ELSEVIER

Brain Research Bulletin 63 (2004) 99–103

BRAIN RESEARCH BULLETIN

www.elsevier.com/locate/brainresbull

Attenuation of mechanical but not thermal hyperalgesia by electroacupuncture with the involvement of opioids in rat model of chronic inflammatory pain

Cheng Huang [a,b], Zhi-Ping Hu [b], Hua Long [a], Yu-Shun Shi [a], Ji-Sheng Han [a], You Wan [a,*]

[a] *Neuroscience Research Institute, Peking University, Key Laboratory of Neuroscience, Ministry of Education, 38 Xueyuan Road, Beijing 100083, PR China*

[b] *Department of Physiology, Gannan Medical College, Ganzhou 341000, PR China*

Received 10 September 2003; received in revised form 20 January 2004; accepted 25 January 2004

Abstract

Opioid peptides have been proven effective in reducing the sign of hyperalgesia associated with inflammation. Electroacupuncture (EA) produces antinociception via release of endogenous opioid peptides in normal rats. Moreover, intrathecal injection of dynorphin has antinociceptive effect in rats. The present study was designed to examine whether EA has effect on the thermal and mechanical hyperalgesia in rat model of complete Freund's adjuvant (CFA)-induced inflammatory pain. The results are the following: (1) single session of 100 Hz EA (0.5–1.0–1.5 mA, 10 min for each intensity) at both Zusanli (ST 36) and Sanyinjiao acupoints (SP 6) significantly increased mechanical withdrawal threshold determined by von Frey filaments but not with thermal withdrawal latency that is determined by hot plate ($52 \pm 0.2\,^{\circ}C$); (2) 100 Hz EA applied twice a week for 4 weeks and showed a significant decrease in the mechanical hyperalgesia at the third and fourth week, with no effect on thermal hyperalgesia; (3) naloxone ($20\,mg\,kg^{-1}$) had the ability to reverse the inhibition of the mechanical hyperalgesia produced by a single session of EA. In conclusion, the present results indicate that a single or repetitive EA could reduce mechanical hyperalgesia, but not thermal hyperalgesia, in CFA-inflammatory pain rats, and the opioid system might be involved in these effects.

Keywords: Inflammatory pain; Chronic pain; Acupuncture; Complete Freund's adjuvant; Naloxone

1. Introduction

Inflammatory pain is one of the most common types of pathological pain in clinical practice. Patients mainly suffer from the ongoing pain (spontaneous pain), evoked pain and hyperalgesia. Injection of complete Freund's adjuvant (CFA) into a rat's hind-paw provides a very good model in order to [2,24] study the mechanism of chronic inflammatory pain and to screen for anti-inflammatory hyperalgesic drugs. NMDA receptor antagonist such as ketamine [8], opioids [1] and non-selective COX inhibitors [21] are effective for the treatment of chronic inflammatory pain. However, after long-term application of these agents will produce some side effects. For instance, ketamine is a psychomimetic and can induce motor dysfunctions. Also, chronic administration of morphine will produce tolerance and dependency. These side effects limit their application in the treatment of chronic inflammatory pain.

It has been reported that endogenous opioid systems has changed during inflammatory pain. For example, CFA that were induced into poly-arthritic rats displays a pronounced increase in the levels and synthesis of dynorphin B in the lumbar spinal cord, which might play a role in the modulation of nociception under chronic pain [22]. CFA induced an up-regulation and increased membrane targeting of δ-opioid receptors in dorsal spinal cord [5]. Taken together, these results may account for the efficacy of opioids on the CFA-induced inflammatory pain.

Acupuncture has been used in China and Asian countries for more than 2000 years. Our previous studies have demonstrated that 2 Hz EA mainly releases β-endorphin, endomorphin, and met-enkephalin, which take effects through activation of μ- and δ-opioid receptors; however, 100 Hz EA mainly releases dynorphin, which plays a role through

* Corresponding author. Tel.: +86-10-8280-1151; fax: +86-10-8280-1111.
E-mail address: ywan@bjmu.edu.cn (Y. Wan).

