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Endomorphins 1 and 2, endogenous μ-opioid receptor agonists, impair passive avoidance learning in mice

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Abstract

The effects of intracerebroventricular administration of endomorphin-1 and endomorphin-2, endogenous μ-opioid receptor agonists, on passive avoidance learning associated with long-term memory were investigated in mice. Endomorphin-1 (10 and 17.5 μg) and endomorphin-2 (17.5 μg) produced a significant decrease in step-down latency in a passive avoidance learning task. β-Funaltrexamine (5 μg) almost completely reversed the endomorphin-1 (17.5 μg)- and endomorphin-2 (17.5 μg)-induced shortening of step-down latency, although neither naltrindole (4 ng) nor nor-binaltorphimine (4 μg) produced any significant effects on the effects of endomorphins 1 and 2. These results suggest that endomorphins 1 and 2 impair long-term memory through the mediation of μ-opioid receptors in the brain.

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

1. Introduction

Zadina et al. (1997) have recently isolated two endogenous, potent, and selective μ-opioid receptor agonists named endomorphin-1 (Tyr-Pro-Trp-Phe-NH_2) and endomorphin-2 (Tyr-Pro-Phe-Phe-NH_2) from bovine frontal cortex. Moreover, immunoreactivity studies have shown the localization of endomorphin-1 in several brain regions, including the thalamus, hypothalamus, striatum and frontal cortex. Recent studies have shown that endomorphins have nitric oxide-dependent vasodilator activity, resulting in a decrease in systemic arterial pressure in rats (Champion and Kadowitz, 1998), while analgesic, orexigenic and anxiolytic effects of endomorphins have been reported in other rodents (Asakawa et al., 1998; Stone et al., 1997).

In contrast, opioid neuronal systems have been demonstrated to play an important role in memory processes. The μ-selective opioid receptor agonists [D-Ala^2, *N*MePhe^4, Gly-ol]enkephalin (DAMGO) and Tyr-D-Arg-Phe-β-Ala-NH_2 (TAPA) and the δ-selective opioid receptor agonists [D-Pen^2,L-Pen^5] enkephalin and [D-Ala^2]deltorphin II impair short- and/or long-term memory processes (Ukai et al., 1993b, 1997; Itoh et al., 1994), whereas the κ-opioid receptor agonist dynorphin A-(1–13) ameliorates the disturbance of learning and memory in aversive and non-aversive tasks (Ukai et al., 1993a). Ukai et al. (2000) have reported that endomorphins 1 and 2 impair spontaneous alternation performance associated with short-term memory. However, the effects of endomorphins on long-term memory have not yet been determined in detail.

Therefore, the present study was designed to examine the effects of intracerebroventricular injection of endomorphins 1 and 2 on passive avoidance learning associated with aversive and long-term memory.

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 at least 5 days in home cages before the experiments were started. The experiments were performed between 1300 and 1700 h in a sound-attenuated room.

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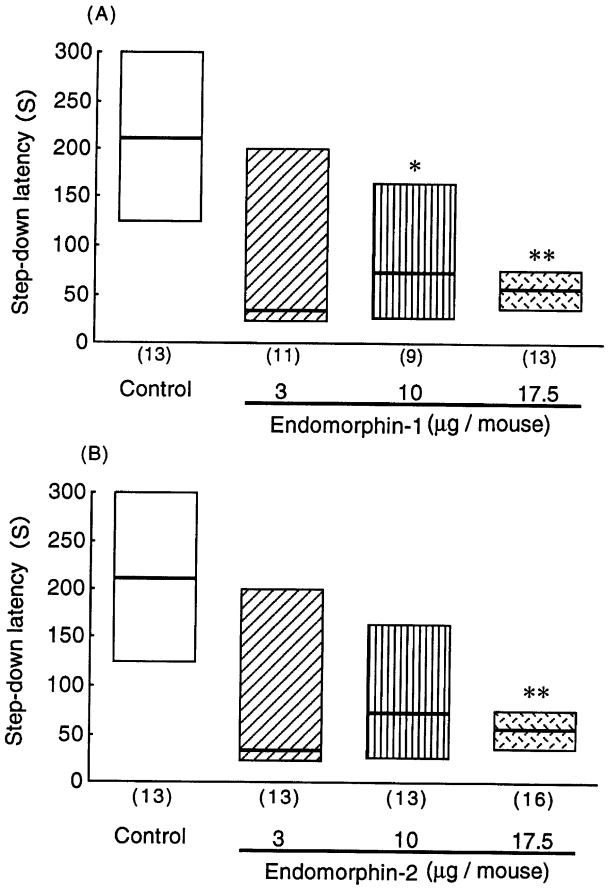

Fig. 1. Effects of endomorphin-1 (A), and endomorphin-2 (B) on step-down latency in a passive avoidance learning task in mice. Endomorphins (i.c.v.) were given to mice immediately after training. Data are shown as the median and interquartile ranges, which are the distances between the first and third quartiles. The number of mice used is shown in parentheses. $^{*}P < 0.05$; $^{**}P < 0.01$ vs. control.

