Blockade of Opioid Receptors in Rostral Ventral Medulla Prevents Antihyperalgesia Produced by Transcutaneous Electrical Nerve Stimulation (TENS)

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ABSTRACT

Although transcutaneous electrical nerve stimulation (TENS) is used extensively in inflammatory joint conditions such as arthritis, the underlying mechanisms are unclear. This study aims to demonstrate an opiate-mediated activation of descending inhibitory pathways from the rostral ventral medulla (RVM) in the antihyperalgesia produced by low- (4 Hz) or high-frequency (100 Hz) TENS. Paw withdrawal latency to radiant heat, as an index of secondary hyperalgesia, was recorded before and after knee joint inflammation (induced by intra-articular injection of 3% kaolin and carrageenan) and after TENS/no TENS coadministration with naloxone (20 μg/1 μl), naltrindole (5 μg/1 μl), or vehicle (1 μl) microinjected into the RVM. The selectivity of naloxone and naltrindole doses was tested against the μ-opioid receptor agonist [d-Ala²,N-Me-Phe⁴,Gly-ol³]-enkephalin (DAMGO) (20 ng, 1 μl) and the δ-opioid receptor agonist deltorphin (5 μg, 1 μl) in the RVM. Naloxone microinjection into the RVM blocks the antihyperalgesia produced by low frequency (p < 0.001), but not that produced by high-frequency TENS (p > 0.05). In contrast, naltrindole injection into the RVM blocks the antihyperalgesia produced by high-frequency (p < 0.05), but not low-frequency (p > 0.05) TENS. The analgesia produced by DAMGO and deltorphin is selectively blocked by naloxone (p < 0.05) and naltrindole (p < 0.05), respectively. Thus, the dose of naloxone and naltrindole used in the current study blocks μ- and δ-opioid receptors, respectively. Hence, low-frequency and high-frequency TENS produces antihyperalgesia by activation of μ- and δ-opioid receptors, respectively, in the RVM.

Transcutaneous electrical nerve stimulation (TENS) is defined by the American Physical Therapy Association as the application of electrical stimulation to the skin for pain control. Although clinical studies support the use of TENS for pain control (for review, see Robinson, 1996), the underlying mechanisms for the analgesia are not fully established. TENS is commonly administered at either high frequencies (>50 Hz) or low frequencies (<10 Hz) and different mechanisms are thought to underlie the actions.

The gate control theory of pain is used to explain the actions of high-frequency TENS (Melzack and Wall, 1965). This theory proposes that stimulation of large diameter afferents by high-frequency TENS attenuates nociceptive fiber-evoked responses in the dorsal horn. Alternatively, Campbell and Taub (1973) suggested that high-frequency stimulation by TENS results in conduction block or fatigue of Aδ fibers. However, Janko and Trontelj (1980) and Lee et al. (1985) demonstrated that afferent barrage evoked by painful stimuli is intact during and after TENS. Moreover, recent data from our laboratory demonstrate that δ-opioid receptors in the spinal cord are activated by high-frequency TENS (Sluka et al., 1999b).

The release of endogenous opioids is typically used to explain the actions of low-frequency TENS analgesia. In human subjects, low-frequency TENS analgesia is reversed by the opioid receptor antagonist naloxone, while high-frequency stimulation analgesia is not (Sjölund and Eriksson, 1979). Spinal blockade of μ-opioid receptors prevents the antihyperalgesia by low-frequency TENS in inflamed rats (Sluka et al., 1999b). However, inhibition of primate spinthalamic cells by TENS is not naloxone reversible (Lee et al., 1985). Furthermore, high-frequency stimulation-induced analgesia is reversed by higher doses of naloxone in rats (Woolf et al., 1980) or spinal administration of a selective δ-opioid receptor antagonist (Sluka et al., 1999b). High-frequency TENS also increases cerebrospinal fluid (Salar et al., 1981) concentrations of β-endorphin in human subjects. These contrasting results are probably due to differences in TENS units that were donated by EMPI, Inc.

