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# Effects of endomorphins-1 and -2, endogenous μ-opioid receptor agonists, on spontaneous alternation performance in mice

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**Abstract**

The effects of intracerebroventricular (i.c.v.) administration of endomorphins-1 and -2, endogenous μ-opioid receptor agonists, on the spontaneous alternation performance associated with spatial working memory were investigated in mice. Endomorphin-1 (10 and 17.5 μg) and endomorphin-2 (10 μg) produced a significant decrease in percent alternation without affecting total arm entries. β-Funaltrexamine (5 μg) almost completely reversed the endomorphin-1 (10 μg)- and endomorphin-2 (10 μg)-induced decrease in percent alternation, although neither naltrindole (4 ng) nor nor-binaltorphimine (4 μg) produced any significant effects on alternation performance. These results suggest that endomorphins impair spatial working memory through the mediation of μ-opioid receptors. 

*Keywords:* Endomorphin-1; Endomorphin-2; μ-Opioid receptor; Spontaneous alternation performance; (Mouse)

## 1. Introduction

It has been reported that the opioid neuronal systems are involved in the memory process. For example, β-endorphin and enkephalins impair the memory process, while naloxone facilitates it (Rigter et al., 1979; Izquierdo, 1980; Castellano and Pavone, 1985; Izquierdo et al., 1985; Izquierdo and Netto, 1985).

Spontaneous alternation performance, a natural tendency to explore a less recently visited arm in a Y-maze, is based upon working memory and is impaired by drugs having amnesic properties such as scopolamine, morphine, and dizocilpine (Sarter et al., 1988; Parada-Turska and Turski, 1990; Stone et al., 1991). [D-Ala$^2$, *N*MePhe$^4$,Gly-ol]enkephalin (DAMGO) and Tyr-D-Arg-Phe-β-Ala-$NH_2$ (TAPA), μ-opioid-selective receptor agonists, have also been reported to impair spontaneous alternation performance (Ukai et al., 1993b; Itoh et al., 1994).

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 demonstrated the localization of endomorphin-1 in several brain regions, including the thalamus, hypothalamus, striatum and frontal cortex. However, the effects of endomorphins-1 and -2 on the memory process have not been assessed in detail.

Consequently, the present study was designed to examine the effects of intracerebroventricular (i.c.v.) injection of endomorphins-1 and -2 on spontaneous alternation performance associated with spatial working 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 starting experiments. The experiments were done between 1300 and 1700 h in a sound-attenuated room.

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### 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. The 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. Spontaneous alternation

A black painted Y-maze made of plywood was used. Each arm was 40 cm long, 12 cm high, 3 cm wide at the bottom and 10 cm wide at the top and positioned at an equal angle. The testing procedure was based upon that of Sarter et al. (1988). Each mouse was placed at the end of one arm and was allowed to move freely through the maze for an 8-min test session. The number of maximum alternations was then the total number of arms entered minus 2, and the percent alternation was calculated as (actual alternations/maximum alternations) × 100. For example, if the three arms were called A, B, and C, and a mouse consecutively entered arms in the sequence of ACBABACBAB, its performance would consist of five alternations (ACB, CBA, BAC, ACB and CBA) out of eight (10 − 2) possible alternations, resulting in a percent alternation of 62.5. In the present study, mice that entered arms less than eight times during the test were not used, because the data obtained from these mice were not considered to reflect precise alternation.

### 2.4. Statistical analysis

All the results were expressed as the means ± S.E.M. All the data were analyzed by Kruskal–Wallis analysis of variance by ranks. If there were significant $H$ values, post-hoc comparisons were made using the non-parametric Bonferroni multiple comparison test (two-tailed).

## 3. Results

### 3.1. Effects of endomorphins

Endomorphin-1 (10 and 17.5 μg) and endomorphin-2 (10 μg) significantly depressed the percent alternation (Kruskal–Wallis analysis: endomorphin-1, $H = 14.65$, $P < 0.05$; endomorphin-2, $H = 13.04$, $P < 0.05$) (Fig. 1). In contrast, endomorphin-1 (3–17.5 μg) or endomorphin-2 (3–17.5 μg) failed to produce any marked effects on total arm entries (Fig. 1).

