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European Journal of Pharmacology 540 (2006) 67–72
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Involvement of spinal μ_1-opioid receptors on the Tyr-D-Arg-Phe-sarcosine-induced antinociception

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Received 13 February 2006; received in revised form 22 April 2006; accepted 25 April 2006
Available online 4 May 2006

Abstract

The involvement of spinal μ-opioid receptor subtypes on the antinociception induced by i.t.-administered Tyr-D-Arg-Phe-sarcosine (TAPS), a N-terminal tetrapeptide analog of dermorphin, was determined in mice tail-flick test. Intrathecal administration of TAPS produced the marked inhibition of the tail-flick response in a dose-dependent manner. The antinociception induced by TAPS was completely eliminated by i.t.-co-administration of Tyr-D-Pro-Phe-Phe-NH_2 (D-Pro^2-endomorphin-2), the μ_1-opioid receptor antagonist, whereas i.t. co-treatment with Tyr-D-Pro-Trp-Phe-NH_2 (D-Pro^2-endomorphin-1) or Tyr-D-Pro-Trp-Gly-NH_2 (D-Pro^2-Tyr-W-MIF-1), the μ_2-opioid receptor antagonists, did not affect the TAPS-induced antinociception. In contrast, the antinociception induced by i.t.-administered [D-Ala^2,*N*-MePhe^4,Gly-ol^5]enkephalin was significantly attenuated by i.t.-co-administration of D-Pro^2-endomorphin-1 or D-Pro^2-Tyr-W-MIF-1, but not D-Pro^2-endomorphin-2. These results suggest that TAPS may stimulate spinal μ_1-opioid receptors to produce the antinociception.
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Keywords: Tyr-D-Arg-Phe-sarcosine; Dermorphin; Antinociception; Spinal cord; μ-Opioid receptor; (Mouse)

1. Introduction

Tyr-D-Ala-Phe-Gly-Tyr-Pro-Ser-NH_2 (dermorphin) is a heptapeptide derived from amphibian skin (Montecucchi et al., 1981). Dermorphin shows high affinity and selectivity to μ-opioid receptors (de Castiglione and Rossi, 1985; Krumins, 1987) and produces potent antinociception (Broccardo et al., 1981; Stevens and Yaksh, 1986). The N-terminal tetrapeptide of dermorphin is a minimum sequence required for its opioid activity, but the short fragment is less potent than the original heptapeptide (Broccardo et al., 1981; Salvadori et al., 1982). However, based on the research for the structure–activity relationship of dermorphin, we developed the dermorphin N-terminal tetrapeptide analog Tyr-D-Arg-Phe-sarcosine (Sar) (TAPS), containing D-Arg^2 and Sar^4 residues instead of D-Ala^2 and Gly^4 residue of dermorphin, as a more potent analgesics than dermorphin (Sasaki et al., 1984; Sato et al., 1984, 1987). The antinociceptive effect produced by i.c.v. and s.c. administrations of TAPS was stronger and more prolonged than that produced by morphine (Sasaki et al., 1984; Sato et al., 1984, 1987). Pretreatment with naloxone resulted in complete antagonism of the antinociceptive effect produced by TAPS, suggesting that TAPS shows the antinociceptive effect through μ-opioid receptor (Sato et al., 1984, 1987).

There is biochemical and pharmacological evidence supporting the existence of μ-opioid receptor subtypes (Elliott et al., 1994; Pasternak, 1993). The major subdivision of μ-opioid receptors is μ_1- and μ_2-opioid receptors, based on the sensitivity to the μ-opioid receptor antagonist naloxonazine, which binds irreversibly to μ_1-opioid receptors, but reversibly to μ_2-opioid receptors (Elliott et al., 1994; Pasternak, 1993). In fact, the antinociception mediated by μ-opioid receptors can be divided into naloxonazine (35 mg/kg,

