Available online at www.sciencedirect.com
SCIENCE DIRECT®
Neuroscience Letters 377 (2005) 185–188
Neuroscience Letters
www.elsevier.com/locate/neulet

Involvement of ionotropic glutamate receptors in low frequency electroacupuncture analgesia in rats

Byung-Tae Choi[a,b,*], Jun-Hyuk Lee[a], You Wan[b], Ji-Sheng Han[b]

[a] *Department of Anatomy, College of Oriental Medicine, Research Institute of Oriental Medicine, Dong-Eui University, Busan 614-052, Korea*
[b] *Neuroscience Research Institute, Peking University Health Science Center, Beijing 100083, China*

Received 20 August 2004; received in revised form 29 November 2004; accepted 30 November 2004

Abstract

The present study was conducted to determine whether blockage of both *N*-methyl-D-aspartate (NMDA) and α-amino-3-hydroxy-5-methylisoxazole-4-proprionic acid/kainate (AMPA/KA) receptors influences the induction of low frequency electroacupuncture (EA) analgesia. Although neither intrathecal injection of NMDA antagonist D-2-amino-5-phosphonopentanoic acid (D-AP-5) or AMPA/KA antagonist 1,2,3,4-tetrahydro-6-nitro-2,3-dioxo-benzo[f]quinoxaline-7-sulfonami-de (NBQX) disodium alone had an effect on analgesia, spinal application of D-AP-5 and NBQX disodium significantly prevented analgesia induced by 2 Hz EA. The intrathecal injection of the excitatory amino acid NMDA produced analgesia for several minutes after intrathecal injection, as did EA stimulation. These results suggest that ionotropic glutamate receptors may be involved in the induction of 2 Hz EA analgesia.

Keywords: Electroacupuncture; NMDA; AMPA; KA

To date, pain modulation has been explained by gate control theory, which results from the balance of activity in nociceptive and nonnociceptive afferents [8]. But unexpected results, such as some forms of prolonged analgesia lasting for hours or days, were found during the stimulation of peripheral nerves [6,7]. Since then, the mechanisms of learning and memory in the brain have been introduced into pain research [12]. Electroacupuncture (EA) as the stimulation of peripheral nerves has been used clinically to relieve acute or chronic pain in human patients. The gate control theory cannot satisfactorily explain the long-lasting analgesia effects of EA [5].

The long-term depression (LTD) of synaptic transmission between fine primary afferent nerve fibers and neurons in the superficial spinal dorsal horn may be involved in long-lasting therapeutic effects following EA or transcutaneous electrical nerve stimulation (TENS) [13,14]. Glutamate and related amino acids, transmitters at the synapse level between primary afferent fibers and neurons in the spinal dorsal horn, are involved in long-lasting pain reduction [3,5]. Recent studies have indicated that long-term synaptic alterations, including LTD, apparently involve activation of both *N*-methyl-D-aspartate (NMDA) and non-NMDA ionotropic receptors [9,11].

Although the mechanism operative in the induction of low frequency EA analgesia has not been definitely identified, this type of EA analgesia may possess similar mechanisms similar to low frequency stimulation-induced LTD. If the activation of glutamate receptors initiates the long-lasting analgesia of low frequency EA analgesia, both NMDA and α-amino-3-hydroxy-5-methylisoxazole-4-proprionic acid/kainate (AMPA/KA) ionotropic receptors are implicated. Therefore, the present study aims to investigate whether ionotropic glutamate receptors are involved in the induction of low frequency EA analgesia.

Male Spraque–Dawley rats weighing 250–300 g were provided by the Animal Care Institute of the Health Science Center, Peking University. All experiments conformed to guidelines approved by the Council of the International

* Corresponding author. Tel.: +82 51 850 8653; fax: +82 51 853 4036.
E-mail address: choibt@deu.ac.kr (B.-T. Choi).

0304-3940/$ – see front matter © 2004 Elsevier Ireland Ltd. All rights reserved.
doi:10.1016/j.neulet.2004.11.095

Association for the Study of Pain in December 1982. Intrathecal (i.t.) catheterization was performed according to the methods of Størkson et al. [15] under 10% chloral hydrate anesthesia (350 mg/kg i.p.). After surgery, only those rats without overt signs of spinal cord or root damage such as paralysis or lameness were used for experimentation.

