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Differential involvement of μ_1-opioid receptors in dermorphin tetrapeptide analogues-induced antinociception

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Abstract

The involvement of putative μ_1-opioid receptors in the antinociception induced by the dermorphin tetrapeptide analogues Try-D-Arg-Phe-β-Ala (TAPA) and Tyr-D-Arg-Phe-β-Ala-NH_2 (TAPA-NH_2) was determined in mice, using a tail-pressure test and a formalin test. TAPA and TAPA-NH_2 injected i.c.v. and i.t. produced dose-dependent antinociception in both assays. In the tail-pressure test, the antinociception induced by i.c.v. or i.t. injected TAPA, but not TAPA-NH_2, was significantly attenuated by pretreatment with naloxonazine, a selective antagonist for putative μ_1-opioid receptors. Moreover, naloxonazine also significantly attenuated the antinociception induced by i.c.v. injected TAPA, but not TAPA-NH_2, in the formalin test. In contrast, the antinociception induced by both TAPA and TAPA-NH_2 given i.t. was significantly attenuated by pretreatment with naloxonazine in the formalin test. The present results suggest that TAPA and TAPA-NH_2 should be considered selective agonists for putative μ_1- and μ_2-opioid receptors, respectively. The C-terminal amidation of TAPA-NH_2 may be critical for distinguishing between putative μ_1- and μ_2-opioid receptors.

Keywords: μ-Opioid receptor; Dermorphin; Tetrapeptide; Naloxonazine; Antinociception; Mouse

1. Introduction

Dermorphin, a heptapeptide (Tyr-D-Ala-Phe-Gly-Tyr-Pro-Ser-NH_2) derived from amphibian skin (Montecucchi et al., 1981), has a potent antinociceptive effect and shows a high selectivity for μ-opioid receptors (Broccardo et al., 1981; Krumins, 1987). The intrinsic activity of dermorphin for G-protein activation is similar to that of [D-Ala^2,NMe-Phe^4,Gly-ol^5]enkephalin (DAMGO) and higher than that of morphine, suggesting that dermorphin is a full agonist at μ-opioid receptors (unpublished observation). The structure–activity relationship of dermorphin analogues clearly shows that the N-terminal tetrapeptide is the minimum sequence for agonistic activity at μ-opioid receptors, even though the short fragment is less potent than the original heptapeptide (Broccardo et al., 1981; Salvadori et al., 1982). We previously reported that Try-D-Arg-Phe-β-Ala (TAPA), the N-terminal tetrapeptide derivative of dermorphin in which D-Ala^2 and Gly^4 residues are replaced by D-Arg^2 and β-Ala^4, respectively, shows potent antinociceptive activity greater than that of morphine, when given by various injection routes, including i.c.v., i.t. and s.c. injections (Chaki et al., 1988; Sasaki et al., 1984; Sato et al., 1985). TAPA retains the high selectivity for μ-opioid receptors and its antinociception is selectively mediated by μ-opioid receptors (Chaki et al., 1988). Sasaki et al. (1991) also reported that Try-D-Arg-Phe-β-Ala-NH_2 (TAPA-NH_2), the C-terminal amidated form of TAPA, shows a potent antinociceptive effect which is mediated by μ-opioid receptors. Interestingly, TAPA-NH_2 has a greater selectivity for μ-opioid receptors than does TAPA (Sasaki et al., 1991).

The μ-opioid receptor had been classically divided into putative μ_1- and μ_2-opioid receptors by Pasternak and his colleagues (Nishimura et al., 1984; Pasternak et al., 1980; Wolozin and Pasternak, 1981). The putative μ_1-opioid receptor shows a high affinity for both opioid peptides and opioid alkaloids. In contrast, the putative μ_2-opioid receptor has a higher affinity for opioid alkaloids than for

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opioid peptides. Putative μ_1- and μ_2-opioid receptors have been identified by their sensitivity for the μ-opioid receptor antagonist naloxonazine, which irreversibly binds to putative μ_1-opioid receptors, but reversibly binds to putative μ_2-opioid receptors (Pasternak, 1993; Elliott et al., 1994). Therefore, the antinociception mediated by the μ-opioid receptor can be divided into naloxonazine (35 mg/kg, s.c.)-sensitive (putative μ_1-opioid receptor-mediated) antinociception and naloxonazine-insensitive (putative μ_2-opioid receptor-mediated) antinociception (Sakurada et al., 1999; Sato et al., 1999).

