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Dynorphin A-(1–13) potently improves scopolamine-induced impairment of passive avoidance response in mice

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Abstract

The effects of intracerebroventricular administration of dynorphin A-(1–13) on scopolamine-induced amnesia were investigated in mice by using a step-down type passive avoidance task. The pre- or post-training, or pre-retention administration of dynorphin A-(1–13) (0.3–10 μg) alone failed to affect step-down latency of the passive avoidance response, while scopolamine (1 mg/kg) significantly shortened step-down latency. Dynorphin A-(1–13) (1 μg) given 15 min before training and retention tests but not immediately after training significantly improved the scopolamine (1 mg/kg)-induced shortening of step-down latency of the passive avoidance response, indicating antiamnesic effects of dynorphin A-(1–13) (1 μg). A lower dose (1 mg/kg) of the κ-opioid receptor antagonist, (−)-(1*R*,5*R*,9*R*)-5,9-diethyl-2-(3-furyl-methyl)-2′-hydroxy-6,7-benzomorphan, reversed the antiamnesic effects of dynorphin A-(1–13) (1 μg). These results suggest that the antiamnesic effects of dynorphin A-(1–13) depend on the timing of drug treatments.

Keywords: Dynorphin A-(1–13); Scopolamine; Passive avoidance response; Memory; Amnesia; (Mouse)

1. Introduction

A growing body of evidence has demonstrated that opioids influence the memory process. In general, opioid receptor agonists such as β-endorphin, enkephalins and morphine impair learning and memory, while the opioid receptor antagonist, naloxone, facilitates them (Olson et al., 1986, 1989). In contrast, the effects of dynorphins, endogenous κ-opioid receptor agonists on learning and memory appear to be dependent upon injection schedules, behavioral tasks and dynorphin fragments. For example, dynorphin A-(1–13) impairs step-through passive avoidance learning (Introini-Collison et al., 1987), while it fails to affect inhibitory and shuttle avoidance learning (Izquierdo et al., 1985). The effects of dynorphin A-(1–17) on the inhibitory avoidance response depend on the strength of electroshocks during training (Del Cerro and Borrell, 1990). Dynorphin A-(1–8) injected into the hippocampus impairs water maze performance (McDaniel et al., 1990).

On the other hand, radioimmunoassay has revealed increased dynorphin A-(1–8)-like immunoreactivity in the hippocampal formation and frontal cortex of aged rats (Jiang et al., 1989). In particular, the increase in dynorphin A-(1–8)-like immunoreactivity in the aged hippocampus is associated with a decline in spatial learning ability (Jiang et al., 1989). The increase in dynorphin A-(1–8) levels may be due to the degradation of dynorphin A-(1–13) or dynorphin A-(1–17). Moreover, the density of cerebral κ-opioid receptors has been demonstrated to increase in the basal forebrain-lesioned rat, resulting from the decrease in dynorphin release in the brain. Actually, intracerebroventricular injection of dynorphin A-(1–13) attenuates basal forebrain-lesion-induced amnesia in rats (Ukai et al., 1993b). Although it has recently been reported that dynorphin A-(1–13) markedly improves the scopolamine-induced deficit in spontaneous alternation performance (Itoh et al., 1993b), it is unclear whether dynorphin A-(1–13) plays a major role in memory processes such as acquisition, consolidation and retrieval in normal and amnesic mice.

Consequently, the present study was designed to examine the effects of intracerebroventricular injection

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of dynorphin A-(1–13) on the passive avoidance response in normal and scopolamine-treated mice. In addition, dynorphin A-(1–13) was administered before training or retention, or immediately after training.

2. Materials and methods

2.1. Animals

Male *ddY* mice (Nihon SLC Co., 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 their home cages before the start of experiments. The experiments were made between 13:00 and 17:00 h in a sound-attenuated room.

2.2. Drugs

Dynorphin A-(1–13) (Peptide Institute, Japan), (−)-(1*R*,5*R*,9*R*)-5,9-diethyl-2-(3-furyl-methyl)-2′-hydroxy-6,7-benzomorphan (Mr2266) (Boehringer Ingelheim, Germany) and scopolamine hydrobromide (Tokyo Kasei Co., Japan) were employed throughout. Dynorphin A-(1–13) was dissolved in sterile isotonic saline (Otsuka Pharmaceutical Co., Japan), whereas scopolamine was dissolved in isotonic saline (0.9% NaCl, pH 7.5). Mr2266 was dissolved in 1 ml 5% w/v (±)-tartaric acid and the volume was made up with isotonic saline. Scopolamine (s.c.) was given 30 min before training, while Mr2266 (i.p.) was given 30 min before training or retention tests. Under light ether anesthesia, dynorphin A-(1–13) was injected into the lateral ventricle of mice 15 min before training or retention tests, or immediately after training. The i.c.v. injection was made with a 4 mm long needle (30 gauge) attached to a 50-μl Hamilton microsyringe according to the method of Haley and McCormick (1957). The injection volume was 5 μl/mouse.