2.2. Drugs

Endomorphins 1 and 2 (Peptide Institute, Minoh, Japan), β-funaltrexamine hydrochloride, naltrindole hydrochloride, and nor-binaltorphimine dihydrochloride (Research Biochemicals, Natick, MA, USA) were used. Endomorphins were dissolved in sterile isotonic saline in polypropylene containers. The injection was made 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).

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 (Ukai et al., 1997). In the training period, each mouse was placed gently onto a wooden platform, and 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. The retention test was done 24 h after training. Each mouse was again placed onto the platform and the step-down latency was measured. An upper cut-off time was set at 300 s.

2.4. Statistical analysis

The step-down latency is expressed as the median and interquartile ranges. All the data were analyzed by a Kruskal–Wallis analysis of variance by ranks. If there were significant H values, post-hoc comparisons were

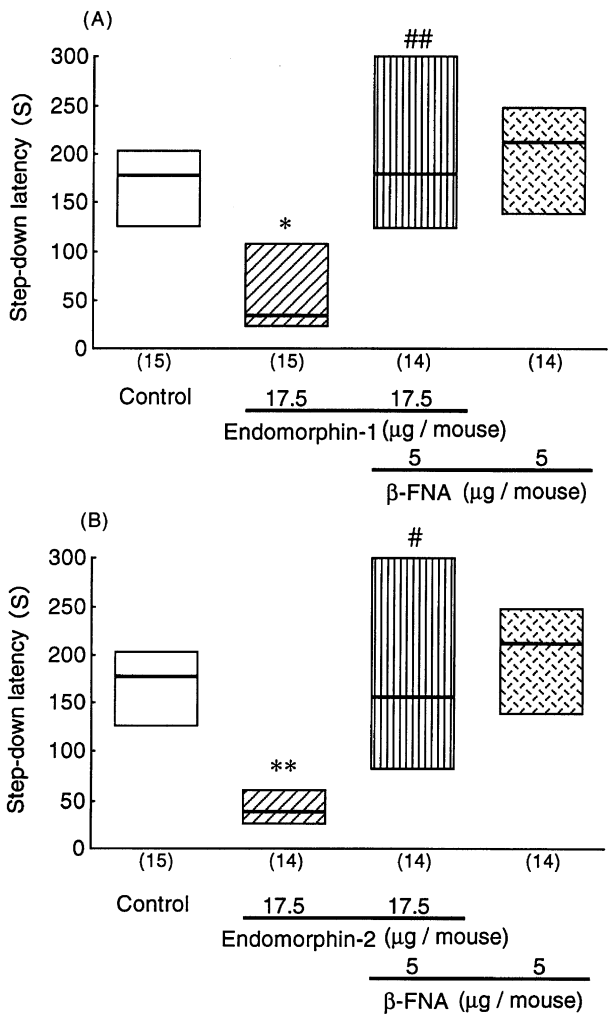

Fig. 2. Effects of endomorphin-1 (A), endomorphin-2 (B) and their combinations with β-funaltrexamine (β-FNA) on step-down latency in a passive avoidance learning task in mice. Endomorphins (i.c.v.) and β-FNA (i.c.v.) were given to mice immediately after and 24 h before training, respectively. Data are shown as the median 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, $\#P < 0.05$; $\#\#P < 0.01$ vs. each of the endomorphins alone.

