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Electroacupuncture combined with MK-801 prolongs anti-hyperalgesia in rats with peripheral inflammation

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Abstract

Our previous study showed that electroacupuncture (EA), an adjuvant to conventional medicine, significantly attenuated hyperalgesia in a rat model of inflammatory pain. In the present study, we evaluated the potential additive and/or synergism of EA and a sub-effective dose of dizocilpine maleate (MK-801), a non-competitive *N*-methyl-D-aspartate (NMDA) receptor antagonist, on hyperalgesia in the same rat model of inflammatory pain.

Hyperalgesia, manifesting as decreased paw withdrawal latency (PWL) to a noxious stimulus, was induced by injecting complete Freund's adjuvant (CFA) into the plantar surface of one hind paw of each rat. EA treatments were given at acupoint GB30 immediately after and 2 h after CFA. MK-801 at 0.001 mg/rat was given (i.t.) 10 min before each of the two EA treatments. PWL was measured prior to and 2.5 and 5 h post-CFA.

Ten and 100 Hz EA significantly inhibited CFA-induced hind paw hyperalgesia. Both 10 and 100 Hz EA combined with the sub-effective dose of 0.001 mg/rat MK-801 showed prolonged anti-hyperalgesia with no side effects. These results demonstrate that EA combined with a sub-effective dose of this NMDA receptor antagonist enhances anti-hyperalgesia, and this combination may provide an effective strategy for pain management.

Keywords: Hyperalgesia; Acupuncture; NMDA receptor; MK-801; Complete Freund's adjuvant; Intrathecal injection

1. Introduction

Various chronic inflammatory diseases affect a large population of patients. Conventional medicine such as non-steroidal anti-inflammatory drugs (NSAIDs) and recently developed COX-2 inhibitors may be associated with significant adverse effects such as gastrointestinal disturbances (Stiel, 2000; Scheiman, 2001; Davies and Jamali, 2004). It is documented that as many as 36–62% of patients use complementary and alternative medicine, including acupuncture, as an adjunct to conventional medicine (Eisenberg et al., 1998; Barnes et al., 2004).

Our study with an animal model of inflammatory pain showed that electroacupuncture (EA) at acupoint Huantiao (GB30) significantly attenuated complete Freund's adjuvant (CFA)-induced hyperalgesia (Lao et al., 2004). It has also been demonstrated that the combination of a sub-effective dose of indomethacin, a classic NSAID drug, or morphine and EA treatment produces greater anti-hyperalgesia than either agent alone and than the sum effects of the individual agents (Zhang et al., 2004a,b).

Additionally, considerable evidence suggests that excessive activation of the *N*-methyl-D-aspartate (NMDA) receptor, an ionotropic glutamate receptor, plays a key role in the development of hyperalgesia and central hyperexcitability (Dubner and Ruda, 1992; Kolhekar et al., 1993; Ren and Dubner, 1993; Sluka and Westlund, 1993), and that

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NMDA antagonists can be used in the management of chronic pain (Dickenson et al., 1997; Ren et al., 1992). However, high dose NMDA antagonists have been shown to cause such side effects as disruption of motor coordination (Hama et al., 2003). Clearly, alternatives or adjunctives to conventional medicine would be clinically useful in the treatment of persistent pain. We hypothesize that the combination of a sub-effective dose of dizocilpine maleate (MK-801), a classic NMDA antagonist, and EA treatment produces greater anti-hyperalgesia than either agent alone in a rat model of inflammatory pain.

2. Methods

2.1. Animal preparation

Male Sprague–Dawley rats weighing 280–350 g (Harlan) were kept under controlled environmental conditions (22 °C ± 0.5 °C, relative humidity 40–60%, 7 a.m. to 7 p.m. alternate light–dark cycles, food and water ad libitum). Animal protocols were approved by the Institutional Animal Care and Use Committee (IACUC) at the University of Maryland School of Medicine. The ethical guidelines for the treatment of animals of the International Association for the Study of Pain were followed in these experiments.

Under pentobarbital sodium anesthesia (50 mg/kg i.p.), the rats were prepared for intrathecal injection. The atlanto-occipital membrane at the level between the head and neck (i.e., approximately the obex level) was exposed and a 7.5-cm length of PE-10 tubing was inserted into the subarachnoid space through a slit made in the membrane. The catheter was advanced to the level of the lumbar spinal cord and filled with saline (approximately 7–10 μl), and the outer end was plugged. The rats were allowed to recover for 7 days after the operation prior to induction of hyperalgesia. Animals with gross signs of motor impairment were excluded from the study. Evans blue was injected via the catheter and the location of the distal end of the catheter was verified.

