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Research Report

Morphine has an antinociceptive effect through activation of the okadaic-acid-sensitive Ser/Thr protein phosphatases PP2A and PP5 estimated by tail-pinch test in mice

Takehiko Maeda[a,*], Wakako Hamabe[a], Yuan Gao[a], Yohji Fukazawa[a], Kazumasa Kumamoto[a], Masanobu Ozaki[b], Shiroh Kishioka[a]

[a]Department of Pharmacology, Wakayama Medical University, Wakayama, 811-1 Kimiidera, Wakayama City, Wakayama 641-0012, Japan
[b]Department of Toxicology, Niigata College of Pharmacy, Niigata, Japan

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Abstract

Although the serine/threonine protein kinases involved in the pharmacological action of morphine are well recognized, the critical contribution of serine/threonine protein phosphatase (PP) has been appreciated on to a slight degree. We examined the involvement of subtypes of serine/threonine protein phosphatase (PP) in the antinociceptive effect of morphine in mice. The antinociceptive effect of subcutaneously administered morphine was attenuated by simultaneously intracerebroventricular (i.c.v.) or intrathecal (i.t.) injection of okadaic acid (OA), a PP inhibitor. To reveal which subtypes of PPs participated in the antinociceptive effect of morphine, mice received i.c.v. or i.t. injections of antisense oligodeoxynucleotide (AS-ODN) directed against either the PP2A or PP5 subtypes of PPs before assessment of morphine-induced antinociception. Pretreatment with AS-ODN against PP2A or PP5 via each route weakened the antinociceptive effect of morphine, accompanied by reduction of expression levels of PP in the periaqueductal gray (PAG) and the spinal cord. Subcutaneously administered morphine increased activity of OA-sensitive PPs in the PAG and the spinal cord in a dose-dependent manner; this was prevented by concurrent administration of naloxone. These results suggest that PP2A and PP5 are involved in the antinociceptive effect of morphine in mice.

Theme: Sensory system
Topic: Pain modulation: pharmacology

Keywords: Antinociception; Antisense oligodeoxynucleotide; Morphine; Okadaic acid; Serine/threonine protein phosphatase

1. Introduction

The nociceptive primary afferent fibers terminate on neurons in the dorsal horn of the spinal cord, and then the nociceptive information is transmitted to the higher center, which is known as ascending pathway. Pain can be also controlled by central mechanisms, of which a representative example is the descending inhibitory pathway arising in the midbrain periaqueductal gray (PAG) and projecting to the dorsal horn of spinal cord through the nucleus raphe magnus and the nucleus reticularis gigantocellularis. Microinjection of low dose of opiates directly into specific regions of the ascending pain pathway, and inhibitory descending pathway produces a powerful antinociception by inhibiting the activity of the nociceptive neurons [20]. PAG and the dorsal horn of spinal cord are the most sensitive regions to morphine of those pathways.

The phosphorylation state of functional protein molecules, catalyzed by protein kinases and protein phosphatases, determines their physiological activity in many cellular events. Although the kinases involved in these

* Corresponding author. Fax: +81 73 446 3806.
E-mail address: tmaeda@wakayama-med.ac.jp (T. Maeda).

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events are well recognized, the critical contribution of serine/threonine protein phosphatase (PP) has only more recently been appreciated. The mammalian PPs which have been isolated so far are PP1, PP2A, PP2B, PP4, PP5, PP6 and PP7 [6]. Much of the knowledge concerning the cellular roles of PPs has been derived from studies using naturally produced toxins that inhibit the activity of certain PPs. Toxins such as okadaic acid (OA) are able to inhibit PP2A, but they have the lower or similar affinity to other PPs, PP1 and PP5 [4]. Studies with these rather non-specific toxins have complicated the understanding of the involvement of individual PP subtypes in cellular events. Therefore, the development of compounds that alter the activity of specific PPs is an important area in elucidation of the role of individual PPs in cellular events.

Only two studies have examined the involvement of PPs in opioid-induced antinociceptive effect; one result is inconsistent with the other. Moncada et al. [16] showed that OA attenuated the antinociceptive effect of morphine in mice. In contrast, Bernstein and Welch [3] reported that relatively high doses of OA did not modify morphine antinociception in mice. A potential approach to resolve this discrepancy is the use of more specific treatments against individual subtypes of PPs.

