Pain 120 (2006) 182–187

PAIN

www.elsevier.com/locate/pain

Transcutaneous electrical nerve stimulation (TENS) reduces chronic hyperalgesia induced by muscle inflammation

Lisa Ainsworth [a], Kendra Budelier [a], Monica Clinesmith [a], Abby Fiedler [a], Rachel Landstrom [a], B.J. Leeper [a], LeAnn Moeller [a], Sarah Mutch [a], Kim O'Dell [a], Jaclyn Ross [a], Rajan Radhakrishnan [a,b,1], Kathleen A. Sluka [a,b,c,*]

[a] *Graduate Program of Physical Therapy and Rehabilitation Science, University of Iowa, Iowa, IA, USA*
[b] *Pain Research Program, University of Iowa, Iowa, IA, USA*
[c] *Neuroscience Graduate Program, University of Iowa, Iowa, IA, USA*

Received 17 June 2005; received in revised form 21 October 2005; accepted 31 October 2005

Abstract

Transcutaneous electrical nerve stimulation (TENS) reduces pain through central mechanisms involving spinal cord and brainstem sites. Since TENS acts through central mechanisms, we hypothesized that TENS will reduce chronic bilateral hyperalgesia produced by unilateral inflammation when applied either ipsilateral or contralateral to the site of muscle inflammation. Sprague–Dawley rats were injected with carrageenan in the left gastrocnemius muscle belly. Mechanical withdrawal threshold was tested bilaterally before and 2 weeks after carrageenan injection. After testing withdrawal thresholds at 2 weeks, rats received TENS treatment either ipsilateral or contralateral to the site of inflammation. In each of these groups, rats were randomized to control (no TENS), low frequency (4 Hz), or high frequency (100 Hz) TENS treatment. TENS was applied for 20 min at sensory intensity under light halothane anesthesia. Mechanical withdrawal thresholds were re-assessed after TENS or 'no TENS' treatment. Unilateral injection of carrageenan to the gastrocnemius muscle significantly reduced the mechanical withdrawal threshold (mechanical hyperalgesia) bilaterally 2 weeks later. Either low or high frequency TENS applied to the gastrocnemius muscle ipsilateral to the site of inflammation significantly reversed mechanical hyperalgesia, both ipsilateral and contralateral to the site of inflammation. Low or high frequency TENS applied to the gastrocnemius muscle contralateral to the site of inflammation also significantly reduced mechanical hyperalgesia, both ipsilateral and contralateral to the site of inflammation. Since ipsilateral or contralateral TENS treatments were effective in reducing chronic bilateral hyperalgesia in this animal model, we suggest that TENS act through modulating descending influences from supraspinal sites such as rostral ventromedial medulla (RVM).

Keywords: TENS; Hyperalgesia; Rat; Inflammation; Muscle; Carrageenan; Chronic

1. Introduction

Chronic pain, especially musculoskeletal pain, is a widespread problem that often goes untreated due to lack of adequate treatment options, and also due to lack of understanding of alternative therapeutic modalities. Apart from pharmacological treatments, there are non-pharmacological modalities, such as transcutaneous electrical nerve stimulation (TENS), which are clinically used to relieve pain. Despite a large number of preclinical studies, including some from our laboratory, the analgesic mechanisms of TENS are not fully understood. Animal models of chronic musculoskeletal pain (Radhakrishnan et al., 2003a, b,c; Sluka et al., 2001) allow an experimental framework to determine the effectiveness and mechanisms of TENS in chronic pain.

* Corresponding author. Address: Graduate Program in Physical Therapy and Rehabilitation Sciences, The University of Iowa, 1-252 Medical Education Building, Iowa City, IA 52242-1190, USA. Tel.: +1 319 335 9791; fax: +1 319 335 9707.
E-mail address: kathleen-sluka@uiowa.edu (K.A. Sluka).
[1] Current address: Department of Pharmaceutical Sciences, College of Pharmacy, Western University of Health Sciences, Pomona, CA, USA.

