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Local transcutaneous electrical stimulation (TENS) effects in experimental inflammatory edema and pain

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Abstract

Few studies in the literature associated transcutaneous electrical stimulation (TENS) use with an antiinflammatory activity. The purpose of this study was to investigate the effects of low (10 Hz)- and high (130 Hz)-frequency TENS on hyperalgesia and edema that occur after injection of carrageenan in rat paw. After induction of inflammation, either low- or high-frequency TENS was applied in the rat paw for 20 min, and the effect of TENS treatment on escape or paw withdrawal and edema was measured. Both low- and high-frequency TENS inhibited by 100% the hyperalgesia but not the edema response. However, low-frequency TENS presented longer lasting effect as compared with high-frequency TENS. Naltrexone-treated animals showed a complete reversion of the analgesic effect induced by low- but not high-frequency TENS. Thus, our data demonstrated absence of an antiinflammatory effect associated to TENS use and confirmed the participation of endogenous opioids on low TENS-induced analgesia.

Keywords: Hyperalgesia; Inflammation; TENS; Carrageenan; Opioid; Electrotherapy

1. Introduction

Transcutaneous electrical nerve stimulation (TENS) is a routine noninvasive treatment used in physiotherapy practice for pain relief in several inflammatory conditions (Levy et al., 1987; Robinson, 1996; Ghoname et al., 1999). The first clinical trial showing TENS efficacy in the control of chronic pain was presented by Wall and Sweet (1967) as early as 1967. From this very first study, many others were followed, which showed that TENS variables, such as frequency, stimulus intensity or pulse duration, could be manipulated to contribute to the analgesic efficacy presented by TENS (Sluka et al., 1998; Gopalkrishnan and Sluka, 2000). Such studies have indicated that differential mechanisms underlie, for instance, high (>100 Hz)- and low (<10 Hz)-frequency TENS-induced analgesia, although both frequencies could decrease dorsal horn neuron activity during stimulation of somatic receptive fields in rats (Ma and Sluka, 2001). In humans, it has been demonstrated reversal by the opioid antagonist naloxone of the analgesia induced by low-frequency TENS, which was not seen following high-frequency TENS application (Sjolund and Eriksson, 1979). At the experimental level, low (4 Hz)- and high (100 Hz)-frequency TENS have been shown to reduce hyperalgesia derived from injection of carrageenan or kaolin in rat paws (Sluka et al., 1998, 1999), two standard models in the study of inflammatory pain mechanisms (Ferreira et al., 1993; Resende et al., 2001; Francischi et al., 2002). These models are also useful in the discovery of new

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analgesic and antiinflammatory drugs (Smith et al., 1998; Hawkey, 1999).

However, few studies in the literature associated TENS use with an antiinflammatory activity. We sought in the present study to verify whether a local application of low (10 Hz)- or high (130 Hz)-frequency TENS in rat paws would also affect paw inflammation (edema), in parallel to the reported reduced hyperalgesic response. In addition, participation of endogenous opioids in this response through the use of a specific opioid antagonist (naltrexone) was also studied.

2. Materials and methods

The protocols used in the present study were approved by a local animal ethics committee (n° 19/2003).

2.1. Animals

Female Holtzman rats, weighing 160–180 g, from Bioterism Center (CEBIO, Federal University of Minas Gerais) were used throughout this study. The animals (four to six per cage) were left to adapt to the new environment in a room under temperature control (23–25 °C) and a light–dark cycle of 12 h, beginning at 7 a.m. with food and water ad libitum.

2.2. Carrageenan model of paw inflammation and pain

In essence, λ-carrageenan (250 μg/0.1 ml physiological saline, pH 7.2–7.4) was subcutaneously injected in one of the plantar surface of rat hind paws (right) at zero time. The contralateral paw was injected with the same volume of saline (vehicle). This carrageenan protocol has proved optimal to induce paw hyperalgesia and edema development in previous studies (Resende et al., 2001; Francischi et al., 2002).

