Original Article

Anti-allodynic Effect of Nefopam and Morphine in a Rat Model of Neuropathic Pain

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Abstract

Background: Neuropathic pain is a chronic pain due to a disorder in the peripheral or central nervous system with different pathophysiological mechanisms. Current treatments are not effective. Here we compared the analgesic effect of nefopam, and morphine in chronic constriction injury (CCI) model of neuropathic pain.

Methods: Male wistar rat (150-200g, n=8) were divided into 3 different groups: 1- Saline-treated CCI group, 2- Saline-treated sham group, and 3- Drug-treated CCI groups. In CCI model of neuropathic pain, the left sciatic nerve was exposed and 4 loose chromic gut ligatures were placed around the nerve proximal to the trifurcation. Ketamine 60mg/kg and xylazine 10 mg/kg were used for anesthesia. Nefopam (10, 20, 30mg/kg), and morphine (1, 3, 5mg/kg) were injected 30 minutes before surgery and continued daily to day 14 post-ligation. Von Frey filaments for mechanical allodynia and acetone test for cold allodynia were respectively used as pain behavioral tests. Experiments were performed on day 0 (before surgery) and days 1, 3, 5,7,10 and 14 post injury. Behavioral studies were performed in a quiet room between 9:00 to 11:00 AM. All experiments followed the IASP guidelines on ethical standards for investigation of experimental pain in animals.

Results: Nefopam (20 and 30mg/kg) blocked mechanical and cold allodynia during the experimental period, but the analgesic effects of morphine (5mg/kg) lasted for 7 days.

Conclusions: It seems that nefopam could effectively reduce pain behavior compared to morphine with reduced adverse effects.

Key Words: neuropathic pain, nefopam, morphine, allodynia.

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Introduction

Neuropathic pain can arise as a result of damage to the peripheral or central nervous system and includes a variety of conditions that differ in etiology as well as location. Sensory abnormalities which manifest as allodynia (pain evoked by normally non-noxious stimuli), and hyperalgesia (an increased response to a noxious stimuli) are routinely observed in human neuropathic pain conditions as well as in relevant animal models [1]. Neuropathic pain affects 2 to 3% of the population in developed countries can be particularly severe and debilitating, and has a profound effect on quality of life [1, 2]. Many drugs are tried to reduce neuropathic pain but the underlying mechanisms of neuropathic pain are multiple and complex, therefore treatment and management of this distressing condition are suggesting the use of more than one type of medication [1].

Among analgesics, morphine is a widely used drug in the treatment of moderate to severe pain. There is considerable controversy for opioid analgesics to treat

chronic pain [3]. Opioids were reported to be ineffective in some patients with neuropathic pain [1], whereas other observations suggest that opioids are effective in attenuating neuropathic pain [2, 3]. The most important point to consider in the use of opioids in neuropathic pain, is their side effects (respiratory depression, sedation, tolerance and constipation) which limit their application [4].

Nefopam, a non opioid analgesic possesses a profile distinct from that of opioids or anti-inflammatory drugs. It does not cause tolerance, withdrawal reactions or physical dependence, and the potential for its abuse is very low. This drug has been demonstrated to induce a rapid and strong depression of the nociceptive reflex in humans, probably through a central mechanism of action. Furthermore, nefopam does not produce respiratory depression even in the post-operative period [5]. Some unpleasant adverse effects consistent with a central mode of action of the drug have also been reported during therapeutic use and include dizziness, headache, nausea, vomiting and sweating. However, the detailed mechanisms underlying the pharmacological actions of nefopam remain unclear [6]. The antinociceptive effect of nefopam has been shown in animal models of acute and chronic pain and in human. Nefopam reduced pain in some behavioral tests **(**the hot plate [7], formalin [8]; carrageenan and incision induced thermal hyperalgesia tests [9]). Moreover, many clinical studies have evaluated the analgesic efficacy of nefopam in postoperative pain [10, 11], and a protective analgesic effect when used as a single dose in the CCI model of chronic neuropathic pain [12].

On this background, our study was designed to evaluate the antiallodynic effect of nefopam in comparison to morphine in chronic constriction injury (CCI) model of neuropathic pain in rat.