0304-3959/$20.00 © 2005 International Association for the Study of Pain. Published by Elsevier B.V. All rights reserved.
doi:10.1016/j.pain.2005.10.030

Transcutaneous electrical nerve stimulation (TENS) is defined as the application of electrical stimulation to the skin for pain control (Clinical Electrophysiology and American Physical Therapy Association, 2001). Wall and Sweet in 1967 promoted the use of TENS for treatment of chronic pain in direct follow up of the gate control theory of pain. The gate control theory proposed that stimulating large diameter afferents with TENS inhibits nociceptive afferent fiber-evoked responses in the dorsal horn (Melzack & Wall, 1965). Clinically, TENS is applied at either a high frequency (>50 Hz) or low frequency (<10 Hz). The intensity varies between subsensory, sensory, or motor. Prior studies from our laboratory show that secondary hyperalgesia induced by acute knee joint inflammation is reduced by either high or low frequency TENS at sensory intensity (King & Sluka, 2001; Sluka et al., 1998). Thus, sensory intensity TENS reduces acute inflammatory hyperalgesia.

TENS produces its analgesic effect in part by activation of endogenous opioids (for review see Sluka & Walsh, 2003). TENS activates opioid receptors in the rostral ventromedial medulla (RVM) and in the spinal cord (Kalra et al., 2001; Sluka et al., 1999). Activation of opioid receptors in the RVM sends increased inhibitory input to the spinal dorsal horn, decreasing activation of dorsal horn neurons (Fields & Basbaum, 1999; Fields et al., 1983). These data suggest that the analgesic effects of TENS utilize both supraspinal and spinal mechanisms.

The animal model of chronic muscle pain used in this study exhibits chronic bilateral hyperalgesia (Radhakrishnan et al., 2003). Since TENS activates spinal and supraspinal sites (Kalra et al., 2001; Sluka et al., 1999), and supraspinal pathways project bilaterally to the spinal cord (Antal et al., 1996; Zemlan et al., 1984), we hypothesize that chronic bilateral hyperalgesia in this model will be reduced by TENS irrespective of the side of application, i.e. ipsilateral or contralateral to inflammation.

2. Materials and methods

2.1. Animals

Male Sprague–Dawley rats ($n=45$, Harlan, St Louis, MO), weighing 250–450 g were used for the experiments. The rats were housed in a 12 h light–dark cycle, three rats per cage, with free access to standard rat food and water. All experiments were approved by the University of Iowa Animal Care and Use Committee and were carried out according to the guidelines of the International Association for the Study of Pain and National Institute of Health (Zimmerman, 1983).

2.2. Pain behavior testing

The animals were kept in a plastic cubicle (16×8×8 cm) on an elevated platform with a wire mesh screen. Threshold to mechanical stimuli was tested using von Frey filaments (North Coast Medical, Morgan Hill, CA) with increasing bending force as previously described (Sluka et al., 2001). von Frey filaments were applied twice, at least 1 s apart, to the plantar aspect of the hind paw, bilaterally. A paw withdrawal in response to the filament application was noted as a response to noxious mechanical stimulus. Testing was started with the filament with the lowest bending force. If there was no response, the next higher filament force was applied. The bending force of the filament, which causes a withdrawal response was interpreted as the mechanical withdrawal threshold (MWT). The following bending forces were assessed: 9.86, 12.9, 16.6, 33.8, 53.2, 71.7, 89.1, 129, 207, and 474 mN. All animals had a baseline mechanical threshold of 129 mN or higher in this study. A decrease from baseline in withdrawal threshold was interpreted as mechanical hyperalgesia in this study. The reliability and validity of this testing method has been previously established (Gopalkrishnan & Sluka, 2000). Behavior testing was done with the tester blinded to treatment group.