The present study was designed to identify the involvement of μ-opioid receptor subclasses (putative μ_1- and μ_2-opioid receptors) in the antinociception induced by the dermorphin tetrapeptide analogues, TAPA and TAPA-NH_2, in the tail-pressure test and the formalin test, using a selective antagonist for the putative μ_1-opioid receptor, naloxonazine.

2. Material and methods

All experiments were approved by and conformed to the guideline of the Committee of Animal Experiments in Tohoku Pharmaceutical University. Every effort was made to minimize the number of animals used and animal suffering.

2.1. Animals

Male ddY mice weighing 22–25 g (SLC, Shizuoka, Japan) were used. Animals were housed in the room maintained at 23 °C with an alternating 12-h light/dark cycle. Food and water were available ad libitum. Mice were used only once.

2.2. Assessment of antinociception

Antinociception was determined by the tail-pressure test (Sakurada et al., 1986) and the formalin test (Sato et al., 1999). In the tail-pressure test, the base of the mouse tail was pressed, and the pressure threshold for biting and licking was recorded. The pressure was increased at a rate of 10 mm Hg/s and the cut-off pressure was set at 100 mm Hg to avoid tissue damage. Only mice that showed the behavioral response against the mechanical nociceptive stimuli (40–50 mm Hg) were used. The antinociceptive effect in the tail-pressure test is expressed as a percentage of the maximum possible effect (% MPE), which was calculated as follows: $[(P_1 - P_0)/(100 - P_0)] \times 100$, where P_0 and P_1 are the pressure threshold before and after the treatment. In the formalin test, mice were placed in transparent cages ($22.0 \times 15.0 \times 12.5$ cm high), which also served as observation chambers, and were allowed to adapt to the environment for 1 h before testing. After habituation, mice were injected with 20 μl of formalin (2.0% in saline) s.c. in the plantar surface of the hind-paw, using a Hamilton microsyringe with a 26-gauge needle. Only biting or licking of the formalin-injected hind-paw was defined as a nociceptive response and the total time of the response was measured for 10 min. Opioid peptides were administered i.c.v. and i.t. to mice 10 and 5 min before the formalin treatment, respectively. The antinociceptive effect in the formalin test is expressed as % MPE and was calculated as follows: $[(T_0 - T_1)/T_0] \times 100$, where T_0 and T_1 is the total time of the nociceptive response in mice treated with vehicle and opioid peptide, respectively.

2.3. I.c.v. and i.t. injection

I.c.v. and i.t. injections were performed following the methods described by Haley and McCormick (1957) and Hylden and Wilcox (1980), respectively, using a Hamilton syringe with a 29-gauge needle. The injection volume for i.c.v. and i.t. injections was 5 μl.

2.4. Drugs

The drugs used were TAPA (synthesized in our laboratory), TAPA-NH_2 (synthesized in our laboratory), naloxonazine (RBI, Natick, MA, USA) and β-funaltrexamine (RBI). TAPA and TAPA-NH_2 were dissolved in sterile artificial cerebrospinal fluid (ACSF) containing 126.6 mM NaCl, 2.5 mM KCl, 2.0 mM $MgCl_2$ and 1.3 mM $CaCl_2$. Naloxonazine and β-funaltrexamine were dissolved in sterile saline and injected in mice 24 h before the treatment with opioid peptides.

