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# Buprenorphine alleviates neuropathic pain-like behaviors in rats after spinal cord and peripheral nerve injury

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**Abstract**

We have studied and compared the antinociceptive and anti-hyperalgesic effect of the partial opioid receptor agonist buprenorphine in normal and neuropathic rats. In normal rats, systemic buprenorphine produced dose-dependent antinociception on the hot plate test. In rats with peripheral nerve or spinal cord injury, buprenorphine markedly alleviated neuropathic pain-related behaviors, including mechanical and cold allodynia/hyperalgesia at doses comparable to that producing antinociception. The results suggest that buprenorphine may be a useful analgesic for treating neuropathic pain and thus is an atypical opioid since morphine tends to be less potent after nerve injury.


*Keywords:* Morphine; Opioid; Sciatic nerve; Spinal cord

## 1. Introduction

The relief of neuropathic pain after injury to the peripheral or central nervous system is difficult in clinical pain management (Bennett, 1994). The effect of opioids in neuropathic pain is controversial (Arnér and Meyerson, 1988; Portenoy et al., 1990; Rowbotham, 1998). The most generally held current opinion is that opioid analgesics are effective in some conditions of neuropathic pain, such as postherpetic neuralgia and diabetic neuropathy (Moulin, 1998; Rowbotham, 1998). There is, however, both clinical (Moulin, 1998; Arnér, 2000) and experimental (Xu and Wiesenfeld-Hallin, 1991; Xu et al., 1992; Mao et al., 1995; Ossipov et al., 1995; Xu and Wiesenfeld-Hallin, 1998) evidence indicating that neuropathic pain may be relatively insensitive to typical μ-opioid analgesics such as morphine. Decrease in opioid sensitivity after nerve injury has been linked to several mechanisms, including loss of opioid receptors on primary afferents and spinal cord (Zhang et al., 1998), a relative ineffectiveness of opioids in treating pain arising from activity in large diameter myelinated afferents and increased activity of anti-opioid systems after nerve injury (Xu and Wiesenfeld-Hallin, 1998).

Buprenorphine is a synthetic opioid that has been traditionally regarded as a partial μ-opioid receptor agonist. Recent evidence suggests, however, that the effect of buprenorphine may be mediated by mechanisms that are very different from those of classical μ-opioid receptor agonists such as morphine (see McCormack et al., 1998; McCormack, 1999 for review). Thus, buprenorphine exhibited considerable affinity to both δ- and κ-opioid receptors (Gourlay, 1998) and unlike most μ-opioids, buprenorphine-induced antinociception is not sensitive to pretreatment of pertussis toxin, which uncouples many G-proteins (Wheeler-Aceto and Cowan, 1991). Moreover, buprenorphine is particularly sensitive to changes in an ATP-sensitive $K^+$ channel (Ocana et al., 1995) and receptor binding of buprenorphine is not typical for an opioid (Rothman et al., 1995). Finally, in several assays, buprenorphine exhibited unexpectedly strong efficacy, such as in the formalin test in neonatal rats (McLaughlin and Dewey, 1994), the cold tail flick test (Wang et al., 1995) and inhibition of diffuse noxious inhibitory control (Guirimand et al., 1995).

These and other unique properties of buprenorphine lead to the suggestion that buprenorphine may be effective in treating neuropathic pain, which was largely based on the

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assumption that the neonatal formalin test and intrathecal pertussis toxin-induced hyperalgesia may be models of neuropathic pain (McCormack et al., 1998; McCormack, 1999). However, to our knowledge, there have been no published reports on the effect of buprenorphine in models of neuropathic pain after nerve injury. We have, thus, carried out experiments examining and comparing the antinociceptive effect of buprenorphine and its effect on neuropathic pain-like behaviors in rats after partial sciatic nerve ischemic injury (Kupers et al., 1998). We have also tested the effect of buprenorphine in spinal cord-injured rats, a model of central neuropathic pain that is particularly resistant to systemic morphine (Xu et al., 1992; Yu et al., 1997).