2.3. Induction of inflammation

The rats were injected with 0.1 ml of 3% lambda carrageenan (Type IV, Sigma Chemical Co., St Louis, MO, dissolved in sterile saline) into the left gastrocnemius muscle. Anesthesia was induced in rats using 5% halothane in oxygen in an anesthetic chamber, and maintained with 1–3% halothane, continuously administered through a nose cone. The skin overlying left gastrocnemius muscle belly was cleaned with alcohol prep-pads, muscle located by palpation, and 3% carrageenan was injected with sterile hypodermic needles (26 G, 3/8″) into the muscle belly, percutaneously. Following injection, the animal was removed from the halothane and returned to the cage, after it recovered from anesthesia. A 2-week duration was allowed for the development of chronic inflammation and bilateral hyperalgesia. Two-week time point was selected for this study because distinct bilateral mechanical hyperalgesia occurs at this time (Radhakrishnan et al., 2003).

2.4. TENS application

Commercially available TENS units and electrodes (EMPI Inc., Minnesota, USA) were used in this study. The current study examined the effects of TENS in rats using two different TENS application paradigms: ipsilateral application (left gastrocnemius; control, $n=5$; high frequency TENS, $n=8$; low frequency TENS, $n=7$) to the inflammation site, and contralateral application (right gastrocnemius; control, $n=8$; high frequency TENS, $n=8$; low frequency TENS, $n=8$) to the inflammation site. Within these groups, rats were randomly assigned to either a ‘no TENS’ control group (anesthesia only), a high frequency (100 Hz) TENS group, or a low frequency (4 Hz) TENS group. Rats were lightly anesthetized with 1–2% halothane, via a vaporizer, for application of TENS to minimize movement. Rats still had withdrawal reflexes while under anesthetic and recovered from anesthetic within 5 min after removal of the halothane. Importantly, 2–4 rats, always including a sham control and a TENS treatment, were treated simultaneously receiving anesthetic through the same vaporizer. This ensures that the same dose of anesthetic is administered to rats that received sham treatment and TENS treatment further minimizing

effects of anesthetic. Prior studies show that the effects of TENS are long lasting, and that light halothane anesthetic has no effect on the decreased withdrawal thresholds produced by inflammation (Sluka et al., 1998).

After the rats were anesthetized, two circular, pregelled electrodes (trimmed to 1 cm in diameter) were placed on the skin overlying the gastrocnemius muscle, one proximal close to the knee joint and one distal near the Achilles tendon. This resulted in the electrodes being placed 2 cm apart (center to center), approximately 1 cm distance between the edges of the two electrodes. Either low or high TENS was applied just below threshold to produce a motor contraction; we previously designated this as 'sensory intensity' (Sluka et al., 1998). The pulse duration was kept at 100 μs in all groups and the TENS treatments were of 20 min duration. The 'no TENS' control group received 20 min of halothane anesthesia along with application site preparation and the electrode placement, except stimulation. These stimulation parameters reduce mechanical and heat hyperalgesia in models of acute inflammation (King & Sluka, 2001; Sluka et al., 1998).

2.5. Protocol

The animals were brought down from the Animal Care Unit and allowed to acclimate to their testing environment at least 1 h prior to behavioral testing. Behavioral tests were done between 8 a.m. and 4 p.m. On the first day, paw withdrawal threshold to mechanical stimuli, as described above, was measured prior to induction of inflammation (baseline, pre-injection). After baseline withdrawal testing, rats were injected with 3% carrageenan into the left gastrocnemius muscle under halothane anesthesia. The animals were returned to their cages and housed in the animal care facility for 2 weeks to allow for development of chronic inflammation and hyperalgesia.

After 2 weeks, animals were returned to the testing room for acclimation. In both TENS application groups (ipsilateral TENS or contralateral TENS), the rats were randomly assigned to (1) a control 'no TENS' group (anesthetic only), (2) a high frequency (100 Hz) TENS group, or (3) a low frequency (4 Hz) TENS group. Paw withdrawal threshold was measured before and after treatment in groups with TENS or 'no TENS' (anesthetic only). Behavior testing was performed 15–30 min after removal from anesthetic.

Following pain behavior testing, rats were euthanized and the ipsilateral and contralateral gastrocnemius muscles were dissected. Examination for signs of inflammation was done by visual comparison with the non-inflamed muscle, and inspection and palpation for edema.

