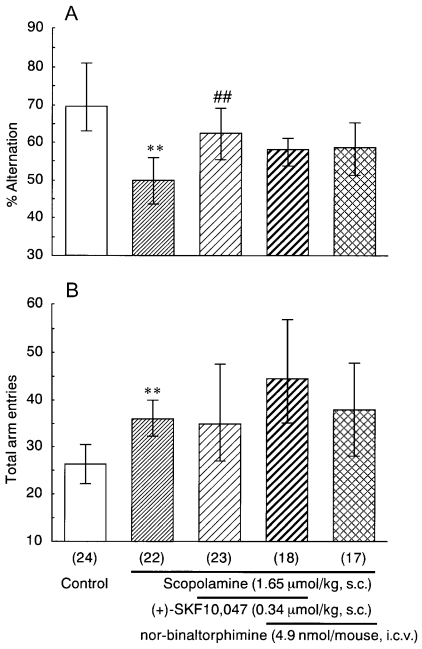

Fig. 7. Effects of (+)-SKF10,047 and its combination with nor-binaltorphimine on scopolamine-induced impairment of spontaneous alternation (A) and total arm entries (B) in the Y-maze test. Mice were treated with (+)-SKF10,047 (0.34 μmol/kg, s.c.) and nor-binaltorphimine (4.9 nmol/mouse, i.c.v.) 25 and 25 min before testing, respectively. Scopolamine (1.65 μmol/kg, s.c.) was injected 30 min before testing. Data are shown as median (vertical column) and first and third quartiles (vertical line). The number of mice used is shown in parentheses. Significance levels: $^{**}P<0.01$ vs. control (Mann–Whitney's *U*-test). $^{\#\#}P<0.01$ vs. scopolamine alone (Bonferroni's test).

and (+)-SKF10,047 (0.34 μmol/kg, s.c.) had no effects on the improvement (Fig. 5).

3.3. Effects of NE-100 and nor-binaltorphimine on the ameliorating effects of U-50,488H and (+)-SKF-10,047 on the scopolamine-induced impairment of spontaneous alternation behavior

To determine whether σ and κ-opioid receptor-mediated systems are involved in the ameliorating effects of U-50,488H and (+)-SKF-10,047, respectively, a selective σ receptor antagonist, NE-100, and a selective κ-opioid receptor antagonist, nor-binaltorphimine, were administered. The ameliorating effect of U-50,488H (0.64 μmol/kg, s.c.) was not antagonized by NE-100 (2.6 μmol/kg, i.p.) (Fig. 6), and that of (+)-SKF10,047 (0.34 μmol/kg, s.c.) was not antagonized by nor-binaltorphimine (4.9 nmol/kg, i.c.v.) (Fig. 7).

3.4. Antinociceptive effects of coadministration of U-50,488H and (+)-SKF10,047 in the acetic acid-induced writhing test

In the acetic acid-induced writhing test, control mice showed about 14–19 writhing responses during the 10-min observation period beginning 10 min after injection of 0.7% acetic acid solution. A dose-related and significant antinociceptive effect was observed after s.c. injection of U-50,488H (2.15 and 6.45 μmol/kg, s.c.) 25 min before the writhing test (Fig. 8).

As described in Introduction, σ_1 receptor antisense treatment enhanced the analgesic activity of U-50,488H (King et al., 1997). However, the antinociceptive effect of U-50,488H (6.45 μmol/kg, s.c.) was not affected by (+)-SKF-10,047 (0.34 μmol/kg, s.c.) or NE-100 (2.6 μmol/kg, i.p.) as shown in Fig. 8B and C, respectively. (+)-SKF-10,047 (0.1–3.4 μmol/kg, s.c.) alone or coadministered with a subactive dose of U-50,488H (0.64 μmol/kg, s.c.) did not induce the antinociceptive effect (data not shown).