Methods

Animals

Experiments were carried out on male Wistar rats (150-200g), that were housed one rat per cage and placed under a 12 hour light/dark cycle in a temperature-controlled room (22 ± 1ºC). Animals had free access to food and water. Rats were divided randomly into several experimental groups, each made-up of 8 animals. All experiments followed the IASP guidelines on ethical standards for investigation of experimental pain in animals [13]. The animals were allowed to habituate to the housing facilities for one week before the experiments began. Behavioral studies were performed in a quiet room between 9:00 to 11:00 AM. Efforts were made to limit distress and use the minimum number of animals necessary to achieve statistical significance.

Surgery

We used the CCI model of neuropathic pain [14]. The surgical procedure was performed under ketamine (60 mg/kg) and xylazine (10 mg/kg) anaesthesia. The left sciatic nerve was exposed and 4 loose chromic gut ligatures were placed around the nerve proximal to the trifurcation**.** The distance between the two adjacent ligatures was 1 mm. The wound was irrigated with saline (0.9%) and closed in two layers with 4-0 silk (facial plane) and surgical skin staples. In the saline-treated sham group, rats underwent the same surgical procedure except for the ligation.

Drug preparation

Nefopam (Biocodex Laboratories, France) and morphine (Sigma, USA) were dissolved in saline 0.9%. Ketamine hydrochloride (Sigma, USA) and xylazine hydrochloride (Sigma, USA) were used for anesthesia. All drugs were injected by the intra-peritoneal (i.p.) route.

Drug administration

Animals were randomly divided into three experimental groups: 1- Saline-treated CCI group, 2- Saline-treated sham group, and 3- Drug-treated CCI groups. Animals received morphine (1, 3, 5 mg/kg) [15], nefopam (10, 20, 30 mg/kg) (12). Drugs were injected 30 minutes before surgery and continued daily to day 14 post-ligation. All behavioral tests were recorded on day 0 (control day) before the surgery and on days 1, 3, 5, 7, 10, and 14 post-nerve injury. The order of pain testing was mechanical and cold allodynia respectively (the interval between each test was 30 minutes).

Behavioral tests and experimental design

The sciatic nerve territory (mid-plantar hind paw) was tested for sensitivity to noxious and innocuous stimuli using standard behavioural assays done sequentially at several intervals up to 14 days following surgery. Animals were acclimated to the testing chambers for

30 min prior to testing. Mechanical and cold allodynia were evaluated in the animals.

Mechanical allodynia

Mechanical sensitivity to non-noxious stimuli was measured by applying a set of calibrated nylon monofilaments (Stoelting, USA). The von Frey methodology was used to assess the sensitivity of the skin to tactile stimulation. Von Frey filaments are calibrated to have a characteristic bending force when pressure is applied. Each rat was placed under a transparent plexiglass cage on an elevated metal screen surface with 1 cm mesh openings. Increasing strengths of von Frey filaments were applied sequentially to the plantar surface of the left hind paw of each animal. The minimum paw withdrawal threshold (PWT), defined as the minimum gram strength eliciting two sequential responses with 3 min intervals between them (withdrawal from pressure), and was recorded for the left paw. The intensity of mechanical stimulation was increased from 2 to 60 g in a graded manner using successively greater diameter filaments until the hind paw was withdrawn. For successive tests, the placement of these stimuli was varied slightly from one trial to the next to avoid sensitization of the hind paw [16].

Cold allodynia

The acetone test [17] was used to determine the reactivity to acetone stimulus. Rats were placed under a transparent plexiglass cage, as described previously, and an acetone bubble was formed at the end of a piece of small polyethylene tubing that was connected to a syringe; then, the bubble was lightly touched to the heel. The acetone was applied 5 times with an interval of 1 min between application, and the number of paw lifts from surface was the response measured. The response was calculated as the percent of paw withdrawal frequency (%PWF) using the following equation: (Number of paw withdrawals/5 trials) × 100.

Statistical analysis

Parametric data were analyzed for significance using an analysis of variance (ANOVA) followed by a post-hoc Tukey's test. Non-parametric data were analyzed using 2 related samples followed by the Wilcoxon test. In all cases, P<0.05 was considered significant.