2.5. Statistical analysis

The antinociceptive responses are presented as means ± S.E.M. The dose–response curves and ED_{50} values with their 95% confidence intervals were calculated with a computer-associated curve-fitting program (GraphPad Prism, GraphPad Software, San Diego, CA, USA). For the statistical significance of differences between groups, the entire dose–response curves were compared using the *F*-test, according to the instruction provided with GraphPad Prism.

3. Results

3.1. Effect of naloxonazine on the antinociception induced by TAPA and TAPA-NH_2 given i.c.v. and i.t. in the tail-pressure test

TAPA and TAPA-NH_2 injected i.c.v. and i.t. produced dose-dependent antinociception in the tail-pressure test (Figs. 1 and 2). The ED_{50} value (with its 95% confidence interval) for the antinociception induced by i.c.v. and i.t.

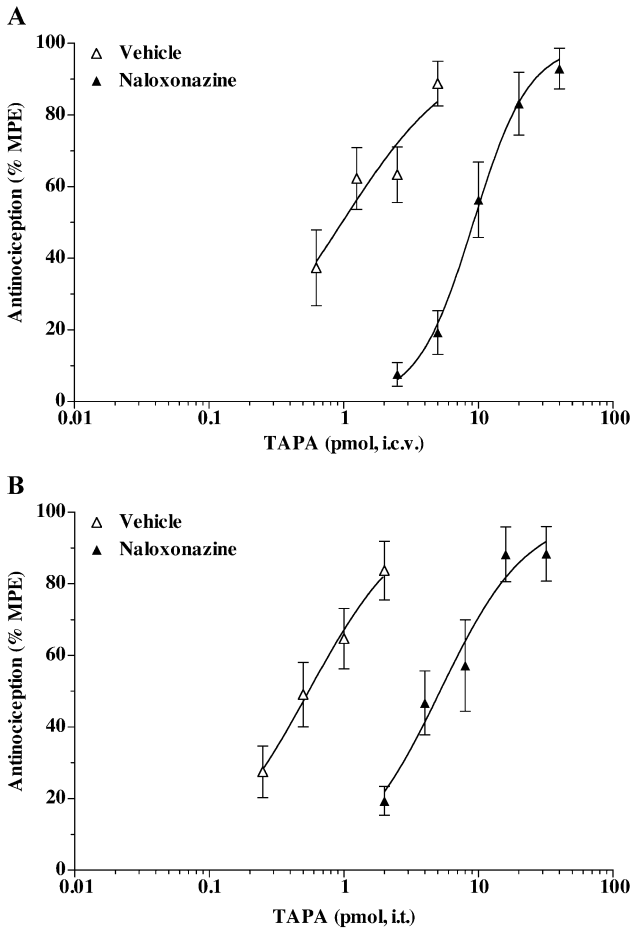

Fig. 1. The antinociception induced by TAPA given i.c.v. (A) or i.t. (B) in the tail-pressure test. Groups of mice pretreated s.c. with vehicle or naloxonazine (35 mg/kg) 24 h before were injected i.c.v. or i.t. with various doses of TAPA, and the antinociception induced by TAPA was measured in the tail-pressure test 10 min later. The antinociceptive responses (% MPE) are presented as the means ± S.E.M. The dose–response curves were calculated with a computer-associated curve-fitting program (GraphPad Prism). The statistical significance of differences between the groups was assessed with *F*-test. (A) The *F*- and *P*-values for TAPA given i.c.v. are 79.53 and 0.0002, respectively. (B) The *F*- and *P*-values for TAPA given i.t. are 50.36 and 0.0005, respectively.

