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Endomorphins 1 and 2 induce amnesia via selective modulation of dopamine receptors in mice

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Abstract

The involvement of dopamine receptors in the amnesic effects of the endogenous μ-opioid receptor agonists endomorphins 1 and 2 was investigated by observing step-down type passive avoidance learning in mice. Although the dopamine D1 receptor agonist *R*(+)-1-phenyl-2,3,4,5-tetrahydro-1*H*-3-benzazepine-7,8-diol hydrochloride (*R*(+)-SKF38393) (0.05 and 0.1 mg/kg), the dopamine D1 receptor antagonist *R*(+)-7-chloro-8-hydroxy-3-methyl-1-phenyl-2,3,4,5-tetrahydro-1*H*-3-benzazepine hydrochloride (*R*(+)-SCH23390) (2.5 and 5 μg/kg) or the dopamine D2 receptor agonist *N*-n-phenethyl-*N*-propylethyl-p-(3-hydroxyphenyl)-ethylamine (RU24213) (0.3 and 1 mg/kg) had no significant effects on the endomorphin-1 (10 μg)- or endomorphin-2 (10 μg)-induced decrease in step-down latency of passive avoidance learning, (−)-sulpiride (10 mg/kg), a dopamine D2 receptor antagonist, significantly reversed the decrease in step-down latency evoked by endomorphin-2 (10 μg), but not by endomorphin-1 (10 μg). Taken together, it is likely that stimulation of dopamine D2 receptors results in the endomorphin-2-but not endomorphin-1-induced impairment of passive avoidance learning. © 2002 Elsevier Science B.V. All rights reserved.

Keywords: Endomorphin-1; Endomorphin-2; μ-Opioid receptor; Passive avoidance learning; Dopamine receptor; (Mouse)

1. Introduction

The dopamine receptor is classified into D1–D5 (Seeman and Van Tol, 1994). In particular, dopamine receptors play an important role in learning and memory (Ukai et al., 1997a,b, 1998). The stimulation of dopamine D2 but not D1 receptors reverses the beneficial effects of substance P and neurokinin A on the scopolamine-induced impairment of spontaneous alternation behavior associated with spatial working memory (Ukai et al., 1998). The dopamine D3 receptor agonist, *R*(+)-7-hydroxy-*N*,*N*-di-*n*-propyl-2-aminotetralin, has been reported to produce memory dysfunction (Ukai et al., 1997b).

Opioid receptors are found in high density in dopaminergic pathways in the corpus striatum and nucleus accumbens where there is a close interaction between opioids and dopaminergic neurotransmission. For example, the intracerebroventricular injection of μ- and δ-opioid receptor agonists facilitates dopamine release in the brain (Di Chiara and Imperato, 1988; Spanagel et al., 1990). Dynorphin A-(1-13), a κ-opioid receptor agonist has been reported to improve the galanin-induced amnesia resulting from stimulation of dopamine D1 receptors (Ukai et al., 1997a). Dynorphin A-(1-13) and [D-Ala^2, *N*MePhe^4, Gly-ol]enkephalin (DAMGO), a μ-opioid receptor agonist, produce a marked decrease in methamphetamine-induced behavioral sensitization in mice (Toyoshi et al., 1996).

Zadina et al. (1997) have recently reported the isolation of endomorphins 1 and 2 from the bovine brain substances which are believed to be the endogenous ligands for μ-opioid receptors. Binding studies in mice have revealed that endomorphins exhibit extraordinarily high affinity and selectivity for μ-opioid receptors, well above the range of other exogenous and endogenous opioids (Goldberg et al., 1998). In the same study, the in vivo activity of endomorphins as antinociceptive agents following i.c.v. administration in mice was comparable to that of morphine and DAMGO (Goldberg et al., 1998). Moreover, endomorphins have been reported to impair spontaneous alternation behavior associated with short-term memory (Ukai et al., 2000) and passive avoidance learning associated with long-

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term memory (Ukai et al., 2001b; Ukai and Lin, 2002). However, it is not certain whether the endomorphin-induced impairment of learning and memory is involved in dopaminergic neurotransmission.

