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Attenuation of memory with Tyr-D-Arg-Phe-β-Ala-NH_2, a novel dermorphin analog with high affinity for μ-opioid receptors

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Abstract

The involvement of μ-opioid receptors in memory retrieval was examined in mice by using Tyr-D-Arg-Phe-β-Ala-NH_2 (TAPA), a novel dermorphin analog with high affinity for μ-opioid receptors, and passive avoidance learning. TAPA was intracerebroventricularly administered to mice before retention tests of passive avoidance learning. A 0.3-ng dose of TAPA markedly shortened step-down latency of passive avoidance learning, and the shortening of step-down latency was reversed by treatment with β-funaltrexamine (5 μg), a μ-opioid receptor antagonist, indicating that TAPA (0.3 ng) attenuates memory retrieval. Although the attenuating dose (0.3 ng) of TAPA failed to affect horizontal or vertical locomotor activity, a 3-ng dose of TAPA showed a tendency to decrease vertical locomotor activity. A 30-ng dose of TAPA produced a significant increase in horizontal locomotor activity accompanied by a marked reduction of vertical locomotor activity. TAPA (3 ng) produced a significant increase in step-down latency of passive avoidance learning with lower intensity of electroshock or without electroshock during training. These results suggest that the activation of μ-opioid receptors impairs memory retrieval.

Keywords: μ-Opioid receptor; Tyr-D-Arg-Phe-β-Ala-NH_2; β-Funaltrexamine; Memory; Passive avoidance; Locomotor activity; (Mouse)

1. Introduction

A large number of studies have demonstrated that endogenous opioids modulate the process of learning and memory. Opioid receptor agonists such as endorphins and enkephalins impair learning and memory, while opioid receptor antagonists facilitate both (Gallagher, 1982; Izquierdo and Netto, 1985; Olson et al., 1989). Because opioid receptors are classified into three types, i.e., μ-, δ- and κ-opioid receptors, the effects of opioids on learning and memory should be observed by using selective opioid receptor agonists (Itoh et al., 1994; Schulteis et al., 1988; Pavone et al., 1990). However, it seems that the involvement of opioid receptor types in learning and memory depends upon animal strain, injection schedules and behavioral tasks. For example, post-training administration of morphine impairs learning and memory, while pre-retention administration of morphine facilitates both in step-through type passive avoidance learning (Shiigi et al., 1990).

Tyr-D-Arg-Phe-β-Ala-NH_2 (TAPA) has recently been introduced as a novel dermorphin analog which is isolated from amphibian skin (Sasaki et al., 1991). TAPA has much higher selectivity and affinity for μ-opioid receptors than [D-Ala^2,*N*MePhe^4,Gly-ol]enkephalin (DAMGO) which is often used as a μ-selective opioid receptor agonist (Sasaki et al., 1991).

Therefore, we designed the first experiment to examine whether pre-retention administration of TAPA affects learning and memory in a step-down type passive avoidance task, and whether the effects of TAPA are antagonized by the μ-selective opioid receptor antagonist, β-funaltrexamine (Portoghese et al., 1980; Ukai et al., 1991). Furthermore, μ-opioid receptors are involved in a variety of behavioral responses such as antinociception and locomotor activity (Toyoshi et al.,

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1991; Ukai et al., 1991, 1993a,b). Consequently, we investigated the effects of TAPA on locomotor activity to clarify the relationship between memory and locomotor activity in the second experiment.

2. Materials and methods

2.1. Animals

Male *ddY* mice (Nihon SLC Co., Hamamatsu, Japan) weighing between 30 and 35 g were used. The animals were housed in groups of 4 or 5 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 their home cages before the start of the experiments. The experiments were done between 13:00 and 17:00 h in a sound-attenuating room. The mice were used only once.