0361-9230/$ – see front matter © 2004 Elsevier Inc. All rights reserved.
doi:10.1016/j.brainresbull.2004.01.006

κ-opioid receptors [11]. Since 15 Hz is between 2 and 100 Hz logarithmically, so 15 Hz EA can release both endorphins and dynorphins in the central nervous system [7,13]. Electroacupuncture (EA, a modern version of traditional manual acupuncture) has shown to induce analgesia via accelerating the release of endogenous opioid peptides and their subsequent interaction with opioid receptors in normal rats and humans [13]. Thus, the aim of the present study is to investigate whether single or repetitive administration of EA could reduce thermal and mechanical hyperalgesia in a rat model with the use of CFA-induced inflammatory pain along with the possible mechanism concerning opioid involvement.

2. Materials and methods

2.1. Animals and chemicals

Female Sprague–Dawley rats weighing 200–250 g were provided by the Department of Animal Sciences of our university. They were housed 4–5 per cage with food pellets and water ad libitum according to University Animal Care and Use Committee Guidelines adopted from NIH, USA. In all experiments, all measures were taken to minimize pain and/or discomfort. CFA and naloxone hydrochloride were products of Sigma Chemicals Company (USA). Naloxone was dissolved in normal saline (NS). Naloxone (20 mg kg^{-1}) or NS were injected intraperitoneally (i.p.) 20 min prior to EA. All injections were in a volume of 1 ml kg^{-1}.

2.2. Establishment of CFA model

Rats were anesthetized with 10% chlorohydrate (0.3 ml per 100 g body weight). One hundred microliter of CFA was injected into the plantar surface of the left hind-paw using a syringe with a 28-gauge needle. Rats were kept at room temperature ($22 \pm 1\,^{\circ}C$) to recover after CFA injection and were used to perform further experiments 48 h after injection.

2.3. Electroacupuncture stimulation

Each rat was kept in a specially designed holder, with their hind legs and tails exposed. Two stainless-steel needles with 0.4 mm in diameter, 4 mm in length were inserted into each leg, one at the Zusanli acupoint (ST 36), 5 mm lateral to the anterior tubercle of the tibia, which was marked by a notch and the other at the Sanyinjiao acupoint (SP 6), 3 mm proximal to the medial melleolus, at the posterior border of the tibia. EA frequency was 100 Hz with pulse width of 0.2 ms. Square waves generated from a Han's Acupoint Nerve Stimulator (HANS, LH series, manufactured in our institute) were applied to both legs simultaneously. The intensity of stimulation was increased in a stepwise manner at 0.5–1.0–1.5 mA, each lasting for 10 min.

2.4. Hot plate test

The hot plate test was carried out to evaluate the thermal hyperalgesia latency. Rats were placed onto the $52 \pm 0.2\,^{\circ}C$ hot plate. The latency period was recorded in either response to the thermal hyperalgesia by lifting hind-paw licking or commences jumping. In order to avoid tissue injury, the cut-off limit was set at 60 s. Each hind-paw was measured for three times and the average value from the three measurements were taken as the thermal hyperalgesia latency.

2.5. von Frey filaments test

To determine mechanical withdrawal thresholds, each rat was placed in an individual plexiglass housing (18 cm × 8 cm × 8 cm) with wire mesh floor, and allowed to explore and groom until they settled down. A set of von Frey filaments (Stoeling Company, USA) with bending forces ranging from 1.2 to 28 g were applied in ascending order to the plantar surface of the inflammatory hind-paw. Hind-paw withdrawal was considered as positive response. When there was a lack in response, the filament with the next greater bending force was applied. The bending force of the von Frey filaments triggering the withdrawal of the hind-paw was recorded. Each hind-paw was measured for three times and the average values from the three measurements were recorded of the mechanical paw withdrawal threshold [6].

2.6. Statistical analysis

The experimental data were expressed as mean ± S.E.M., and analyzed with non-parametric one-way or two-way analyses of variance (ANOVA) where appropriate, and followed by Dunn's post hoc test when needed. $P < 0.05$ was used as significance level.

3. Results

3.1. Effect of single session EA on CFA-induced mechanical and thermal hyperalgesia

After 48 h of CFA injection, 24 rats were randomly divided into three groups including control (no needle insertion, restrained in holder only), needling (needle insertion without electrical stimulation) and EA (needling insertion plus electrical stimulation). The mechanical hyperalgesia thresholds and thermal withdrawal latencies were measured before EA administration ("pre-EA"). Fifteen minutes after EA treatment ("post-EA"), EA significantly increased mechanical withdrawal thresholds of left hind-paw (CFA injection side) compared with the corresponding pre-EA ($P < 0.01$). However, in the control and needling groups mechanical withdrawal thresholds of post-EA did not show any obvious change compared to that of pre-EA (Fig. 1A). Nevertheless, EA had no significant effect on thermal withdrawal latency of the left hind-paw (Fig. 1B).