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

Endomorphin-1 (10 μg) and endomorphin-2 (10 μg) again decreased percent alternation, while β-funaltrexamine (5 μg), naltrindole (4 ng) or nor-binaltorphimine (4

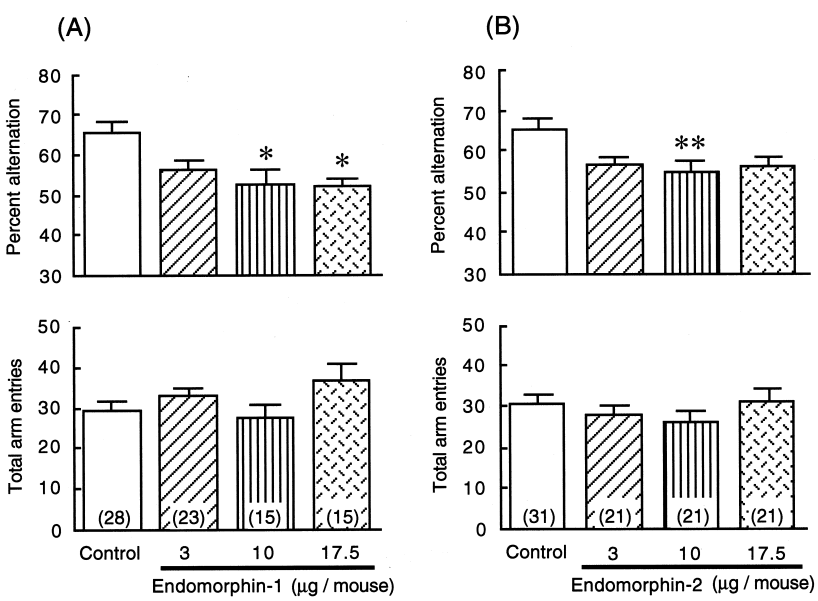


Fig. 1. Effects of endomorphins on spontaneous alternation performance in mice. Each value represents the mean ± S.E.M. Endomorphins (i.c.v.) were given to mice 15 min before measurement. The number of mice used is shown in parentheses. $^{*}P < 0.05$; $^{**}P < 0.01$ vs. control.

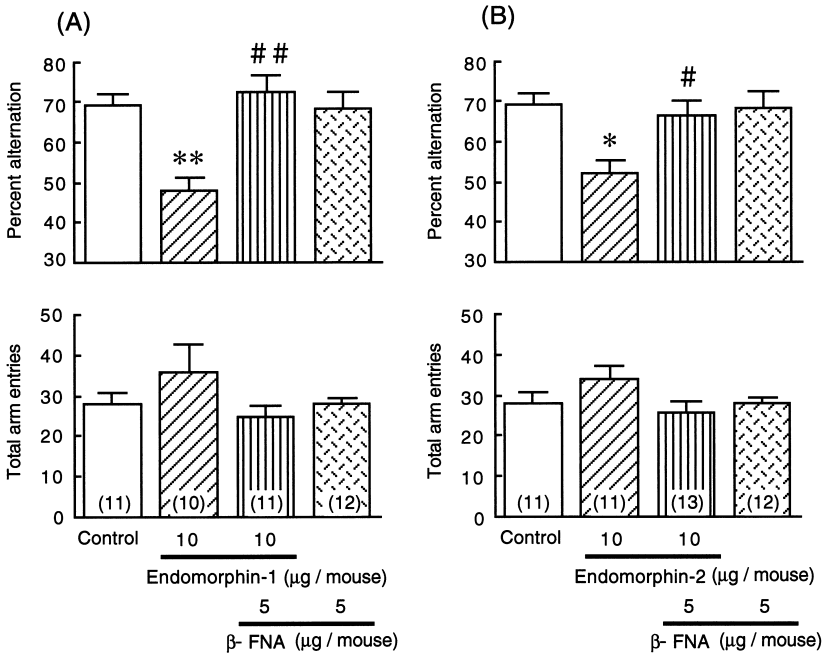


Fig. 2. Effects of endomorphins and their combination with β-funaltrexamine (β-FNA) on spontaneous alternation performance in mice. Each value represents the mean ± S.E.M. Endomorphins (i.c.v.) and β-FNA (i.c.v.) were given to mice 15 min and 24 h before measurement, respectively. The number of mice 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.