ABBREVIATIONS: TENS, transcutaneous electrical nerve stimulation; RVM, rostral ventral medulla; NRM, nucleus raphe magnus; NGC, nucleus reticularis gigantocellularis pars alpha; 5-HT, L-5-hydroxytryptophan; PWL, paw withdrawal latency; DAMGO, [d-Ala²,N-Me-Phe⁴,Gly-ol³]-enkephalin; DELT, [d-Ala²]-deltorphin II; HBC, 2-hydroxypropyl-β-cyclodextrin; ANOVA, analysis of variance; PAG, periaqueductal gray.
parameters, doses of naloxone used and its route of administration. The release of endogenous opioids by TENS could be due to activation of local spinal circuits and/or activation of descending inhibitory pathways.

The rostral ventral medulla (RVM) in the brainstem includes the nucleus raphe magnus (NRM), nucleus reticularis gigantocellularis pars alpha (NGCo), and nucleus reticularis paragigantocellularis lateralis (Fields and Basbaum, 1994). These nuclei project to the spinal dorsal horn with the highest density of projections to the substantia gelatinosa (Wang and Wessendorf, 1999). Electrical (Zorman et al., 1982; Aimone and Gebhart, 1986; Aimone et al., 1987) or chemical (Dickenson et al., 1979; Rossi et al., 1994) stimulation of the RVM inhibits reflex and behavioral responses to noxious stimuli and also inhibits neurons in the spinal dorsal horn that receive nociceptive input (Gebhart, 1993). This inhibition is naloxone reversible (Zorman et al., 1982). Microinjection of morphine into the RVM produces naloxone reversible antinociception (Dickenson et al., 1979; Aimone and Gebhart, 1986) and lesions of NRM block systemic morphine antinociception (Young et al., 1984). The raphe spinal pathway uses the neurotransmitter serotonin, among others, and the antinociception induced by stimulation of the RVM can be inhibited by serotonin receptor antagonists (Dickenson et al., 1979; Aimone et al., 1987). Hence, the RVM plays a role in opioid-mediated antinociception.

Several studies support a role of descending inhibitory pathways in TENS analgesia. Electrical stimulation-induced antinociception is significantly enhanced by administration of L-5-hydroxytryptophan (5-HT), a serotonin precursor, and abolished by the opiate receptor antagonist naloxone and 5-HT receptor blocker methysergide (Shimizu et al., 1981). Depletion of 5-HT, a neurotransmitter of the raphe-spinal pathway, diminishes the antinociceptive effect of high-frequency stimulation in the intact animal but not in the spinal animal (Woolf et al., 1980). This suggests a role of raphespinal projections in electrical stimulation-induced antinociception. Thus, we hypothesize that both low- and high-frequency TENS produce antinociceptive effects by activation of descending inhibitory pathways. If TENS antinociception is naloxone reversible (Zorman et al., 1982). The sites plotted show the area of maximum concentration of the dye.

Experimental Procedures

General Methods. All experiments were approved by the Animal Care and Use Committee at the University of Iowa (Iowa City, IA). Adult male Sprague-Dawley rats (n = 126) (220–300 g; Harlan, Indianapolis, IN) were used for all experiments. The animals were housed in a 12-h dark/light cycle, and the testing was done only in the light cycle. Food and water were available to the animals ad libitum.

Cannula Implantation. The animals were anesthetized with sodium pentobarbital (60 mg/kg i.p.) and then placed in a stereotaxic frame (David Kopf Instruments, Inc.). A dummy cannula (33 gauge; Plastics One, Roanoke, VA) was inserted into the guide cannula to maintain its patency. The animals were allowed to recover for 4 to 6 days post-surgery before behavioral testing.

Behavioral Assessment. Rats were placed in clear plastic chambers on an elevated glass table and allowed to acclimatize for approximately 30 to 40 min. The time taken by the rat to withdraw the hind paw (paw withdrawal latency (PWL)) in response to a radiant heat source was recorded bilaterally as an index of the nociceptive heat threshold (Hargreaves et al., 1988; Sluka and Westlund, 1993). The heat source was a high-intensity light beam that was shone through the glass table that the animals were placed on, and was directed to the mid-plantar surface of the weight-bearing hind paw. The light box was attached to a timer that measures to the hundredth of a second. Latencies of five trials taken at 5-min intervals were averaged to give the baseline paw withdrawal latency for each hind paw. Each hind paw was tested independently. Twenty seconds was the cut-off point for the PWL to avoid damaging dermal tissue. The validity (Hargreaves et al., 1988) and test-retest reliability of this method have previously been established (r2 = 0.7, p = 0.0001) (Sluka et al., 1999a).