2.3. Hyperalgesia measurement

Assessment of algesia consisted of measurement of the threshold stimulus for reaction (escape or paw withdrawal) using a weight (maximum limit of 500 g) applied to the pads of hind paws by an experimenter using an Ugo Basile apparatus; this is essentially the Randall and Selitto (1957) method. The threshold for pain sensation was measured before (time 0) and 1, 2, 3, 4, 6 and 24 h after the intraplantar injection of carrageenan. Results are presented as the difference in threshold between the test (carrageenan-injected) paw and the contralateral, control (saline-injected) paw. The more negative the value in *y*-axis, the greater hyperalgesia intensity is show by the animal. Groups of *N* rats yielded a mean (±S.E.M.) value for this *difference in threshold; this is the value shown in the figures as hyperalgesia in grams.

2.4. Edema measurements

The volume (ml) of the hind paws from control and treated animals was measured with a hydroplethysmometer (Ugo Basile 1750) at the same time points used for hyperalgesia measurements, i.e., time 0 and 1, 2, 3, 4, 6 and 24 h after stimulus injection. Results are presented as the difference in volume between the test paw and the control paw for each animal with means (±S.E.M) derived from the treatment groups, as before.

2.5. Transcutaneous electrical stimulation (TENS)

Electrical stimulation of rat paws with TENS was made through a Neurodyn III apparatus (IBRAMED, Brazil). This apparatus has been previously calibrated to release the following parameters: high- and low-frequency stimulation of 130 and 10 Hz, respectively, with pulse duration of 130 μs. Limit for sensory intensity was considered immediately below the motor threshold. Stimulation of test groups (N=6 per group) was released during 20 min by means of two specially constructed electrods (1-cm^2 size), each one fixed in the dorsal and plantar surface of rat paws at 2 h and 30 min of carrageenan injection. A control (placebo) group (N=6), constituted by animals in which electrods were fixed in carrageenan-injected paws for 20 min but with no stimulation release, was also used. Animals were freely moving in the cages during placebo and TENS delivery.

2.6. Drug treatment

The opioid antagonist naltrexone (3 mg/kg) was injected subcutaneously (s.c.) 2 h before carrageenan administration to verify involvement of endogenous opioid system during TENS treatment.

2.7. Statistics

Animals were randomly ascribed to TENS or placebo treatments. Mean group hyperalgesia and edema measurements (±S.E.M.) at depicted times were compared under the various treatments, with accepted differences when $P<0.05$ given by analysis of variance (one-way ANOVA), using the Sigma Stat 1.0 software in a Windows environment.

3. Results

3.1. Hyperalgesia and edema development following intraplantar injection of carrageenan in rat paws

Experimental conditions of inflammation (hyperalgesia and paw edema) in rat paws were established using the intraplantar administration of different doses of carrageenan

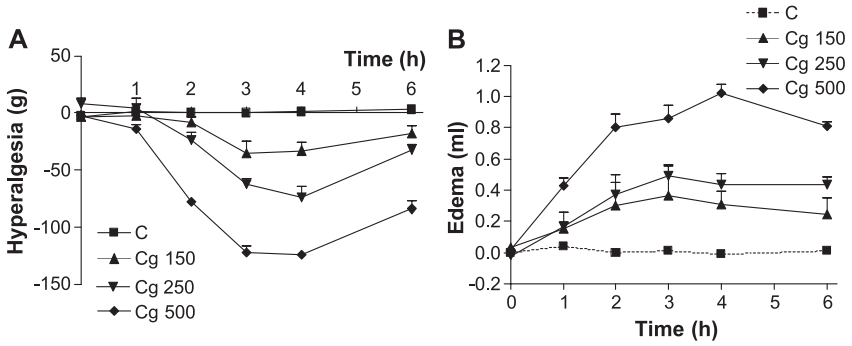

Fig. 1. Hyperalgesia (A) and edema (B) development following intraplantar injection of carrageenan in rat paws. ■ Saline (C—control), ▲ carrageenan (Cg, 150 μg/paw), ▼ carrageenan (Cg, 250 μg/paw), ◆ carrageenan (Cg, 500 μg/paw). Hyperalgesia (A) is described as the minimal weight in grams applied to pads necessary to provoke an escape movement by the animals at the various time points using a Randall-Selitto apparatus. The greater the negative *y*-axis values, the most intense the hyperalgesic response is. Edema (B) was obtained by volume displacement of hind paws (ml) using a plethysmometer apparatus. Results were presented as the difference between test and control paw values±S.E.M. in groups of five animals.