Results

Response to mechanical allodynia (von Frey Filament test)

Figure 1: In the von Frey test, all saline-treated CCI groups were strongly allodynic, at the fifth day post-ligation compared to the control day (P<0.001); this effect was sustained until the end of the study. In the contrary, the saline-treated sham group did not show pain behavior during the period of the study, in the drug-treated CCI groups, nefopam reduced mechanical allodynia at 20 and 30 mg/kg doses, but not at the dose of 10 mg/kg (p<0.05) (Figure 1A); morphine (5 mg/kg) decreased tactile allodynia until day 7, but not at the 1 and 3 mg/kg doses (p<0.05) (Figure 1B).

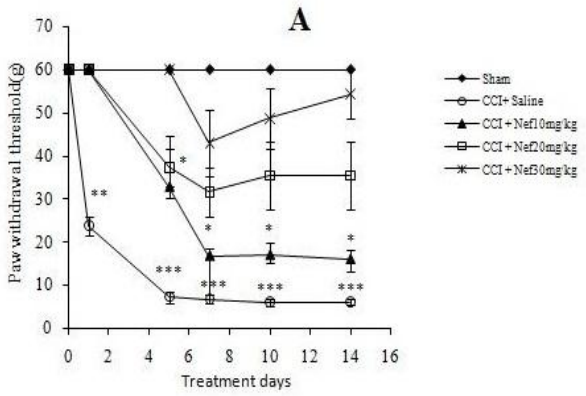

Figure 1A: Paw withdrawal threshold in response to von Frey filaments before and at several time points after surgery in saline-treated CCI group, saline-treated sham group and drug-treated CCI group. Nefopam (10, 20, 30mg/kg) was injected i.p. Results are expressed as the mean±SEM of 8 animals per group. Nef: Nefopam, Asterisks indicate a significant difference between post-surgery days compared with day 0 (* p<0.05, ** p<0.01, ***p<0.001).

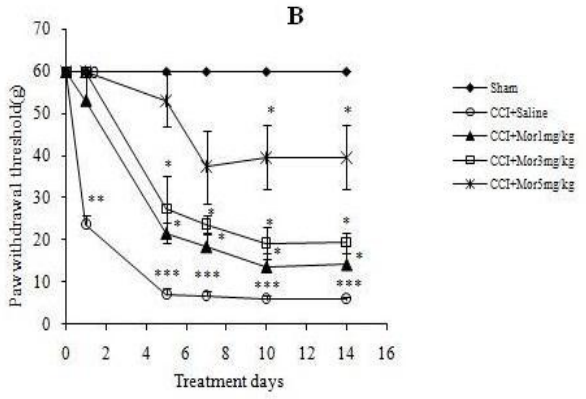

Figure 1B: Paw withdrawal threshold in response to von Frey filaments before and at several time points after surgery in saline-

treated CCI group, saline-treated sham group and drug-treated CCI group. Morphine (1, 3, 5mg/kg) was injected i.p. Results are expressed as the mean±SEM of 8 animals per group. Mor: Morphine. Asterisks indicate a significant difference between post-surgery days compared with day 0 (* p<0.05, ** p<0.01, ***p<0.001).

Response to cold allodynia (Acetone test)

Figure 2: In the acetone test, the saline-treated CCI group, showed a significant difference in pain behavior (P<0.001) at the fifth day post-injury compared to day 0; this effect continued until the end of the study. However, cold allodynia was not observed in the saline-treated sham group. In the drug-treated CCI group, nefopam reduced cold allodynia at the dose of 20 and 30 mg/kg, but not at the 10 mg/kg dose (p<0.05) (Figure 2A). The antiallodynic effect of morphine (5mg/kg) lasted for 5 days, but not at the dose of 1 and 3mg/kg (P<.0.01) (Figure 2B).