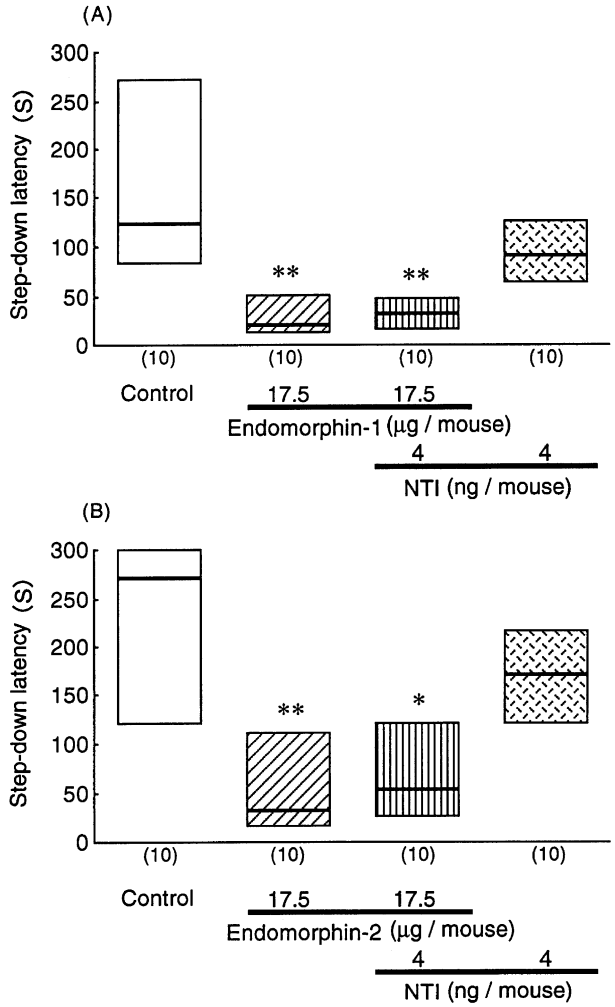

Fig. 3. Effects of endomorphin-1 (A), endomorphin-2 (B) and their combinations with naltrindole (NTI) on step-down latency in a passive avoidance learning task in mice. Endomorphins (i.c.v.) and NTI (i.c.v.) were given to mice immediately after and 20 min before training, respectively. Data are shown as the median 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.

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 endomorphins

Endomorphin-1 (10 and 17.5 μg) and endomorphin-2 (17.5 μg) significantly shortened the step-down latency in a passive avoidance learning task (Kruskal–Wallis analysis: endomorphin-1, $H = 12.71$, $P < 0.05$; endomorphin-2, $H = 18.41$, $P < 0.05$) (Fig. 1).

3.2. Effects of β-funaltrexamine, naltrindole and nor-binaltorphimine

Endomorphin-1 (17.5 μg) and endomorphin-2 (17.5 μg) again shortened the step-down latency, while β-funaltrexamine (5 μg), naltrindole (4 ng) or nor-binaltorphimine (4 μg) alone had no significant effects on the step-down latency (Figs. 2–4). β-Funaltrexamine (5 μg) almost completely reversed the effects of endomorphin-1 (17.5 μg) (Kruskal–Wallis analysis: $H = 16.72$, $P < 0.05$) and endomorphin-2 (17.5 μg) (Kruskal–Wallis analysis: $H = 16.22$, $P < 0.05$) (Fig. 2), although naltrindole (4 ng) (Kruskal–Wallis analysis: $H = 22.33$, $P < 0.05$ for endomorphin-1, and $H = 15.58$, $P < 0.05$ for endomorphin-2) and nor-binaltorphimine (4 μg) (Kruskal–Wallis analysis: $H = 27.02$, $P < 0.05$ for endomor-