(150 to 500 μg/paw). As shown in panels (A) and (B) in Fig. 1, carrageenan induced a significant and dose-dependent hyperalgesia and paw edema development, respectively. Compared with control (saline-injected) animals, the hyperalgesic and paw edema responses to carrageenan administration reached a maximal level by 3 h of injection and lasted for the further 3 h, as also shown in Fig. 1. A return to basal conditions occurred 24 h later in animals injected with the two smaller carrageenan doses, i.e., 150 and 250 μg/paw (data not shown). The dose of 250 μg/paw carrageenan was chosen as the internal standard to induce hyperalgesia and paw edema in further studies.

3.2. Effect of low and high transcutaneous electrical stimulation (TENS) in the carrageenan model of pain and inflammation

The effectiveness of TENS in the chosen experimental model of inflammation was verified by submitting rats to a local 20-min application of 10- or 130-Hz intensity TENS at 2 ½ h of carrageenan response. As shown in panels (A) and (B) in Fig. 2, both TENS intensities completely reverted the hyperalgesia presented by carrageenan-treated animals in the following time point studied, i.e., at 3 h, indicating development of an analgesic effect by TENS treatment. However, the analgesic response due to TENS was of a longer lasting nature in low frequency (10 Hz)—as compared with the higher frequency-treated animals (130 Hz), as it was also detected following 4 h of carrageenan administration in the former animals or 1½ h after TENS application. Surprisingly, paw edema responses were not modified by TENS treatment of the animals at any frequency (Fig. 3A and B). Otherwise, switching off the TENS delivery apparatus did not alter control (hyperalgesia and paw edema) responses (shown by carrageenan injected animals; Fig. 4A and B).

3.3. Effect of naltrexone on low- and high-frequency TENS-induced analgesia

To test the effect of a pure opioid antagonist on TENS-induced analgesia, animals were systemically treated with 3 mg/kg naltrexone 30 min before TENS. Naltrexone-treated animals have shown a complete reversion of the analgesic effect induced by low-frequency TENS at the 3rd and 4th h of observation, as shown in panel (A), Fig. 5. Strikingly, the

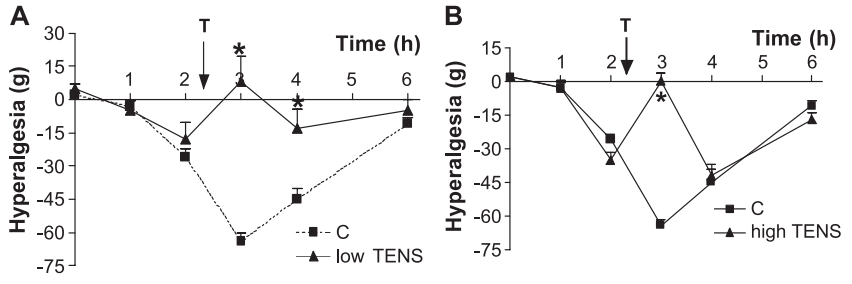

Fig. 2. Effect of low (A)- and high (B)-frequency TENS on hyperalgesia induced by carrageenan in rat paws. ■ C—controls (with no electrical stimulation). ▲ TENS (low-frequency TENS-10 Hz and high-frequency TENS, 130 Hz). T—stimulation of test group at 2 h and 30 min of carrageenan injection. Hyperalgesia is described as the minimal weight in grams applied to pads necessary to provoke an escape movement by the animals at the various time points using a Randall-Selitto apparatus. Results were presented as the difference between test and control values±S.E.M. in groups of five to six animals. * indicates $p < 0,05$ given by one-way ANOVA.