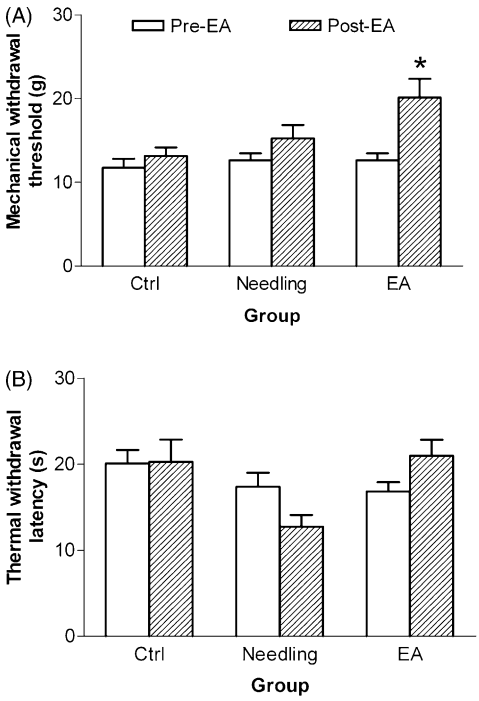

Fig. 1. The effect of single session EA on mechanical hyperalgesia and thermal hyperalgesia in CFA-induced inflammatory pain rats. (A) The mechanical thresholds before EA administration (pre-EA) (open column) and 15 min after EA treatment (post-EA) (striped column). $^{*}P < 0.05$ compared with corresponding pre-EA. (B) The thermal thresholds before EA administration (open column) and 15 min after treatment (striped column). No significance was observed between pre- and post-EA.

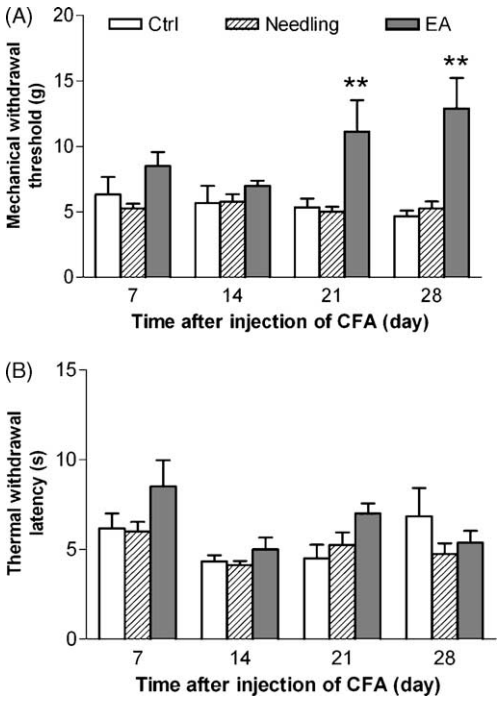

Fig. 2. The effect of repetitive EA on mechanical hyperalgesia and thermal hyperalgesia in inflammatory pain rats at Days 7, 14, 21 and 28 after CFA injection. (A) The mechanical thresholds. $^{**}P < 0.01$ compared with the corresponding control group (open column) at the same day of treatment. (B) The thermal hyperalgesia. No significant effect was observed between EA group and the corresponding control group at the same day.

3.2. Effect of repetitive EA on CFA-induced mechanical and thermal hyperalgesia

Animals were randomly divided into three groups as above mentioned. EA was given twice a week for 4 weeks. Briefly, EA was applied at Days 3 and 6 of each week, respectively, and the thermal and mechanical hyperalgesia thresholds were measured at Days 7, 14, 21 and 28. The mechanical withdrawal thresholds of left hind-paw increased significantly at Days 21 and 28 after EA treatment as compared with the control group ($P < 0.01$). No difference in mechanical thresholds was observed at Days 7 and 14 after EA. However, thermal withdrawal latencies of left hind-paw were unchanged within 28 days of treatment in all three groups (Fig. 2).

3.3. Naloxone blockade on the inhibitory effect of single session EA on mechanical hyperalgesia

Rats received i.p. injection of either naloxone (20 mg kg^{-1}) or NS 20 min before EA application. The mechanical withdrawal thresholds were measured at 15, 30, 45, 60 min after EA. It was found that i.p. injection of naloxone significantly blocked the inhibitory effect of EA on mechanical hyperalgesia after EA application at the time period of 15 min compared with NS plus EA group ($P < 0.05$). No significant difference was observed at other time periods (Fig. 3). This reversible effect of naloxone on the suppression of EA was diminished 60 min after the termination of EA.