Inflammatory hyperalgesia was induced by injecting 0.08 ml of CFA, which was suspended in an 1:1 oil/saline emulsion and contained 40 μg *Mycobacterium tuberculosis* (Sigma), subcutaneously into the plantar surface of one hind paw of each rat using a 25 gauge hypodermal needle. Hyperalgesia was determined by a decrease in PWL to a noxious thermal stimulus (Hargreaves et al., 1988). The effect of the hyperalgesia on the normal behavior of the CFA-inflamed rats appeared to be minimal, and they showed normal grooming behavior and levels of activity (Lao et al., 2001, 2004).

2.2. Experimental design

Rats were randomly divided into the following groups (n = 7–9 per group): (1) MK-801 (Sigma), 0.001 (n = 8), 0.005 (n = 9) and 0.01 (n = 8) mg/rat (10 μl i.t.); (2) saline control (10 μl i.t., n = 9); (3) MK-801 (0.001 mg/rat, n = 9) or saline (n = 7) plus 10 Hz EA; (4) MK-801 (0.001 mg/rat, n = 8) or saline (n = 8) plus 100 Hz EA; and (5) sham EA (n = 9). The MK-801 was dissolved in saline and administered (i.t.) 10 min before each of two EA treatments.

2.3. Acupuncture treatment

Detailed EA procedure has been described previously (Lao et al., 2004). To maximize the anti-hyperalgesia, animals were given two 20-min EA treatments, one immediately after CFA administration and the second 2 h post-CFA. Our previous study tested a single EA treatment, administered at the time of injection, but it produced no anti-hyperalgesia in this animal model (Lao et al., 2004). Previously determined EA parameters of low frequency (10 Hz) and high frequency (100 Hz) at 3 mA and 0.1 ms pulse width, each of which showed significant anti-hyperalgesic effects in the rat inflammation model (Lao et al., 2004), were used in the present study. The equivalent of human acupoint GB30 on the rat's hind limbs was treated bilaterally. In humans, GB30 is located at the junction of the lateral 1/3 and medial 2/3 of the distance between the greater trochanter and the sacral hiatus; underneath are the sciatic nerve, inferior gluteal nerve and gluteal muscles (Cheng, 1999). Anatomically comparable landmarks were used to locate GB30.

GB30 was chosen based on traditional Chinese medicine (TCM) meridian theory (O'Connor and Bensky, 1981) and its successful use in previous studies (Xu et al., 1993; Lao et al., 2004; Zhang et al., 2004a,b). Our previous study (Lao et al., 2004) showed that EA at acupoint GB30, but not at TE5 (the 5th acupoint on the Triple Energizer Meridian) or at sham points, including the point at the opposite aspect of GB30 and an unnamed abdominal point, showed significant anti-hyperalgesia.

The animals were gently handled for 30 min each day for 2–3 days to habituate them before acupuncture treatment. After cleaning the skin with alcohol swabs, one investigator swiftly inserted disposable acupuncture needles (gauge #32, 0.5 in. in length) with electrodes soldered to their handles bilaterally, approximately 0.5 in. into GB30 while another held the animal gently. The needles and the electrodes were stabilized with adhesive tape (Lao et al., 2004). The procedure typically lasted less than 20 s and caused little apparent distress to the animal.

During EA treatment, each rat was placed under an inverted clear plastic chamber (approximately 5 in.×8 in.×11 in.) but was neither restrained nor given any anesthetic. EA was delivered by an electrical stimulator (A300 Pulsemaster, World Precision Instruments) via an isolator (A360D Stimulus Isolator, World Precision Instruments), which converts electrical voltage into electrical current. While EA frequency was held constant, intensity was adjusted slowly over the period of approximately 2 min