The aim of our study is to reveal which subtype of PPs is involved in the antinociceptive effect of morphine in mice. We first examined the effect of intracerebroventricular (i.c.v.) and intrathecal (i.t.) injection of OA on morphine antinociception in mice to compare with the previously reported results. Next, we examined the effects of i.c.v. and i.t. administered antisense oligodeoxynucleotide (AS-ODN) directed against PP2A and PP5, possibly more specific treatments, on the antinociceptive effect of morphine. To determine whether PPs participate in intracellular signaling following opioid receptor stimulation, we also measured PP activity in the spinal and supraspinal tissue prepared from mice given morphine.

2. Materials and methods

2.1. Analgesic test

Male ICR mice (SLC, Hamamatsu, Japan) weighing 20–30 g were used. They were housed 5 to a cage in an air-conditioned (23–24 °C, 60% humidity) and light-controlled (lights on from 7:00 to 19:00 h) room. The antinociceptive effect was evaluated by the tail-pinch method as we reported previously [14]. In brief, a flattened clip (approximately 6 mm wide) was placed at the base of the tail. The pressure produced by the clip on the tail was adjusted to approximately 500 g. The nociceptive response was indicated by the time (latency) required for the mouse to respond to this pressure by vocalizing or biting at the clip. The clip was never applied for longer than 15 s. The percentage of maximum possible effect (%MPE) was calculated using the formula: %MPE = 100 × (each latency − baseline latency) / (15 − baseline latency). The magnitude of antinociception was evaluated as area under the curve (AUC) calculated from the time course of %MPE. Mice were given i.c.v. or i.t. injection of either 0.01 pg–10 ng OA (dissolved in 5 μl saline: Sigma-Aldrich, Tokyo, Japan) or saline immediately before subcutaneous administration of either 5 mg/kg morphine hydrochloride (Takeda, Osaka, Japan) or saline. The i.c.v. and i.t. administration was followed by the methods as reported previously [10,12]. Mice were killed by decapitation for Western blot and phosphatase assay. All procedures were approved by Animal Research Committee of Wakayama Medical University in accordance with Japanese Government Animal Protection and Management Law, Japanese Government Notification on Feeding and Safekeeping of Animals and The Guidelines for Animal Experiments in Wakayama Medical University (approval number 64).

2.2. Design of antisense oligodeoxynucleotide

AS-ODN and mismatch oligodeoxynucleotide (MM-ODN) were directed against PP mRNA (Sigma-Aldrich, Tokyo, Japan). AS-ODN consisted of phosphorothioate of the following complementary sequence of bases: for mouse PP2A catalytic subunit α mRNA (188–207), 5′-CTT-CTCGTCCATGATGCCGC-3′ [11]; for mouse PP5 (141–159), 5′-CTGTGTCTTGAGCTCCTCT-3′ [15]. MM-ODN, which served as control, had the following sequence: for mouse PP2A catalytic subunit α mRNA, 5′-TCCTG-TCCTGCACGACTGCT-3′; for mouse PP5, 5′-TCTG-TCTCGTCATCGTCTG-3′. Mice received i.c.v. or i.t. injection of 1 μg of AS-ODN or MM-ODN, dissolved in 5 μl sterile saline, twice a day for 3 consecutive days, and, on the following day, the analgesic test was performed.

2.3. Western blot

The brain and the spinal cord of mice were quickly removed immediately after the analgesic test, and thick coronal sections of the midbrain and the spinal cord were made. The periaqueductal gray (PAG) and the lumbar segments of spinal cord were isolated from the coronal section by the following method. For isolation of the PAG, the midbrain coronal section, 1 mm thick, was dissected at the anterior–posterior coordinates at bregma, −3.0 to −4.0 mm according to the atlas of Paxinos and Franklin [18], and then the PAG was isolated. For preparation of total fraction sample, the dissected tissues were lysed by brief sonication in ice-cold buffer: 20 mM HEPES, 0.4 M NaCl, 20% glycerol, 1% Nonidet P-40, 5 mM $MgCl_2$, 1 mM EDTA, 0.4 mM EGTA, 0.1 mM *p*-aminobenzamide (PABA), 1 mM phenylmethylsulfonyl fluoride (PMSF) and 5 mM dithiothreitol (DTT). The homogenate was centrifuged at 20,000 × *g* for 30 min. The protein concentration of the supernatant was evaluated by Bradford method (Coomassie