2.6. Statistical analysis

A Kruskal Wallis ANOVA examined variability between groups for differences in mechanical withdrawal threshold at baseline, 2 weeks after carrageenan (post-inflammation, before TENS) and after TENS. Post hoc analysis with a signed rank test analyzed differences between control, low frequency, and high frequency treatment groups. Data were considered significant if $P<0.05$. Data are represented as the median with the 25th and 75th percentiles.

3. Results

3.1. Ipsilateral TENS treatment

Two weeks after induction of inflammation, the animals show no overt behavioral signs, i.e. limping or guarding of the limb. However, the mechanical withdrawal threshold to von Frey filaments significantly decreased in both the left paw (ipsilateral, $P=0.01$) and right paw (contralateral, $P=0.01$) 2 weeks after induction of inflammation when compared to baseline values (Fig. 1). The mechanical withdrawal threshold was not significantly different between groups for either the ipsilateral or the contralateral paw before or after inflammation.

TENS applied to the ipsilateral muscle at the site of inflammation significantly increased the mechanical withdrawal threshold on the ipsilateral ($\chi^2=7.851$, $P=0.02$) and the contralateral paw ($\chi^2=7.795$, $P=0.02$) (Fig. 1). Post hoc testing revealed that 20 min treatment with either low frequency ($P=0.03$) or high frequency ($P=0.01$)

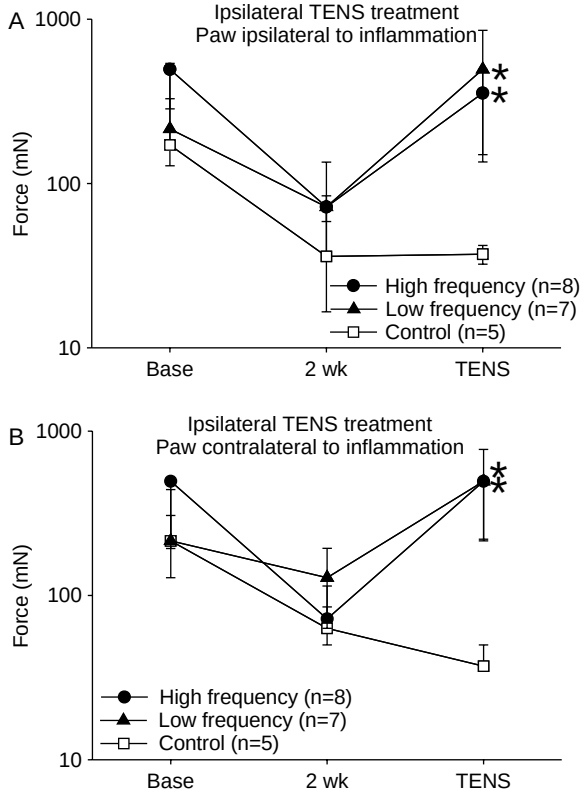

Fig. 1. Graph shows the mechanical withdrawal thresholds (MWT) to von Frey filaments for the hind paw ipsilateral to the site of inflammation (A) and the hindpaw contralateral to the site of inflammation (B) after low or high TENS treatment ipsilateral to the site of inflammation. MWT was measured at baseline, 2 weeks after carrageenan injection, and after TENS treatment. When applied on the ipsilateral side, both low and high TENS reverse the reduction in MWT in the ipsilateral as well as the contralateral hindpaw. Data points represent median values, error bars represent 25th and 75th percentiles. Asterisks (*) indicate significant difference from controls ($P<0.05$).

TENS increased the mechanical withdrawal threshold on the side ipsilateral to treatment and inflammation when compared to sham controls. Similarly, both low frequency ($P=0.016$) and high frequency ($P=0.019$) TENS increased the mechanical withdrawal threshold on the side contralateral to treatment and inflammation when compared to sham controls.

