Short Communication

Antihyperalgesic Effect of Buprenorphine Involves Nociceptin/Orphanin FQ Peptide–Receptor Activation in Rats With Spinal Nerve Injury–Induced Neuropathy

Tomoko Takahashi1, Kazumasa Okubo1, Shota Kojima1, Hiroyuki Nishikawa1, Motohide Takemura2, Maho Tsubota-Matsunami1, Fumiko Sekiguchi1, and Atsufumi Kawabata1,*

1Division of Pharmacology & Pathophysiology, Kinki University School of Pharmacy, 3-4-1 Kowakae, Higashi-Osaka 577-8502, Japan
2Department of Oral Anatomy and Neurobiology, Osaka University Graduate School of Dentistry, 1-1 Yamada-oka, Suita 565-0871, Japan

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Abstract. We evaluated the effect of buprenorphine, a mixed agonist for μ-opioid receptors and nociceptin/orphanin FQ peptide (NOP) receptors, in neuropathic rats, using the paw pressure test. Buprenorphine, administered i.p. at 50, but not 20, μg/kg, exhibited naloxone-reversible analgesic activity in naïve rats. In contrast, buprenorphine at 0.5 – 20 μg/kg produced a naloxone-sensitive antihyperalgesic effect in the L5 spinal nerve–injured neuropathic rats. Intrathecal injection of [N-Phe1]nociceptin(1-13)NH2, a NOP-receptor antagonist, reversed the effect of buprenorphine in neuropathic rats, but not in naïve rats. Together, buprenorphine suppresses neuropathic hyperalgesia by activating NOP and opioid receptors, suggesting its therapeutic usefulness in treatment of neuropathic pain.

Keywords: buprenorphine, nociceptin/orphanin FQ peptide receptor, neuropathic pain

Buprenorphine is a partial μ-opioid receptor agonist with antagonistic activity toward κ-opioid receptors (1). Buprenorphine is also capable of activating nociceptin/orphanin FQ peptide (NOP) receptors (2). The analgesic activity of buprenorphine is mediated by activation of μ-opioid receptors, and unaffected or reduced by activation of NOP receptors in rodents (3, 4). Buprenorphine is now considered a broad spectrum analgesic, since it exhibits analgesic activity in a broad range of rodent models of acute and chronic pain including neuropathic pain (5, 6) and in neuropathic pain patients (1, 7). Although the effectiveness of typical opioid analgesics such as morphine in neuropathic pain is controversial in general, the complex and unique pharmacological properties of buprenorphine including the ceiling effect on respiratory depression may provide some advantages over other μ-opioid agonists (1, 7). Most interestingly, there is recent evidence that coactivation of μ-opioid receptors and NOP receptors produces synergistic analgesia in primates (4). We thus hypothesized that the potent effect of buprenorphine on neuropathic pain might result from dual activation of μ-opioid receptors and NOP receptors. In the present study, we therefore evaluated the antihyperalgesic effect of buprenorphine in rats with spinal nerve injury–induced neuropathy and examined the roles of NOP receptors in addition to opioid receptors in the buprenorphine-induced antihyperalgesic and analgesic effects in neuropathic and naïve animals, respectively.

Male Wistar rats (7-week-old at the beginning of the study) were purchased from Japan SLC, Inc. (Shizuoka). All animals were used with approval by the Kinki University School of Pharmacy’s Committee for the Care and Use of Laboratory Animals, and all experiments were performed according to the Guiding Principles approved by The Japanese Pharmacological Society. Mechanical nociceptive threshold was assessed by the paw pressure test, using an analgesia meter (MK-300; Muromachi Kikai Co., Tokyo), as described previously (8). Nociceptive thresholds are presented as the percentage
of the baseline threshold and/or as AUC (area under the curve) for the time course of the nociceptive threshold. For creation of a neuropathy pain model, the right L5 spinal nerve of the rat was tightly ligated and cut under anesthesia with pentobarbital (40 mg/kg, i.p.), as described elsewhere (9) with some modifications (10). The rat was used 14 – 21 days after the operation. For intrathecal administration, a PE-10 tube was inserted into the lumber subarachnoid cavity from the space between the L5-L6 vertebrae in rats under pentobarbital anesthesia, as reported previously (8, 11). Chemicals in a volume of 10 μL were injected through the catheter, followed by flushing with 10 μL of saline. Buprenorphine hydrochloride, morphine hydrochloride, and naloxone hydrochloride were purchased from Johnson Matthey Macfarian Smith (London, UK), Takeda Pharma Co., Ltd. (Osaka) or Sigma-Aldrich (St. Louis, MO, USA), respectively. [N-Phe¹]nociceptin(1-13)NH₂, a NOP-receptor antagonist, was obtained from Sigma-Aldrich. Buprenorphine hydrochloride was dissolved in saline containing 0.2% Tween 20 (Kishida Chemical Co., Ltd.), and all other chemicals were dissolved in saline. [N-Phe¹] nociceptin(1-13)NH₂ was administered intrathecally 25 min after i.p. injection of buprenorphine, considering the previous evidence for analgesic roles of spinal, but not supraspinal, NOP receptors in neuropathic animals (12, 13). Naloxone was administered i.p. 25 min after i.p. buprenorphine because both spinal and supraspinal opioid receptors are known to mediate the analgesia caused by narcotic agents. All data are expressed as mean ± S.E.M. The data were analyzed by Student’s \( t \)-test for two group comparisons or by Tukey’s test for comparisons of three groups or more. A value of \( P < 0.05 \) was considered statistically significant.