The 1,2,3,4-tetrahydro-6-nitro-2,3-dioxo-benzo[f]quinoxaline-7-sulfonamide disodium (NBQX disodium, AMPA/KA antagonist), D-2-amino-5-phosphonopentanoic acid (D-AP-5, NMDA antagonist) and NMDA were purchased from Sigma Chemical Co. Chemicals were dissolved in sterile saline and injected intrathecally in a volume of 10 μl via catheter within 1 min and then filled with 8 μl of saline for flushing followed by EA. Two stainless-steel needles of 0.25 mm diameter were inserted in each hind limb at those acupoints corresponding to Zusanli and Sanyinjiao in man. The needles were connected to a HANS electric stimulator (Beijing Aviation Institute, Beijing, China), which delivered square wave pulses of 0.3 ms duration at a frequency of 2 Hz. The intensity was set at 1 mA and increased stepwise to 2 mA and 3 mA, each step lasting for 10 min.

The nociceptive threshold of the animals was measured by the tail flick latency (TFL) elicited by radiant heat. At the beginning of the experiments, TFL was assessed three times at 5 min intervals, and the mean values from the first three assessments were taken as the basal pain threshold, usually in the range of 4–6 s. The values of subsequent measurements of the EA and/or vehicle and i.t. injections were expressed as percentage changes from baseline. An increase of over 150% of the baseline tail flick latency was taken as the cut-off limit to avoid unnecessary damage to the tail skin. Data are expressed as mean ± S.E. and comparisons were made using Student's *t*-test.

I.t. injection of the NMDA antagonist D-AP-5 was started one day after surgery. EA stimulation started immediately after the termination of D-AP-5 injection. D-AP-5 at doses of 0.3 μg, 1 μg and 5 μg produced significant changes in TFL. Following spinal blockage of NMDA receptors by D-AP-5, EA analgesia induced by 2 Hz EA was strongly reduced compared to the vehicle group as illustrated in Fig. 1. With the same experimental design, the antagonistic effects of i.t. injection of NBQX disodium at doses of 0.3 μg, 1 μg and 5 μg were measured. As shown in Fig. 2, TFL changes in rats treated with NBQX disodium showed a similar antagonistic pattern as D-AP-5. To test whether the application of D-AP-5 and NBQX disodium reduces the TFL, the effect of an i.t. injection of D-AP-5 and NBQX disodium at a dose of 1 μg was examined without EA stimulation. Spinal application of both D-AP-5 and NBQX disodium increased TFL compared to the vehicle-injected group as shown in Fig. 3.

Excitatory amino acid antagonists provide a new class of analgesic agents, which might be selected for pain states [1]. EA and glutamate receptor antagonists have synergetic anti-nociceptive effects against carrageenan-induced hyperalgesia [17]. Co-application of EA and D-AP-5 or NBQX disodium significantly decreased TFL in normal rats of the

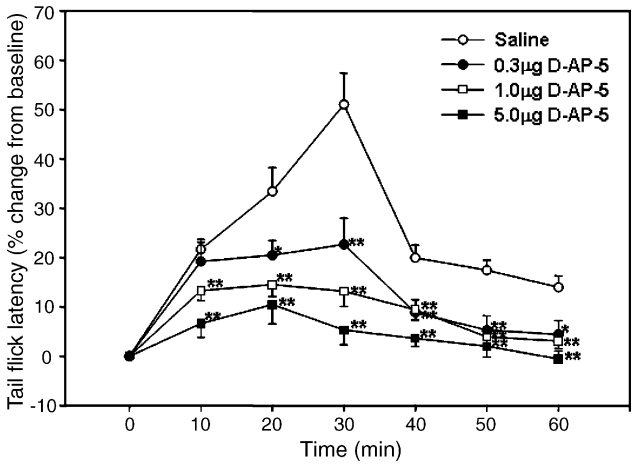

Fig. 1. The effects of NMDA antagonist D-AP-5 at different doses on 2 Hz EA-induced analgesia in rats. A significant difference was found between the saline and any of the three doses of D-AP-5. All values are expressed as mean percentage increase over baseline TFL. The vertical line represents the mean ± S.E. ($n = 10$). $^{*}P < 0.05$ and $^{**}P < 0.005$ compared to the vehicle control.

present study. But those contrary reports are based on a different experimental design between normal and pain animal models.