3.2. Contralateral TENS treatment

TENS applied to the gastrocnemius muscle contralateral to the side of inflammation (Fig. 2) significantly increased the mechanical withdrawal threshold (reversed hyperalgesia) on the side ipsilateral to inflammation ($\chi^2=10.149$, $P=0.006$) and the side contralateral to inflammation ($\chi^2=8.765$, $P=0.012$). Post hoc testing revealed that 20 min treatment with either low frequency ($P=0.038$) or high frequency ($P=0.001$) TENS increased the mechanical withdrawal threshold on the side contralateral to treatment when compared to sham controls. Similarly, both low frequency ($P=0.005$) and high frequency ($P=0.021$) TENS also increased the mechanical withdrawal threshold on the side contralateral to inflammation and ipsilateral to treatment when compared to sham controls.

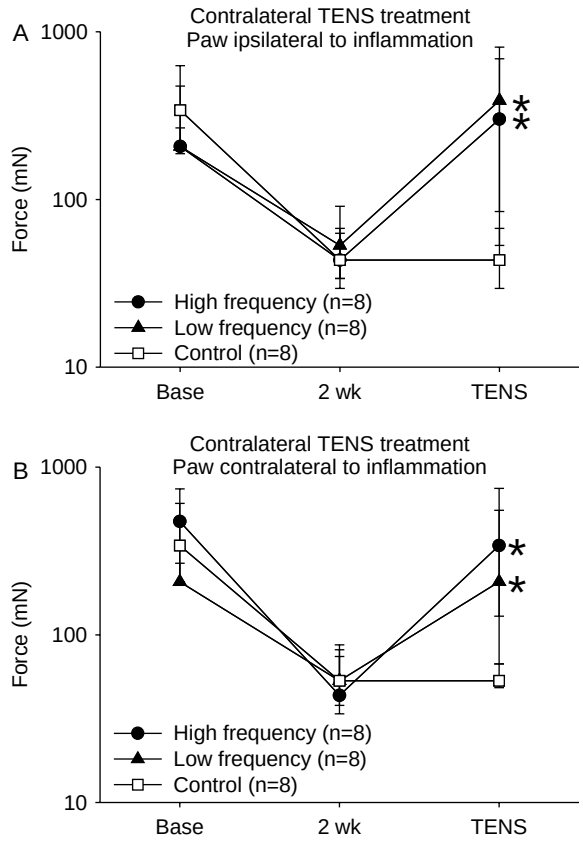

Fig. 2. Graph shows the mechanical withdrawal threshold (MWT) to von Frey filaments for the hind paw ipsilateral to the site of inflammation (A) and the hind paw contralateral to the site of inflammation (B) after low or high TENS treatment contralateral to the site of inflammation. MWT was measured at baseline, 2 weeks after carrageenan injection, and after TENS treatment. When applied on the contralateral side, both low and high TENS reverse the reduction in MWT in the ipsilateral as well as the contralateral hindpaw. Data points represent median values, error bars represent 25th and 75th percentiles. Asterisks (*) indicate significant difference from controls ($P<0.05$).

4. Discussion

The results from the present study show that injection of carrageenan into one gastrocnemius muscle decreases mechanical withdrawal threshold of the paw bilaterally. We interpret this decrease in mechanical withdrawal threshold as cutaneous mechanical hyperalgesia. People with deep tissue pain syndromes such as fibromyalgia and osteoarthritis similarly show increased cutaneous sensitivity (Berglund et al., 2002; Kosek & Ordeberg, 2000). TENS applied to either the ipsilateral or contralateral side of inflammation reduces cutaneous mechanical hyperalgesia bilaterally. Therefore, these results support our hypothesis that unilateral application of TENS to either the ipsilateral or contralateral side, reduces cutaneous mechanical hyperalgesia bilaterally, indicating that TENS acts through a central mechanism.