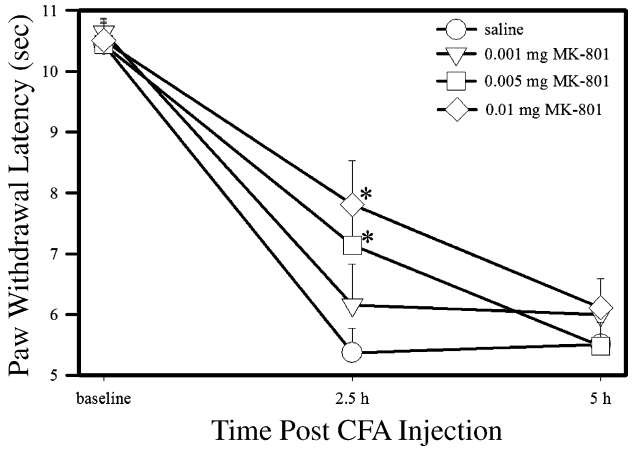

Fig. 1. Effects of MK-801 on CFA-induced hyperalgesia. MK-801 at 0.001 ($n=8$), 0.005 ($n=9$) and 0.01 ($n=8$) mg/rat (i.t.) dose-dependently increased the paw withdrawal latency compared to saline control ($n=9$). *$P<0.05$ compared to saline.

to the designated level of 3 mA, which is the maximum EA current intensity that a conscious animal can tolerate. Mild muscle twitching was observed. The animals remained awake and still during treatment and gave no observable signs of distress.

For sham control, acupuncture needles were inserted bilaterally into GB30 without electrical stimulation or manual needle manipulation. This control showed little anti-hyperalgesia in our previous study (Lao et al., 2004) and seems to be an appropriate control for non-specific needling effects. Sham treatment and EA-treated animals were handled identically.

2.4. Behavioral test

Rats were tested for hind paw thermal hyperalgesia pre-CFA and 2.5 and 5 h post-CFA (Hargreaves et al., 1988; Zhang et al., 2004a). Because the half-life of MK-801 is 2.05 h in the brain (Vezzani et al., 1989), we believe that MK-801 has little effect after 5 h. Therefore, the effect of the EA/MK-801 combination was only tested at 2.5 h (i.e. immediately after the second 20-min EA treatment) and 5 h post-CFA in the present study. The rats were placed under a clear plastic chamber on the glass surface of the Paw Thermal Stimulator System (UCSD, San Diego) and allowed to acclimatize for 30 min. A radiant heat stimulus was applied with a high intensity projector lamp bulb located beneath the glass floor (CXL/CXR, 8 V, 50 W). The heat stimulus was directed onto the plantar surface of each hind paw, and PWL to the nearest 0.1 s was automatically determined. The intensity of the thermal stimulus was adjusted to derive an average baseline PWL of approximately 10.0 s in naive animals. A 20-s cut-off was used to prevent tissue damage (Hargreaves et al., 1988; Zhang et al., 2004a). Mean paw withdrawal latency (PWL) was established by averaging the latency of four tests with a 5-min interval between each test. The investigator who conducted the measurement was blind to the treatment assignments.

2.5. Data analysis

The data were presented as mean±S.E.M. and were analyzed using repeated measures analysis of variance (ANOVA) followed by Fisher's protected least significant difference post-hoc analysis. $P<0.05$ was considered significant in all cases.

3. Results

3.1. Effect of MK-801 on hyperalgesia

Before CFA injection, the overall mean baseline PWL to noxious heat stimuli was similar in all groups of rats, and there was no significant difference in PWL between left (10.23±0.38 s) and right (10.48±0.48 s) hind paws. Following injection of 0.08 ml CFA into the left hind paw, its latency was significantly shorter than that of the contralateral hind paw, which was unchanged. After pretreatment of 0.001 ($n=8$), 0.005 ($n=9$) and 0.01 ($n=8$) mg/rat MK-801 and saline ($n=9$), hyperalgesia was observed using PWL test. A 4×2 repeated measures ANOVA revealed a main effect of drug treatment ($F_{(3,30)}=2.07$, $p=0.13$) and time ($F_{(1,30)}=9.65$, $P<0.01$) and an interaction between drug treatment and time ($F_{(3,30)}=3.21$, $p<0.05$). Post-hoc mean comparisons revealed that MK-801 at 0.05–0.1 mg/ rat (i.t.) significantly ($P<0.05$) increased PWL 2.5 h post-CFA compared to saline control. The analytical results indicate that MK-801 at 0.05–0.1 mg/rat alleviated inflammation-induced hyperalgesia and that this effect diminished over time (Fig. 1).