In naïve rats, i.p. administration of buprenorphine at 50 μg/kg, but not 20 μg/kg, significantly elevated the mechanical nociceptive threshold (Fig. 1A), an effect reversed by i.p. naloxone, an opioid receptor antagonist, at 1 mg/kg (data not shown). In the rats with L5 spinal nerve injury, the nociceptive threshold in the ipsilateral, but not contralateral, hindpaw decreased to approximately 60% of the baseline (Fig. 1B). Buprenorphine at 0.5, 2, and 20 μg/kg reversed the neuropathic hyperalgesia in a dose-dependent manner (Fig. 1B). The AUC of the time-threshold curves for 2 h showed that the effective dose range of buprenorphine was 2 – 20 μg/kg in the ipsilateral hindpaw, while no significant effect of buprenorphine at the same doses was detected in the contralateral hindpaw (Fig. 1: C, D). On the other hand, morphine at 1 mg/kg produced a significant analgesic effect in both ipsilateral and contralateral hindpaws (Fig. 1: C, D). In the neuropathic rats, intrathecal administration of [N-Phe¹]nociceptin(1-13)NH₂, a NOP-receptor antagonist, at 20 nmol/rat (27.6 μg/rat) as well as i.p. administration of naloxone at 1 mg/kg reversed the antihyperalgesic effect of i.p. buprenorphine at 20 μg/kg.

![Fig. 1.](image-url)
in the ipsilateral hindpaw (Fig. 2A). In contrast, intrathecal [N-Phe 1]nociceptin(1-13)NH₂ at the same dose did not affect the analgesic effect of i.p. buprenorphine at 50 μg/kg in naïve rats (Fig. 2B).

Our data clearly show that buprenorphine potently suppresses neuropathic hyperalgesia in a dose range, 2 – 20 μg/kg, that is lower than the minimal effective dose, 50 μg/kg, in naïve rats. This increased analgesic potency of buprenorphine is not attributable to functional upregulation of opioid receptors in neuropathic rats, since the analgesic potency of morphine in the ipsilateral hindpaw of the neuropathic rats was similar to that in the contralateral hindpaw of the neuropathic rats (see Fig. 1: C, D) and also in the hindpaw of the naïve rats (data not shown). Therefore, acting points including NOP receptors other than opioid receptors should contribute to the antihyperalgesic effect of buprenorphine in neuropathic rats. Actually, our study demonstrates that the antihyperalgesic effect of buprenorphine at the lower doses involves activation of both NOP receptors and opioid receptors in neuropathic rats, while the analgesic effect of buprenorphine at the higher doses is mediated predominantly by opioid receptor activation, but independent of NOP receptors, in naïve rats. Synergistic analgesia caused by coactivation of NOP receptors and μ-opioid receptors has been reported in primates (4) and rodents (12). Interestingly, NOP receptors in the dorsal root ganglia (DRG) are upregulated in rats with neuropathy induced by partial sciatic nerve transection (14). It has also been reported that intrathecal administration of NOP at 0.1 – 10 μg/rat had no effect on nocicep-
3 Yamamoto T, Shono K, Tanabe S. Buprenorphine activates mu and opioid receptor like-1 receptors simultaneously, but the analgesic effect is mainly mediated by mu receptor activation in the rat formalin test. J Pharmacol Exp Ther. 2006;318:206–213.