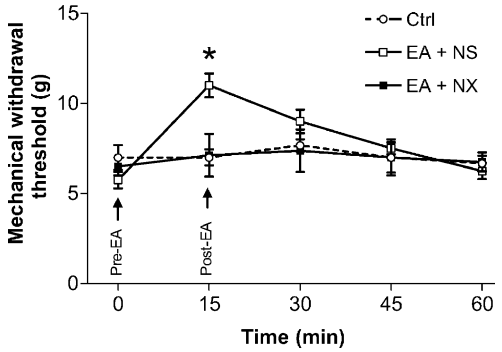

Fig. 3. Naloxone blockade of the inhibitory effect of single session EA on CFA-induced mechanical hyperalgesia. The basal thresholds (g) of rats were evaluated prior to EA administration (pre-EA). Rats received i.p. injection of either naloxone (20 mg kg^{-1}) or NS 20 min before EA, the mechanical withdrawal thresholds were measured 15, 30, 45, 60 min after EA. $^{*}P < 0.05$ compared with NS plus EA group or needling control group at the corresponding points.

4. Discussion

The present study demonstrated that EA attenuated mechanical but not thermal hyperalgesia in CFA-induced

inflammatory pain, and this effect could be blocked by large dose of naloxone. The mechanisms underlying EA analgesia are very complicated and many different analgesic neurotransmitter systems are involved [13], especially opioid peptides. Exogenously i.t. injection of dynorphin produced analgesia [15] and 100 Hz EA produced analgesia via accelerating the release of endogenous dynorphin and interaction with κ-receptor in the spinal cord [13]. The effects of opioids in animal models of inflammatory pain have been investigated in great detail. Exogenous administration of opioid agonists showed antinociceptive activity under inflammatory conditions. For example, i.c.v. administration of μ-opioid receptor agonists DAMGO or morphine, δ-opioid receptor agonist deltorphin, or κ-opioid receptor agonist dynorphin (1–17) significantly reversed the hyperalgesia associated with peripheral inflammation in a dose-dependent manner [2,4,9]. The i.t. injection of nor-BNI, a κ-opioid receptor antagonist, produced a dose-dependent increase in arthritic flexion pain scores, suggesting that spinal dynorphin/kappa system have various effects in suppressing arthritic pain [20]. In several chronic arthritic studies, dynorphin B was elevated significantly in discrete brain areas and lumbar region of the spinal cord, and CFA induced an up-regulation of prodynorphin and preprodynorphin mRNA content and dynorphin [2,23]. The induction of preprodynorphin mRNA was parallel to the development of behavioral hyperalgesia. This rise possibly reflected an enhancement of opioid biosynthesis during chronic arthritic pain [22].

On the other hand, the effect of dynorphin on chronic pain was controversial. Other published literature reported that dynorphin A administered spinally produced a long-lasting allodynia in neuropathic pain. The sign of allodynia was attenuated by pre-treatment with the NMDA receptor antagonist, MK-801 and LY235959, but not the opioid antagonist, naloxone [18,25]. It is hypothesized that dynorphin has both physiological and pathological roles in acute and chronic pain states [17].

It was reported that the required blockade dose of naloxone for 100 Hz EA-induced analgesia was at 20 mg kg^{-1} [13]. Goldstein et al. [10] reported the dose of naloxone blockade on κ-receptor was 20-fold higher than that on μ- and δ-receptors in vitro. Here the large dose of naloxone blockade on inhibitory effect of single session EA on mechanical hyperalgesia in CFA induced inflammatory pain further supported the possible involvement of opioid systems, especially dynorphin and κ-receptor system in this process. This result is consistent with that from the normal rats [10,13,14]. In this present study, we also observed the effect of a single session of EA on the mechanical hyperalgesia which was diminished 1 h after administration of 100 Hz EA. This was similar with that from normal rat, so we speculated that a single session 100 Hz of EA produced transient antinociception via release of opioid peptides. Although we did not observe the effect of naloxone on repetitive EA, but it was found that the suppressive effects of naloxone has diminished 60 min after i.p. injection. So the mechanisms for a single session or repetitive of EA might be similar.