4.1. Chronic bilateral hyperalgesia

Unilateral injection of 3% carrageenan into the gastrocnemius muscle induces chronic bilateral hyperalgesia of the paw 2 weeks after induction of inflammation, which confirms previous findings from our laboratory (Radhakrishnan et al., 2003). The contralateral spread of hyperalgesia ('mirror' hyperalgesia) induced by unilateral muscle inflammation is considered secondary hyperalgesia. Similar spread of hyperalgesia to the contralateral side of injury has been observed by other investigators using different noxious agents (Aloisi et al., 1993; Chacur et al., 2001; Rees et al., 1996; Sluka et al., 2001). Although the mechanisms for the development of bilateral hyperalgesia are not clear, various theories have been suggested (see review by Koltzenburg et al., 1999). Most hypotheses regarding bilateral spread of hyperalgesia suggest neuronal changes in the central nervous system responsible for the bilateral spread, and there are some reports indicating a role for glial cells in the central nervous system (Chacur et al., 2001; see review by Wieseler-Frank et al., 2004). Descending influences originating in the rostral ventromedial medulla (RVM) can be either inhibitory or facilitatory (Fields & Basbaum, 1999; Kaplan & Fields, 1991; Porreca et al., 2002; Urban & Gebhart, 1999). The RVM is a group of key nuclei that mediate descending inhibition and facilitation, and likely represent the final common pathway(s) to the spinal cord. Descending facilitation is a probable mechanism that is responsible for causing secondary bilateral hyperalgesia and maintaining chronic hyperalgesia. For example, inactivation of the RVM by lidocaine reverses, and lesion of RVM by

pre-treatment with ibotenic acid completely blocks, secondary heat hyperalgesia produced by knee joint injection of carrageenan (Urban et al., 1999). These manipulations in the RVM do not affect the primary hyperalgesia produced by carrageenan injected into the plantar paw. Thus, supraspinal centers play a major role in the production and maintenance of secondary hyperalgesia. Spinal projections from the RVM are bilateral and the neurons in RVM have widespread receptive fields that include the contralateral limb (Antal et al., 1996; Basbaum et al., 1978; Hama et al., 1997; Hurley & Hammond, 2000; Zemlan et al., 1984). Thus, it is highly possible that the contralateral hyperalgesia observed in the current study could be mediated by RVM or other supraspinal centers through descending facilitatory pathways, although the current study does not directly provide data to support this hypothesis.

4.2. Bilateral effect of unilateral TENS

A significant reduction in cutaneous mechanical hyperalgesia in both the ipsilateral and contralateral paws following unilateral TENS application on either the ipsilateral or contralateral side of inflammation supports our hypothesis that TENS produces its antihyperalgesic effect through central mechanisms. There was no difference between the effectiveness of low or high frequency TENS in reducing secondary cutaneous mechanical hyperalgesia, when applied either ipsilateral or contralateral to the site of inflammation. These findings are similar to those of Sluka et al. (1998) where low and high frequency TENS reduces acute, secondary heat hyperalgesia induced by joint inflammation. Therefore, TENS appears to be equally effective for secondary heat and mechanical hyperalgesia induced by either acute or chronic inflammation.

The bilateral decrease in hyperalgesia by TENS supports the theory that TENS modulates descending influences, either activating inhibitory pathways or inhibiting facilitatory pathways. Previous works further support this interpretation. Specifically, TENS analgesia is prevented by blockade of opioid receptors in the RVM (Kalra et al., 2001). In the spinal cord, TENS utilizes opioid, serotonin, and muscarinic receptors (Radhakrishnan & Sluka, 2003; Radhakrishnan et al., 2003; Sluka et al., 1999), all of which are implicated in analgesia mediated by descending inhibition from the RVM (Atweh & Kuhar, 1977; Ding et al., 1996; Fields & Basbaum, 1999; Jensen & Yaksh, 1984). When the nuclei of RVM are stimulated electrically or chemically, changes in nociceptive thresholds occur bilaterally in the hindpaws (Hurley & Hammond, 2000; Terayama et al., 2002). In particular, opioid agonists microinjected into the RVM produce bilateral increases in the response to radiant heat (Hurley & Hammond, 2000). Taken together, these findings suggest that TENS likely produces its analgesic effect by modulating descending influences from the RVM. However, since secondary hyperalgesia is mediated by descending facilitation from the RVM (Urban et al., 1999; see review by Porreca et al., 2002) an alternative hypothesis is that that TENS reduces descending facilitation.