2.2. Drugs

TAPA, which was synthesized by a solid-phase method (Sasaki et al., 1991), and β-funaltrexamine (Research Biochemicals, Natick, MA, USA) were used throughout. All drugs were dissolved in sterile isotonic saline (0.9% NaCl, pH 7.5) in polypropylene containers and were injected intracerebroventricularly (i.c.v.). The i.c.v. injections were made according to the method of Haley and McCormick (1957). The unilateral injection site was 2 mm from either side of the midline on a line drawn through the anterior roots of the ears. The injection was made with a needle (30-gauge; 4 mm long) attached to a 50-μl microsyringe (705 LT; Hamilton Co., Reno, NV, USA). The needle was inserted perpendicularly through the skull and into the lateral ventricle of the brain. The mouse was anesthetized with ether, and solutions were injected in a volume of 5 μl per mouse over a period of 20 s as previously described (Kameyama and Ukai, 1983). The site was checked by injecting a 1:10 dilution of India ink in isotonic saline. Histological examination revealed particles of the ink in the lateral and third ventricles but not in the others. As previously described, neither insertion of the needle nor injection of 5 μl of isotonic saline solution had a significant influence on behavioral responses (Kameyama and Ukai, 1983) or cognitive functions (Itoh et al., 1994).

2.3. Passive avoidance task

The apparatus for the step-down type passive avoidance task has been described in detail (Kameyama et al., 1986). In a 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, 1 Hz, 0.5 s duration) was delivered. The retention test was done 24 h after training, with each mouse again being placed on the platform, and the step-down latency being measured. An upper cut-off time was set at 300 s. TAPA and β-funaltrexamine were administered 15 min and 24 h before the retention tests, respectively.

2.4. Locomotor activity

Locomotor activity was measured by means of animal movement-analyzing systems (Scanet SV-10, Toyo Sangyo Co., Toyama, Japan) (Ukai et al., 1994). This apparatus was equipped with 144 pairs of photosensors (*X*-axis: 88 pairs, *Y*-axis: 56 pairs) set at 5-mm intervals, covering a measurement area of 480 × 300 mm. Prior to the start of recording, the animals (one animal per testing cage) were placed in Plexiglas cages (400 × 250 × 200 mm). Five sets of Scanet SV-10 were connected to a personal computer (PC 9801 RX, Nihondenki Co., Tokyo, Japan). Locomotor activity was measured between 15 and 20 min after injection of TAPA.

2.5. Statistical analysis

The data were expressed as the median and interquartile ranges (passive avoidance learning) or the means ± S.E. (locomotor activity). The Kruskal-Wallis non-parametric one-way analysis of variance (passive avoidance learning) and the parametric one-way analysis of variance (ANOVA) (locomotor activity) were first applied, and further statistical analyses for individual groups were done with the two-tailed Bonferroni test (passive avoidance learning) and Student's *t*-test (locomotor activity). In addition, the Mann-Whitney *U*-test was used for step-down latency without electroshock during training.

3. Results

3.1. Effects of pre-retention administration of TAPA on passive avoidance response

The pre-retention administration of TAPA (0.3 ng) significantly shortened step-down latency in retention tests (Kruskal-Wallis analysis: $H = 15.71$, $P < 0.01$), indicating the amnesic effects of TAPA (Fig. 1A), whereas increasing the dose to 3 ng showed a tendency to prolong step-down latency. β-Funaltrexamine (5 μg) significantly reversed the effects of TAPA (0.3 ng) (Kruskal-Wallis analysis: $H = 23.47$, $P < 0.01$) (Fig. 1B).

3.2. Effects of TAPA on step-down latency of passive avoidance response induced by weaker electroshock and on step-down latency without electroshock during training

TAPA (0.3 ng) did not influence step-down latency of the passive avoidance response induced by a weaker electroshock, although TAPA (3 ng) significantly prolonged step-down latency (Kruskal-Wallis analysis: $H = 21.69$, $P < 0.01$) (Fig. 2A). Furthermore, TAPA (3 ng) significantly prolonged the step-down latency without electroshock during training (Mann-Whitney U-test: $U = 3.54$, $P < 0.01$) (Fig. 2B).

3.3. Effects of TAPA on locomotor activity

TAPA (0.3–30 ng) had a significant effect on locomotor activity (ANOVA: $F(3,38) = 8.28$, $P < 0.01$). The amnesic dose (0.3 ng) of TAPA failed to affect horizontal or vertical locomotor activity, but a higher dose (3 ng) of TAPA showed a tendency to decrease vertical locomotor activity (Fig. 3). A 30-ng dose of TAPA produced a significant increase in horizontal locomotor activity (Fig. 3A) accompanied by a marked reduction of vertical locomotor activity ($F(3,38) = 3.51$, $P < 0.05$) (Fig. 3B).