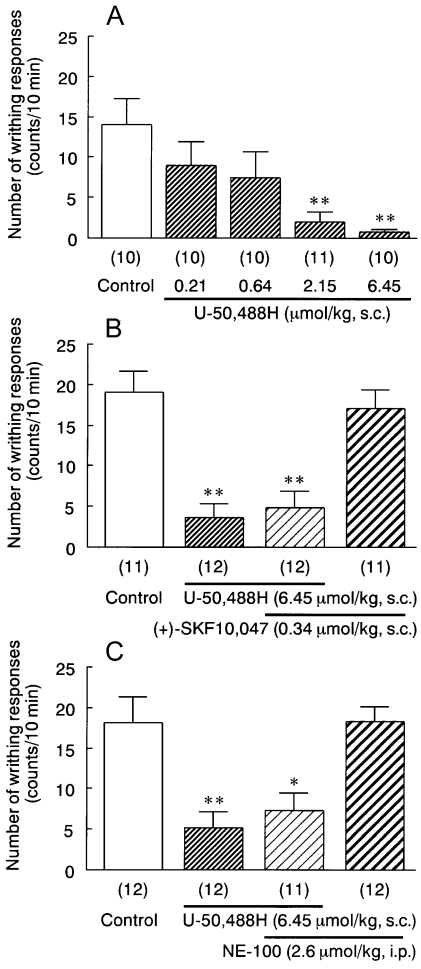

Fig. 8. Antinociceptive effects of U-50,488H in the acetic acid-induced writhing test. (A) Mice were treated with U-50,488H (0.21, 0.64, 2.15 and 6.45 μmol/kg, s.c.) 25 min before testing. (B) Mice were treated with U-50,488H (6.45 μmol/kg, s.c.) and (+)-SKF10,047 (0.34 μmol/kg, s.c.) 25 and 25 min before testing, respectively. (C) Mice were treated with NE-100 (2.6 μmol/kg, i.p.) and U-50,488H (6.45 μmol/kg, s.c.) 30 and 25 min before testing, respectively. Acetic acid (0.7%) was injected 10 min before testing and the number of writhing responses was counted for 10 min. Data are shown as means ± S.E.M. The number of mice used is shown in parentheses. Significance levels: $^{*}P<0.05$, $^{**}P<0.01$ vs. control (Bonferroni's test).

4. Discussion

(±)-Pentazocine is widely used clinically to treat mild to moderate pain as a racemic compound. It has been reported that the enantiomers of pentazocine act on different receptors and each has a distinct pharmacology (Bowen et al., 1989; Pasternak, 1988). (−)-Pentazocine shows analgesic effects by acting on κ-opioid receptors in mice and humans (Pasternak, 1988, 1993), and (+)-pentazocine improves learning and memory impairments in mice, acting on σ receptors (Maurice et al., 1994; Hoshino et al., 2000). On the other hand, we previously reported that U-50,488H, a selective κ-opioid receptor agonist, improved learning and memory impairment in various animal models (Hiramatsu et al., 1996a,b, 1998b). The ameliorating effect of U-50,488H was reversed by nor-binaltorphimine, a selective κ-opioid receptor antagonist, suggesting that the effects of U-50,488H on impairment of learning and memory are mediated via κ-opioid receptors (Hiramatsu et al., 1998b). If (±)-pentazocine is to be used for the treatment of learning and memory disorders, we require information about the pharmacology of each enantiomer and of their combination. In this study, therefore, we investigated whether κ-opioid and/or σ receptors were activated simultaneously, using spontaneous alternation behavior in a Y-maze.

We now confirmed that a κ-opioid receptor agonist, U-50,488H, improved the impairment of spontaneous alternation behavior induced by scopolamine even when the dose was less than that showing antinociceptive effects in the acetic acid-induced writhing test. Furthermore, (+)-SKF 10,047, a selective σ receptor agonist, also improved the impairment of spontaneous alternation behavior. This observation is consistent with previous reports that (+)-SKF10,047 attenuated the impairment of learning and memory mediated via σ receptors (Maurice et al., 1994; Senda et al., 1997). Other σ receptor agonists, SA-4503, (+)-pentazocine, DTG and (+)-3-PPP, also improved learning and memory impairments in various behavioral tests (Matsuno et al., 1995; Maurice et al., 1994; Senda et al., 1997). These agonists enhanced acetylcholine release in guinea pig cerebral slices (Siniscalchi et al., 1987), and extracellular acetylcholine levels in the rat frontal cortex (Matsuno et al., 1992, 1993) and hippocampus (Matsuno et al., 1995). Therefore, σ receptors in the central nervous system play an important role in modulating learning and memory function via cholinergic neuronal systems. We also reported that κ-opioid receptor agonists, U-50,488H and dynorphin A-(1–13), improved learning and memory impairment by suppressing the decrease in acetylcholine release induced by mecamylamine, galanin or carbachol in the hippocampus. However, these κ-opioid receptor agonists showed no significant effect in normal animals (Hiramatsu et al., 1996a,b, 1998a,b). These findings suggest that both κ-opioid receptors and σ receptors might have a common mechanism. When cholinergic neuronal transmission is impaired, κ-opioid and σ receptor agonists enhance this neuronal transmission, and as a result, the learning and memory impairment is improved. In agreement with our results, post-training administration of dynorphin A-(1–13) had no effect at doses between 1.25 and 125 ng/rat, i.c.v. in the inhibitory avoidance task (Izquierdo et al., 1985). However, bilateral infusion of dynorphin A-(1–8) (10–20 μg/side) into the dorsal hippocampus resulted in a dose-related impairment of spatial working memory in a radial maze task (McDaniel et al., 1990). In a nonspatial task, post-training dynorphin injection in the dorsal hippocampus had no effect on the retention of step-through passive avoidance (McDaniel et al., 1990). These results suggest that dynorphin specifically affects different types of memory at different dose ranges.