μg) alone had no significant effects on percent alternation (Figs. 2–4). β-Funaltrexamine (5 μg) almost completely reversed the effects of endomorphin-1 (10 μg) (Kruskal–Wallis analysis: $H = 17.64$, $P < 0.05$) and endomorphin-2 (10 μg) (Kruskal–Wallis analysis: $H = 12.55$, $P < 0.05$) (Fig. 2), although naltrindole (4 ng) (Kruskal–Wallis analysis: $H = 17.65$, $P < 0.05$ for endomorphin-1, and $H = 11.29$, $P < 0.05$ for endomorphin-2) or nor-binaltorphi-

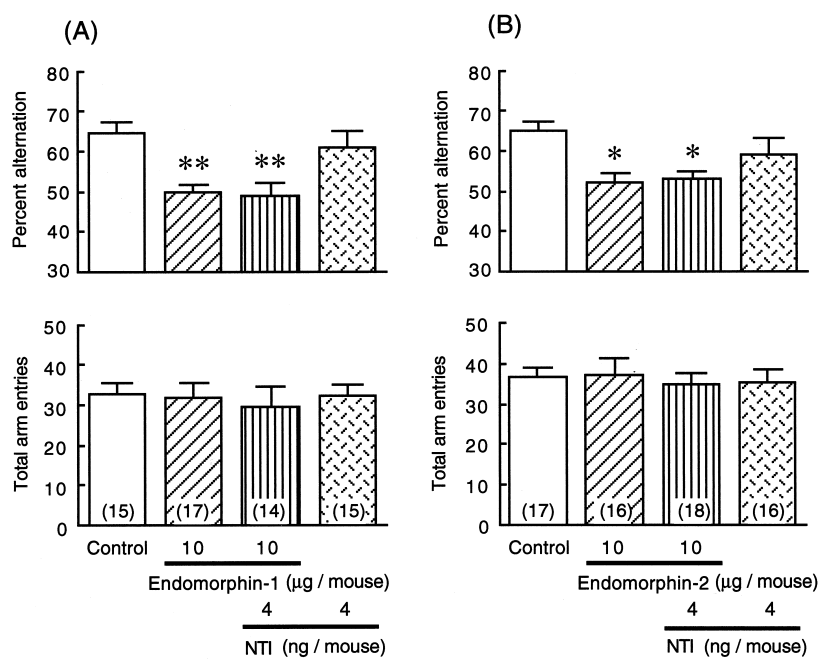


Fig. 3. Effects of endomorphins and their combination with naltrindole (NTI) on spontaneous alternation performance in mice. Each value represents the mean ± S.E.M. Endomorphins (i.c.v.) and NTI (i.c.v.) were given to mice 15 and 20 min before measurement, respectively. The number of mice used is shown in parentheses. $^{*}P < 0.05$; $^{**}P < 0.01$ vs. control.

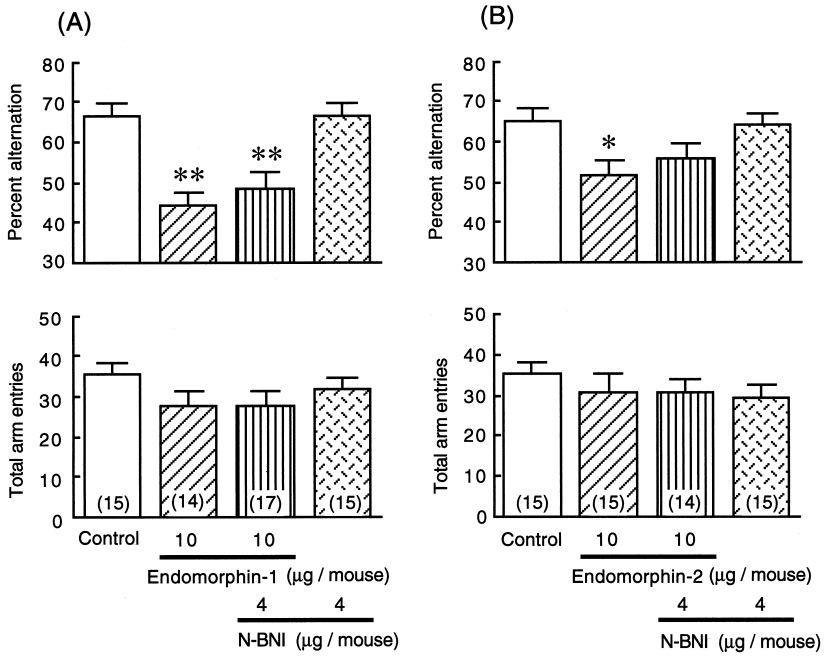


Fig. 4. Effects of endomorphins and their combination with nor-binaltorphimine (N-BNI) on spontaneous alternation performance in mice. Each value represents the mean ± S.E.M. Endomorphins (i.c.v.) and N-BNI (i.c.v.) were given to mice 15 and 60 min before measurement, respectively. The number of mice used is shown in parentheses. $^{*}P < 0.05$; $^{**}P < 0.01$ vs. control.