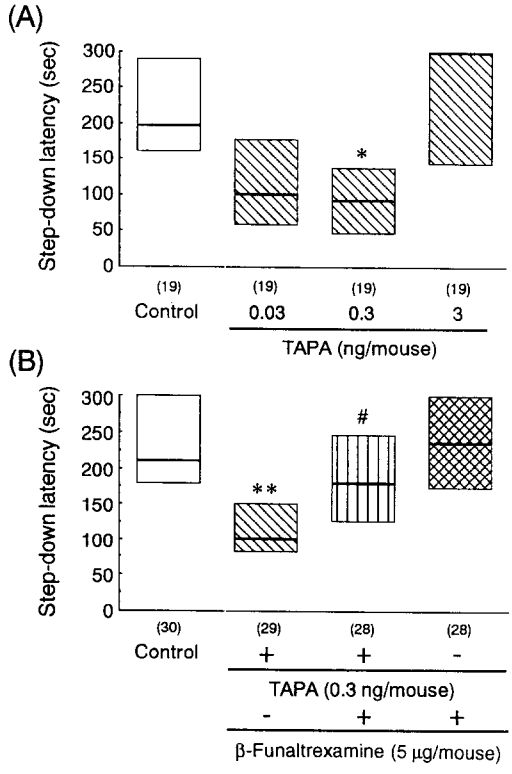

Fig. 1. Effects of Tyr-D-Arg-Phe-β-Ala-NH_2 (TAPA) (A) and its combination with β-funaltrexamine (B) on step-down latency of passive avoidance learning in mice. TAPA and β-funaltrexamine were administered 15 min and 24 h before retention tests, respectively. Each value represents the median and interquartile 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. * $P < 0.05$; ** $P < 0.01$ vs. controls, # $P < 0.05$ vs. TAPA (0.3 ng) alone.

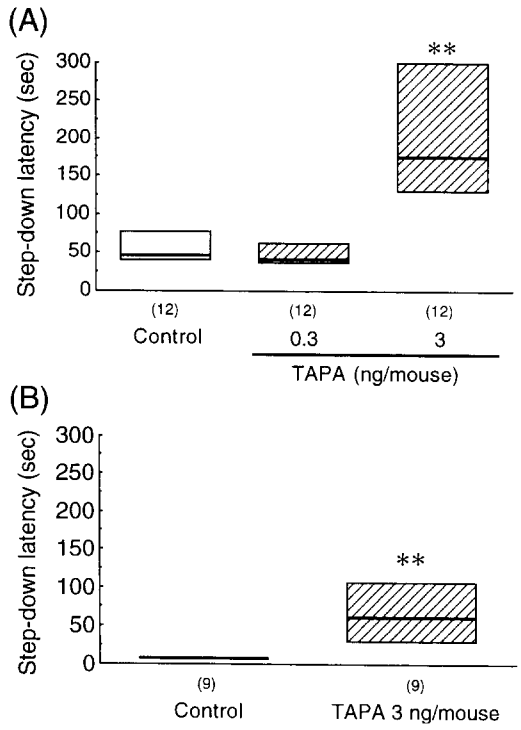

Fig. 2. Effects of Tyr-D-Arg-Phe-β-Ala-NH_2 (TAPA) on step-down latency of passive avoidance response induced by weaker electroshocks (60 V, DC, 1 Hz, 0.5 s×3) (A) and on step-down latency without electroshock during training (B). TAPA was administered 15 min before retention tests. Each value represents 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.

4. Discussion

TAPA is reportedly one of the most selective and potent μ-opioid receptor agonists compared with DAMGO which is often used as a μ-opioid receptor agonist. Furthermore, incubation of TAPA with rat brain homogenate produces no detectable degradation product, demonstrating good stability of the peptide against degradation enzymes in the brain. TAPA is thus considered to be an excellent reagent for assessing biological functions mediated via μ-opioid receptors (Sasaki et al., 1991). It has been reported that a variety of opioids influence memory processes in rodents (Gallagher, 1982; Izquierdo and Netto, 1985; Izquierdo et al., 1985; Izquierdo, 1989). TAPA impairs passive avoidance learning when administered before and immediately after training. Moreover, the effects of TAPA