2.3. Apparatus and procedure of passive avoidance response

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 (Kameyama et al., 1986). 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 a grid floor, an intermittent electroshock (60 V, DC, 0.5 s, 1 Hz) was delivered. The retention tests were 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.

Table 1
Effects of dynorphin A-(1–13) on the step-down latency in passive avoidance task in mice

Treatments	Dose (μg)	*n*	Step-down latency (s)
(A)			
Control		9	300 (180–300)
Dynorphin A-(1–13)	0.3	9	300 (65–300)
	1	9	300 (183–300)
	3	10	300 (209–300)
(B)			
Control		15	296 (120–300)
Dynorphin A-(1–13)	0.3	14	267 (149–300)
	1	15	300 (212–300)
	3	13	243 (124–300)
	10	14	172 (135–300)
(C)			
Control		11	210 (132–300)
Dynorphin A-(1–13)	0.3	11	140 (84–300)
	1	11	263 (91–300)
	3	10	173 (125–289)
	10	10	153 (108–300)

Dynorphn A-(1–13) was given to mice 15 min before training (A), immediately after (B) and 15 min before retention test (C). *n*: the number of mice used. Each value represents the median and interquartile ranges.

2.4. Statistical analysis

Data were expressed as the median and interquartile ranges and statistically analyzed by means of a Kruskal-Wallis non-parametric one-way analysis of variance. Further statistical analysis for individual groups was done with a two-tailed Bonferroni's test.

3. Results

3.1. Effects of dynorphin A-(1–13) on passive avoidance response

The pre-training administration of dynorphin A-(1–13) (0.3–3 μg) did not change behavioral responses (flinch, vocalization and jump) to electroshock during training. Pre- or post-training, or pre-retention administration of dynorphin A-(1–13) (0.3–10 μg) failed to affect step-down latency in retention tests (Table 1). Additionally, pre-retention administration of dynorphin A-(1–13) (0.3–10 μg) did not affect step-down latency in retention tests in mice when no electroshocks were given during training (data not shown).

3.2. Effects of pre-training administration of dynorphin A-(1–13) on scopolamine-induced amnesia

Scopolamine (1 mg/kg) alone markedly reduced step-down latency when administered 30 min before

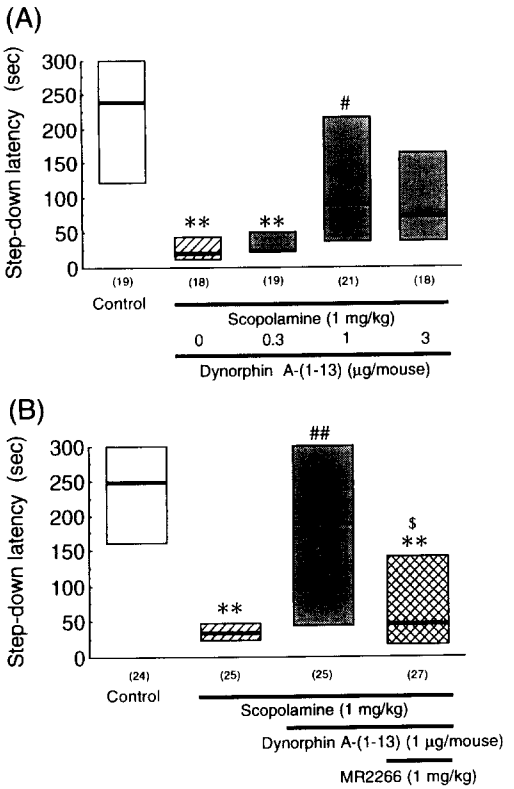

Fig. 1. Effects of dynorphin A-(1–13) (A) and its combination with Mr2266 (B) on step-down latency of passive avoidance response in mice treated with scopolamine. Dynorphin A-(1–13) (i.c.v.), scopolamine (s.c.) and Mr2266 (i.p.) were administered 15, 30 and 30 min before training, respectively. Data were expressed as the median and interquartile ranges, which are the distances between the first quartile (25th percentile) and the third quartile (75th percentile). The number of mice used is shown in parentheses. $^{**}P < 0.01$ vs. controls, $^{\#}P < 0.05$; $^{\#\#}P < 0.01$ vs. scopolamine 1 mg/kg, $^{\$}P < 0.05$ vs. scopolamine 1 mg/kg plus dynorphin A-(1–13) 1 μg/mouse.

training. Dynorphin A-(1–13) (1 μg) significantly inhibited the scopolamine (1 mg/kg)-induced reduction of step-down latency (Fig. 1). The inhibitory effects of dynorphin A-(1–13) (1 μg) were reversed by treatment with a lower dose (1 mg/kg) of the opioid antagonist Mr2266 (Fig. 1). In contrast, Mr2266 (1 mg/kg) alone did not affect step-down latency of the passive avoidance response in normal or scopolamine-treated mice (data not shown).