Drug Injection. Drugs were microinjected into the RVM through a 33-gauge injection cannula that extended 3 mm below the guide cannula tip (Urban and Smith, 1994). The injection cannula was attached to a 10-μl Hamilton syringe via a length of PE-10 tubing. All drug injections were made in a volume of 1 μl to affect a sufficient volume of tissue in the RVM (Urban and Smith, 1994). The drugs were microinjected over a period of 30 s, and the needle was left in position for a minute to allow diffusion of drug before the needle was withdrawn.

Drugs. Drugs used in the present experiments were μ-opioid receptor agonist [D-Ala2,N-Me-Phe4,Gly(ol)-enkephalin (DAMGO) (Sigma/Research Biochemicals, Inc., Natick, MA); δ-opioid receptor agonist [D-Ala2]-deltorphin II (DELT) (Sigma Chemical Co., St. Louis, MO); 0.9% saline (1 μl) (Abbott Laboratories, North Chicago, IL); naloxone hydrochloride (C17H21NO4Cl) (Sigma Chemical Co.); and naltrindole hydrochloride [17-(cyclopropylmethyl)-6,7-dehydro-4,5α-epoxy-3,14-dihyrox-6,7-2,3′-indolomorphinan] (Tocris Cookson, Baldwin, MO), a selective δ-opioid receptor antagonist. Naloxone and DAMGO were dissolved in 0.9% saline, DELT in 45% w/v HBC (Sigma/Research Biochemicals, Inc.) in distilled water, and naltrindole in 10% dimethyl sulfoxide.

Histology. At the end of the experiment, 1% methylene blue was microinjected through the cannula for verification of the injection sites. The animals were euthanized by an overdose of pentobarbital sodium (150 mg/kg i.p.). The brain was removed, frozen, cut in serial 40-μm-thick sections on a cryostat and examined for the injection sites. The sites plotted show the area of maximum concentration of the dye.

Experimental Design for Experiment 1. The ability of naloxone (20 μg, 1 μl) and naltrindole (5 μg, 1 μl) to block the antinociceptive effects of DAMGO, a μ-opioid receptor agonist, and DELT, a δ-opioid receptor agonist, was tested. Naloxone (20 μg, 1 μl) (Aimone and Gebhart, 1986) or naltrindole (5 μg, 1 μl) (Rossi et al., 1994) was microinjected into the RVM followed 5 min later by microinjections of the μ-opioid receptor agonist DAMGO (20 ng, 1 μl) (Rossi et al., 1994) or the δ-opioid receptor agonist DELT (5 μg, 1 μl) (Thorat and Hammond, 1997; Hurley et al., 1999). Antagonism of the agonist-induced antinociception by naloxone/naltrindole was tested by measuring changes in PWL to radiant heat in normal rats.

An equivalent volume of vehicle was injected into the RVM instead of naloxone or naltrindole along with DAMGO or DELT as controls. Effects of HBC (45% w/v, 1 μl) on baseline PWL was also observed in normal animals as controls (n = 4). Previous studies show that dimethyl sulfoxide (10%, 5 μl i.v.) had no effects on opioid-induced analgesia in the tail-flick test in mice (Horan et al., 1993). All injections were done while the animals were awake.

After determining the baseline PWL, the rats were randomly
assigned to six groups (n = 25): 1) DAMGO + naloxone (n = 4), 2) DELT + naloxone (n = 4), 3) DAMGO + saline (n = 5), 4) DELT + saline (n = 4), 5) DAMGO + naltrindole (n = 4), and 6) DELT + naltrindole (n = 4). PWL to radiant heat was recorded bilaterally: 1) pre-DAMGO or DELT (baseline), and 2) postantagonist (naloxone/saline/naltrindole) 1 DAMGO or DELT. The opioid receptor agonist was only injected once (Horan et al., 1993).