In this study, the involvement of dopamine receptors in the endomorphin-induced impairment of learning and memory was investigated by using the dopamine D1 receptor agonist *R*(+)-1-phenyl-2,3,4,5-tetrahydro-1*H*-3-benzazepine-7,8-diol hydrochloride (*R*(+)-SKF38393), the dopamine D1 receptor antagonist *R*(+)-7-chloro-8-hydroxy-3-methyl-1-phenyl-2,3,4,5-tetrahydro-1*H*-3-benzazepine hydrochloride (*R*(+)-SCH23390), the dopamine D2 receptor agonist *N*-n-phenethyl-*N*-propylethyl-p-(3-hydroxyphenyl)-ethylamine (RU24213), and the dopamine D2 receptor antagonist (−)-sulpiride (Ukai et al., 1997a,b, 1998).

2. Materials and methods

2.1. Animals

Male ddY mice (Nihon SLC, Japan), weighing between 30 and 35 g, were used. The animals were housed in standard plastic cages in a temperature-controlled room (23 ± 1 °C). Food and water were freely available and a 12-h light/dark cycle was set. The mice were kept for at least 5 days in home cages before the experiments were started. The experiments were performed between 13:00 and 17:00 in a sound-attenuated room. In addition, all efforts were made to minimize animal suffering, and to reduce the number of animals used according to the Guiding Principles for the Care and Use of Laboratory Animals approved by the Faculty of Pharmacy, Meijo University.

2.2. Drugs

Endomorphins 1 and 2 (Peptide Institute, Minoh, Japan) were used. Endomorphins were dissolved in sterile isotonic saline in polypropylene containers. The intracerebroventricular injection of endomorphins was made under ether anesthesia with a 4-mm-long needle (30 gauge) attached to a 50-μl microsyringe (Hamilton, Reno, NV, USA) according to the method of Haley and McCormick (1957). *R*(+)-1-phenyl-2,3,4,5-tetrahydro-1*H*-3-benzazepine-7, 8-diol hydrochloride (*R*(+)-SKF38393) and *N*-n-phenethyl-*N*-propylethyl-p-(3-hydroxyphenyl)-ethylamine (RU24213) were dissolved in 0.9% saline as free base. *R*(+)-7-Chloro-8-hydroxy-3-methyl-1-phenyl-2,3,4,5-tetrahydro-1*H*-3-benzazepine hydrochloride (*R*(+)-SCH23390) was dissolved in distilled water (Otsuka Pharm., Tokyo) as free base. (−)-Sulpiride was dissolved in 8.5% lactic acid solution diluted with 0.9% saline, and prepared with 1.0 N NaOH solution adjusted to pH 6.0–7.0. All of the dopaminergic drugs were injected i.p. or s.c. in a volume of 10 ml/kg.

2.3. Apparatus and procedure

The passive avoidance apparatus consisted of a Plexiglas inner box (30 × 30 × 40 cm high) with a grid floor and a sound-attenuated wooden outer box (30 × 30 × 90 cm) with a 15-W light. The grid floor consisted of 30 parallel steel rods (0.3 cm in diameter) set 1 cm apart. A wooden platform (4 × 4 × 4 cm) was placed in the center of the grid floor. In the training period, each mouse was placed gently onto a wooden platform. When the mouse stepped down from the platform and placed all its paws on the grid floor, an intermittent electroshock (60 V, dc, 0.5 s, 1 Hz) was delivered for 15 s (Ukai et al., 1997a,b). The retention test was done 24 h after training. Each mouse was again placed on the platform and the step-down latency was measured. An upper cut off time was set at 300 s. Endomorphins (i.c.v.) were administered immediately after training while *R*(+)-SKF38393 (s.c.), *R*(+)-SCH23390 (i.p.), RU24213 (s.c.) and (−)-sulpiride (i.p.) were administered 15, 30, 15 and 30 min before training, respectively.