injected TAPA in mice pretreated with vehicle was 0.97 (0.38–2.52) and 0.55 (0.44–0.68) pmol, respectively, whereas the ED_{50} value for the antinociception induced by i.c.v. and i.t. injected TAPA-NH_2 in mice pretreated with vehicle was 4.40 (3.31–5.85) and 7.74 (4.91–12.20) pmol, respectively. In mice pretreated s.c. with naloxonazine (35 mg/kg), the antinociception induced by TAPA given i.c.v. and i.t. was significantly attenuated, and the dose–response curve of TAPA given i.c.v. or i.t. was significantly shifted to the right by 9.49- and 9.44-fold, respectively (Fig. 1). The ED_{50} value for the antinociception induced by i.c.v. and i.t. injected TAPA in mice pretreated with naloxonazine was 9.21 (8.21–10.32) and 5.19 (3.50–7.70) pmol, respectively. In contrast, the antinociception induced by TAPA-NH_2 given i.c.v. and i.t. was not affected by the pretreatment with naloxonazine (Fig. 2). The ED_{50} value for the antinociception induced by TAPA-NH_2 given i.c.v. and i.t. in mice pretreated with naloxonazine was 5.86 (4.41–7.79) and 12.71 (5.91–27.33) pmol, respectively. However, the antinociception induced by TAPA-NH_2 given i.c.v. and i.t. in the tail-pressure test was completely blocked by s.c. pretreatment with selective μ-opioid receptor antagonist β-funaltrexamine, which irreversibly binds to both putative μ_1- and μ_2-opioid receptors (Fig. 2).

3.2. Effect of naloxonazine on the antinociception induced by TAPA and TAPA-NH_2 given i.c.v. and i.t. in the formalin test

In the formalin test, TAPA and TAPA-NH_2 injected i.c.v. and i.t. produced antinociception in a dose-dependent man-

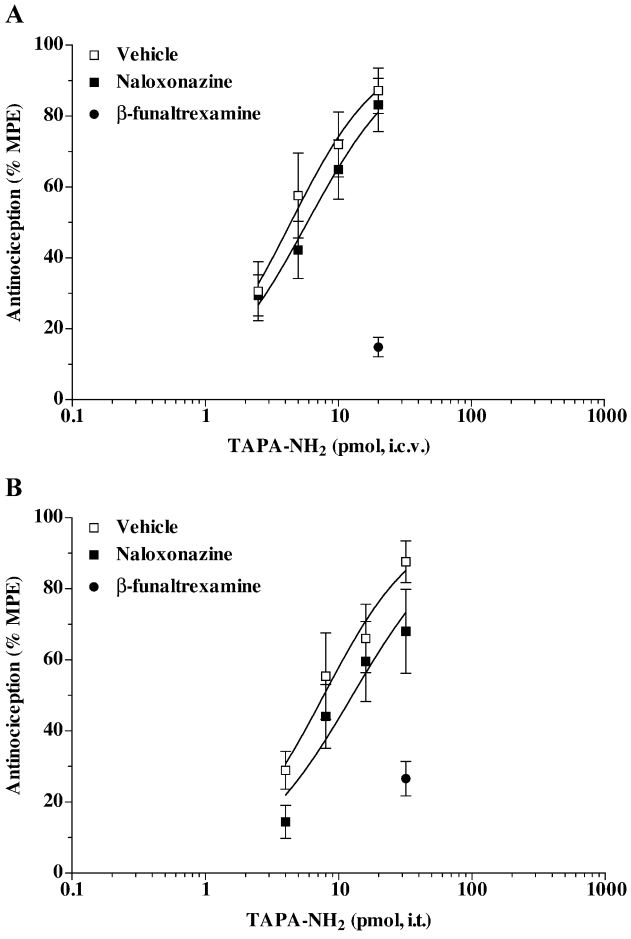

Fig. 2. The antinociception induced by TAPA-NH_2 given i.c.v. (A) or i.t. (B) in the tail-pressure test. Groups of mice pretreated s.c. with vehicle, naloxonazine (35 mg/kg) or β-funaltrexamine (40 mg/kg) 24 h before were injected i.c.v. or i.t. with various doses of TAPA-NH_2, and the antinociception induced by TAPA-NH_2 was measured in the tail-pressure test 10 min later. The antinociceptive responses (% MPE) are presented as the means ± S.E.M. The dose–response curves were calculated with a computer-associated curve-fitting program (GraphPad Prism). The statistical significance of differences between the groups was assessed with *F*-test. (A) The *F*- and *P*-values for TAPA-NH_2 given i.c.v. are 5.384 and 0.07, respectively. (B) The *F*- and P-values for TAPA-NH_2 given i.t. are 3.158 and 0.15, respectively.