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0014-2999/$ - see front matter © 2006 Elsevier B.V. All rights reserved.
doi:10.1016/j.ejphar.2006.04.029

s.c. or 5.5 nmol, i.t.)-sensitive antinociception and naloxonazine-insensitive antinociception, which are mediated by μ_1-opioid receptors and μ_2-opioid receptors, respectively. As a μ_1-opioid receptor antagonist, naloxonazine can discriminate the antinociceptive effect of μ_1-opioid receptor agonist endomorphin-2 from that of μ_2-opioid receptor agonist endomorphin-1 (Sakurada et al., 1999, 2000a, 2001). In contrast to μ_1-opioid receptor, the selective antagonist for μ_2-opioid receptor had not been found. However, we recently developed Tyr-D-Pro-Trp-Gly-NH_2 (D-Pro^2-Tyr-W-MIF-1) and Tyr-D-Pro-Trp-Phe-NH_2 (D-Pro^2-endomorphin-1) as the selective antagonists for μ_2-opioid receptors, and Tyr-D-Pro-Phe-Phe-NH_2 (D-Pro^2-endomorphin-2) as new selective antagonist for μ_1-opioid receptors (Sakurada et al., 2002; Watanabe et al., 2005).

The present study was designed to describe the involvement spinal μ_1-opioid receptor on the antinociceptive effect of TAPS, using the recently developed selective μ_1-opioid receptor antagonist D-Pro^2-endomorphin-2 and selective μ_2-opioid receptor antagonists D-Pro^2-Tyr-W-MIF-1 and D-Pro^2-endomorphin-1.

2. Materials and methods

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

2.1. Animals

Male ddY mice (SLC, Hamamatsu, Japan) weighting 22–25 g were used. The animals were housed in a light- and temperature-controlled room (light on at 9:00 and off at 21:00; 23 °C). Food and water were available ad libitum. Animals were used only once.

2.2. Drugs

TAPS (Peptide Institute Inc., Osaka Japan), [D-Ala^2,*N*-MePhe^4,Gly-ol^5]enkephalin (DAMGO: Sigma Chemical Co., St. Louis, MO), D-Pro^2-endomorphin-1 (synthesized in our laboratory), D-Pro^2-endomorphin-2 (synthesized in our laboratory) and D-Pro^2-Tyr-W-MIF-1 (synthesized in our laboratory) were used. All drugs were dissolved in sterile artificial cerebrospinal fluid containing 7.4 g of NaCl, 0.19 g of KCl, 0.19 g of $MgCl_2$, 0.14 g of $CaCl_2$ in 1 l.

2.3. Intrathecal administration

The i.t. administration was performed according to the procedure described by Hylden and Wilcox (1980) using a 10-μl Hamilton microsyringe with a 29-gauge needle. The injection volume was 2 μl.

2.4. Assessment of nociceptive response

The antinociceptive response was assessed with the thermal tail-flick test, using an automated tail-flick unit (BM kiki, Tokyo, Japan). Mice were adapted to the testing environment for at least 1 h before any stimulation. Each animal was restrained with a soft cloth to reduce visual stimuli, and the light beam as a noxious radiant heat stimulation was applied from underneath toward the tail. The light beam focused on the tail surface, and the latency for the tail-flick response against the noxious radiant heat stimulation was measured. The intensity of the noxious radiant heat stimulation was adjusted so that the predrug latency for the tail-flick response was 2.5–3.5 s. The antinociceptive effect was expressed as percent of the maximum possible effect (%MPE), which was calculated with the following equation: $[(T_1-T_0)/(10-T_0)]\times 100$, where T_0 and T_1 are the predrug and postdrug latencies for the tail-flick response, respectively. To prevent tissue damage in the tail, the noxious radiant heat stimulation was terminated automatically if the mouse did not flick the tail within 10 s. The measurement of the tail-flick latency was performed by only one individual who was uninformed about the drug treatment for each mouse.

2.5. Statistical analysis

The data are expressed as the mean±S.E.M. The statistical significance of the differences between groups was assessed with a one-way analysis of variance (ANOVA) followed by Dunnett's test. The ED_{50} and ID_{50} values with their 95% confidence intervals were calculated by computer-associated curve-fitting program (GraphPad Prism, GraphPad Software, Inc., San Diego, CA, USA).