In the present study, we observed that EA has suppressed the mechanical but not the thermal hyperalgesia that was induced by CFA. The exact mechanisms are not clear. The possible explanations might be as the following. (1) As mentioned above, 100 Hz of EA accelerated the release of endogenous dynorphin at the spinal cord [13]. This suppressive effect was not strong enough as that of the exogenously injected morphine. (2) During CFA-induced inflammation, the peripheral mechanism that is responsible for thermal hyperalgesia might be different from that of the mechanical hyperalgesia. This was supported by the following reports: the mechanical allodynia induced by CFA can be reversed by MK-801, but not by CNQX, suggesting that CFA-induced mechanical hyperalgesia was mediated via peripheral activation of NMDA, but not by non-NMDA receptors [19]. However, thermal hyperalgesia following carrageenan-induced inflammation was mediated through activation of peripheral NMDA as well as non-NMDA receptors [16]. Histogranin, a peptide NMDA receptor antagonist, had modest effect on mechanical hyperalgesia, but not on thermal hyperalgesia in a rat model of CFA-induced inflammatory pain [12]. In a recent report, it has been suggested that thermal hyperalgesia involved both spinal and supraspinal circuits, while mechanical allodynia depended on a supraspinal loop. This difference might reflect that afferent inputs might be associated with different fiber types. Another observation indicated that the thermal hyperalgesia was likely dependent on opioid-sensitive small-diameter primary afferent fibers, whereas, mechanical allodynia may be largely independent of small-fiber input in neuropathic pain [3,26].

In conclusion, our present study showed that either a single session or a repetitive EA administration could attenuate a mechanical but not thermal hyperalgesia in a rat model of CFA-induced inflammatory pain, and the blockade of naloxone on inhibitory effect of EA on mechanical hyperalgesia suggested that opioid system might be involved in this effect.

Acknowledgements

The project was supported by grants from the National Natural Science Foundation of China (30170319, 30240059 and 30330026), the National Basic Research Program of China (G1999054000) and the Scientific and Technological Program of the Department of Education, Jiangxi Province, China. The authors thank Dr. Hassan Dib for his help in editing the manuscript.

References

[1] N. Andreev, L. Urban, A. Dray, Opioids suppress spontaneous activity of polymodal nociceptors in rat paw skin induced by ultraviolet irradiation, Neuroscience 58 (1994) 793–798.