The PWL was recorded bilaterally five times at 5-min intervals in normal rats and was averaged to yield a single value of PWL. Repeated measures analysis of variance (ANOVA) tested for differences in PWL across time and within groups for DAMGO and DELT (p < 0.05). Post hoc Tukey’s test compared differences in PWL between groups (p < 0.05). For the HBC group repeated measures ANOVA tested for differences in PWL pre- and post-HBC (p = 0.05). The PWL values are represented as mean ± S.E.M.

Experimental Design for Experiment 2. This experiment determined whether μ- and/or δ-opioid receptors in the RVM contribute to TENS antihyperalgesia. Rats were injected intra-articularly with 3% kaolin and 3% carrageenan (0.1 ml in sterile saline, pH 7.2–7.4), in the left knee joint under anesthesia (2–4% halothane with oxygen) (Sluka and Westlund, 1993). Intra-articular injection of 3% kaolin and 3% carrageenan in the rat’s knee is an established model of inflammation that induces secondary heat hyperalgesia (Sluka and Westlund, 1993).

The degree of joint inflammation was assessed by knee joint circumference measurements bilaterally with a flexible tape measure.

Fig. 1. Histological verification of cannula placements in the RVM. A–F, distribution of microinjection sites in RVM for the drugs used in this study, where DAMGO (20 ng, 1 μl) (n = 13) (A); DELT (5 μg, 1 μl) (n = 12) (B); saline (1 μl) (n = 29) (C); naloxone (20 μg, 1 μl) (n = 30) (D); naltrindole (5 μg, 1 μl) (n = 26) (E); HBC (45% w/v) (n = 4) (F); and injections were outside the RVM sites and animals received naloxone (20 μg, 1 μl) and low-frequency TENS (n = 8) (G). The rostral-to-caudal coordinate is with respect to the bregma suture (Paxinos and Watson, 1998). Sp5, spinal trigeminal nucleus; VII, facial nucleus; Pyr, pyramidal tract; A5, A5 catecholamine cell group.
around the center of the knee joint as the knee was extended. Circumferential measurements were recorded before inflammation and approximately 5 h after induction of inflammation, just before TENS was administered. Intra-articular presence of kaolin and carrageenan was confirmed by a post-mortem dissection of the knee joint. All animals demonstrated an inflammatory exudate within the knee joint capsule on dissection.

Parameters of TENS selected in this study mimic those used clinically (Robinson, 1996) and produce significant and prolonged inhibition of secondary heat hyperalgesia in this model of inflammation (Sluka et al., 1998). Commercially available TENS units (Eclipse +; EMPI, Inc., Minneapolis, MN) and electrodes (EMPI, Inc.) were used for all tests. Pregelled electrodes (2.5 cm in diameter) were placed on the inflamed knee joint, one medially and one laterally. Rats received 1) high-frequency TENS at sensory intensity (100 Hz, 100 µs, 10–18 mA, 20 min), 2) low-frequency TENS at sensory intensity (4 Hz, 100 µs, 10–18 mA, 20 min), or 3) halothane without TENS (20 min, control). Sensory intensity was defined as inducing a muscle contraction and then reducing the intensity to just below this level (Sluka et al., 1998). The waveform was balanced asymmetrical and biphasic. Halothane in the absence of TENS has no effect on the decreased PWL postinflammation (Sluka et al., 1998). TENS (high and low frequency) applied to the knee joint does not alter the PWL to heat in the absence of inflammation (Sluka et al., 1998). After determining the baseline and postinflammation (4 h) PWL, rats (n = 85) were randomly divided into nine groups: 1) saline + no TENS (n = 11), 2) naloxone + no TENS (n = 9), 3) naltrindole + no TENS (n = 8), 4) saline + low-frequency TENS (n = 7), 5) naloxone + low-frequency TENS (n = 9), 6) naltrindole + low-frequency TENS (n = 9), 7) saline + high-frequency TENS (n = 11), 8) naloxone + high-frequency TENS (n = 12), and 9) naltrindole + high-frequency TENS (n = 9). Rats were lightly anesthetized with 1 to 2% halothane and oxygen, the drug was injected into the RVM and TENS was applied to the inflamed knee joint. PWL was recorded bilaterally before and after inflammation (4 h), and after drug (naloxone, naltrindole, or saline) + TENS. Joint circumference measurements were recorded bilaterally before and after inflammation.