3. Results

3.1. Antinociceptive effects of i.t.-administered TAPS and DAMGO

Groups of mice were treated i.t. with TAPS (0.5–2 pmol) and DAMGO (2.5–10 pmol), and antinociceptions induced by TAPS and DAMGO were measured 5, 10, 15, 30, 45, 60 and 90 min after the treatment. TAPS and DAMGO produced dose-dependent antinociception with a peak effect at 10 and 5 min, respectively. ED_{50} values of TAPS and DAMGO for antinociception at their peak time were 0.84 pmol (0.61–1.17 pmol) and 4.49 pmol (1.52–13.28 pmol), respectively (Fig. 1). The antinociception induced by

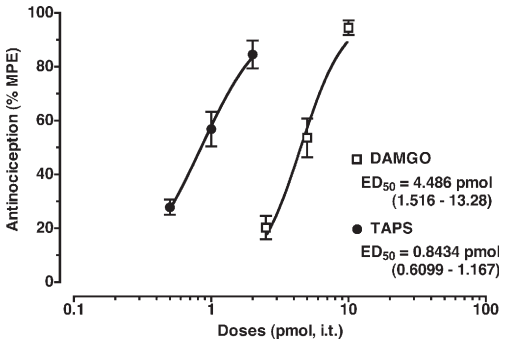

Fig. 1. Antinociceptive effects of TAPS and DAMGO in the mouse tail-flick test. Groups of mice were treated i.t. with TAPS (0.5–2 pmol) or DAMGO (2.5–10 pmol), and antinociceptions induced by TAPS and DAMGO were measured 10 and 5 min after the treatment, respectively. Each data represents the mean± S.E.M. for 10 mice. The dose–response curves and the ED_{50} values with their 95% confidence intervals were calculated with a computer-associated curve-fitting program (GraphPad Prism).

TAPS is 5.35 times more potent than that by DAMGO. In the following experiments, 2 pmol of TAPS or 10 pmol of DAMGO, which produced approximately 82% or 92% of MPE, respectively, at their peak time, was used.

3.2. Effect of i.t.-co-administration of μ^1-opioid receptor antagonist D-Pro^2-endomorphin-2 on the antinociceptions induced by TAPS and DAMGO

Groups of mice were co-administered i.t. various doses of μ_1-opioid receptor antagonist D-Pro^2-endomorphin-2 (25–200 pmol) with TAPS (2 pmol) or DAMGO (10 pmol), and the antinociceptions induced by TAPS and DAMGO were measured 10 min and 5 min after the treatment, respectively. D-Pro^2-endomorphin-2 at any doses used did not show any antinociceptive or hyperalgesic effect by itself after the treatment (data not shown). Co-administration of D-Pro^2-endomorphin-2 dose-dependently attenuated the antinociception induced by TAPS (Fig. 2A). The ID_{50} value of D-Pro^2-endomorphin-2 against TAPS-induced antinociception was 79.25 pmol (67.90–92.50 pmol). In contrast, co-administered D-Pro^2-endomorphin-2 at a dose of 200 pmol, which

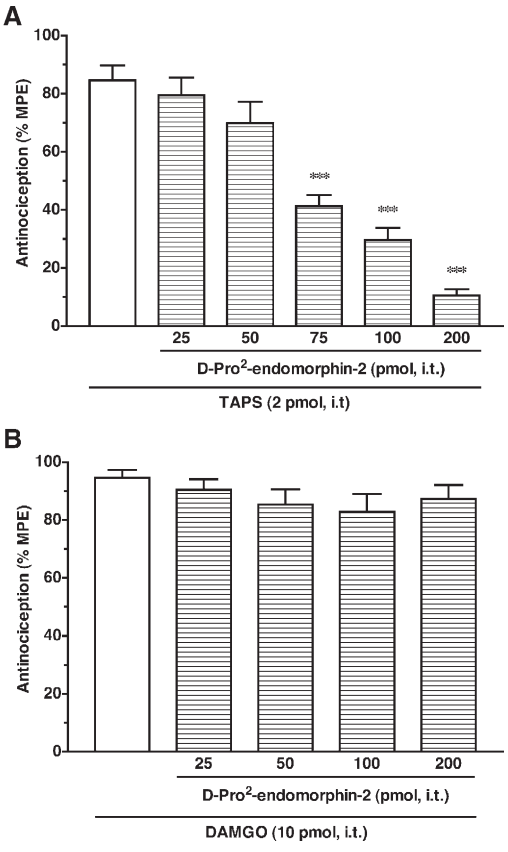

Fig. 2. Effect of D-Pro^2-endomorphin-2 on the antinociception induced by TAPS (A) and DAMGO (B). Groups of mice were co-administered i.t. D-Pro^2-endomorphin-2 (25–200 pmol) with TAPS (2 pmol) or DAMGO (10 pmol), and the antinociceptive effects of TAPS and DAMGO were measured 10 min and 5 min after the treatment, respectively. Each data represents the mean±S.E.M. for 10 mice. The statistical significance of the differences between groups was assessed with a one-way ANOVA followed by the Dunnett's test. (A) The F-value of the one-way ANOVA for TAPS was $F[5,54]=34.64$ ($P<0.001$). ***$P<0.001$ compared with TAPS alone group.