## 2. Methods

Male and female Sprague–Dawley rats (Mollegård, Denmark) weighing 200–250 g at the start of the experiments were used. All experimental procedures were approved by the local research ethics committee.

### 2.1. Hot plate test

A hot plate (IITC; Woodland Hills, CA, USA) maintained at 54 ± 0.1 °C was used to assess the antinociceptive effect of buprenorphine in normal male rats. The latency of licking a hindpaw was measured with an accuracy of 0.1 s and the cut-off time was 30 s. The rats were trained on the hot plate for 4 days before drug administration in order to obtain a stable baseline response of 3–6 s. On the day of the experiment, two control latencies were measured before the rats were injected subcutaneously (s.c.) with saline or buprenorphine at 0.03, 0.1 or 0.3 mg/kg. The animals were tested 15, 30, 60, 90, 120 and 180 min after drug administration.

### 2.2. Photochemically induced ischemic spinal cord injury

Female Sprague–Dawley rats were subjected to an ischemic spinal cord injury according to methods described previously (Xu et al., 1992). Briefly, the rats were anaesthetized with chloral hydrate (300 mg/kg, i.p.) and one jugular vein was cannulated. A midline incision was made in the skin overlying vertebral segments T12–L1. The animals were positioned beneath an argon laser beam and irradiated for 10 min with the beam directed towards vertebral segment T12 or T13 (spinal segments L3–5). Immediately prior to and 5 min after the start of the irradiation, erythrosin B (Red No. 3, Aldrich-Chemie, Steinheim, Germany) dissolved in 0.9% saline was injected i.v. at a dose of 32.5 mg/kg. A tunable argon ion laser (Innova model 70, Coherent Laser Product Division, Palo Alto, CA) operating at 514 nm was used. The average beam output power was 160 mW. During irradiation, the temperature of the rats was maintained at 37–38 °C.

We have previously reported that a subset of spinally injured rats developed a chronic pain syndrome, including marked mechanical and cold allodynia. Sixteen rats injured in 3–6 months were previously used to examine the effect of buprenorphine. The mechanical allodynia was assessed by examining the vocalization thresholds to graded mechanical touch/pressure applied with von Frey hairs. During testing, the rats were gently restrained in a standing position and the von Frey hair was pushed onto the skin until the filament became bent. The frequency of the stimulation was about 1/s and at each intensity, 5–10 stimuli were applied. The intensity of stimulation that induced consistent vocalization (>75% response rate) was considered as a pain threshold. The response to cold was tested with ethyl chloride spray (Medikema, Perstrop, Sweden) applied to the shaved allodynic skin area. Response was graded with a score of 0 = no observable response; 1 = localized response (skin twitch and contraction), no vocalization; 2 = transient vocalization, moderate avoidance; and 3 = sustained vocalization and avoidance.

### 2.3. Photochemically induced peripheral nerve injury

Male Sprague–Dawley rats were anesthetized with chloral hydrate and the common sciatic nerve was exposed at mid-thigh level, gently dissected from the surrounding tissue over a distance of about 1 cm proximal to trifurcation. The part of the nerve just proximal to the trifurcation was irradiated for 2 min with the laser using parameters identical to spinal cord irradiation after i.v. administration of erythrosin B. After irradiation, the wound was closed in layers. The contralateral nerve was exposed but not irradiated.

Behavioral tests were conducted before, 3 and 7 days after irradiation. The rats were put in chambers with metal mesh floors. Von Frey hairs were used to assess mechanical allodynia. They were applied in ascending order on the plantar surface of the hindpaw at a frequency of 1/s. The lowest force at which the animal withdrew the paw in at least two of the three trials was taken as mechanical withdrawal threshold. The strongest mechanical stimulus was 63 g as stronger von Frey hairs lifted the paw. The response to cold was tested with ethyl chloride, which was briefly (< 1 s) sprayed on the plantar surface of the hindpaw. The responses were scored as the following: 0 = no response, 1 = startle-like response, no hindpaw withdrawal, 2 = brief withdrawal of the stimulated hindpaw, 3 = sustained or repeated withdrawal of the stimulated hindpaw, brief licking or shaking, and 4 = prolonged withdrawal, shaking and licking of the hindpaws, vocalization and generalized aversive reactions.