Differences in PWL and joint circumference were assessed across time (at each time: baseline, 4 h postinflammation and post-TENS) and between groups (high-frequency TENS, low-frequency TENS, no TENS) by repeated measures ANOVA. Post hoc Tukey’s test compared for differences in PWL between individual groups (p ≤ 0.05). Differences in joint circumference were assessed across time and within groups by repeated measures ANOVA (p ≤ 0.05) followed by post hoc Tukey’s test. A paired t test (p ≤ 0.05) compared for differences in PWL between baseline and postinflammation values. The PWL values are expressed as mean ± S.E.M.

**Results**

**Distribution of Microinjection Sites in RVM.** Histological analysis revealed that the microinjection sites were distributed predominantly (92%) in the NRM and NGCα. Figure 1, A–G, summarizes the injection sites in all groups. Sites outside the RVM included the cerebellum (n = 4), superior or inferior cerebellar peduncle (n = 3), NGC (n = 8), the fourth ventricle (n = 2), the medial longitudinal fasciculus (n = 1), the vestibular nuclei (n = 1), principle sensory and spinal nucleus of V (n = 1), and the pyramids (n = 1). The sites plotted show the area of maximum concentration of the dye. There was considerable overlap between injection sites, hence the number of sites in the schematics appears less than the number of sites plotted. Previous studies showed no difference in the magnitude or onset to the increase in latency produced by opioids in the NRM, NGCα, and NGC in the tail-flick assay and the hot-plate tests (Thorat and Hammond, 1997).

Hence, injections within the NRM (n = 99), NGCα (n = 6), and NGC (n = 8) were pooled for analysis.

**Selectivity of Naloxone and Naltrindole.** A significant increase in the PWL was observed following administration of saline + DAMGO into the RVM (p < 0.001). No differences were observed between groups in the PWL at baseline (p > 0.05). The increase in PWL produced by DAMGO was blocked by naloxone compared with saline (p < 0.05), but not by naltrindole compared with the group injected with saline prior to DAMGO (p > 0.05) (Fig. 2A). Furthermore, the PWL for the group that received naloxone + DAMGO was significantly less than the PWL following naltrindole + DAMGO (p < 0.01).

There was a significant increase in the PWL to heat following administration of saline + DELT into the RVM (p = 0.05). There was no difference between groups in the PWL at baseline (p > 0.05). Naltrindole prevented the increase in PWL produced by DELT compared with saline (p < 0.05) (Fig. 2B). DELT produced a similar increase in the PWL to heat when coadministered with saline or with naloxone (p > 0.05).

There was no change in PWL after 45% w/v HBC (F1,3 = 0.001, p > 0.05). The PWL remained unchanged with a

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**Fig. 2.** Effects on PWL to heat of saline (1 µl), naloxone (20 µg, 1 µl), or naltrindole (5 µg, 1 µl) co-injected into the RVM with DAMGO (20 ng, 1 µl) (A) or DELT (5 µg, 1 µl) (B). Closed columns represent PWL before injection of agonist and open columns represent the PWL after injection of the agonist. DAMGO and DELT significantly increase PWL when coadministered with saline. The increase produced by DAMGO is blocked by naloxone and that of DELT is blocked by naltrindole. The values are expressed as mean ± S.E.M. *, significantly increased from baseline, p < 0.05; #, significantly less than saline, p < 0.05.
Antihyperalgesia. An increase in joint circumference occurred in the inflamed knee joint \((F_{1,69} = 1069.14, p < 0.001)\), but not in the contralateral (noninflamed) knee joint \((F_{1,69} = 1.6, p > 0.05)\) in all groups tested 4 h after induction of inflammation. There were no time \(*\) group effect in the ipsilateral (inflamed) hindlimb \((F_{5,69} = 0.297, p > 0.05)\). The joint circumference measurements across all groups, before and after inflammation are shown in Table 1.