It was reported that the effects of σ receptor ligands might be partly mediated by the opioid receptors (Kobayashi et al., 1996). κ-Opioid receptor agonists inhibited σ_1 receptor binding in guinea-pig brain, liver and spleen (Brent, 1996). As described in Introduction, a tonically active anti-opioid σ receptor system markedly influences the sensitivity of mice to opioid analgesics, particularly κ-opioid drugs with the analgesic actions of opioids (Chien and Pasternak, 1993, 1994, 1995). σ_1 Receptor antisense enhanced the analgesic activities of both the κ_1- and κ_3-opioid receptor analgesics, of U-50,488H and of naloxone benzoylhydrazone, respectively (King et al., 1997), and haloperidol enhanced κ_1 and κ_3 analgesia more dramatically than did morphine through σ mechanisms (Chien and Pasternak, 1994). These results suggest that an interaction between κ-opioid receptors and σ receptors regarding memory function might exist in the central nervous system. In the present study, however, coadministration of U-50,488H and a subactive dose of (+)-SKF10,047 had no significant effects compared to (+)-SKF10,047 alone, and coadministration of (+)-SKF10,047 and a subactive dose of U-50,488H did not show significant effects, compared to U-50,488H alone. Coadministration of the active dose of U-50,488H and that of (+)-SKF10,047 had no additive or antagonizing effects. Furthermore, the ameliorating effect of U-50,488H was not antagonized by a selective σ receptor antagonist, NE-100, and the effect of (+)-SKF10,047 was not antagonized by a selective κ-opioid receptor antagonist, nor-binaltorphimine.

We tested whether there is a similarity between U-50,488H and (+)-SKF10,047 using the acetic acid-induced writhing test. U-50,488H showed dose-dependent antinociceptive effects, and (+)-SKF10,047 showed no antinociceptive effects, which was consistent with previous reports (Chien et al., 1994; Kolesnikov et al., 1996; Pasternak, 1993). When U-50,488H and (+)-SKF10,047 were coadministered, no interactive effect was observed in the writhing assay. Chien and Pasternak (1994) reported that the σ system functionally antagonized opioid analgesia without affecting other effects on gastrointestinal transit or lethality. Taken together with our findings, these modulations are observed only under definite conditions, such as the tail flick assay using radiant heat.

In conclusion, κ-opioid and σ receptor agonists independently stimulated cholinergic neural transmission and improved learning and memory impairment. Furthermore, since (+)-SKF 10,047 did not interfere with the antinociceptive effects of U-50,488H, (±)-pentazocine acting on both κ-opioid and σ receptors might be useful for the treatment of learning and memory disorders.

Acknowledgements

We are grateful to Taisho Pharmaceutical (Tokyo) for supplying the NE-100. This study was supported in part by grants from the Japan Smoking Research Foundation and by Grants-in-Aid for Scientific Research (No. 12672230 for M.H., High-Tech Research Center Project and Scientific Frontier Research Project of Meijo University) from the Ministry of Education, Science, Sports and Culture, Japan.

References

Bartus, R.T., Dean, R.L., Beer, B., Lippa, A.S., 1982. The cholinergic hypothesis of geriatric memory dysfunction. Science 217, 408–414.

Beninger, R.J., Wirsching, B.A., Jhamands, K., Boegman, R.J., 1989. Animal studies of brain acetylcholine and memory. Arch. Gerontol. Geriatr., Suppl. 1, 71–89.