### 2.4. Drugs

Buprenorphine (Tamgesic, Schering-Plough, Stockholm, Sweden) was diluted in 0.9% physiological saline and injected s.c. in a volume of 1 ml/kg. All experiments were

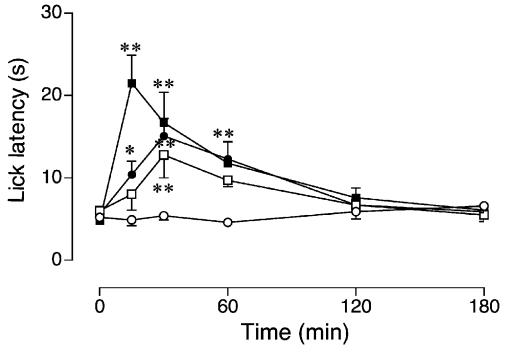


Fig. 1. Effects of systemic saline (open circles), buprenorphine at 0.03 (open squares), 0.1 (filled circles) and 0.3 (filled squares) mg/kg on the lick latency of the hot plate test in rats ($n=8$ in each group). The data are expressed as mean ± S.E.M. ANOVA with repeated measures indicated a significant overall increase in lick latency after all three doses of buprenorphine compared to saline. Individual comparison is made with Dunnett's test, $*P<0.05$ and $**P<0.01$ compared to baseline value at time 0.

conducted in a blind fashion with physiological saline as control.

### 2.5. Statistics

The data from the von Frey hair test and cold test are expressed as median ± median absolute deviation (M.A.D.) and analysed with Friedman analysis of variance (ANOVA) followed by Wilcoxon signed-ranks' test and the other data are presented as mean ± S.E.M. and analysed with ANOVA followed by Fisher's Protected Least Significant Difference (PLSD) test.

## 3. Results

### 3.1. Effects of s.c. buprenorphine on hot plate latency in rats

S.c. administration of buprenorphine, but not saline, produced a dose-dependent increase in lick latency in normal rats on the hot plate test (Fig. 1). At 0.03 mg/kg, buprenorphine already produced a significant increase in lick latency at 30 min. The effect of buprenorphine at the two higher doses was more potent and prolonged (Fig. 1). The peak effect of 0.3 mg/kg buprenorphine was stronger than 0.1 mg/kg, but the effect was only significant from baseline at 15 and 30 min, whereas the effect of 0.1 mg/kg buprenorphine was significant up to 60 min after administration. No signs of side effects such as sedation or motor impairment were noted following buprenorphine administration at any dose.

### 3.2. Effect of buprenorphine on sciatic nerve injury-induced mechanical and cold hypersensitivity

Mechanical hyperalgesia was observed following photochemically induced ischemia in the sciatic nerve (Fig. 2A). There was a significant decrease in paw withdrawal threshold to von Frey hairs starting 1 day following the nerve injury and peaked at 3 and 7 days. The response to cold was also increased with a time course similar to mechanical hypersensitivity (not shown). The changes in mechanical and cold sensitivity occurred bilaterally, although the hypersensitivity was more severe and consistent on the injured side.

Repeated s.c. saline administration had no effect, whereas cumulative doses of buprenorphine effectively