<table>
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<tr>
<th>Drug</th>
<th>TENS</th>
<th>Baseline</th>
<th>After Ipsilateral Knee Injection</th>
<th>Baseline</th>
<th>After Ipsilateral Knee Injection</th>
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<td>Naloxone</td>
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<td></td>
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<td></td>
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<td>6.00 ± 0.06</td>
<td>5.97 ± 0.07</td>
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<td>5.70 ± 0.06</td>
<td>5.67 ± 0.07</td>
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<td>7.07 ± 0.3</td>
<td>5.80 ± 0.12</td>
<td>5.74 ± 0.11</td>
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### Discussion

DAMGO is a selective \(\mu\)-opioid receptor agonist, while DELT is selective for \(\delta\)-opioid receptors. The results of this study show that the dose of naloxone used (20 \(\mu\)g, 1 \(\mu\)l) antagonizes the antinociceptive effects of DAMGO but not that of DELT. Naltrindole (5 \(\mu\)g, 1 \(\mu\)l) antagonizes the antinociceptive effects of DELT, but not that of DAMGO. Hence, the data are interpreted on the assumption that the doses of naloxone and naltrindole used in the current study are selective for \(\mu\)- and \(\delta\)-opioid receptors, respectively.

The present study shows local microinjections of naloxone, but not that of saline or naltrindole, in the RVM block low-frequency TENS antihyperalgesic effects. Hence, the antihyperalgesic effects of low-frequency TENS are mediated by \(\mu\)-opioid receptors in the RVM. Also since the effects of high-frequency TENS were blocked by naltrindole, but not by naloxone or saline, it is concluded that high-frequency TENS activates \(\delta\)-opioid receptors in the RVM. Studies show that low-frequency, but not high-frequency TENS is reversed by low doses of naloxone that would be expected to block \(\mu\)-opioid receptors selectively (Sjölund and Eriksson, 1979; Sluka et al., 1999b). Furthermore, higher, nonselective doses of naloxone and the selective \(\delta\)-opioid receptor antagonist naltrindole reverse high-frequency TENS stimulation (Han et al., 1991; Sluka et al., 1999b) confirming a role of opioid receptors other than \(\mu\) in mediating high-frequency TENS analgesia. Autoradiographic (Bowker and Dilts, 1988), im-
Blockade of spinal $\mu$- or $\delta$-opioid receptors prevents the antihyperalgesia produced by low- and high-frequency TENS, respectively (Sluka et al., 1999b). Taken together, these studies indicate activation of $\mu$-opioid receptors both at the spinal and supraspinal level and $\delta$-opioid receptors at the spinal and supraspinal level by low- and high-frequency TENS, respectively. Previous studies have demonstrated that there is a synergistic interaction between $\mu$-$\mu$- (Yeung and Rudy, 1980) and $\delta$-$\delta$-opioid receptors (Hurley et al., 1999; Kovelowski et al., 1999) at the spinal and supraspinal level. Morphine is believed to mediate antinociceptive effects by activation of both spinal and supraspinal $\mu$-opioid receptors (Yeung and Rudy, 1980). Similarly, TENS seems to activate both spinal and supraspinal opioid receptors.

This study was designed to effect a large area of the RVM to try to block activation in these nuclei through TENS that is applied to the skin at the site of inflammation. We thus used a 1-$\mu$l injection to accomplish affecting this large of an area. Studies using intracerebral injection of radiolabeled substances found 1-$\mu$l injections diffuse approximately 1.0 mm from the injection site (Myers and Hoch, 1978). In support, 1-$\mu$l injection of lidocaine into the RVM produces a functional neuronal block having a radius of approximately 1 mm (Sandkuler et al., 1987), and numerous studies used 1 $\mu$l of intra-RVM lidocaine injections to produce a selective functional block of the RVM (Urban and Smith, 1994; Mansikka and Pertovaara, 1997; Pertovaara, 1998; Kovelowski et al., 2000). Additionally, studies examining the pharmacology of descending systems from the RVM typically use 1-$\mu$l intra-RVM injections of receptor antagonists to selectively affect a large volume of tissue within the RVM (Urban et al., 1999; Kovelowski et al., 2000). Furthermore, in the current study, when injection sites were outside the RVM no effect was observed on the analgesia produced by TENS.