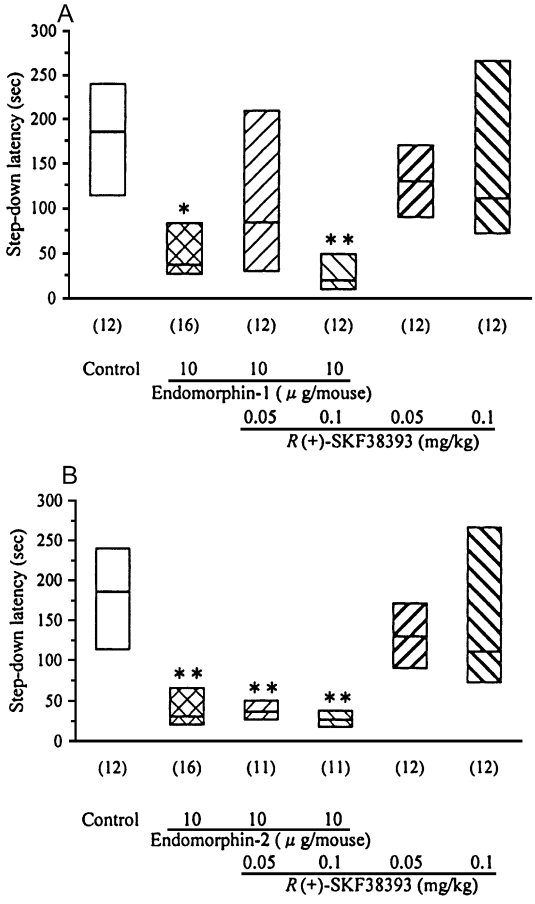

Fig. 1. Effects of endomorphin-1 (A), endomorphin-2 (B) and their combination with *R*(+)-SKF38393 on passive avoidance learning in mice. Endomorphins (i.c.v.) and *R*(+)-SKF38393 (s.c.) were administered immediately after and 15 min before training, respectively. Data are shown as medians and interquartile ranges which are the distances between the first and third quartiles. The number of mice used is shown in the parentheses. * $P < 0.05$; ** $P < 0.01$ vs. control.

2.4. Statistical analyses

The step-down latency is expressed as the median with interquartile ranges. All of the data were analyzed by a Kruskal-Wallis analysis of variance by ranks. If there were significant H values, post-hoc comparisons were made using a Bonferroni's multiple comparison test (two-tailed). The criterion for statistical significance was $P<0.05$ in all evaluations.

3. Results

3.1. Effects of R(+)-SKF38393 and R(+)-SCH23390

Endomorphin-1 (10 μg) and endomorphin-2 (10 μg) significantly shortened the step-down latency of passive avoidance learning. Although a 0.05 mg/kg dose of *R*(+)-SKF38393 had some tendency to reverse the endomorphin-1 (10 μg)-induced shortening of step-down latency of passive avoidance learning, *R*(+)-SKF38393 (0.05 and/or 0.1 mg/kg) or *R*(+)-SCH23390 (2.5 and 5 μg/kg) did not influence the endomorphin-1 (10 μg)- or endomorphin-2 (10 μg)-induced shortening. Furthermore, such drugs alone were without significant effects (Figs. 1 and 2).