ner (Figs. 3 and 4). The ED_{50} value for the antinociception induced by TAPA given i.c.v. and i.t. in mice pretreated with vehicle was 0.15 (0.11–0.23) and 0.34 (0.10–1.18) pmol, respectively, whereas the ED_{50} value for the antinociception induced by TAPA-NH_2 given i.c.v. and i.t. in mice pretreated with vehicle was 4.18 (2.45–7.13) and 1.47 (0.95–2.29) pmol, respectively. In mice pretreated with naloxonazine, the antinociception induced by TAPA given i.c.v. and i.t. was significantly attenuated, and the dose–response curve of TAPA given i.c.v. and i.t. was markedly shifted to the right by 26.60- and 18.15-fold, respectively (Fig. 3). The ED_{50} value for the antinociception induced by i.c.v. and i.t. injected TAPA in mice pretreated with naloxonazine was 3.99 (3.24–4.91) and 6.17 (4.89–7.79) pmol, respectively. In contrast, naloxonazine only attenuated the antinociception induced by i.t. injected TAPA-NH_2, but not that induced by i.c.v. injected TAPA-NH_2 (Fig. 4). The dose–response curve of TAPA-NH_2 given i.t. was significantly shifted to the right by 4.32-fold (Fig. 4B). The ED_{50} value for the antinociception induced by TAPA-NH_2 given i.c.v. and i.t. in mice pretreated with naloxonazine was 3.12 (1.81–5.38) and 6.35 (5.07–7.96) pmol, respectively. The antinociception induced by TAPA-NH_2 given i.c.v. and i.t. in formalin test was markedly attenuated by s.c. pretreatment with β-funaltrexamine (Fig. 4).