3.3. Effects of post-training administration of dynorphin A-(1–13) on scopolamine-induced amnesia

Scopolamine (1 mg/kg) itself markedly shortened step-down latency. However, dynorphin A-(1–13) (0.3–10 μg) had no effects on the scopolamine (1 mg/kg)-induced reduction of step-down latency (Fig. 2).

3.4. Effects of pre-retention administration of dynorphin A-(1–13) on scopolamine-induced amnesia

Scopolamine (1 mg/kg) itself markedly reduced step-down latency. Dynorphin A-(1–13) (1 μg) significantly inhibited the scopolamine (1 mg/kg)-induced shortening of step-down latency (Fig. 3). The effects of dynorphin A-(1–13) (1 μg) were significantly reversed by treatment with Mr2266 (1 mg/kg). In contrast, Mr2266 (1 mg/kg) alone failed to influence step-down

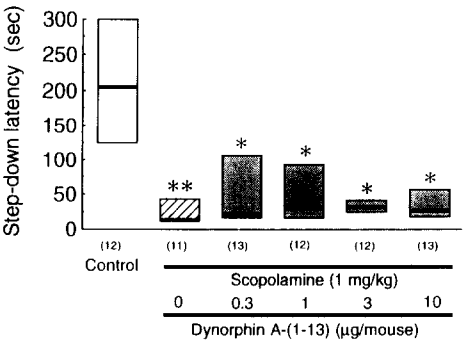

Fig. 2. Effects of dynorphin A-(1–13) on step-down latency of passive avoidance response in mice treated with scopolamine. Dynorphin A-(1–13) (i.c.v.) and scopolamine (s.c.) were administered immediately after and 30 min before training, respectively. Data were expressed as the median and interquartile ranges, which are the distances between the first quartile (25th percentile) and the third quartile (75th percentile). $^{*}P < 0.05$; $^{**}P < 0.01$ vs. controls.

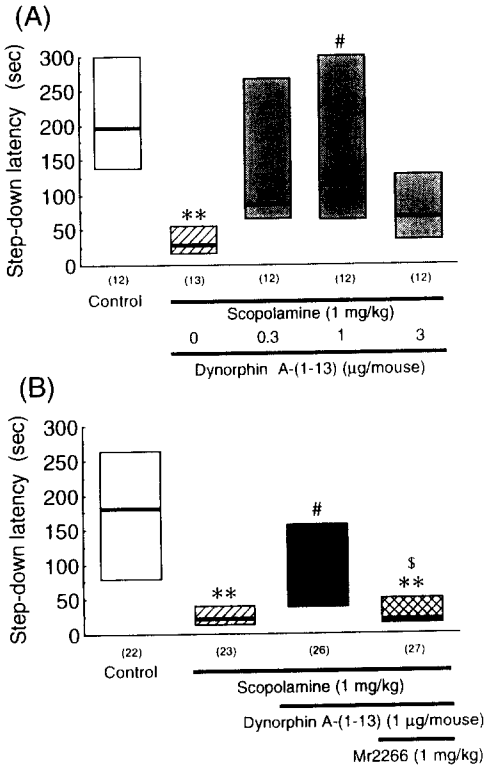

Fig. 3. Effects of dynorphin A-(1–13) (A) and its combination with Mr2266 (B) on step-down latency of passive avoidance response in mice treated with scopolamine. Dynorphin A-(1–13) (i.c.v.) and scopolamine (s.c.) were administered 15 min before retention tests and 30 min before training, respectively. Data were expressed as the median and interquartile ranges which are the distances between the first quartile (25th percentile) and the third quartile (75th percentile). $^{**}P < 0.01$ vs. controls, $^{\#}P < 0.05$ vs. scopolamine 1 mg/kg, $^{\$}P < 0.05$ vs. scopolamine 1 mg/kg plus dynorphin A-(1–13) 1 μg/mouse.

latency of the passive avoidance response in normal or scopolamine-treated mice (data not shown).