The presently described inhibition of 2 Hz EA analgesia by both ionotropic antagonist D-AP-5 and NBQX disodium may underlie some involvement of ionotropic receptors in the spinal dorsal horn induced by low frequency EA as in LTD mechanisms. This result is in line with the observation that spinal LTD could be induced by changes of glutamatergic excitatory transmission in the spinal dorsal horn, and inhibited by the antagonism of NMDA [2,3,5].

Two induction protocols of LTD, low (1–5 Hz, 5–15 min) and high frequency stimulation (50–100 Hz for 5 s), are proposed for the neuron of the spinal dorsal horn [5]. Condi-

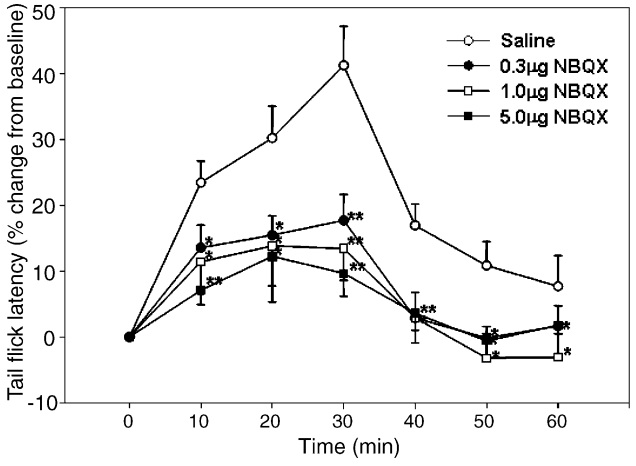

Fig. 2. The effects of AMPA/KA antagonist NBQX disodium at different doses on 2 Hz EA-induced analgesia in rats. Inhibition induced by NBQX disodium was observed through all test points of experiments ($n = 10$). $^{*}P < 0.05$ and $^{**}P < 0.005$ compared to vehicle control.

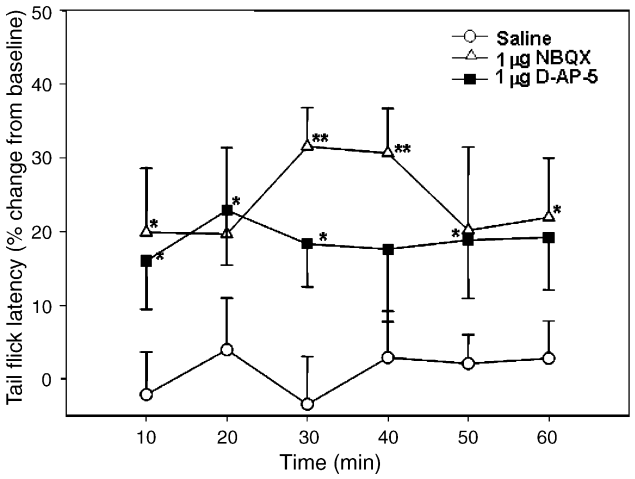

Fig. 3. Effects of i.t. injection of NMDA antagonist D-AP-5 and AMPA/KA antagonist NBQX disodium at a dose of 1 μg on TFL in rats. D-AP-5 and NBQX disodium showed significant analgesic effects ($n = 8$). $^{*}P < 0.05$ and $^{**}P < 0.005$ compared to vehicle control.

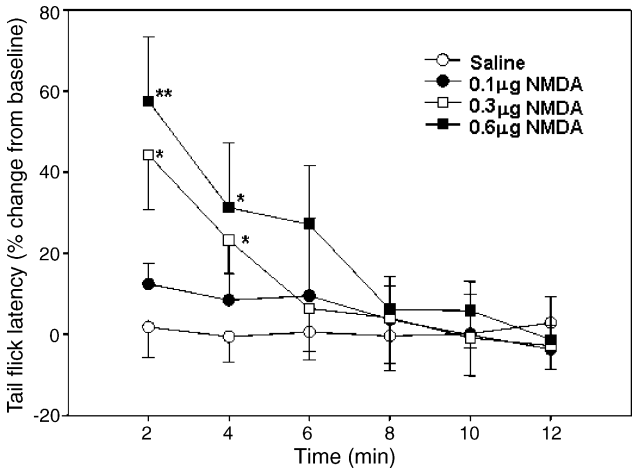

Fig. 4. The effects of NMDA at different doses in rats. Significant analgesic effects were noted for several minutes after i.t. injection ($n = 8$). $^{*}P < 0.05$ and $^{**}P < 0.005$ compared to the vehicle control.