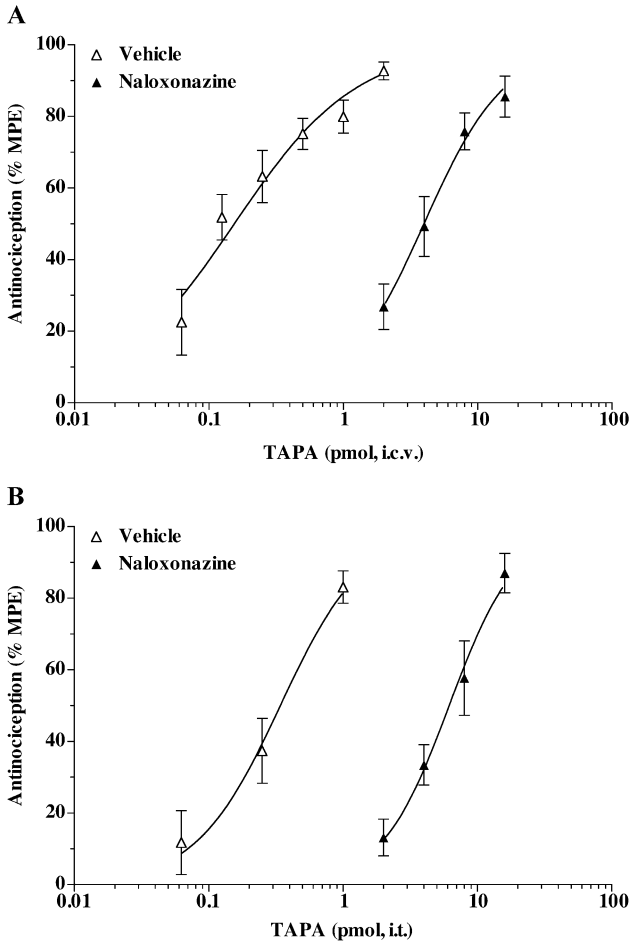

Fig. 3. The antinociception induced by TAPA given i.c.v. (A) or i.t. (B) in the formalin test. Groups of mice pretreated s.c. with vehicle or naloxonazine (35 mg/kg) 24 h before were injected i.c.v. or i.t. with various doses of TAPA. Ten and five minutes after the treatment with TAPA given i.c.v. or i.t., respectively, mice were injected with 2.0% formalin s.c. in the plantar surface of the hind-paw, and the time spent biting or licking the formalin-injected hind-paw was measured for 10 min. The antinociceptive responses (% MPE) induced by TAPA are presented as the means ± S.E.M. The dose–response curves were calculated with a computer-associated curve-fitting program (GraphPad Prism). The statistical significance of differences between the groups was assessed with *F*-test. (A) The *F*- and *P*-values for TAPA given i.c.v. are 76.73 and 0.0001, respectively. (B) The *F*- and *P*-values for TAPA given i.t. are 149.9 and 0.001, respectively.

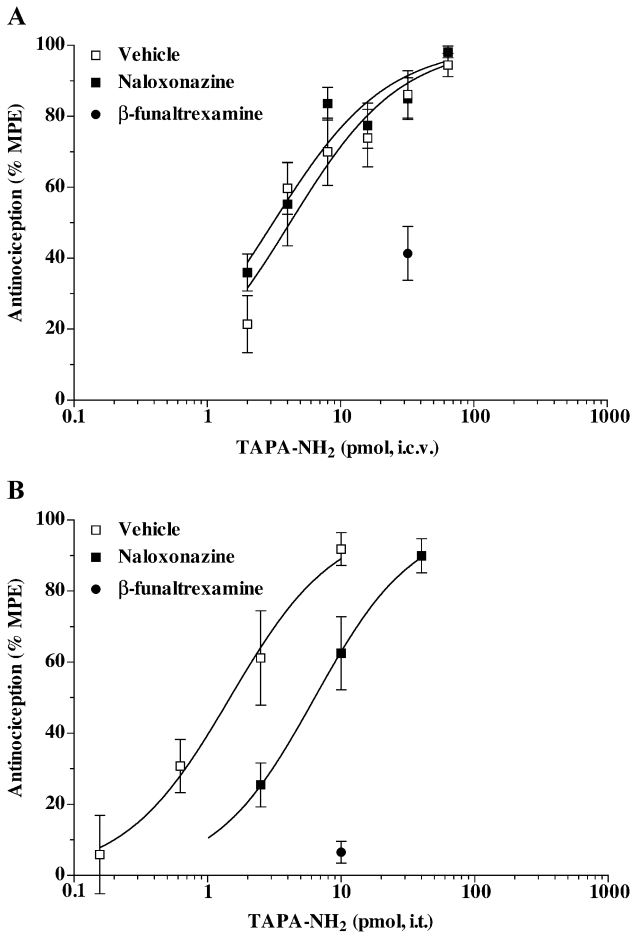

Fig. 4. The antinociception induced by TAPA-NH_2 given i.c.v. (A) or i.t. (B) in the formalin test. Groups of mice pretreated s.c. with vehicle, naloxonazine (35 mg/kg) or β-funaltrexamine (40 mg/kg) 24 h before were injected i.c.v. or i.t. with various doses of TAPA-NH_2. Ten and five minutes after the treatment with TAPA-NH_2 given i.c.v. or i.t., respectively, mice were injected with 2.0% formalin s.c. in the plantar surface of the hind-paw, and time spent biting or licking the formalin-injected hind-paw was measured for 10 min. The antinociceptive responses (% MPE) induced by TAPA-NH_2 are presented as the means ± S.E.M. The dose–response curves were calculated with a computer-associated curve-fitting program (GraphPad Prism). The statistical significance of differences between the groups was assessed with *F*-test. (A) The *F*- and *P*-values for TAPA-NH_2 given i.c.v. are 0.653 and 0.55, respectively. (B) The *F*- and *P*-values for TAPA-NH_2 given i.t. are 63.48 and 0.004, respectively.