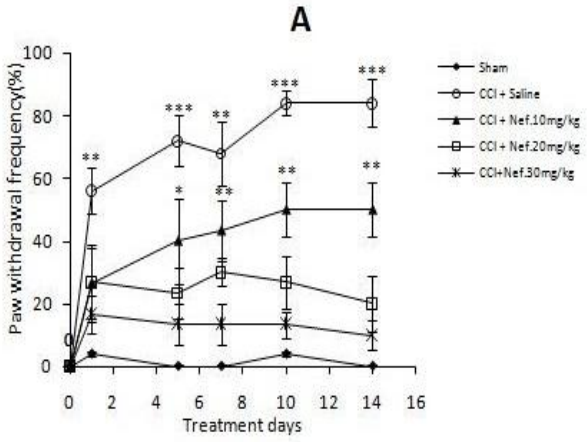

Figure 2A: The frequency of paw withdrawal in response to acetone before and at several time points after surgery in saline-treated CCI group, saline-treated sham group and drug-treated CCI group. Nefopam (10, 20, 30mg/kg) was injected i.p. Results are expressed as the mean±SEM of 8 animals per group. Nef: Nefopam. Asterisks indicate a significant difference between post-surgery days compared with day 0 (p<0.05, ** p<0.01, ***p<0.001).

Discussion

In this study the analgesic effects of nefopam, and morphine were investigated preemptively, in a rat model of neuropathic pain. Pre-emptive analgesia can provide an effective treatment which prevents the establishment of pain. Based on the animal model studies, it was suggested that pre-emptive and early treatment can be more effective than treatment of established pain [18]. We used CCI model of nerve injury which is reported to mimic types of neuropathic pain found in human [14].

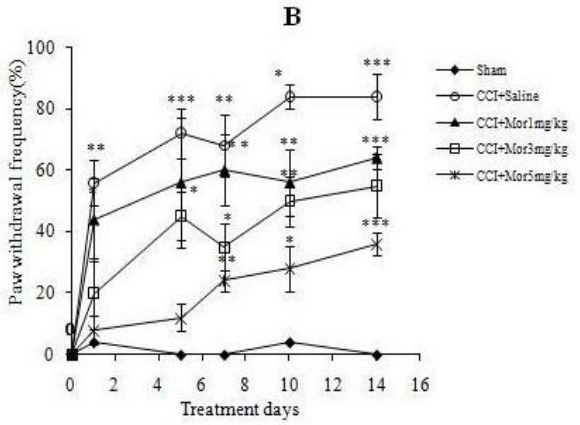

Figure 2B: The frequency of paw withdrawal in response to acetone before and at several time points after surgery in saline-treated CCI group, saline-treated sham group and drug-treated CCI group. Morphine (1, 3, 5mg/kg) was injected i.p. Results are expressed as the mean±SEM of 8 animals per group. Mor: Morphine. Asterisks indicate a significant difference between post-surgery days compared with day 0 (p<0.05, ** p<0.01, ***p<0.001).

The management of neuropathic pain remains a major clinical challenge due to the relative absence of clinically effective treatments [19]. This is in part due to an inadequate understanding of the mechanisms involved in the etiology of the disease.

Opioids currently represent the best option for the management of moderate to severe trauma induced perioperative, cancer pain and also they are increasingly used for non-cancer associated chronic pathological pain. Although a large number of clinical studies have reported that opioids, particularly morphine, had weak analgesic efficacy in neuropathic pain in humans [20, 21]. However, prolonged administration of opioids is associated with significant problems including the development of tolerance to its analgesic effects, and as a result higher doses of the drug are required over time to elicit the same degree of analgesia. It was reported that repeated administration of morphine or fentanyl also results in increasing pain sensitivity, a syndrome clinically known as opioid-induced hyperalgesia [22, 23]. Several mechanisms have been proposed to explain the reduced analgesic efficacy of opioids in animal models of neuropathic