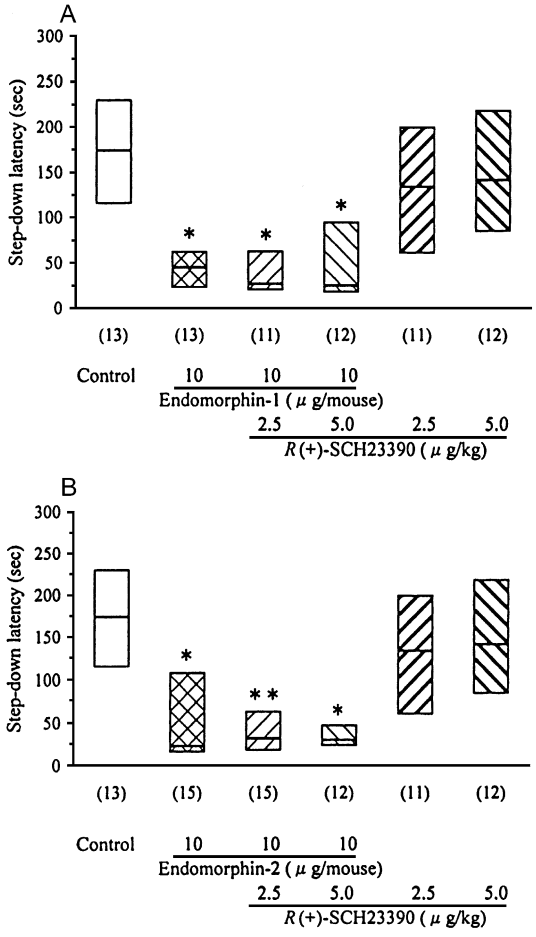

Fig. 2. Effects of endomorphin-1 (A), endomorphin-2 (B) and their combination with *R*(+)-SCH23390 on passive avoidance learning in mice. Endomorphins (i.c.v.) and *R*(+)-SCH23390 (i.p.) were administered immediately after and 30 min before training, respectively. Data are shown as medians and interquartile ranges which are the distances between the first and third quartiles. The number of mice used is shown in the parentheses. * $P<0.05$; ** $P<0.01$ vs. control.

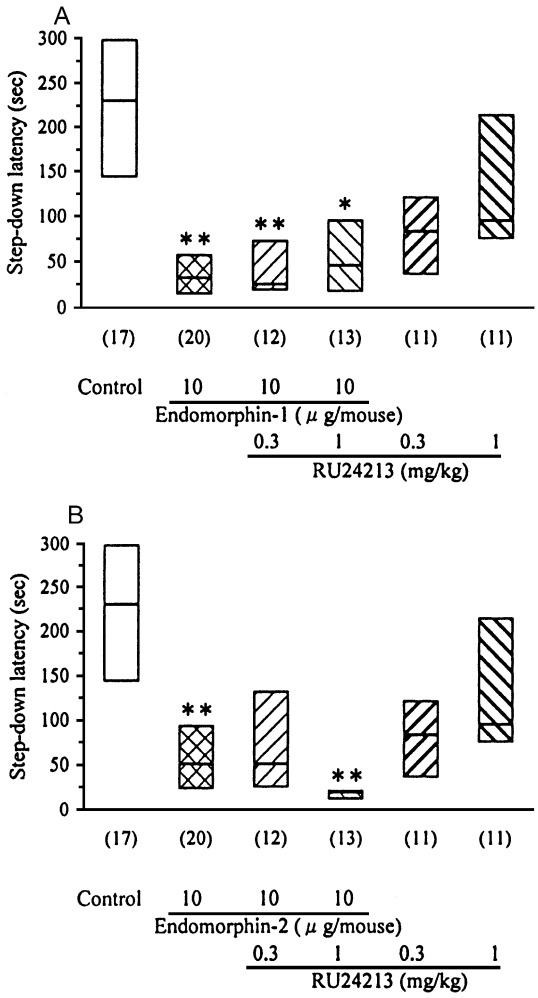

Fig. 3. Effects of endomorphin-1 (A), endomorphin-2 (B) and their combination with RU24213 on passive avoidance learning in mice. Endomorphins (i.c.v.) and RU24213 (s.c.) were administered immediately after and 15 min before training, respectively. Data are shown as medians and interquartile ranges which are the distances between the first and third quartiles. The number of mice used is shown in parentheses. * $P<0.05$; ** $P<0.01$ vs. control.