4.3. Clinical implications

The current study demonstrates a bilateral effect of TENS in decreasing secondary cutaneous mechanical hyperalgesia. Since ipsilateral or contralateral TENS treatments were equally effective in reducing chronic hyperalgesia in this animal model, this study provides some prospects of utilizing TENS in reducing generalized chronic pain conditions in humans. The data support the postulate that TENS decreases hyperalgesia through activation of central sites. The current study is unique in that the effect of TENS on hyperalgesia was studied after application on the ipsilateral or the contralateral side to inflammation, and it was found that TENS is effective in both modes of application. If this finding translates clinically, TENS can be applied contralaterally to the site of injury in cases of severe maceration, amputation, or cutaneous allodynia, where contact sites in the primary area of injury may be difficult to achieve.

4.4. Conclusions

The current study shows that both low and high frequency TENS reverse chronic, secondary hyperalgesia bilaterally when applied to the ipsilateral or the contralateral site of injury. These findings should, however, be confirmed in humans by randomized controlled clinical trials.

Acknowledgements

The authors wish to thank Ms. Tammy L. Lisi for technical support. Supported by K0202201 to KAS.

References

Aloisi AM, Porro CA, Cavazzuti M, Baraldi P, Carli G. 'Mirror pain' in the formalin test: behavioral and 2-deoxyglucose. Pain 1993;55:267–73.

Antal M, Petko M, Polgar E, Heizmann CW, Storm-Mathisen J. Direct evidence of an extensive GABAergic innervation of the spinal dorsal horn by fibres descending from the rostral ventromedial medulla. Neuroscience 1996;73:509–18.

Atweh SF, Kuhar MJ. Autoradiographic localization of opiate receptors in rat brain. I. Spinal cord and lower medulla. Brain Res 1977;124:53–67.

Basbaum AI, Clanton CH, Fields HL. Three bulbospinal pathways from the rostral medulla of the cat: an autoradiographic study of pain modulating systems. J Comp Neurol 1978;178:209–24.

Berglund B, Harju EL, Kosek E, Londblom U. Quantitative and qualitative perceptual analysis of cold dysesthesia and hyperalgesia in fibromyalgia. Pain 2002;96:177–87.

Chacur M, Milligan ED, Gazda LS, Armstrong C, Wang H, Tracey KJ, Maier SF, Watkins LR. A new model of sciatic inflammatory neuritis

(SIN): induction of unilateral and bilateral mechanical allodynia following acute unilateral peri-sciatic immune activation in rats. Pain 2001;94:231–44.

Ding YQ, Kaneko T, Nomura S, Mizuno N. Immunohistochemical localization of mu-opioid receptors in the central nervous system of the rat. J Comp Neurol 1996;367:375–402.

Electrotherapy Standards Committee: Electrotherapeutic Terminology in Physical Therapy (Report). Section on Clinical Electrophysiology and American Physical Therapy Association, Alexandria, VA, 2001.

Fields, HL, Basbaum, AI. Central nervous system mechanisms of pain modulation. Wall PD, Melzack R, editors. Textbook of pain, New York, NY; 1999. p. 243–57. (Chapter 12).

Fields HL, Vanegas H, Hentall ID, Zorman G. Evidence that disinhibition of brain stem neurones contributes to morphine analgesia. Nature 1983; 306:684–6.

Gopalkrishnan P, Sluka KA. Effect of varying frequency, intensity, and pulse duration of transcutaneous electrical nerve stimulation on primary hyperalgesia in inflamed rats. Arch Phys Med Rehab 2000;81:984–9.

Hama AT, Fritschy JM, Hammond DL. Differential distribution of (GABA) A receptor subunits on bulbospinal serotonergic and nonserotonergic neurons of the ventromedial medulla of the rat. J Comp Neurol 1997; 384:337–48.

Hurley RW, Hammond DL. The analgesic effects of supraspinal mu and delta opioid receptor agonists are potentiated during persistent inflammation. J Neurosci 2000;20:1249–59.