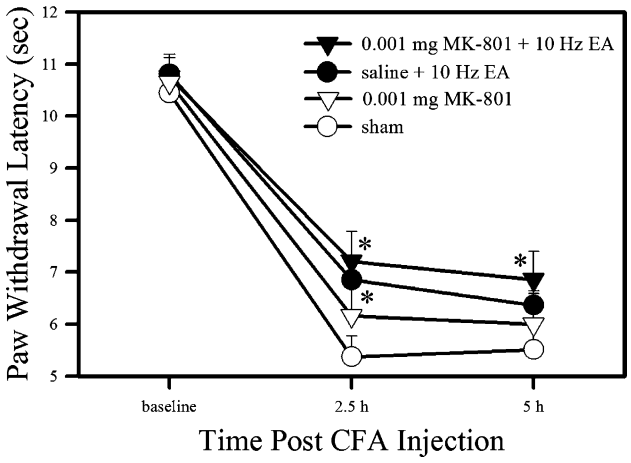

Fig. 2. Effect of a combination of 10 Hz EA and a sub-effective dose of 0.001 mg MK-801 on CFA-induced hyperalgesia. EA plus saline ($n=7$) significantly increased PWL only at 2.5 h post-CFA, while EA plus MK-801 ($n=9$) significantly increased PWL at both 2.5 h and 5 h post-CFA. MK-801 alone ($n=8$) showed little effect. *$P<0.05$ compared to sham control ($n=9$).

3.2. Effects of EA and its combinations with MK-801 on hyperalgesia

No differences were found for baseline PWL among groups. The sub-effective dosage of MK-801 (0.001 mg/rat) was combined with both 10 and 100 Hz EA. In the 10 Hz study (Fig. 2), $2\times2\times2$ repeated measures ANOVA revealed a main effect of drug treatment ($F_{(1,29)}=1.93$, $p=0.17$), 10 Hz EA treatment ($F_{(1,29)}=7.34$, $P<0.05$) and time ($F_{(1,29)}=0.48$, $P=0.49$); it also revealed interactions between EA treatment and time ($F_{(1,31)}=0.43$, $p=0.50$), between drug and time ($F_{(1,29)}=0.01$, $P=0.92$) and among drug, EA treatment and time ($F_{(2,29)}=0.10$, $P=0.91$). Post-hoc mean comparisons revealed that EA significantly ($P<0.05$) increased PWL of the CFA-injected hind paw 2.5 h post-CFA injection compared to sham control, while EA plus MK-801 significantly ($P<0.05$) increased PWL not only at 2.5 h but also 5 h post-injection compared to sham control (Fig. 2).

In the 100 Hz study, $2\times2\times2$ repeated measures ANOVA revealed a main effect of drug treatment ($F_{(1,29)}=2.20$, $P=0.15$), 100 Hz EA treatment ($F_{(1,29)}=4.99$, $P<0.05$) and time ($F_{(1,29)}=0.25$, $P=0.62$); it also revealed an interactions between EA treatment and time ($F_{(1,29)}=0.30$, $P=0.58$), between drug and time ($F_{(1,29)}=0.69$, $P=0.41$) and among drug, EA treatment and time ($F_{(2,29)}=0.98$, $P=0.38$). Post-hoc mean comparisons revealed that 100 Hz EA significantly increased PWL of the CFA-injected hind paw 2.5 h post-CFA injection compared to sham control, while EA plus MK-801 significantly ($P<0.05$) increased PWL at both 2.5 and 5 h post-CFA compared to sham control (Fig. 3). These analytical results indicate that both 10 Hz and 100 Hz EA significant alleviate inflammation-induced hyperalgesia. Considering that drug and EA effects on PWL did not interact, and that a significant effect of EA was observed, it may be suggested that the combination of MK-801 with EA at both 10 Hz and 100 Hz produces an additive effect.