3.2. Effects of RU24213 and (−)-sulpiride

Although the significant effect of endomorphin-2 (10 μg) disappeared after treatment with a 0.3 mg/kg dose of RU24213, this drug (0.3 and/or 1 mg/kg) produced no significant effects on the endomorphin-1 (10 μg)- or endomorphin-2 (10 μg)-induced decrease in step-down latency. (−)-Sulpiride (10 mg/kg) reversed the endomorphin-2 (10 μg)- but not endomorphin-1 (10 μg)-induced decrease in step-down latency of passive avoidance learning. In addi-

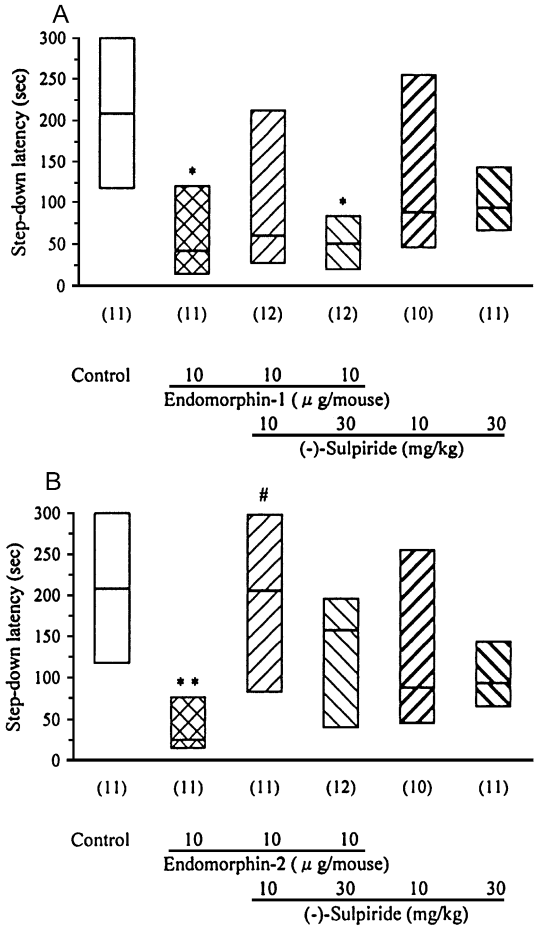

Fig. 4. Effects of endomorphin-1 (A), endomorphin-2 (B) and their combination with (−)-sulpiride on passive avoidance learning in mice. Endomorphins (i.c.v.) and (−)-sulpiride (i.p.) were administered immediately after and 30 min before training, respectively. Data are shown as medians and interquartile ranges which are the distances between the first and third quartiles. The number of mice used is shown in parentheses. * $P<0.05$; ** $P<0.01$ vs. control, # $P<0.05$ vs. endomorphin-2.

tion, RU24213 (0.3 and 1 mg/kg) or (−)-sulpiride (10 and 30 mg/kg) alone has no significant effects on step-down latency (Figs. 3 and 4).

4. Discussion

Endomorphins 1 and 2 are considered to be endogenous agonists for μ-opioid receptors (Zadina et al., 1997). Binding studies in mice have revealed that the endomorphins exhibit extraordinarily high affinity and selectivity for μ-opioid receptors, well above the range for other exogenous and endogenous opioids (Goldberg et al., 1998). Endomorphins 1 and 2 have been demonstrated to produce analgesic, anxiolytic, orexigenic and hypotensive effects (Zadina et al., 1997; Asakawa et al., 1998; Champion et al., 1997). Ukai et al. (2000, 2001b), and Ukai and Lin (2002) have recently demonstrated that endomorphins 1 and 2 significantly impair short- and long-term memory assessed by spontaneous alternation behavior and passive avoidance learning, respectively. In addition, the post-training amnestic effects of endomorphins may be induced by state-dependency, particularly in the paradigm of passive avoidance learning (Nishimura et al., 1990) whereas the amnestic effects of endomorphins have already been confirmed in spontaneous alternation behavior without special reference to state-dependency (Ukai et al., 2000).