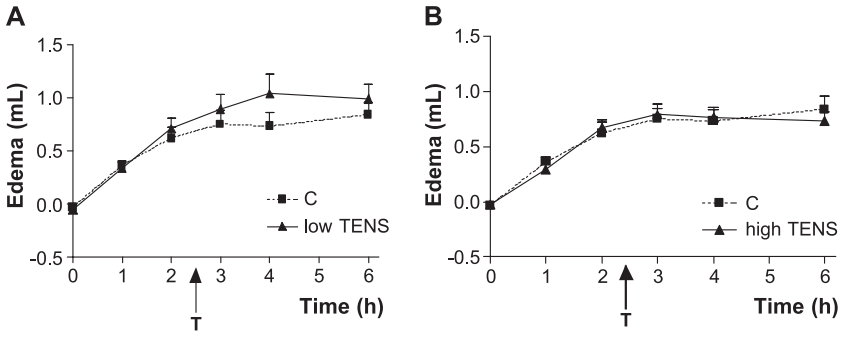

Fig. 3. Effect of low (A)- and high (B)-frequency TENS on edema induced by carrageenan in rat paws. ■ C—controls (no electrical stimulation). ▲ Low TENS (low-frequency TENS,10 Hz, and high frequency, 130 Hz). T—stimulation of test group at 2 h and 30 min of carrageenan injection. Edema was obtained by volume displacement of hind paws using a plethysmometer apparatus. Results were presented as the difference between test and control values±S.E.M. in groups of five to six animals. *indicates $p<0,05$ given by one-way ANOVA.

analgesic effect of high-frequency TENS was neither modified by previous treatment of the animals with the same naltrexone dose (Fig. 5B) nor paw edema responses shown by these animals (data not shown).

4. Discussion

Much of the success of the physiotherapy, while a clinical therapy, relies on the adequacy of the physical methods employed by the physiotherapists to treat their patients (Prentice, 1998). It was long ago established in either experimental animals and humans that one of such methods, transcutaneous electrical stimulation (TENS), induces analgesia through endogenous opioids and non-opioid release mechanisms, and that TENS-induced analgesia is dependent on stimulation parameters (Sjolund and Eriksson, 1979). Progress with TENS use in clinics made available many methods for its application under various pathological conditions presented by patients (Robinson, 1996). However, basic information for a more efficacious use of TENS in humans is still lacking inasmuch as sparse and often poorly controlled experiments using TENS are found in the literature. In addition, few studies have been developed to demonstrate TENS efficacy under inflammatory conditions. In the present study, we sought to verify the efficacy of two-frequency intensities of TENS (10 or 130 Hz) in a standard experimental model of inflammation in rats (Sluka et al., 1994), which allows concomitant pain and paw edema development evaluation. As it has been shown, low- and high-frequency TENS were highly effective as analgesic treatments, as both abolished inflammatory pain (hyperalgesia) presented by the animals, thus confirming and extending previous reports (Gopalkrishnan and Sluka, 2000). Furthermore, low frequency compared with high-frequency TENS has shown a better analgesic profile as its effect was of longer duration under our experimental conditions. However, no antiedematogenic efficacy has been observed following either high- or low-frequency TENS used. In fact, paw edema and hyperalgesia responses seem to subserve independent mechanisms of activation, as observed in various models of acute and chronic inflammation by our and other groups (Tatsuo et al., 1994; Resende et al., 2001; Lorenzetti and Ferreira, 1985). Moreover, our data strongly suggest that the analgesic mechanism of low-frequency TENS but not that of high-

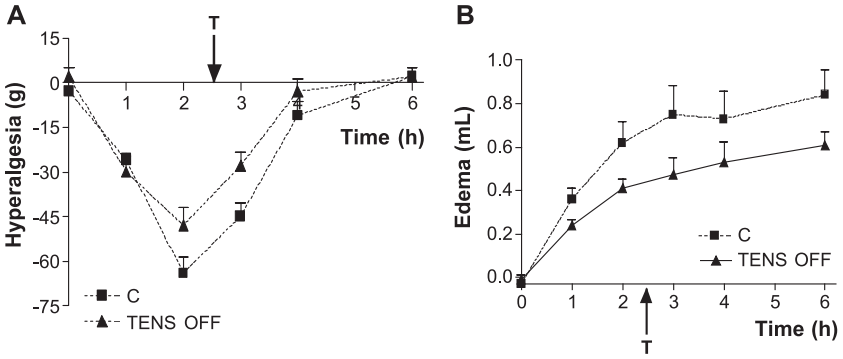

Fig. 4. Effect of switched-off TENS on hyperalgesia (A) and edema (B) induced by carrageenan ■ C—Cg-injected animals (A and B). ▲ Switched-off TENS (placebo group, panels A and B). T—switched-off TENS was applied at 2 h and 30 min after intraplantar injection of carrageenan. Results (in g [A] and in ml [B]) depicted in the figure refer to the mean difference between test and control paw values±S.E.M. in groups of five animals.