Bowen, W.D., Hellewell, S.B., McGarry, K.A., 1989. Evidence for a multi-site model of the rat brain sigma receptor. Eur. J. Pharmacol. 163, 309–318.

Brent, P.J., 1996. Kappa opioid receptor agonists inhibit sigma-1 (sigma 1) receptor binding in guinea-pig brain, liver and spleen: autoradiographical evidence. Brain Res. 725, 155–165.

Chien, C.-C., Pasternak, G.W., 1993. Functional antagonism of morphine analgesia by (+)-pentazocine: evidence for an anti-opioid σ_1 system. Eur. J. Pharmacol. 250, R7–R8.

Chien, C.-C., Pasternak, G.W., 1994. Selective antagonism of opioid analgesia by a sigma system. J. Pharmacol. Exp. Ther. 271, 1583–1590.

Chien, C.-C., Pasternak, G.W., 1995. (−)-Pentazocine analgesia in mice: interaction with a σ receptor system. Eur. J. Pharmacol. 294, 303–308.

Chien, C.-C., Brown, G., Pan, Y.-X., Pasternak, G.W., 1994. Blockade of U50,488H analgesia by antisense oligonucleotides to a κ-opioid receptor. Eur. J. Pharmacol. 253, R7–R8.

Coyle, J.T., Price, D.L., DeLong, M.R., 1983. Alzheimer's disease: a disorder of cortical cholinergic innervation. Science 219, 1184–1190.

Haley, T.J., McCormick, W.G., 1957. Pharmacological effects produced by intracerebral injection of drugs in the conscious mouse. Br. J. Pharmacol. 12, 12–15.

Hiller, J.M., Itzhak, Y., Simon, E.J., 1987. Selective changes in mu, delta and kappa opioid receptor binding in certain limbic regions of the brain in Alzheimer's disease patients. Brain Res. 406, 17–23.

Hiramatsu, M., Sasaki, M., Kameyama, T., 1995. Effects of dynorphin A-(1–13) on carbon monoxide-induced delayed amnesia in mice studied in a step-down type passive avoidance task. Eur. J. Pharmacol. 282, 185–191.

Hiramatsu, M., Hyodo, T., Kameyama, T., 1996a. U-50,488H, a selective κ-opioid receptor agonist, improves carbon monoxide-induced delayed amnesia in mice. Eur. J. Pharmacol. 315, 119–125.

Hiramatsu, M., Mori, H., Murasawa, H., Kameyama, T., 1996b. Improvement by dynorphin A-(1–13) of galanin-induced impairment of memory accompanied by blockade of reductions in acetylcholine release in rats. Br. J. Pharmacol. 118, 255–260.

Hiramatsu, M., Sasaki, M., Nabeshima, T., Kameyama, T., 1997. Effects of dynorphin A-(1-13) on carbon monoxide-induced delayed amnesia in mice. Pharmacol. Biochem. Behav. 56, 73–79.

Hiramatsu, M., Murasawa, H., Mori, H., Kameyama, T., 1998a. Reversion of muscarinic autoreceptor agonist-induced acetylcholine decrease and learning impairment by dynorphin A-(1–13), an endogenous κ-opioid receptor agonist. Br. J. Pharmacol. 123, 920–926.

Hiramatsu, M., Murasawa, H., Nabeshima, T., Kameyama, T., 1998b. Effects of U-50,488H on scopolamine-, mecamylamine- and dizocilpine-induced learning and memory impairment in rats. J. Pharmacol. Exp. Ther. 284, 858–867.

Hiramatsu, M., Inoue, K., Kameyama, T., 2000. Dynorphin A-(1–13) and (2–13) improve β-amyloid peptide-induced amnesia in mice. NeuroReport 11, 431–435.

Hoshino, T., Hiramatsu, M., Kameyama, T., Nabeshima, T., 2000. Improvement of memory impairment by (+)- and (−)-pentazocine via kappa opioid and/or sigma receptors in mice. Neurosci. Abstr., 30.

Itoh, J., Ukai, M., Kameyama, T., 1993. Dynorphin A-(1–13) markedly improves scopolamine-induced impairment of spontaneous alternation performance in mice. Eur. J. Pharmacol. 236, 341–345.

Izquierdo, I., De Almeida, M.A., Emiliano, V.R., 1985. Unlike beta-endorphin, dynorphin 1–13 does not cause retrograde amnesia for shuttle avoidance or inhibitory avoidance learning in rats. Psychopharmacology 87, 216–218.