tioning low frequency stimulation for afferent Aδ-fibers can depress synaptic strength between primary afferent Aδ- or C-fibers and neurons in the spinal dorsal horn for long periods of time [2,3,14]. This form of LTD is also hypothesized to be relevant to antinociception following afferent stimulation such as EA [2,12].

Although the stimulation parameters for the induction of LTD of C-fiber-evoked potentials in this in vivo study differs from those in in vitro work, EA also requires stimulation at an intensity that produces tolerable pain and involves afferent Aδ-fibers [5,13]. In the present study, electrical stimulation (frequency at 2 Hz; pulse with 0.3 ms; intensity, 1–3 mA) was delivered to the stainless-steel needle inserted into the acupoints. These stimulations induce an excitation of Aα,β-fibers and a subset of Aδ- and C-fibers, but Aβ- and Aδ-fibers mainly work to produce an antinociception effect of EA [4].

Sandkühler [13] proposed a cellular mechanism in the spinal dorsal horn that may underlie the long-lasting analgesia following EA. The conditioning stimulation of Aδ-fibers induces the release of glutamate from nerve terminals in the spinal dorsal horn that activates NMDA ionotropic glutamate receptors. This leads to a moderate increase in free cytosolic Ca^{2+} concentration sufficient for active protein phosphates. Subsequently, long-lasting depression of synaptic strength occurs as a result of the dephosphorylation of synaptic proteins such as the AMPA receptor. To confirm that the LTD can be formed by EA stimulation, we may need further experiments, especially on various receptors participating in the calcium influx or in the intracellular calcium release.

If ionotropic receptors are involved in the induction of low frequency EA analgesia, the spinal application of excitatory amino acids itself should produce the analgesic effect as did EA stimulation. Because NMDA has been documented as playing an important role in pain signaling in the spinal cord [16], we applied NMDA into the spinal cord as agonist for ionotropic glutamate receptors. The NMDA at doses of 0.3 μg, 1.0 μg and 3.0 μg, especially at higher doses, produced vocalization, scratching and biting of the hindlimbs after i.t. injection. When TFL was measured at 10 min intervals for 1 h after i.t. injection of NMDA, NMDA at all doses examined produced hyperalgesic effects (data not present). But when we checked the TFL at 2 min intervals for 12 min after i.t. injection of NMDA at doses of 0.1 μg, 0.3 μg and 0.6 μg, i.t. injection of NMDA produced analgesia effects for several minutes, as illustrated in Fig. 4.

The injection of the excitatory amino acid NMDA into the spinal subarachnoid space produces both hyperalgesic and analgesic effects [10]. In the present study, i.t. injection of NMDA produced hyperalgesic effects and behavioral signs of spontaneous pain from 10 min after injection. But brief analgesia effects induced by NMDA were also detected. These results suggest involvement of NMDA receptors in the mediation of spinal pain inhibitory mechanisms as reported by Raigorodsky and Urca [10]. Although a temporary analgesia by NMDA agonist is different from a long-lasting EA analgesia, NMDA agonist was injected once only whereas and EA analgesia is the result of continuous treatment of EA for 30 min. If NMDA agonist of lower concentration was injected continuously, we may get a result similar to the long-lasting EA analgesia effects.

Activation of ionotropic glutamate receptors is necessary to induce low frequency stimulation-induced LTD in the spinal dorsal horn [3]. The present data shows that ionotropic glutamate receptors may be involved in the induction of 2 Hz EA analgesia in the spinal dorsal horn and share the same process as LTD of excitatory synaptic transmission. If we have a proper understanding of the mechanism of LTD by EA stimulation, new forms of afferent stimulation for pain modulation can be introduced into clinical practice. Further studies of long-lasting EA analgesia would be worthwhile in

order to examine the roles of ionotropic glutamate receptors in endogenous antinociception.