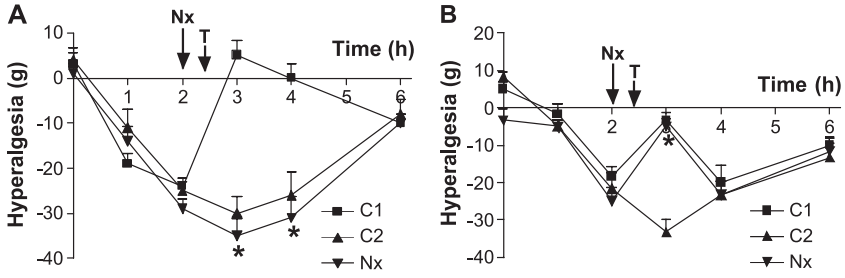

Fig. 5. Effect of naltrexone (Nx) on low (A)- and high (B)-frequency TENS following hyperalgesia induced by carrageenan (Cg). Nx and T indicate treatment with naltrexone and TENS, respectively. C1—saline was administered subcutaneously at 2 h after Cg and associated at 2 h and 30 min with low (10 Hz)- or high (130 Hz)-frequency TENS. ▲ C2—saline was administered subcutaneously at 2 h after carrageenan and associated at 2 h and 30 min with switched-off TENS. ▼ Nx—naltrexone was administered at 2 h after Cg and associated with low (10 Hz) or high (130 Hz) at 2 h and 30 min after injection of Cg in rat paw. N=6–8 animals/group *indicates p<0,05 given by one-way ANOVA.

frequency TENS is related to the endogenous release of opioids inasmuch as it has been reverted by a systemic low dose of naltrexone, a long-lasting and full opioid antagonist (Reisine and Pasternack, 1996). This result was consistent with those from Watkins et al. (1984) and Terman et al. (1984), whose studies used both higher doses of naltrexone and a more aggressive or stressful stimulation, such as that given by inescapable foot shock in rats. In addition, our data are in agreement with findings from Sluka et al. (1999), who showed similar results to ours using naloxone, another classical full opioid antagonist. Other studies have also demonstrated that the analgesic effect produced by low-frequency stimulation is naloxone reversible, while high-frequency stimulation is not (Lee and Beitz, 1992; Guo et al., 1996). However, controversy still remains, as several other studies have demonstrated a participation of endogenous opioids in analgesia induced by both high- and low-frequency TENS (Hughes et al., 1984; Han et al., 1991; Kalra et al., 2001). In our opinion, the controversy may reflect differential experimental conditions used, such as the site of TENS stimulation or the own frequency chosen by the different research groups. Perhaps, more importantly, other endogenous substances at the central nervous system, such as histamine, serotonin, noradrenaline, etc., may also mediate TENS-induced analgesia (Terman et al., 1984).

Giving further support to our working hypothesis, i.e., the involvement of endogenous opioids in the analgesic efficacy of low-frequency TENS is the existence of spinal opioid circuits and/or activation of descending inhibitory pathways (Kalra et al., 2001), whose neurons contain mu, delta and κappa opioid receptors. These receptors have been localized presynaptically on primary afferent fibers and postsynaptically on dorsal horn neurons (Lamotte et al., 1976; Fields et al., 1980). Furthermore, the presence of opioid peptides met-enkephalin-Arg-Phe (MEAP) and dynorphin A in the cerebral spinal fluid of humans after application of either high- or low-frequency TENS have been demonstrated (Han et al., 1991). Localization of these receptors and the presence of endogenous opioids in the spinal cord provide both an anatomic and a pharmacological basis to understand the modulation of inflammatory pain-driven inputs by low-frequency TENS under our experimental conditions.