mine (4 μg) (Kruskal–Wallis analysis: $H = 28.89$, $P < 0.05$ for endomorphin-1, and $H = 10.87$, $P < 0.05$ for endomorphin-2) was without significant effects. In contrast, there were no marked changes in total arm entries (Figs. 3 and 4).

## 4. Discussion

μ-Opioid receptor binding has been reported to decrease in the brain of Alzheimer's disease patients (Hiller et al., 1987). Moreover, lesioning of the nucleus basalis of Meynert produces a significant decrease in μ-opioid receptor binding of the rat cerebral cortex (Ofri et al., 1992) and elicits amnesia (Ukai et al., 1993a). It thus appears that disorder of μ-opioid neuronal systems is involved in cognitive malfunctions. Itoh et al. (1994) have reported that DAMGO impairs spontaneous alternation performance associated with spatial working memory. TAPA impairs passive avoidance learning, while TAPA has much higher selectivity and affinity for μ-opioid receptors than DAMGO. Therefore, it is possible that μ-opioid receptor agonists elicit amnesia.

The endogenous opioid receptor agonist, endomorphin-1, is more effective than DAMGO in vitro (Zadina et al., 1997). A second peptide, endomorphin-2, which differs by one amino acid, was also isolated (Zadina et al., 1997). These new peptides have the highest specificity and affinity for μ-opioid receptors of any endogenous substances so far described and they are considered to be natural ligands for the receptor.

Endomorphins-1 and -2 have hypotensive activity in the rabbit (Champion et al., 1997), although they act as partial agonists in this assay system compared with DAMGO (Hosohata et al., 1998). In addition, endomorphins-1 and -2 have been demonstrated to have analgesic, anxiolytic and orexigenic effects (Zadina et al., 1997; Asakawa et al., 1998). These findings suggest that endomorphins have the potential to modulate neuronal activity in vivo.

Endomorphins-1 (3 μg) and -2 (3 μg) were inactive, while increasing their doses to 10 or 17.5 μg inhibited the spontaneous alternation performance. The inhibitory effects of endomorphins were almost completely antagonized by β-funaltrexamine (5 μg) but not by naltrindole (4 ng) (Kameyama et al., 1998) or nor-binaltorphimine (4 μg), indicating that the effects of endomorphins are mediated via opioid receptors. The effects of endomorphins were not mediated via δ- or κ-opioid receptors, confirming the evidence from receptor binding (Zadina et al., 1997).

It has been reported that 5- to 20-μg doses of endomorphins produce antinociception (Zadina et al., 1997). However, the antinociception would not affect behavioral responses, because endomorphins failed to affect total arm entries as indexed by locomotor activity. Therefore, these results provide further support for the reports that μ-opioid receptor agonists lead to amnesia related to the dysfunction of spatial working memory (Ukai et al., 1993b; Itoh et al., 1994).

Spatial alternation performance is associated with septo-hippocampal cholinergic activity (Givens and Olton, 1990). DAMGO reportedly inhibits the high $K^{+}$-induced release of acetylcholine from slices of the nucleus accum-

bens (Heijna et al., 1990; 1992) and hippocampus (Lapchak et al., 1989). The DAMGO-induced impairment of alternation performance is significantly improved by systemic injection of physostigmine (Itoh et al., 1994). Endomorphins-1 and -2 inhibit the acetylcholine release evoked by electrical field stimulation in longitudinal muscle preparations in guinea pig ileum (Nishiwaki et al., 1998). The inhibitory effects on acetylcholine release are abolished by a μ-opioid receptor antagonist. Therefore, the endomorphin-induced impairment of spontaneous alternation performance may be due to the inhibition of hippocampal cholinergic activity through the stimulation of μ-opioid receptors.

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