4. Discussion

It has been reported that stimulation of μ- and δ-opioid receptors impairs the memory process in various behavioral tasks (Patterson et al., 1989; Ukai et al., 1993a). However, the role of κ-opioid receptors in learning and memory is unclear. The present study showed that the endogenous κ-opioid receptor agonist, dynorphin A-(1–13) (0.3–10 μg), administered alone before training or retention, or immediately after training failed to affect the passive avoidance response in normal mice. The results are consistent with the finding that dynorphin A-(1–13) does not cause retrograde amnesia for shuttle avoidance or inhibitory avoidance learning in rats (Izquierdo et al., 1985). However, Introini-Collison et al. (1987) have reported that dynorphin induces task-specific impairment of memory. In particular, immediate post-training administration of dynorphin A-(1–13) (0.1–1 μg/kg i.p.) significantly impairs 24-h retention of a one-trial inhibitory avoidance task in normal mice. The discrepancy appears to result from differences in animal strain, experimental apparatus or route of administration. On the other hand, microinjection of dynorphin A-(1–8) into the hippocampus impairs spatial learning in rats (McDaniel et al., 1990). Additionally, dynorphin A-(1–8) does not affect step-down latency of the passive avoidance response in normal or scopolamine-treated mice (data not shown). In fact, dynorphin A-(1–8) has been shown to possess a higher affinity for μ-opioid receptors than dynorphin A-(1–13) (Leslie, 1987). A change in dynorphin A-(1–8) levels may be involved in the degradation of dynorphin A-(1–13) or dynorphin A-(1–17).

It is possible that κ-opioid receptor agonists, unlike other types of opioids, are aversive in animals. If dynorphin was aversive in this situation, it might have promoted learning, particularly when given immediately after training. The fact that this did not happen (Fig. 2) strengthens the conclusion that dynorphin affects the learning process rather than the aversive quality of the training stimulus.

In particular, dynorphin A-(1–13) (1 μg) inhibited the scopolamine-induced impairment of the passive avoidance response when administered before training and retention but not immediately after training, suggesting that dynorphin A-(1–13) improves the impairment of acquisition and retrieval processes resulting from cholinergic dysfunctions, although the dose-response curve for the antiamnesic effects of dynorphin A-(1–13) was U-shaped. Lesioning of the basal forebrain reportedly decreases κ-opioid binding in the occipital cortex and hippocampal CA_3 region (Ofri et al., 1992). In postmortem brains from Alzheimer's disease patients the change in opioid receptor binding is similar to that in the basal forebrain-lesioned rat brain (Hiller et al., 1987). We have recently reported that dynorphin A-(1–13) attenuates the basal forebrain lesion-induced impairment of the passive avoidance response when administered before training (Ukai et al., 1993b). More recent evidence shows that dynorphin A-(1–13) improves the scopolamine-induced deficit of spontaneous alternation performance associated with spatial working memory in mice (Itoh et al., 1993b). It is possible that signals from ascending cholinergic neurons originating from the nucleus basalis of Meynert increase opioid neuronal activity in the cortex, which would lead to an increase in endogenous opioid release (Ofri et al., 1992), suggesting that dynorphin A-(1–13) improves amnesia resulting from cholinergic dysfunctions.

The pre-training and pre-retention administration of dynorphin A-(1–13) (1 μg equivalent to 0.62 nmol) produced a significant improvement of the scopolamine (1 mg/kg)-induced amnesia. Further, its antiamnesic effects were almost fully reversed by a lower dose (1 mg/kg) of the opioid antagonist, Mr2266, with relatively high affinity for κ-opioid receptors (Oka et al., 1982; Ukai and Kameyama, 1985). These results suggest that dynorphin A-(1–13) improves the scopolamine-induced impairment of the passive avoidance response through the mediation of κ-opioid receptors.

Dynorphin A-(1–13) appears to be more effective in that the 0.3-μg dose seemed to improve retention when administered before retention, although the result did not reach statistical significance, suggesting that κ-opioid systems are much more involved in the retention process of memory.

Ukai et al. (1992) have reported that dynorphin A-(1–13) at a 12.5 μg dose modulates the behavioral responses induced by the indirect dopamine receptor agonist, methamphetamine. The attenuating effects of dynorphin A-(1–13) on the scopolamine-induced deficit of the passive avoidance response may be involved in the blockade of dopamine D_2 receptors in the brain, because the antiamnesic effects of dynorphin A-(1–13) are inhibited by treatment with the dopamine D_2 receptor agonist, RU24213 (Itoh et al., 1993a).

In conclusion, dynorphin A-(1–13) improves the impairment of memory processes such as acquisition and retrieval resulting from cholinergic dysfunctions.

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