[2] A. Beyer, M. Schafer, C. Stein, Antinociceptive effects of dynorphin peptides in a model of inflammatory pain, Pain 70 (1997) 141–147.
[3] D. Bian, M.H. Ossipov, C. Zhong, T.P. Malan, F. Porreca, Tactile allodynia, but not thermal hyperalgesia, of the hindlimbs is blocked by spinal transection in rats with nerve injury, Neurosci. Lett. 24 (1998) 79–82.
[4] P.J. Cabot, L. Carter, M. Schafer, C. Stein, Methionine-enkephalin and Dynorphin A release from immune cells and control of inflammatory pain, Pain 93 (2001) 207–212.
[5] C.M. Cahill, A. Morinville, C. Hoffert, D. O'Donnell, A. Beaudet, Up-regulation and trafficking of delta opioid receptor in a model of chronic inflammation: implications for pain control, Pain 101 (2003) 199–208.
[6] S.R. Chaplan, F.W. Bach, J.W. Pogrel, J.M. Chung, T.L. Yaksh, Quantitative assessment of tactile allodynia in the rat paw, J. Neurosci. Meth. 53 (1994) 55–63.
[7] X.H. Chen, J.S. Han, Analgesia induced by electroacupuncture of different frequencies is mediated by different types of opioid receptors: another cross-tolerance study, Behav. Brain Res. 47 (1992) 143–149.
[8] K. Fisher, T.J. Coderre, N.A. Hagen, Targeting the *N*-methyl-D-aspartate receptor for chronic pain management. Preclinical animal studies, J. Pain Symptom. Manage. 20 (2000) 358–373.
[9] G.L. Fraser, G.A. Gaudreau, P.B. Clarke, D.P. Menard, M.N. Perkins, Antihyperalgesic effects of delta opioid agonists in a rat model of chronic inflammation, Br. J. Pharmacol. 129 (2000) 1668–1672.
[10] A. Goldstein, S. Tachibana, L.I. Lowney, M. Hunkapiller, L. Hood, Dynorphin-(1-13), an extraordinarily potent opioid peptide, Proc. Natl. Acad. Sci.: USA 76 (1979) 6666–6670.
[11] H.F. Guo, J. Tian, X. Wang, Y. Fang, Y. Hou, J.S. Han, Brain substrates activated by electroacupuncture (EA) of different frequencies (II): role of Fos/Jun proteins in EA-induced transcription of preproenkephalin and preprodynorphin genes, Brain Res. Mol. Brain Res. 43 (1996) 167–173.
[12] A. Hama, J. Sagen, Selective antihyperalgesic effect of [Ser1] histogranin on complete Freund's adjuvant-induced hyperalgesia in rats, Pain 95 (2002) 15–21.
[13] J.S. Han, Acupuncture: neuropeptide release produced by electrical stimulation of different frequencies, Trends Neurosci. 26 (2003) 17–22.
[14] J.S. Han, Q. Wang, Mobilization of specific neuropeptides by peripheral stimulation of identified frequencies, News Physiol. Sci. 7 (1992) 176–180.
[15] J.S. Han, G.X. Xie, A. Goldstein, Analgesia induced by intrathecal injection of dynorphin B in the rat, Life Sci. 34 (1984) 1573–1579.
[16] D.L. Jackson, C.B. Graff, J.D. Richardson, K.M. Hargreaves, Glutamate participates in the peripheral modulation of thermal hyperalgesia in rats, Eur. J. Pharmacol. 284 (1995) 321–325.
[17] T.M. Laughlin, A.A. Larson, G.L. Wilcox, Mechanisms of induction of persistent nociception by dynorphin, J. Pharmacol. Exp. Ther. 299 (2001) 6–11.
[18] T.M. Laughlin, T.W. Vanderah, J. Lashbrook, M.L. Nichols, M. Ossipov, F. Porreca, G.L. Wilcox, Spinally administered dynorphin A produces long-lasting allodynia: involvement of NMDA but not opioid receptors, Pain 72 (1997) 253–260.
[19] J.W. Leem, J.H. Hwang, S.J. Hwang, H. Park, M.K. Kim, Y. Choi, The role of peripheral *N*-methyl-D-aspartate receptors in Freund's complete adjuvant induced mechanical hyperalgesia in rats, Neurosci. Lett. 297 (2001) 155–158.
[20] H.X. Liu, Repeated 100 Hz TENS for the treatment of chronic inflammatory pain in rats: optimal parameters and possible neurochemical mechanisms, Prog. Physiol. Sci. 30 (1999) 35–37.
[21] W. Ma, W. Du, J.C. Eisenach, Role for both spinal cord COX-1 and COX-2 in maintenance of mechanical hypersensitivity following peripheral nerve injury, Brain Res. 937 (2002) 94–99.
[22] M.J. Millan, A. Czlonkowski, C.W. Pilcher, O.F. Almeida, M.H. Millan, F.C. Colpaert, A. Herz, A model of chronic pain in the rat: functional correlates of alterations in the activity of opioid systems, J. Neurosci. 7 (1987) 77–87.
[23] M. Spetea, G. Rydelius, I. Nylander, M. Ahmed, I. Bileviciute-Ljungar, T. Lundeberg, S. Svensson, A. Kreicbergs, Alteration in endogenous opioid systems due to chronic inflammatory pain conditions, Eur. J. Pharmacol. 435 (2002) 245–252.
[24] J.H. Tian, W. Zhang, Y. Fang, W. Xu, D.K. Grandy, J.S. Han, Endogenous orphanin FQ: evidence for a role in the modulation of electroacupuncture antinociception and the development of tolerance to antinociception produced by morphine and electroacupuncture, Br. J. Pharmacol. 124 (1998) 21–26.
[25] T.W. Vanderah, T. Laughlin, J.M. Lashbrook, M.L. Nichols, G.L. Wilcox, M.H. Ossipov, T.P. Malan, F. Porreca, Single intrathecal injections of dynorphin A or des-Tyr-dynorphins produce long-lasting allodynia in rats: blockade by MK-801 but not naloxone, Pain 68 (1996) 275–281.
[26] S. Wegert, M.H. Ossipov, M.L. Nichols, D. Bian, T.W. Vanderah, T.P. Malan, F. Porreca, Differential activities of intrathecal MK-801 or morphine to alter responses to thermal and mechanical stimuli in normal or nerve-injured rats, Pain 71 (1997) 57–64.