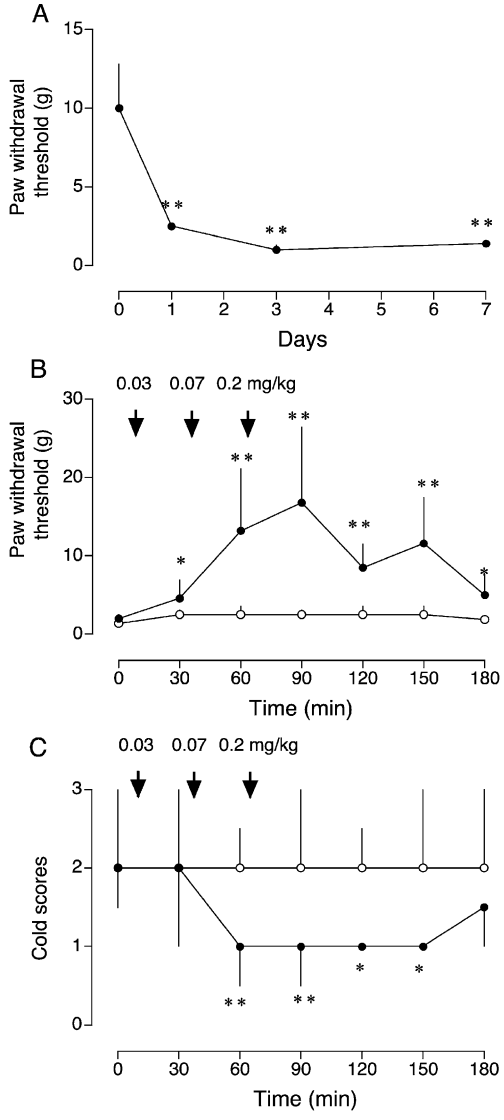


Fig. 2. (A) Development of mechanical hypersensitivity in the paw ipsilateral to partial sciatic nerve ischemic injury. The data represent the median paw withdrawal threshold to stimulation with von Frey hairs just before (day 0), 1, 3 and 7 days after nerve irradiation in 12 rats. Variability is expressed as median absolute deviation (M.A.D.). $**P<0.01$ compared to pre-irradiation level with Wilcoxon signed-ranks test. B and C show the effect of cumulative s.c. buprenorphine (filled circles) or repeated s.c. saline (open circles) on mechanical (B) and cold (C) hypersensitivity for the ipsilateral paw on post irradiation day 7. The data are expressed as median ± M.A.D. and there were 12 rats in each group. $*P<0.05$ and $**P<0.01$ compared to predrug value (time 0).

alleviated mechanical and cold hypersensitivity after nerve ischemia in rats (Fig. 2B and C). At 0.03 mg/kg, the effect on mechanical hypersensitivity was modest but significant, and at two higher doses, cumulative 0.1 and 0.3 mg/kg, buprenorphine totally normalized paw withdrawal threshold to mechanical stimulation and normalized the cold response (Fig. 2B and C). The effect after 0.3 mg/kg cumulative dose of buprenorphine lasted 90–120 min. Again, no sedation or motor impairments were noted.

### 3.3. Effect of buprenorphine on spinal cord injury-induced mechanical and cold hypersensitivity

Spinally injured rats developed chronic pain-related behavior, manifested as reduction in vocalization threshold to mechanical stimulation (from normal values of 73–95 to 0.7–5.5 g) and as increased response to cold stimulation (Fig. 3). As in peripheral nerve-injured rats, s.c. cumulative doses of buprenorphine markedly alleviated the mechanical and cold allodynia-like behaviors in spinally injured rats (Fig. 3). At 0.03 mg/kg, buprenorphine significantly increased vocalization threshold to mechanical stimuli and normalized the cold response. Higher doses of buprenorphine raised the mechanical vocalization threshold above normal level and at 0.3 mg/kg, buprenorphine totally abolished cold hyperalgesia. In fact, the cold response was reduced below normal level after the highest dose of buprenorphine (Fig. 3). The effect of buprenorphine at 0.3 mg/kg lasted more than 120 min and no sedation or motor impairment was seen. Repeated s.c. saline produced no effect (Fig. 3).