pain. These include loss of functional spinal μ-and δ-opioid binding sites, NMDA receptor-induced excitation of spinal neurons, antagonism of inhibitory opioid actions by cholecystokinin and activation of descending facilitatory controls [24, 25]. While it is thought that opioids modulate tactile hyperalgesia solely by acting at neuronal opioid receptors, chronic morphine administration is also known to induce a rapid increase in the expression of the proinflammatory cytokines such as TNFα, IL-1β and IL-6 in a number of cell types within the nervous system [26]. These proinflammatory cytokines which are powerful pain enhancing proteins that may, in turn, suppress acute opioid analgesia and contribute to the apparent loss of opioid analgesia upon repeated opioid administration (''tolerance'') [27, 28]. There are some controversies about relative efficacy of opiate analgesics against neuropathic pain in clinical and experimental researches [29]. Various studies have reported reduced antinociceptive efficacy of morphine in animal models of peripheral or central nerve injury. However, conflicting results regarding the efficacy of opioids in different animal models of neuropathic pain have been reported. While systemic administration of morphine attenuates allodynia and hyperalgesia in chronic constriction injury and spinal nerve ligation (SNL) models, intrathecal morphine is apparently ineffective. In contrast, intrathecal morphine dose-dependently reversed mechanical allodynia in a rat model of central pain, whereas systemic morphine had little effect on this measure [30]. Recently, these findings have been challenged by Zhao et al who reported antiallodynic effects of both systemic and intrathecally administered morphine in the spinal nerve injury (SNI), SNL and spinal cord injury (SCI) animal models of neuropathic pain. These results indicate that the efficacy of opioids in neuropathic pain is variable and seems to depend on several factors (e.g., the kind of nerve injury and the route of drug administration). Contradictory evidence about the efficacy of opioids in mechano-allodynia comes from studies on CCI model of neuropathic pain [14]. The multiple mechanisms involved in neuropathic pain is only one explanation for the controversial results with opioids in treating neuropathic pain patients [31]. In systemic injection, mechanical allodynia was reduced only when a higher concentration of morphine (5 mg/kg) was used. It is suggested that systemic morphine has limited effect on mechanical allodynia [32]. The preventive efficacy of morphine has been investigated and evidence showed that preemptive use of morphine produced a slight antiallodynic effect in CCI model of neuropathic pain [20].

In our research, lower doses of morphine (1 and 3 mg/kg) did not reduce pain behavior (mechanical and cold allodynia) during the experimental period. The antiallodynic effect of morphine was produced only in high dose (5 mg/kg) which lasted for 7 days, this result is consistent with the above mentioned studies. It seems that the reduced analgesic effect of morphine may be due to the tolerance of its analgesic effects.

On the other hand, preemptive administration of nefopam, produced a long lasting analgesic effect compared to morphine. The mechanism of action of nefopam is not precisely known, several mechanistic studies have suggested its inhibitory effect on the catecholamines and serotonin reuptake in the central nervous system [33]. Placebo-controlled trials suggest that nefopam was more analgesic than acetaminophen and equianalgesic with non-steroidal anti-inflammatory drugs. Indeed, nefopam appeared to be more analgesic than proparacetamol [34], and equianalgesic with ketamine [11]. It was reported that in CCI model of neuropathic pain, a single dose of nefopam, significantly reduced pain behavior. Moreover it was shown that nefopam has preventive analgesic effect [12]. Acute administration of nefopam exhibited a dose dependent attenuation of pain behavior in hot plate and plantar tests [7]. Given preemptively, nefopam may be effective at improving postoperative pain management and at reducing the risk of developing postoperative chronic pain, because the drug has both analgesic and antihyperalgesic properties [35]. In our experiment**,** we used nefopam preemptively and in a dose dependent manner. Nefopam 20 and 30 mg/kg showed pain reducing effects. Our data are in agreement with above mentioned studies. However it should be noted that nefopam 30 mg/kg produced a slight hyperexcitability state lasting for 15 min after drug administration. Therefore we suggest that nefopam 20 mg/kg could reduce pain behavior with lower incidence of side effects. Moreover nefopam was regarded as a generally well tolerated drug. It does not cause tolerance,

withdrawal reactions or physical dependence, and the potential for its abuse is very low [36].

Conclusion

Our results confirm previous findings concerning the analgesic efficacy of systemically administered morphine and nefopam in animal models of neuropathic pain. Based on previous studies on morphine analgesia in neuropathic pain, we also found that there is controversy in morphine efficacy in controlling pain in CCI model of neuropathic pain in rats. Nefopam a non opioid drug, effectively reduced pain behavior. It was reported that nefopam causes less adverse effects than morphine. Further studies are needed to evaluate the exact mechanism of action of nefopam in neuropathic pain.