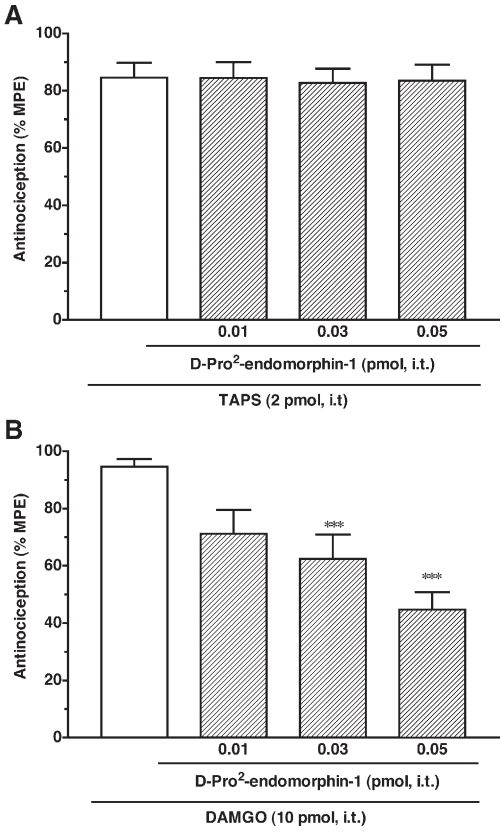

Fig. 3. Effect of D-Pro^2-endomorphin-1 on the antinociception induced by TAPS (A) and DAMGO (B). Groups of mice were co-administered i.t. D-Pro^2-endomorphin-1 (0.01–0.05 pmol) with TAPS (2 pmol) or DAMGO (10 pmol), and the antinociceptive effects of TAPS and DAMGO were measured 10 min and 5 min after the treatment, respectively. Each data represents the mean±S.E.M. for 10 mice. The statistical significance of the differences between groups was assessed with a one-way ANOVA followed by the Dunnett's test. (B) The F-value of the one-way ANOVA for DAMGO was $F[3,36]=9.159$ ($P<0.001$). ***$P<0.001$ compared with DAMGO alone group.

almost completely eliminated TAPS-induced antinociception, did not affect the antinociception induced by DAMGO (Fig. 2B).

3.3. Effects of i.t.-co-administration of μ^2-opioid receptor antagonists D-Pro^2-endomorphin-1 and D-Pro^2-Tyr-W-MIF-1 on the antinociceptions induced by TAPS and DAMGO

Groups of mice were co-administered i.t. various doses of μ_2-opioid receptor antagonists D-Pro^2-endomorphin-1 (0.01–0.05 pmol) or D-Pro^2-Tyr-W-MIF-1 (50–800 pmol) with TAPS (2 pmol) or DAMGO (10 pmol), and the antinociceptions induced by TAPS and DAMGO were measured 10 min and 5 min after the treatment, respectively. D-Pro^2-endomorphin-1 and D-Pro^2-Tyr-W-MIF-1 at any doses used did not show any antinociceptive or hyperalgesic effect by itself after the treatment (Data not shown). Co-administration of D-Pro^2-endomorphin-1 and D-Pro^2-Tyr-W-MIF-1 dose-dependently attenuated the antinociception induced by DAMGO (Figs. 3B and 4B). The ID_{50} values of D-Pro^2-endomorphin-1 and D-Pro^2-Tyr-W-MIF-1 against DAMGO-induced antinociception were 0.05241 pmol (0.0007319–3.752) and 2156 pmol (821.6–5659 pmol), respectively. In contrast, co-administration with D-Pro^2-endomorphin-1 (0.05 pmol) and D-