Stress during manipulation of the animals could be another source contributing to the pain relief given by TENS in our assays, as previously considered. So, to evaluate the contribution of stress in the detected TENS-induced analgesia, animals were exposed to switched-off TENS. As shown, no analgesic effect was detected under such condition, thus discarding a stress component to the analgesic effect observed in animals treated either with low- or high-frequency TENS. However, mechanisms underlying the detected differences between analgesia induced by low- and high-frequency TENS, apart (1) the longer duration of analgesic effect and (2) the participation of endogenous opioids in the former case as presently demonstrated, remain unclear. At light of such findings, however, we may suggest that the use of the lower compared with higher frequency TENS may have a better prognostic profile in the treatment of small contusions, for instance, in athletes.

In conclusion, our data clearly demonstrated absence of an antiinflammatory effect associated with TENS use, independent of whether TENS was delivered in high (130 Hz)- or low-frequency (10 Hz) intensities. However, low- and high-frequency TENS were highly effective as a local treatment for inflammatory pain relief, giving further support for TENS use under inflammatory conditions, such as those accompanying small accidents in sports and in daily life. In addition, participation of endogenous opioids on TENS-induced analgesia was confirmed in low- but not in high-frequency (TENS) treated animals, thus confirming and extending previous findings in which concomitant inflammation had not been addressed as an intervening factor.

References

Ferreira, S.H., Lorenzetti, B.B., Poole, S., 1993. Bradykinin initiates cytokines mediated inflammatory hyperalgesia. Br. J. Pharmacol. 110, 1227–1231.

Fields, H.L., Emson, P.C., Leigh, B.K., 1980. Multiple opiate receptor sites on primary afferent fibers. Nature 284, 351–353.

Francischi, J.N., Chaves, C.T., Moura, A.C.L., Lima, A.S., Ferreira-Alves, D.L., Rocha, O.A., Bakhle, Y.S., 2002. Selective inhibitors of cyclo-oxygenase-2 (COX-2) induce hypoalgesia in a rat paw model of inflammation. Br. J. Pharmacol. 137, 837–844.

Ghoname, E.A., Graig, W.F., White, P.F., Ahmed, F.H.E., Hamza, M.A., Gajraj, N.M., Vakharia, A.S., Noe, C.E., 1999. The effect of stimulus frequency on the analgesic response to percutaneous electrical nerve stimulation in patients with chronic low back pain. Anesth. Analg. 88, 841–846.

Gopalkrishnan, P., Sluka, K.A., 2000. Effect of varying frequency, intensity and pulse duration of transcutaneous electrical nerve stimulation on primary hyperalgesia in inflamed rats. Arch. Phys. Med. Rehabil. 81, 984–990.

Guo, H.F., Tian, J., Wang, X., Fang, Y., Hou, Y., Han, J., 1996. Brain substrates activated by electroacupuncture of different frequencies: (I). Comparative study on the expression of oncogene c-*fos* and genes coding for three opioid peptides. Mol. Brain Res. 43, 157–166.

Han, J.S., Chen, X.H., Sun, S.L., Xu, X.J., Yuan, Y., Yan, S.C., Hao, J.X., Terenius, L., 1991. Effect of low- and high-frequency TENS on Met-enkephalin-Arg-Phe and dynorphin A immunorreactivity in human lumbar CSF. Pain 47, 295–298.

Hawkey, C.J., 1999. COX-2 inhibitors. The Lancet 353, 307–314.

Hughes Jr., G.S., Lichstein, P.R., Whitlock, D., Harker, C., 1984. Response of plasma beta-endorphins to transcutaneous electrical nerve stimulation in healthy subjects. Phys. Ther. 64, 1062–1066.

Kalra, A., Urban, M.O., Sluka, K.A., 2001. Blockade of opioid receptors in rostral ventral medulla prevents antyperalgesia produced by transcutaneous electrical nerve stimulation (TENS). J. Pharmacol. Exp. Ther. 298, 257–263.

LaMotte, C., Pert, C.B., Snyder, S.H., 1976. Opiate receptor binding in primate spinal cord. Distribution and changes after dorsal root section. Brain Res. 112, 407–412.

Lee, J.H., Beitz, A.J., 1992. Electroacupuncture modifies the expression of c-*fos* in the spinal cord induced by noxious stimulation. Brain Res. 577, 80–91.