Patients who are tolerant to opioids are also tolerant to the effects of TENS (Solomon et al., 1980). Also low-frequency TENS shows significantly decreased efficacy in reducing secondary heat hyperalgesia in morphine-tolerant rats compared with high-frequency TENS (Sluka et al., 2000). Thus, if the patient is taking, or has taken, opioids in the past, TENS may not be the modality of choice. TENS also reduces the need for postoperative opioids in patients who have not taken opioid analgesics preoperatively (Solomon et al., 1980). Hence, an understanding of the role of opioid-mediated descending inhibitory pathways in the antihyperalgesia induced by low- and high-frequency TENS could have important clinical implications. An opioid-mediated activation of the descending inhibitory pathways by TENS may be ineffective in producing analgesia if patients are tolerant to opioid derivatives. A potential synergistic or additive effect of TENS and opioids may reduce the intake of exogenous opiates for pain control. Both high-and low-frequency TENS shift the dose-response curve for systemic morphine to the left, such that the same dose of morphine in combination with TENS is more efficacious than morphine alone (Sluka, 2000). Hence, many side effects associated with opiate drug intake may be reduced. In support of this, Wang et al. (1997) found a decrease in the morphine-associated nausea, dizziness, and pruritus when combined with high-frequency TENS, owing to a reduction in the morphine intake postoperatively. Thus, an understanding of the mechanism of TENS will help identify the categories of patients who will likely benefit from TENS and optimize the efficacy of the treatment.

**Fig. 3.** Effects of saline, naloxone, and naltrindole microinjected in the RVM on PWL in animals that did not receive TENS (no TENS) (A), received low-frequency TENS (4 Hz) (B), or received high-frequency TENS (100 Hz) (C). Naloxone prevented the increase in PWL by low-frequency TENS compared with saline, or to naltrindole microinjection. Naltrindole significantly attenuated the increase in PWL by high-frequency TENS compared with saline, or to naloxone microinjection. Values are mean ± S.E.M. *p < 0.05.

municytochemical (Kalyuzhny and Wessendorf, 1998), and in situ hybridization methods (Gutstein et al., 1998) have localized $\mu$-opioid receptors to the RVM and to spinoally projecting cells in the RVM (Kalyuzhny et al., 1996; Kalyuzhny and Wessendorf, 1998). $\delta$-Opioid receptors are localized to varicosities and terminals in the RVM (Arvidsson et al., 1995). Microinjection of $\mu$- (Aimone and Gebhart, 1986; Rossi et al., 1994) and $\delta$-opioid receptor ligands (Thorat and Hammond, 1997; Hurley et al., 1999; Kovelowski et al., 1999) in the RVM produce antinociception.
It is possible that TENS activates opioid receptors in other structures, such as the periaqueductal gray (PAG) in the midbrain or the locus coeruleus, that are known to influence nociception via descending pathways (Fields and Basbaum, 1994). The PAG has reciprocal connections with the RVM, and previous studies demonstrate the role of RVM in the antinociception from the PAG (Aimone and Gebhart, 1986; Urban and Smith, 1994). The PAG on activation by TENS could in turn stimulate descending inhibitory pathways from the RVM. The locus coeruleus has direct spinal projections and also receives projections from NGC (Fields and Basbaum, 1994). Activation of descending inhibitory pathways from the locus coeruleus could also mediate TENS antihyperalgesia. Thus, although this study demonstrates an opioid-mediated activation of the descending inhibitory pathways involving the RVM by TENS, activation of other descending inhibitory pathways by opiate or nonopiate mechanisms cannot be ruled out. Also there could be other alternative descending inhibitory pathways that are opioid-mediated, but do not include the RVM.

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