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References

1. Arner S, Meyerson BA. Lack of analgesic effect of opioids on neuropathic and idiopathic
forms of pain. Pain 1988 Apr;33(1):11-23.
2. Raja SN, Haythornthwaite JA, Pappagallo M, Clark MR, Travison TG, Sabeen S, et al.
Opioids versus antidepressants in postherpetic neuralgia: a randomized, placebo-controlled trial. Neurology 2002 Oct 8;59(7):1015-21.
3. Wu CL, Tella P, Staats PS, Vaslav R, Kazim DA, Wesselmann U, et al. Analgesic effects of intravenous lidocaine and morphine on postamputation pain: a randomized double-blind, active placebo-controlled, crossover trial. Anesthesiology 2002 Apr;96(4):841-8.
4. Dobecki DA, Schocket SM, Wallace MS. Update on pharmacotherapy guidelines for the
treatment of neuropathic pain. Curr Pain Headache Rep 2006 Jun;10(3):185-90.
5. Fernandez-Sanchez MT, Diaz-Trelles R, Groppetti A, Manfredi B, Brini AT, Biella G, et al. Nefopam, an analogue of orphenadrine, protects against both NMDA receptor-dependent and independent veratridine-induced neurotoxicity. Amino Acids 2002;23(1-3):31-6.
6. Durrieu G, Olivier P, Bagheri H, Montastruc JL. Overview of adverse reactions to nefopam: an analysis of the French Pharmacovigilance database. Fundam Clin Pharmacol 2007 Oct;21(5):555-8.
7. Girard P, Pansart Y, Coppe MC, Gillardin JM. Nefopam reduces thermal hypersensitivity in acute and postoperative pain models in the rat. Pharmacol Res 2001 Dec;44(6):541-5.
8. Girard P, Coppe MC, Verniers D, Pansart Y, Gillardin JM. Role of catecholamines and serotonin receptor subtypes in nefopam-induced antinociception. Pharmacol Res 2006 Sep;54(3):195-202.
9. Girard P, Pansart Y, Gillardin JM. Nefopam potentiates morphine antinociception in allodynia and hyperalgesia in the rat. Pharmacol Biochem Behav 2004 Apr;77(4):695-703.
10. Beloeil H, Delage N, Negre I, Mazoit JX, Benhamou D. The median effective dose of nefopam and morphine administered intravenously for postoperative pain after minor surgery: a prospective randomized double-blinded isobolographic study of their analgesic action. Anesth Analg 2004 Feb;98(2):395-400, table of contents.
11. Kapfer B, Alfonsi P, Guignard B, Sessler DI, Chauvin M. Nefopam and ketamine comparably enhance postoperative analgesia. Anesth Analg 2005 Jan;100(1):169-74.
12. Biella GE, Groppetti A, Novelli A, Fernandez-Sanchez MT, Manfredi B, Sotgiu ML. Neuronal sensitization and its behavioral correlates in a rat model of neuropathy are prevented by a cyclic analog of orphenadrine. J Neurotrauma 2003 Jun;20(6):593-601.
13. Zimmermann M. Ethical guidelines for investigations of experimental pain in conscious animals. Pain 1983 Jun;16(2):109-10.
14. Bennett GJ, Xie YK. A peripheral mononeuropathy in rat that produces disorders of pain sensation like those seen in man. Pain 1988 Apr;33(1):87-107.
15. Zhao C, Tall JM, Meyer RA, Raja SN. Antiallodynic effects of systemic and intrathecal morphine in the spared nerve injury model of neuropathic pain in rats. Anesthesiology 2004 Apr;100(4):905-11.
16. Stuesse SL, Crisp T, McBurney DL, Schechter JB, Lovell JA, Cruce WL. Neuropathic pain in aged rats: behavioral responses and astrocytic activation. Exp Brain Res 2001 Mar;137(2):219-27.
17. Choi Y, Yoon YW, Na HS, Kim SH, Chung JM. Behavioral signs of ongoing pain and cold allodynia in a rat model of neuropathic pain. Pain 1994 Dec;59(3):369-76.
18. Vo T, Rice AS, Dworkin RH. Non-steroidal anti-inflammatory drugs for neuropathic pain: how do we explain continued widespread use? Pain 2009 Jun;143(3):169-71.
19. Dworkin RH, Backonja M, Rowbotham MC, Allen RR, Argoff CR, Bennett GJ, et al. Advances in neuropathic pain: diagnosis, mechanisms, and treatment recommendations. Arch Neurol 2003 Nov;60(11):1524-34.
20. Hamidi GA, Manaheji H, Janahmadi M, Noorbakhsh SM, Salami M. Co-administration of MK-801 and morphine attenuates neuropathic pain in rat. Physiol Behav 2006 Jul 30;88(4-5):628-35.
21. Dellemijn PL, van Duijn H, Vanneste JA. Prolonged treatment with transdermal fentanyl in neuropathic pain. J Pain Symptom Manage 1998 Oct;16(4):220-9.
22. Angst MS, Koppert W, Pahl I, Clark DJ, Schmelz M. Short-term infusion of the mu-opioid agonist remifentanil in humans causes hyperalgesia during withdrawal. Pain 2003 Nov;106(1-2):49-57.
23. Singla A, Stojanovic MP, Chen L, Mao J. A differential diagnosis of hyperalgesia, toxicity, and withdrawal from intrathecal