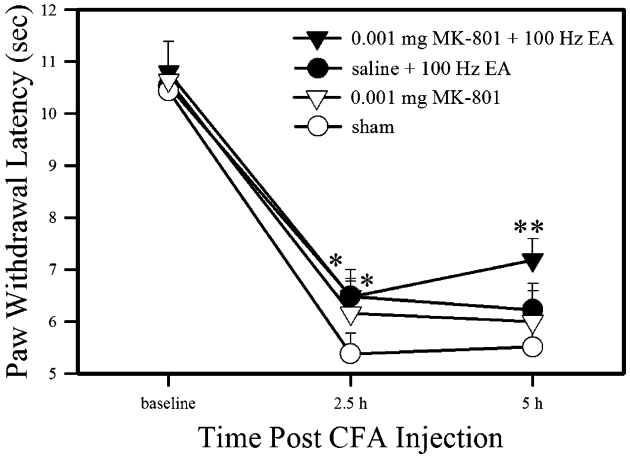

Fig. 3. Effect of a combination of 100 Hz EA and a sub-effective dose of 0.001 mg MK-801 on CFA-induced hyperalgesia. EA plus saline ($n=8$) significantly increased PWL only at 2.5 h post-CFA, while EA plus MK-801 ($n=8$) significantly increased PWL at both 2.5 h and 5 h post-CFA. MK-801 alone ($n=8$) showed little effect. *$P<0.05$ and **$P<0.01$ compared to sham control ($n=9$).

4. Discussion

EA combined with a sub-effective dose of MK-801 showed enhanced anti-hyperalgesia. The combination potentially decreases the side effects of drug therapy and suggests a novel strategy for pain management. Previous studies on a transient pain animal model showed that 2 Hz EA induces endomorphin and enkephalin release and 100 Hz EA induces dynorphin release, while 15 Hz induces release of all of three (Han, 2003). However, transient pain is distinctly different from persistent pain, which is associated with long-lasting alterations of the nervous system (Ren and Dubner, 1999), and thus findings from the uninjured animal model may not apply to persistent pain. Moreover, previous studies with an uninjured animal model show only brief (20–60 min) acupuncture analgesia (Bossut and Mayer, 1991; Romita et al., 1997; Han et al., 1999), but acupuncture has been found to have long-term pain relieving effects in patients with nociceptive pain (Carlsson, 2002; Berman et al., 2004) and in an animal model of chronic inflammatory pain (Lao et al., 2004). These date demonstrated that EA effects differ in healthy and pathological conditions.

Our previous study demonstrated that both μ and δ opioid receptor antagonists, but not κ opioid receptor antagonists, blocked the anti-hyperalgesia of both 10 Hz and 100 Hz EA in the CFA-induced peripheral inflammatory pain rat model (Zhang et al., 2004a). The findings of that study suggest that the significant anti-hyperalgesia produced by both 100 Hz and 10Hz EA is the result of the activation of endorphin/endomorphin (for μ receptors) and enkephalin (for δ receptors) systems but not dynorphin (for κ receptors) systems at the spinal level. Thus, 10 Hz and 100 Hz EA seem to share the same mechanisms of anti-hyperalgesia in the persistent pain animal model. However, we do not exclude the possible differences of 10 Hz and 100 Hz in treating other conditions such as anti-inflammation (Zhang et al., 2005).

The enhanced effect of the combination may result from combined effects of MK-801, a NMDA antagonist, and opioids induced by EA. It is known that noxious inputs activate the NMDA receptor-containing spinal neurons (Zhang et al., 1998) and NMDA receptor antagonists inhibit the excitatory activities of nociceptive spinal neurons (Dickenson et al., 1997). One of the ways in which EA exerts its effects is by inducing the central release of endogenous opioids (Han, 2003). As aforementioned, our recent study with the same inflammatory pain rat model demonstrates that EA anti-hyperalgesia is blocked by pretreatment with μ and δ opioid receptor antagonists, which indicates that the EA effect on persistent pain is mediated by opioid receptors (Zhang et al., 2004a). It has

been anatomically demonstrated that μ and κδ opioid receptors are distributed on both presynaptic primary afferent fibers and postsynaptic dorsal horn neurons (Besse et al., 1990; Abbadie et al., 2002). Previous research demonstrates that opioids reduce the release of the excitatory amino acid from the presynaptic terminals (Kangrga and Randic, 1991). Electrophysiologically, an agonist with μ- or δ-activity shows inhibitory actions on excitatory transmission in spinal postsynaptic dorsal horn neurons (Glaum et al., 1994). Therefore, we postulate that the enhanced anti-hyperalgesia of the EA/MK-801 combination may be due to a functional potentiation between the MK-801 and the opioids.