Acknowledgement

This study was supported by a grant of the Oriental Medicine R&D Project, Ministry of Health and Welfare, Republic of Korea (02-PJ9-PG3-20600-0005).

References

[1] C. Advokat, D. Rutherford, Selective antinociceptive effect of excitatory amino acid antagonists in intact and acute spinal rats, Pharmacol. Biochem. Behav. 51 (1995) 855–860.
[2] J. Chen, B. Heinke, J. Sandkühler, Activation of group I metabotropic glutamate receptors induces long-term depression at sensory synapses in superficial spinal dorsal horn, Neuropharmacology 39 (2000) 2231–2243.
[3] J. Chen, J. Sandkühler, Induction of homosynaptic long-term depression at spinal synapses of sensory Aδ-fibers requires activation of metabotropic glutamate receptors, Neuroscience 98 (2000) 141–148.
[4] S.C. Fan, X.Z. Xie, J.S. Han, Topical application of capsaicin on sciatic nerve for the study of afferent fibers mediating electroacupuncture signals, Physiol. Sci. 6 (1986) 322–323.
[5] X.G. Liu, C.R. Morton, J.J. Azkue, M. Zimmermann, J. Sandkühler, Long-term depression of C-fibre-evoked spinal field potentials by stimulation of primary afferent Aδ-fibres in the adult rat, Eur. J. Neurosci. 10 (1998) 3069–3075.
[6] R. Melzack, Prolonged relief of pain by brief intense transcutaneous somatic stimulation, Pain 1 (1975) 357–373.
[7] R. Melzack, P. Vetere, L. Finch, Transcutaneous electrical nerve stimulation for low back pain, Phys. Ther. 63 (1983) 489–493.
[8] R. Melzack, P.D. Wall, Pain mechanisms: a new theory, Science 150 (1965) 971–979.
[9] G. Miletic, V. Miletic, Long-term changes in sciatic-evoked A-fiber dorsal horn field potentials accompany loose ligation of the sciatic nerve in rats, Pain 84 (2000) 353–359.
[10] G. Raigorodsky, G. Urca, Intrathecal *N*-methyl-D-aspartate (NMDA) activates both nociceptive and antinociceptive systems, Brain Res. 422 (1987) 158–162.
[11] M. Randić, M.C. Jiang, R. Cerne, Long-term potentiation and long-term depression of primary afferent neurotransmission in the rat spinal cord, J. Neurosci. 13 (1993) 5228–5241.
[12] J. Sandkühler, Neurobiology of spinal nociception: new concepts, in: G. Carli, Z. Zimmermann (Eds.), Prog. Brain Res., Towards the Neurobiology of Chronic Pain, vol. 110, Elsevier, Amsterdam, 1996, pp. 207–224.
[13] J. Sandkühler, Long-lasting analgesia following TENS and acupuncture: Spinal mechanisms beyond gate control, in: M. Dever, M.C. Rowbotham, Z. Wiesenfeld-Hallin (Eds.), Proceedings of the 9th World Congress on Pain, Progress in Pain Research and Management, vol. 16, WA, IASP, Seattle, 2000, pp. 359–369.
[14] J. Sandkühler, J.G. Chen, G. Cheng, M. Randić, Low-frequency stimulation of afferent Aδ-fibers induces long-term depression at primary afferent synapses with substantia gelatinosa neurons in the rat, J. Neurosci. 17 (1997) 6483–6491.
[15] R.V. Størson, A. Kjørsvik, A. Tjølsen, K. Hole, Lumbar catheterization of the spinal subarachnoid space in the rat, J. Neurosci. Methods 65 (1996) 167–172.
[16] M.O. Urban, G.F. Gebhart, Supraspinal contributions to hyperalgesia, Proc. Natl. Acad. Sci. U.S.A. 96 (1999) 7687–7692.
[17] Y.Q. Zhang, G.C. Ji, G.C. Wu, Z.Q. Zhao, Excitatory amino acid receptor antagonists and electroacupuncture synergetically inhibit carrageenan-induced behavioral hyperalgesia and spinal fos expression in rats, Pain 99 (2002) 525–535.