morphine infusion. Anesth Analg 2007 Dec;105(6):1816-9, table of contents.
24. Porreca F, Ossipov MH, Gebhart GF. Chronic pain and medullary descending facilitation. Trends Neurosci 2002 Jun;25(6):319-25.
25. Wiesenfeld-Hallin Z, Xu XJ, Hokfelt T. The role of spinal cholecystokinin in chronic pain states. Pharmacol Toxicol 2002 Dec;91(6):398-403.
26. Johnston IN, Milligan ED, Wieseler-Frank J, Frank MG, Zapata V, Campisi J, et al. A role for proinflammatory cytokines and fractalkine in analgesia, tolerance, and subsequent pain facilitation induced by chronic intrathecal morphine. J Neurosci 2004 Aug 18;24(33):7353-65.
27. Wilson NM, Jung H, Ripsch MS, Miller RJ, White FA. CXCR4 signaling mediates morphine-induced tactile hyperalgesia. Brain Behav Immun 2011 Mar;25(3):565-73.
28. Hutchinson MR, Coats BD, Lewis SS, Zhang Y, Sprunger DB, Rezvani N, et al. Proinflammatory cytokines oppose opioid-induced acute and chronic analgesia. Brain Behav Immun 2008 Nov;22(8):1178-89.
29. Erichsen HK, Hao JX, Xu XJ, Blackburn-Munro G. Comparative actions of the opioid analgesics morphine, methadone and codeine in rat models of peripheral and central neuropathic pain. Pain 2005 Aug;116(3):347-58.
30. Choi JI, Kim WM, Yoon MH, Lee HG. Antiallodynic Effect of Thalidomide and Morphine on Rat Spinal Nerve Ligation-induced Neuropathic Pain. Korean J Pain 2010 Sep;23(3):172-8.
31. Dellemijn P. Are opioids effective in relieving neuropathic pain? Pain 1999 Apr;80(3):453-62.
32. Kim J, Jung JI, Na HS, Hong SK, Yoon YW. Effects of morphine on mechanical allodynia in a rat model of central neuropathic pain. Neuroreport 2003 May 23;14(7):1017-20.
33. Gomaa AA, Aly SA, Badary MS, Ahmed EA. The immunopotentiator effects of nefopam. Int Immunopharmacol 2007 Feb;7(2):266-71.
34. Mimoz O, Incagnoli P, Josse C, Gillon MC, Kuhlman L, Mirand A, Soilleux H, Fletcher D. Analgesic efficacy and safety of nefopam vs. propacetamol following hepatic resection. Anaesthesia 2001 Jun;56(6):520-5.
35. Laboureyras E, Chateauraynaud J, Richebe P, Simonnet G. Long-term pain vulnerability after surgery in rats: prevention by nefopam, an analgesic with antihyperalgesic properties. Anesth Analg 2009 Aug;109(2):623-31.
36. Villier C, Mallaret MP. Nefopam abuse. Ann Pharmacother 2002 Oct;36(10):1564-6.