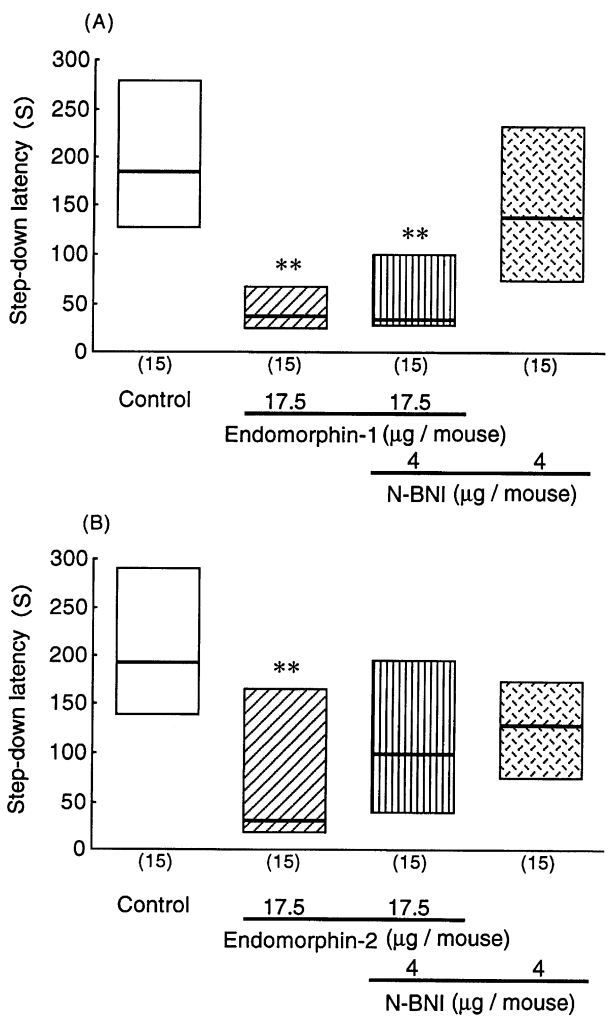

Fig. 4. Effects of endomorphin-1 (A), endomorphin-2 (B) and their combinations with nor-binaltorphimine (N-BNI) on step-down latency in a passive avoidance learning task in mice. Endomorphins (i.c.v.) and N-BNI (i.c.v.) were given to mice immediately after and 60 min before training, respectively. Data are shown as the median and interquartile ranges, which are the distances between the first and third quartiles. The number of mice used is shown in parentheses. $^{**}P < 0.01$ vs. control.

phin-1, and $H = 12.86$, $P < 0.05$ for endomorphin-2) were without significant effects. In addition, the general behavior was not markedly changed after drug treatments.

4. Discussion

μ-Opioid receptor binding has been reported to decrease in the brains of Alzheimer's disease patients (Hiller et al., 1987). Moreover, lesioning of the nucleus of basalis of Meynert produces a significant decrease in μ-opioid receptor binding in the rat cerebral cortex (Ofri et al., 1992) and evokes amnesia (Ukai et al., 1993a). The μ-opioid receptor agonists DAMGO and TAPA impair spontaneous alternation performance in association with short-term memory and passive avoidance learning in association with long-term memory, respectively (Ukai et al., 1993b; Itoh et al., 1994). TAPA has much a higher selectivity and affinity for μ-opioid receptors than DAMGO.

Endomorphins 1 and 2 are considered to be endogenous agonists for μ-opioid receptors (Zadina et al., 1997). In addition, endomorphins 1 and 2 have been demonstrated to produce analgesic, anxiolytic, orexigenic and hypotensive effects (Zadina et al., 1997; Asakawa et al., 1998; Champion and Kadowitz, 1998). These findings suggest that endomorphins have the potential to modulate neuronal activity in the brain. The effects of endomorphins 1 and 2 on long-term memory are inconclusive, although Ukai et al. (2000) have recently demonstrated that endomorphins 1 and 2 significantly impair short-term memory assessed by spontaneous alternation performance.

Endomorphins 1 (3 μg) and 2 (3 and 10 μg) were inactive but after their doses were increased to 10 and/or 17.5 μg, they inhibited passive avoidance learning associated with long-term memory. The inhibitory effects of endomorphins were almost completely antagonized by β-funaltrexamine (5 μg) but not by naltrindole (4 ng) or nor-binaltorphimine (4 μg) (Ukai et al., 2000), indicating that the impairing effects of endomorphins 1 and 2 on long-term memory are mediated by μ-opioid receptors. The effects of endomorphins were not mediated by δ- or κ-opioid receptors, confirming the evidence of receptor binding (Zadina et al., 1997) and short-term memory experiments (Ukai et al., 2000). Moreover, the doses of the opioid receptor antagonists used have been validated as causing the blockade of opioid receptors such as μ, δ and κ, as indicated in previous reports (Sofuoglu et al., 1991; Itoh et al., 1994; Ukai et al., 1995, 2000), although only one dose of the antagonist was used.

It has been reported that 5 to 20 μg doses of endomorphins produce antinociception (Zadina et al., 1997). However, the antinociception and other effects (e.g. attention, motivation, motor activity, state dependence, etc.) that indirectly affect learning and memory would not have been influential during training, because the endomorphins were administered immediately after training. Therefore, these results provide further support for the finding that μ-opioid receptor agonists lead to amnesia related to the dysfunction of long-term memory (Ukai et al., 1993b; Itoh et al., 1994).

DAMGO has been reported to inhibit the high K^+-induced release of acetylcholine from slices of the nucleus accumbens (Heijna et al., 1990, 1992) and hippocampus (Lapchak et al., 1989). Endomorphins 1 and 2 inhibit acetylcholine release evoked by electrical field stimulation in longitudinal muscle preparations of the guinea pig ileum through the mediation of μ-opioid receptors (Nishiwaki et al., 1998). The DAMGO-induced impairment of alternation performance is significantly improved by systemic injection of physostigmine (Itoh et al., 1994). Therefore, the endomorphin-induced impairment of passive avoidance learning may be due to the inhibition of hippocampal cholinergic activity through the stimulation of μ-opioid receptors. Furthermore, (−)-sulpiride, a dopamine D_2 receptor antagonist, inhibited the amnesic effects of endomorphins 1 and 2 (unpublished observation), suggesting the involvement of dopamine receptors in the effects of endomorphins.

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