Levy, A., Dalith, M., Abramovici, A., Pinkhas, J., Weinberger, A., 1987. Transcutaneous electrical nerve stimulation in experimental acute arthritis. Arch. Phys. Med. Rehabil. 68, 75–78.

Lorenzetti, B.B., Ferreira, S.H., 1985. Mode of analgesic action of dipyrone: direct antagonism of inflammatory hyperalgesia. Eur. J. Pharmacol. 114, 375–381.

Ma, Y.T., Sluka, K.A., 2001. Reduction in inflammation-induced sensitization of dorsal horn neurons by transcutaneous electrical nerve stimulation in anesthetized rats. Exp. Brain Res. 13, 94–102.

Prentice, W.E., 1998. Therapeutic modalities in rehabilitation. In: Prentice, W.E. (Ed.), Rehabilitation Techniques in Sports Medicine. Mosby, St. Louis, pp. 204–218.

Randall, L.D., Selitto, J.J., 1957. A method for measurement of analgesic activity on inflamed tissues. Arch. Int. Pharmacodyn. 113, 233–249.

Reisine, T., Pasternack, G., 1996. Opioid analgesics and antagonists. In: Goodman, A., Gilman, H.J.G., Limbird, L.E., Molinoff, P.B., Raymond, W.R. (Eds.), The Pharmacological Basis of Therapeutics. The McGraw-Hill, México, pp. 521–555.

Resende, M.A., Reis, W.G.P., Pereira, L.S.M., Ferreira, W., Garcia, M.H.L.P., Santoro, M.M., Francischi, J.N., 2001. Hyperalgesia and edema responses induced by rat peripheral blood mononuclear cells incubated with carrageenin. Inflammation 25, 277–285.

Robinson, A.J., 1996. Transcutaneous electrical nerve stimulation for the control of pain in musculoskeletal disorders. JOSPT 24, 208–226.

Sluka, K.A., Jordan, H.H., Westlund, K.N., 1994. Reduction in joint swelling and hyperalgesia following post treatment with a non-NMDA glutamate receptor antagonist. Pain 59, 95–100.

Sluka, K.A., Bailey, K., Bogush, J., Olson, R., Ricketts, A., 1998. Treatment with either high or low frequency TENS reduces the secondary hyperalgesia observed after injection of kaolin and carrageenan into the knee joint. Pain 77, 97–102.

Sluka, K.A., Deacon, M., Stibal, A., Strissel, S., Terpstra, A., 1999. Spinal blockade of opioid receptors prevents the analgesia produced by TENS in arthritic rats. J. Pharmacol. Exp. Ther. 289, 840–846.

Smith, C.J., Zhang, Y., Koboldt, C.M., Muhammad, J., Zweifel, B.S., Shaffer, A., Talley, J.J., Masferrer, J.L., Seibert, K., Isakson, P.C., 1998. Pharmacological analysis of cyclooxygenase-1 in inflammation. Proc. Natl. Acad. Sci. U. S. A. 95, 13313–13318.

Sjolund, B.H., Eriksson, B.E., 1979. The influence of naloxone on analgesia produced by peripheral conditioning stimulation. Brain Res. 137, 295–301.

Tatsuo, M.A.K.F., Carvalho, W.M., Silva, C.V., Miranda, A.E.G., Ferreira, S.H., Francischi, J.N., 1994. Analgesic and anti-inflammatory effects of dipirone in rat adjuvant arthritis model. Inflammation 18, 399–405.

Terman, G.W., Shavit, Y., Lewis, J.W., Cannon, J.T., Liebeskind, J.C., 1984. Intrinsic mechanisms of pain inhibition: activation by stress. Science 226, 1270–1277.

Wall, P.D., Sweet, W.H., 1967. Temporary abolition of pain in man. Science 155, 108–109.

Watkins, L.R., Drugan, R., Hyson, R.L., Moye, T.B., Ryan, S.M., Mayer, D.J., Maier, S.F., 1984. Opiate and non-opiate analgesia induced by inescapable tail-shock: effects of dorsolateral funiculus lesions and decerebration. Brain Res. 291, 325–336.