King, M., Pan, Y.-X., Mei, J., Chang, A., Xu, J., Pasternak, G.W., 1997. Enhanced κ-opioid receptor-mediated analgesia by antisense targeting the σ_1 receptor. Eur. J. Pharmacol. 331, R5–R6.

Kobayashi, T., Ikeda, K., Ichikawa, T., Togashi, S., Kumanishi, T., 1996. Effects of sigma ligands on the cloned mu-, delta- and kappa-opioid receptors co-expressed with G-protein-activated K^+(GIRK) channel in Xenopus oocytes. Br. J. Pharmacol. 119, 73–80.

Kolesnikov, Y., Jain, S., Wilson, R., Pasternak, G.W., 1996. Peripheral κ_1-opioid receptor-mediated analgesia in mice. Eur. J. Pharmacol. 310, 141–143.

Litchfield, S.T., Wilcoxon, F., 1949. A simplified method of evaluating dose effect experiments. J. Pharmacol. Exp. Ther. 96, 99–113.

Mathieu-Kia, A.M., Fan, L.Q., Kreek, M.J., Simon, E.J., Hiller, J.M., 2001. Mu-, delta- and kappa-opioid receptor populations are differentially altered in distinct areas of postmortem brains of Alzheimer's disease patients. Brain Res. 893, 121–134.

Matsuno, K., Matsunaga, K., Mita, S., 1992. Increase of extracellular acetylcholine level in rat frontal cortex induced by (+)-*N*-allylnormetazocine as measured by brain microdialysis. Brain Res. 575, 315–319.

Matsuno, K., Matsunaga, K., Senda, T., Mita, S., 1993. Increase in extracellular acetylcholine level by sigma ligands in rat frontal cortex. J. Pharmacol. Exp. Ther. 265, 851–859.

Matsuno, K., Senda, T., Kobayashi, T., Mita, S., 1995. Involvment of σ_1 receptor in (+)-*N*-allylnormetazocine-stimulated hippocampal cholinergic functions in rats. Brain Res. 690, 200–206.

Matsuno, K., Nakazawa, M., Okamoto, K., Kawashima, Y., Mita, S., 1996. Binding properties of SA4503, a novel and selective σ_1 receptor agonist. Eur. J. Pharmacol. 306, 271–279.

Martin, W.R., Eades, C.G., Thompson, J.A., Huppler, R.E., Gilbert, P.E., 1976. The effect of morphine and morphine-like drugs in the non-dependent and morphine-dependent chronic spinal dog. J. Pharmacol. Exp. Ther. 197, 517–532.

Maurice, T., Hiramatsu, M., Kameyama, T., Hasegawa, T., Nabeshima, T., 1994. Behavioral evidence for a modulating role of σ ligands in memory processes: II. Reversion of carbon monoxide (CO)-induced amnesia. Brain Res. 647, 57–64.

McDaniel, K.L., Mudy, W.R., Tilson, H.A., 1990. Microinjection of dynorphin into the hippocampus impairs spatial learning in rats. Pharmacol. Biochem. Behav. 35, 429–435.

Newhouse, A., 1990. Cholinergic drug studies in dementia and depression. Adv. Exp. Med. Biol. 282, 65–76.

Pasternak, G.W., 1988. Early studies of opioid binding. In: Pasternak, G.W. (Ed.), The Opioid Receptors. Humana Press, Clifton, NJ, pp. 59–94.

Pasternak, G.W., 1993. Pharmacological mechanisms of opioid analgesia. Clin. Neuropharmacol. 16, 1–18.

Quirion, R., Bowen, W.D., Itzhak, Y., Junien, J.L., Musacchio, J.M., Rothman, R.B., Su, T.-P., Tam, S.W., Taylor, D.P., 1992. A proposal for the classification of sigma binding sites. Trends Pharmacol. Sci. 13, 85–86.

Senda, T., Matsuno, K., Kobayashi, T., Mita, S., 1997. Reduction of the scopolamine-induced impairment of passive-avoidance performance by σ receptor agonist in mice. Physiol. Behav. 61, 257–264.

Siniscalchi, A., Cristofori, P., Veratti, E., 1987. Influence of *N*-allyl-normetazocine on acetylcholine release from brain slices: involvement of muscarinic receptors. Naunyn-Schmiedeberg's Arch. Pharmacol. 336, 425–429.