Although endomorphins appear to be more potent than β-endorphin at μ-opioid receptors (Goldberg et al., 1998), a quite large dose of endomorphins (10 μg) was needed in order to induce amnesia in this study. This is approximately 400 times more than the dose (25 ng) of β-endorphin given i.c.v. in rats (De Almeida and Izquierdo, 1984). In particular, β-endorphin has been shown to enhance retrieval in memory processes (Izquierdo, 1980; Kovacs et al., 1983; De Almeida and Izquierdo, 1984). It thus appears that μ-opioid receptors do not play a major role in endomorphin-induced amnesia. Interestingly, our recent study revealed that a lower dose (30 ng) of endomorphin-1 but not of endomorphin-2 improves the scopolamine-induced impairment of short-term memory while the peptide alone is without a significant effect on short-term memory (Ukai et al., 2001a).

Although the dopamine D1 receptor agonist *R*(+)-SKF38393 (0.05 and 0.1 mg/kg) and the dopamine D1 receptor antagonist *R*(+)-SCH23390 (2.5 and 5 μg/kg) failed to affect the endomorphin-1 (10 μg)- or endomorphin-2 (10 μg)-induced shortening of step-down latency of passive avoidance learning, the dopamine D2 receptor antagonist (−)-sulpiride (10 mg/kg) significantly attenuated the endomorphin-2 (10 μg)-, but not the endomorphin-1 (10 μg)-, induced impairment of passive avoidance learning. Therefore, the results suggest that the endomorphin-2-induced impairment of passive avoidance learning results from stimulation of dopamine D2 receptors. The doses of dopamine receptor agonists and antagonists used in this study were sufficient to clarify the contribution of dopamine receptors to the effects of endomorphins (Itoh et al., 1993; Ukai et al., 1997a,b, 1998), although (−)-sulpiride at a higher dose (30 mg/kg) was without any significant effects, indicating that the nonspecific effects of (−)-sulpiride may have occurred at a 30 mg/kg but not a 10 mg/kg dose. In addition, the endomorphin-1 (10 μg)-induced shortening of step-down latency seems not to be associated with dopamine D_2 receptors. Differential responses to endomorphins 1 and 2 have been reported for nociception and behavioral sensitization with amphetamine (Ohsawa et al., 2000; Chen et al., 2001).

In contrast, the dopamine D2 receptor agonist RU24213 (0.3 and 1 mg/kg) should have enhanced the amnestic effects of endomorphin-2. However, it did not significantly influence the endomorphin-2 (10 μg)-induced shortening of step-down latency. This may be caused by the modest effect of endomorphins alone.

Galanin, a neuropeptide, has been found to impair passive avoidance learning (Ukai et al., 1997a). The gal-

anin-induced impairment is attenuated by SKF38393, but not by RU24213, SCH23390 or (−)-sulpiride, suggesting that the involvement of dopamine receptors in the effects of endomorphins is different from that of galanin. In contrast, there is a similarity between the endomorphin-2- and scopolamine-induced memory impairment, because scopolamine impairs memory through the mediation of dopamine D2 receptors (Ukai et al., 1998). This supports the evidence that physostigmine, a cholinesterase inhibitor, ameliorates the endomorphin-1- and endomorphin-2-induced memory impairment (Ukai and Lin, 2002).

The hippocampus is believed to be important for converting new memories into long-term memories. It is possible that the endomorphin-induced impairment of memory results from functional disintegration of the hippocampus because passive avoidance learning is based on long-term memory (Ukai et al., 2001b). Actually, the microinjection of endomorphin-2 into the CA3 region of the rat hippocampus has been reported to significantly impair spatial learning (Sandin et al., 2000). Furthermore, endomorphin-2 was administered immediately after training, whereas (−)-sulpiride was administered before training, suggesting that the interaction between endomorphin-2 and dopamine D_2 receptors develops in the process of acquisition and/or consolidation of memory.

This is the first demonstration that endomorphin-2, but not endomorphin-1, produces memory impairment resulting from activation of dopamine D2 receptors, although the involvement of dopamine receptors in the pharmacological effects of endomorphins had not been determined.

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