Previous studies support this hypothesis. For example, sub-effective doses of an NMDA receptor antagonist combined with morphine produced significant anti-nociceptive responses (Advokat and Rhein, 1995; Kauppila et al., 1998) and markedly suppressed spinal neuronal excitation (Chapman and Dickenson, 1992). Microiontophoretic application of selective μ and δ opioid receptor agonists significantly reduce NMDA-evoked responses of nociceptive neurons in the superficial and deeper dorsal horn of the trigeminal nucleus caudalis (Zhang et al., 1996). Additionally, NMDA receptor antagonist pain sensitivity inhibition is prevented by naloxone (Bernardi et al., 1996). These data suggest enhanced effect is produced when opioids and NMDA receptor antagonist are present simultaneously.

Our findings suggest that the combination of EA with low doses of an NMDA receptor antagonist may provide improved strategies for pain management, thus potentially decreasing drug side effects in patients with persistent pain.

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References

Abbadie C, Lombard MC, Besson JM, Trafton JA, Basbaum AI. Mu and delta opioid receptor-like immunoreactivity in the cervical spinal cord of the rat after dorsal rhizotomy or neonatal capsaicin: an analysis of pre- and postsynaptic receptor distributions. Brain Res 2002;930:150–62.

Advokat C, Rhein FQ. Potentiation of morphine-induced antinociception in acute spinal rats by the NMDA antagonist dextrorphan. Brain Res 1995;699:157–60.

Barnes P, Powell-Griner E, McFann K, Nahin R. Complementary and alternative medicine use among adults: United States, 2002. CDC Adv Data Rep 2004;343:1–20.

Berman MB, Lao L, Langenberg P, Lee WL, Gilpin AMK, Hochberg MC. Effectiveness of acupuncture as adjunctive therapy in osteoarthritis of the knee: a randomized, controlled trial. Ann Intern Med 2004;141: 901–10.

Bernardi M, Bertolini A, Szczawinska K, Genedani S. Blockade of the polyamine site of NMDA receptors produces antinociception and enhances the effect of morphine in mice. Eur J Pharmacol 1996;298: 51–5.

Besse D, Lombard MC, Zajac JM, Roques BP, Besson JM. Pre- and postsynaptic distribution of mu, delta and kappa opioid receptors in the superficial layers of the cervical dorsal horn of the rat spinal cord. Brain Res 1990;521:15–22.

Bossut DF, Mayer DJ. Electroacupuncture analgesia in naive rats: effects of brainstem and spinal cord lesions, and role of pituitary-adrenal axis. Brain Res 1991;549:52–8.

Carlsson C. Acupuncture mechanisms for clinically relevant long-term effects—reconsideration and a hypothesis. Acupunct Med 2002;20: 82–99.

Chapman V, Dickenson AH. The combination of NMDA antagonism and morphine produces profound antinociception in the rat dorsal horm. Brain Res 1992;573:321–3.

Cheng X. Chinese acupuncture and moxibustion. Beijing: Foreign Languages Press; 1999.

Davies NM, Jamali F. COX-2 selective inhibitors cardiac toxicity: getting to the heart of the matter. J Pharm Pharm Sci 2004;7:332–6.

Dickenson AH, Chapman V, Green GM. The pharmacology of excitatory and inhibitory amino acid-mediated events in the transmission and modulation of pain in the spinal cord. Gen Pharmacol 1997;28:633–8.

Dubner R, Ruda MA. Activity-dependent neuronal plasticity following tissue injury and inflammation. Trends Neurosci 1992;15:96–103.

Eisenberg DM, Davis RB, Ettner SL, Appel S, Wilkey S, Van Rompay M, et al. Trends in alternative medicine use in the United States, 1990–1997: results of a follow-up national survey. JAMA 1998;280:1569–75.

Glaum SR, Miller RJ, Hammond DL. Inhibitory actions of delta 1-, delta 2-, and mu-opioid receptor agonists on excitatory transmission in lamina II neurons of adult rat spinal cord. J Neurosci 1994;14:4965–71.

Hama A, Lee JW, Sagen J. Differential efficacy of intrathecal NMDA receptor antagonists on inflammatory mechanical and thermal hyperalgesia in rats. Eur J Pharmacol 2003;459:49–58.

Han JS. Acupuncture: neuropeptide release produced by electrical stimulation of different frequencies. Trends Neurosci 2003;26:17–22.

Han Z, Jiang YH, Wan Y, Wang Y, Chang JK, Han JS. Endomorphin-1 mediates 2 Hz but not 100 Hz electroacupuncture analgesia in the rat. Neurosci Lett 1999;274:75–8.