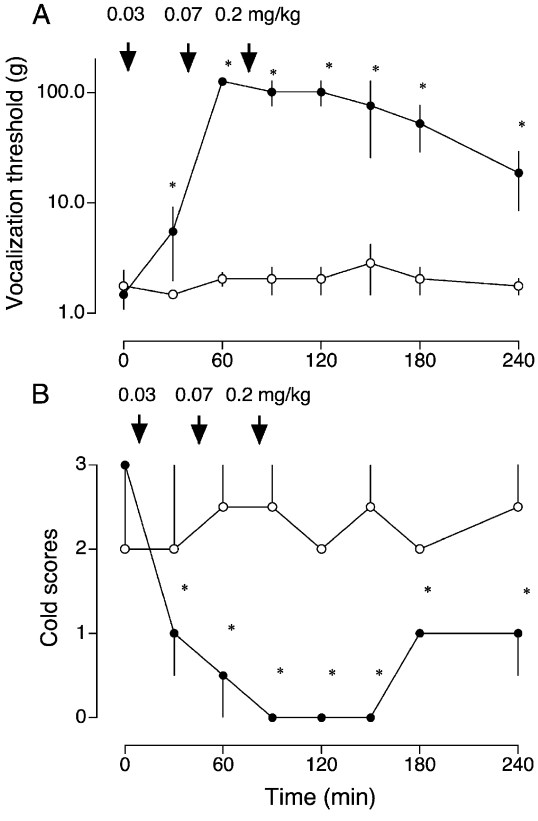


Fig. 3. Effect of cumulative s.c. doses of buprenorphine (filled circles) or repeated saline injection (open circles) on mechanical (A) and cold (B) hypersensitivity in spinally injured rats. The data are expressed as median ± MAD and there were eight rats in each group. $*P<0.05$ compared to predrug sensitivity (time 0) with Wilcoxon signed-ranks test.

## 4. Discussion

In this study, s.c buprenorphine effectively alleviated mechanical and cold hyperalgesia/allodynia-like behaviors in rats with spinal injury and partial sciatic nerve injury. The anti-hyperalgesic effect of buprenorphine was observed at the same doses as those producing moderate antinociception on the hot plate test in normal rats. The effect was already significant at the lowest dose and total alleviation of pain was seen after 0.1 and 0.3 mg/kg. Importantly, there was no sedation associated with the pain-relieving effect of buprenorphine. This is different from the effect of morphine in these two models. At least 5 mg/kg morphine was required to produce anti-hyperalgesia (Xu et al., 1992; Yu et al., 1997; Kauppila et al., 1998; Bulka et al., 2002), which produced sedation. Sedation could interfere with behavioral testing, particularly for the model of spinal cord injury pain where vocalization was used as endpoint. It is also interesting to note that the antinociceptive effect of buprenorphine at these doses did not reach cut-off on the hot plate test, whereas the reversal of abnormal pain-related behaviors in both neuropathic pain models was total and prolonged.

The receptor subtype selectivity, binding characteristics and coupling to G-protein of buprenorphine to opioid receptors is quite different from that of typical μ-opioid receptor agonists (Wheeler-Aceto and Cowan, 1991; Ocana et al., 1995; Rothman et al., 1995; Gourlay, 1998). This has lead to the suggestion that the analgesic mechanisms for buprenorphine may also be different as demonstrated in several functional models (Wheeler-Aceto and Cowan, 1991; McLaughlin and Dewey, 1994; Guirimand et al., 1995; Wang et al., 1995). Our results may support this hypothesis. One of the interesting parallels between our results and that of previous studies is the effect of buprenorphine on cold hyperalgesia in spinally injured rats. Thus, Wang et al. (1995) showed that buprenorphine produced a potent antinociception in the cold tail flick test ( − 20 °C) at doses lower than those required to produce heat antinociception. In the present study, buprenorphine totally abolished cold hyperalgesia in spinally injured rats and actually caused analgesia, which has been observed in this model only following high dose intrathecal DAMGO, a highly efficacious μ-opioid receptor agonist (Hao et al., 1998).

Buprenorphine is used clinically to treat moderate to severe nociceptive pain, such as post-operative pain. It has also been suggested to be useful to treat opioid withdrawal with efficacy comparable to methadone (Johnson et al.,

2002). Our results support the hypothesis of McCormack et al. (1998); thus, buprenorphine may be effective for treating neuropathic pain, including central pain.

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