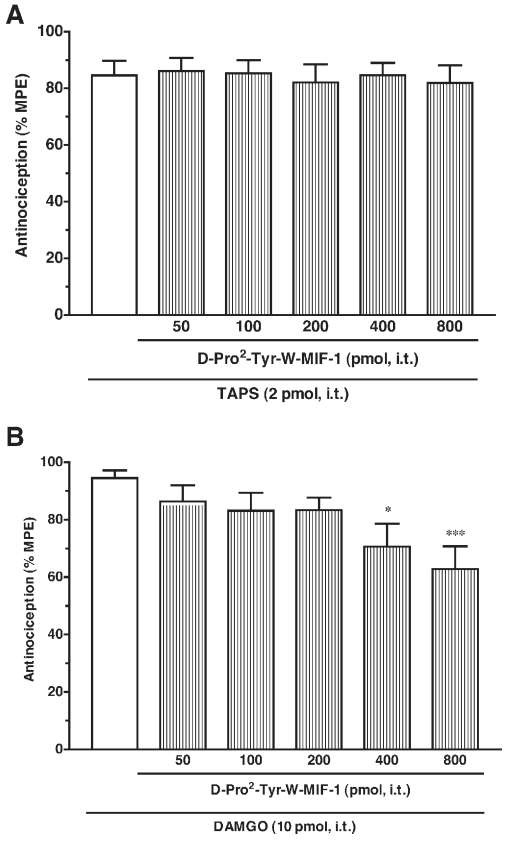

Fig. 4. Effect of D-Pro^2-Tyr-W-MIF-1 on the antinociception induced by TAPS (A) and DAMGO (B). Groups of mice were co-administered i.t. D-Pro^2-Tyr-W-MIF-1 (50–800 pmol) with TAPS (2 pmol) or DAMGO (10 pmol), and the antinociceptive effects of TAPS and DAMGO were measured 10 min and 5 min after the treatment, respectively. Each data represents the mean±S.E.M. for 10 mice. The statistical significance of the differences between groups was assessed with a one-way ANOVA followed by the Dunnett's test. (B) The F-value of the one-way ANOVA for DAMGO was $F[5,54]=3.572$ ($P<0.01$). ***$P<0.001$ and *$P<0.05$, compared with DAMGO alone group.

Pro^2-Tyr-W-MIF-1 (800 pmol), which significantly attenuated the DAMGO-induced antinociception, did not affect the antinociception induced by TAPS (Figs. 3A and 4A).

4. Discussion

TAPS induced a dose-dependent antinociception in the tail-flick tests after i.t. administration. The antinociceptive effect of TAPS reached its peak effect at 10 min after the injection and disappeared by 60 min. The duration of TAPS-induced antinociception is similar to that by DAMGO, but the TAPS-induced antinociception is 5.35 times more potent than that by DAMGO.

We recently developed D-Pro^2-Tyr-W-MIF-1 and D-Pro^2-endomorphin-1, as the selective antagonists for μ_2-opioid receptors, and also developed D-Pro^2-endomorphin-2 as the selective antagonist for μ_1-opioid receptors (Sakurada et al., 2002; Watanabe et al., 2005). We initially found that D-Pro^2-endomorphin-1 and D-Pro^2-endomorphin-2, analogs of the endomorphins containing D-Pro^2, selectively show the antagonistic properties against their parent peptides endomorphin-1 and endomorphin-2, respectively, in the mouse spinal cord. D-Pro^2-endomorphin-2 given i.t. attenuated the antinociception induced by i.t. administration of endomorphin-2, but not those by DAMGO and endomorphin-1, in a dose-dependent manner, indicating that like a naloxonazine, D-Pro^2-endomorphin-2 may act as a selective μ_1-opioid receptor antagonist (Sakurada et al., 2002). In contrast, D-Pro^2-endomorphin-1 given i.t. significantly attenuated the antinociception induced by i.t.-administered endomorphin-1 and DAMGO, but not endomorphin-2, suggesting that D-Pro^2-endomorphin-1 may act as a selective μ_2-opioid receptor antagonist (Sakurada et al., 2002). Based on the evidence with D-Pro^2-endomorphins, we synthesized D-Pro^2-Tyr-W-MIF-1, analog of the selective μ_2-opioid receptor agonist Tyr-W-MIF-1 containing D-Pro^2, and found that D-Pro^2-Tyr-W-MIF-1 is also selective antagonist for μ_2-opioid receptor (Watanabe et al., 2005). However, antagonistic activity of D-Pro^2-Tyr-W-MIF-1 is different with that of D-Pro^2-endomorphin-1, since the antinociception of μ_2-opioid receptor agonist Tyr-W-MIF-1 was significantly and dose-dependently attenuated by D-Pro^2-Tyr-W-MIF-1 at the low doses (100-400 pmol) which did not affect the antinociception induced by other μ_2-opioid receptor agonists endomorphin-1 and DAMGO (Watanabe et al., 2005), indicating that D-Pro^2-Tyr-W-MIF-1 can discriminate the antinociceptive effects of μ_2-opioid receptor agonists endomorphin-1 and DAMGO from that of Tyr-W-MIF-1 which may act the different subtype of the μ_2-opioid receptors.