4. Discussion

The existence of multiple μ-opioid receptors (putative μ_1- and μ_2-opioid receptors) has been predicted on the basis of pharmacological evidence. The putative μ_1-opioid receptor is reported to be implicated in supraspinal analgesia, modulation of acetylcholine and prolactin release and some aspects of feeding. In contrast, the putative μ_2-opioid receptor is considered to mediate different actions, including spinal analgesia, respiratory depression and inhibition of gastrointestinal transit (Heyman et al., 1988; Pasternak and Wood, 1986). In fact, the antinociception induced by the non-selective μ-opioid receptor agonist (putative μ_1- and μ_2-opioid receptor agonist) DAMGO given i.c.v. or i.t. is predominantly mediated by putative μ_1- and μ_2-opioid receptors, respectively (Sakurada et al., 1999, 2000). In the present study, the antinociception induced by TAPA, but not by TAPA-NH_2, given i.c.v. and i.t. in the tail-pressure test was blocked by pretreatment with naloxonazine. The results clearly suggest that the antinociception induced by TAPA and TAPA-NH_2 against mechanical nociception is mediated by the putative μ_1- and μ_2-opioid receptors, respectively, at both supraspinal and spinal sites. The results also provide the first direct evidence to prove the existence of putative μ_1- and μ_2-opioid receptors involved in the production of antinociception at both supraspinal and spinal sites.

In the formalin test, the antinociception induced by TAPA, but not by TAPA-NH_2, given i.c.v. was blocked by pretreatment with naloxonazine. The results of TAPA and TAPA-NH_2 given i.c.v. in the formalin test were consistent with those in the tail-pressure test. In contrast, the antinociception induced by both TAPA and TAPA-NH_2 given i.t. was significantly blocked by pretreatment with naloxonazine. However, the rightward shift of the dose–response curve for i.t. injected TAPA-NH_2 (4.32 times) produced by naloxonazine in the formalin test was much smaller than that for i.t. injected TAPA (18.15 times). We previously found that the antinociception induced by i.t. injected DAMGO, which is naloxonazine-insensitive (the putative μ_2-opioid receptor-mediated), against mechanical nociception (Sakurada et al., 1999) was significantly attenuated by naloxonazine in the formalin test (unpublished observation). Like that of TAPA-NH_2, the rightward shift of the dose–response curve for i.t. injected DAMGO (2.67 times) produced by naloxonazine in the formalin test was also much smaller than that of i.t. injected TAPA. Since the antinociception induced by i.t. injected TAPA-NH_2 and DAMGO in the formalin test was completely blocked by pretreatment with β-funaltrexamine (present data and unpublished observation), the antinociception induced by i.t. injected TAPA-NH_2 and DAMGO in the formalin test may be mediated by both putative μ_1- and μ_2-opioid receptors. The above findings reveal the possibility that a different mechanism is involved in the inhibition of different nociceptive stimuli. The spinal antinociception induced by the μ-opioid receptor agonist against formalin-induced nociception may be predominantly mediated by putative μ_1-opioid receptors, in contrast with that against mechanical nociception.

It is of interest that TAPA-induced antinociception is mediated by putative μ_1-opioid receptors, whereas C-terminal amidation of TAPA TAPA-NH_2-induced antinociception is mediated by putative μ_2-opioid receptors. The C-terminal amidation of dermorphin analogues has been considered to be involved in the stabilization of the peptides, since the existence of C-terminal amidation prevents the enzymatic degradation of dermorphin analogues, which are degraded from the C-terminal end (Chaki et al., 1990; Negri and Improta, 1984). We report here for the first time that the C-terminal amidation of the peptide may determine to which receptor it binds.

In conclusion, TAPA and TAPA-NH_2 are selective agonists for putative μ_1- and μ_2-opioid receptors, respectively. The C-terminal amidation may be the critical portion of TAPA-NH_2 for distinguishing between putative μ_1- and μ_2-opioid receptors.

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