Jensen TS, Yaksh TL. Spinal monoamine and opiate systems partly mediate the antinociceptive effects produced by glutamate at brainstem sites. Brain Res 1984;321:287–97.

Kalra A, Urban MO, Sluka KA. Blockage of opioid receptors in rostral ventral medulla prevents anti-hyperalgesia produced by transcutaneous electrical nerve stimulation (TENS). J Pharmacol Exp Therap 2001; 298:257–63.

Kaplan H, Fields HL. Hyperalgesia during acute opioid abstinence: evidence for a nociceptive facilitating function of the rostral ventromedial medulla. J Neurosci 1991;11:1433–9.

King EW, Sluka KA. The effect of varying frequency and intensity of transcutaneous electrical nerve stimulation on secondary mechanical hyperalgesia in an animal model of inflammation. J Pain 2001;2: 128–33.

Koltzenburg M, Wall PD, McMahon SB. Does the right side know what the left is doing? Trends Neurosci 1999;22:122–7.

Kosek E, Ordeberg G. Abnormalities of somatosensory perception in patients with painful osteoarthritis normalize following successful treatment. Eur J Pain 2000;4:229–38.

Melzack R, Wall PD. Pain mechanisms: a new theory. Science 1965;150: 971–8.

Porreca F, Ossipov MH, Gebhart GF. Chronic pain and medullary descending facilitation. Trends Neurosci 2002;25:319–25.

Radhakrishnan R, Sluka KA. Spinal muscarinic receptors are activated during low or high frequency TENS-induced antihyperalgesia in rats. Neuropharmacol 2003a;45:1111–9.

Radhakrishnan R, Moore S, Sluka KA. Unilateral carrageenan injection into muscle or joint induces chronic bilateral hyperalgesia in rats. Pain 2003b;104:567–77.

Radhakrishnan R, King EW, Dickman JK, Herold CA, Johnston NF, Spurgin ML, Sluka KA. Spinal 5-HT(2) and 5-HT(3) receptors mediate low, but not high, frequency TENS-induced antihyperalgesia in rats. Pain 2003;105:205–2013.

Rees H, Sluka KA, Lu Y, Westlund KN, Willis WD. Dorsal root reflexes in articular afferents occur bilaterally in a chronic model of arthritis in rats. J Neurophysiol 1996;76:4190–3.

Sluka KA, Walsh D. Transcutaneous electrical nerve stimulation: basic science mechanisms and clinical effectiveness. J Pain 2003;4:109–21.

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

Sluka KA, Deacon M, Stibal A, Strissel S, Terpstra A. Spinal blockade of opioid receptors prevents the analgesia produced by TENS in arthritic rats. J Pharmacol Exp Ther 1999;289(2):840–6.

Sluka KA, Kalra A, Moore SA. Unilateral intramuscular injections of acidic saline produce a bilateral, long-lasting hyperalgesia. Muscle Nerve 2001;24:37–46.

Terayama R, Dubner R, Ren K. The roles of NMDA receptor activation and nucleus reticularis gigantocellularis in the time-dependent changes in descending inhibition after inflammation. Pain 2002;97:171–81.

Urban MO, Gebhart GF. Supraspinal contributions to hyperalgesia. Proc Natl Acad Sci USA 1999;96:7687–92.

Urban MO, Zahn PK, Gebhart GF. Descending facilatory influences from the rostral medial medulla mediate secondary, but not primary hyperalgesia in the rat. Neurosci 1999;90:349–52.

Wall PD, Sweet WH. Temporary abolition of pain in man. Science 1967; 155:108–9.

Wieseler-Frank J, Maier SF, Watkins LR. Glial activation and pathological pain. Neurochem Int 2004;45:389–95.

Zemlan FP, Behbehani MM, Beckstead RM. Ascending and descending projections from nucleus reticularis magnocellularis and nucleus reticularis gigantocellularis: an autoradiographic and horseradish peroxidase study in the rat. Brain Res 1984;292:207–20.

Zimmerman M. Ethical guidelines for investigations of experimental pain in conscious animals. Pain 1983;16:109–10.