In the present study, the effects of variety doses of D-Pro^2-endomorphin-1, D-Pro^2-endomorphin-2 and D-Pro^2-Tyr-W-MIF-1 on the antinociception induced by TAPS were determined to identify the μ-opioid receptor subtypes involved in the antinociceptive responses to TAPS. The antinociception induced by i.t. administered TAPS was significantly attenuated by co-administration of D-Pro^2-endomorphin-2, whereas i.t. co-administered D-Pro^2-endomorphin-1 or D-Pro^2-Tyr-W-MIF-1 did not affect the antinociceptive effect of TAPS. On the contrary, the antinociceptive effect of i.t.-administered DAMGO was significantly attenuated by the co-administration of D-Pro^2-endomorphin-1 or D-Pro^2-Tyr-W-MIF-1, but not D-Pro^2-endomorphin-2. The present results suggest that the spinal antinociception induced by TAPS is mediated through the μ_1-opioid receptors, but not μ_2-opioid receptors.

The μ_1- and μ_2-opioid receptors had been considered to be involved in the supraspinal and spinal antinociception, respectively (Pasternak, 1993; Paul et al., 1989). However, recent behavioral pharmacological studies suggest the presence of μ_1-opioid receptors involved in the antinociception in spinal sites as assayed with the formalin test, hot-plate test, tail-pressure test and tail-flick test (Sakurada et al., 1999, 2000b; Sato et al., 1999). Autoradiographic studies show that μ_1- and μ_2-opioid receptors are localized in the spinal structures involved in the modulation of nociception (Moskowitz and Goodman, 1985). In the spinal dorsal horn, the excitatory amino acid glutamate and the tachykinin peptide substance P are considered to be the main neurotransmitters for nociceptive processing. Following the high-intensity nociceptive stimulation, glutamate and substance P are released from primary afferent nerve terminal (Otsuka and Konishi, 1976; Skilling et al., 1988; Smullin et al., 1990). The μ-opioid receptors are localized in the presynaptic site (the primary afferent nerve terminal) and postsynaptic site (the trigeminal dorsal horn) of the spinal dorsal horn (Aicher et al., 2000a,b;

Mansour et al., 1994a,b).To produce the antinociception, the μ-opioid receptor agonists are considered to inhibit the postsynaptic excitation directory at the postsynaptic site, and also inhibit the release of glutamate and substance P from the primary afferent nerve at the primary afferent nerve terminal (Aicher et al., 2000a, b). We recently found that the antinociception induced by i.t.-administered selective μ_1-opioid receptor agonists Tyr-D-Arg-Phe-β-Ala (TAPA) (Mizoguchi et al., 2004; Sakurada et al., 2000b) and endomorphin-2 (Sakurada et al., 1999, 2000a) is predominantly mediated through the inhibition of the release of the excitatory amino acids and the neuropeptides from the primary afferent nerve, rather than through the direct inhibition of postsynaptic excitation in the dorsal horn (Watanabe et al., 2006; unpublished observation). TAPS, which has a structural similarity with TAPA except 4th position amino acid in its sequence, is characterized as a selective μ_1-opioid receptor agonist in the present study. As well as TAPA, the antinociception induced by i.t.-administered TAPS may be predominately mediated through the inhibition of the release of the excitatory amino acids and the neuropeptides from the primary afferent nerve.

In conclusion, the antinociception induced by TAPS administered spinally is mediated by the stimulation of μ_1-opioid receptors in the spinal cord.

Acknowledgements

This work was supported by The Science Research Promotion Fund from The Promotion and Mutual Aid Corporation for Private Schools of Japan, a Grant-in-Aid for Scientific Research (C) (KAKENHI 16590058 and 17590065) from the Japan Society for the Promotion of Science, and a Grant-in-Aid for High Technology Research Program from the Ministry of Education, Culture, Sports, Science and Technology Japan.

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