Hargreaves K, Dubner R, Brown F, Flores C, Joris J. A new and sensitive method for measuring thermal nociception in cutaneous hyperalgesia. Pain 1988;32:77–88.

Kangrga I, Randic M. Outflow of endogenous aspartate and glutamate from the rat spinal dorsal horn in vitro by activation of low-and high-threshold primary afferent fibers. Modulation by mu-opioids. Brain Res 1991;553:347–52.

Kauppila T, Xu XJ, Yu W, Wiesenfeld-Hallin Z. Dextromethorphan potentiates the effect of morphine in rats with peripheral neuropathy. Neuroreport 1998;9:1071–4.

Kolhekar R, Meller ST, Gebhart GF. Characterization of the role of spinal *N*-methyl-aspartate receptors in thermal nociception in the rat. Neuroscience 1993;57:385–95.

Lao L, Zhang G, Wei F, Berman BM, Ren K. Electroacupuncture attenuates behavioral hyperalgesia and selectively reduces spinal Fos protein expression in rats with persistent inflammation. J Pain 2001;2:111–7.

Lao L, Zhang R-X, Zhang G, Wang X, Berman B, Ren K. A parametric study of electroacupuncture on persistent hyperalgesia and Fos protein expression in rats. Brain Res 2004;1020:18–29.

O'Connor J, Bensky D. Acupuncture: a comprehensive text. Chicago: Eastland Press; 1981.

Ren K, Dubner R. NMDA receptor antagonist attenuate mechanical hyperalgesia in rats with unilateral inflammation of the hindpaw. Neurosci Lett 1993;163:22–6.

Ren K, Dubner R. Inflammatory models of pain and hyperalgesia. ILAR J 1999;40:111–8.

Ren K, Hylden JL, Williams GM, Ruda MA, Dubner R. The effects of a non-competitive NMDA receptor antagonist, MK-801, on behavioral hyperalgesia and dorsal horn neuronal activity in rats with unilateral inflammation. Pain 1992;50:331–44.

Romita VV, Suk A, Henry JL. Parametric studies on electroacupuncture-like stimulation in a rat model: effects of intensity, frequency, and duration of stimulation on evoked antinociception. Brain Res Bull 1997; 42:289–96.

Scheiman JM. The impact of nonsteroidal anti-inflammatory drug-induced gastropathy. Am J Manag Care 2001;7(Suppl. 1):S10–4.

Sluka KA, Westlund KN. An experimental arthritis model in rats: the effects of NMDA and non-NMDA antagonists on aspartate and glutamate release in the dorsal horn. Neurosci Lett 1993;149:99–102.

Stiel D. Exploring the link between gastrointestinal complications and over-the-counter analgesics: current issues and considerations. Am J Ther 2000;7:91–8.

Vezzani A, Serafini R, Stasi MA, Caccia S, Conti I, Tridico RV, et al. Kinetics of MK-801 and its effect on quinolinic acid-induced seizures and neurotoxicity in rats. J Pharmacol Exp Ther 1989;249:278–83.

Xu R, Guan X, Wang C. Influence of capsaicin treating sciatic nerve on the pain threshold and the effect of acupuncture analgesia of rats. Acupunct Res 1993;18:280–4.

Zhang KM, Wang XM, Mokha SS. Opioids modulate *N*-methyl-D-aspartic acid (NMDA)-evoked responses of neurons in the superficial and deeper dorsal horn of the medulla (trigeminal nucleus caudalis). Brain Res 1996;719:229–33.

Zhang R-X, Wang H, Ruda MA, Iadarola MJ, Qiao JT. c-Fos expression in NMDA receptor-contained neurons in spinal cord in a rat model of inflammation: a double immunocytochemical study. Brain Res 1998; 795:282–6.

Zhang R-X, Lao L, Wang L, Liu B, Wang X, Ren K, et al. Involvement of opioid receptors in electroacupuncture-produced anti-hyperalgesia in rats with peripheral inflammation. Brain Res 2004;1020:12–7.

Zhang R-X, Lao L, Wang X, Ren K, Berman BM. Electroacupuncture combined with indomethacin enhances anti-hyperalgesia in inflammatory rats. Pharmacol Biochem Behav 2004;78:793–7.

Zhang R-X, Lao L, Wang X, Fan A, Wang L, Ren K, et al. Electroacupuncture attenuates inflammation in a rat model. J Altern Complement Med 2005;11:135–42.