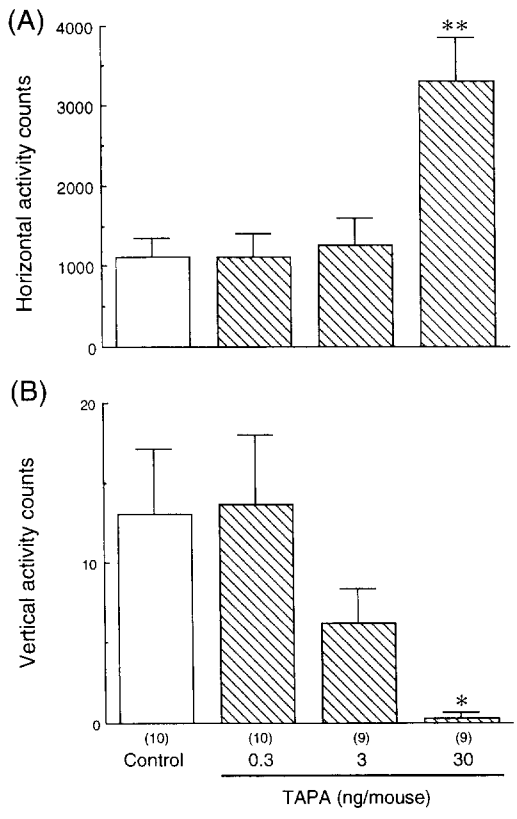

Fig. 3. Effects of Tyr-D-Arg-Phe-β-Ala-NH_2 (TAPA) on locomotor activity in mice. Locomotor activity was observed between 15 and 20 min after injection of TAPA. Each value represents the mean ± S.E.M. The number of mice used is shown in parentheses. * $P < 0.05$; ** $P < 0.01$ vs. controls.

are antagonized by the μ-selective opioid receptor antagonist, β-funaltrexamine (Ukai et al., 1993a), suggesting that the impairing effects of TAPA on memory acquisition and consolidation are mediated via μ-opioid receptors.

TAPA (0.3 ng) also impaired passive avoidance learning when administered before the retention tests, and the effects of TAPA (0.3 ng) were antagonized by β-funaltrexamine (5 μg). The amnesic dose of TAPA (0.3 ng) did not influence horizontal or vertical locomotor activity in the present study, or the nociceptive response in a previous study (Ukai et al., 1993a). These results suggest that the stimulation of μ-opioid receptors impairs memory retrieval without any effects on other behavioral responses. The previous data for morphine or β-endorphin had suggested state dependence (Izquierdo, 1980; Shiigi et al., 1990), whereas the results for TAPA do not suggest it clearly. The difference may result from higher selectivity and affinity of TAPA for μ-opioid receptors than for those of morphine or β-endorphin (Sasaki et al., 1991).

Although morphine is not exclusively selective for μ-opioid receptors, pre-retention administration of morphine has been reported to facilitate memory (Shiigi et al., 1990), suggesting the involvement of μ-opioid receptors in memory retrieval. In fact, TAPA (3 ng) significantly prolonged the step-down latency in passive avoidance learning induced by weaker electroshock. However, TAPA (3 ng) also prolonged step-down latency without electroshock during training. Therefore, the prolonging effects of TAPA (3 ng) may be due to a non-specific effect such as a motor deficit because, although not significantly so, TAPA (3 ng) showed a tendency to decrease vertical locomotor activity to less than half of the control value. In other words, the exclusive stimulation of μ-opioid receptors before retention tests would not facilitate memory retrieval as indicated by passive avoidance learning.

The memory process is associated with septo-hippocampal cholinergic activity (Givens and Olton, 1990). DAMGO reportedly inhibits the high-K^+-induced release of acetylcholine from slices of nucleus accumbens (Heijna et al., 1990, 1992) and hippocampus (Lapchak et al., 1989). The DAMGO-induced impairment of working memory is significantly improved by systemic injection of physostigmine (Itoh et al., 1994). Therefore, these results suggest strongly that the activation of μ-opioid receptors impairs a memory process such as retrieval as a result of cholinergic dysfunction (Itoh et al., 1994).

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