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TRANSCUTANEOUS ELECTRICAL NERVE STIMULATION AT BOTH HIGH AND LOW FREQUENCIES ACTIVATES VENTROLATERAL PERIAQUEDUCTAL GREY TO DECREASE MECHANICAL HYPERALGESIA IN ARTHRITIC RATS

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Abstract

Transcutaneous electric nerve stimulation (TENS) is widely used for the treatment of pain. TENS produces an opioid-mediated antinociception that utilizes the rostroventromedial medulla (RVM). Similarly, antinociception evoked from the periaqueductal grey (PAG) is opioid-mediated and includes a relay in the RVM. Therefore, we investigated whether the ventrolateral or dorsolateral PAG mediates antinociception produced by TENS in rats. Paw and knee joint mechanical withdrawal thresholds were assessed before and after knee joint inflammation (3% kaolin/carrageenan), and after TENS stimulation (active or sham). Cobalt chloride ($CoCl_2$; 5 mM) or vehicle was microinjected into the ventrolateral periaqueductal grey (vlPAG) or dorsolateral periaqueductal grey (dlPAG) prior to treatment with TENS. Either high (100 Hz) or low (4 Hz) frequency TENS was then applied to the inflamed knee for 20 min. Active TENS significantly increased withdrawal thresholds of the paw and knee joint in the group microinjected with vehicle when compared to thresholds prior to TENS ($P<0.001$) or to sham TENS ($P<0.001$). The increases in withdrawal thresholds normally observed after TENS were prevented by microinjection of $CoCl_2$ into the vlPAG, but not the dlPAG prior to TENS and were significantly lower than controls treated with TENS ($P<0.001$). In a separate group of animals, microinjection of $CoCl_2$ into the vlPAG temporarily reversed the decreased mechanical withdrawal threshold suggesting a role for the vlPAG in the facilitation of joint pain. No significant difference was observed for dlPAG. We hypothesize that the effects of TENS are mediated through the vlPAG that sends projections through the RVM to the spinal cord to produce an opioid-mediated analgesia.

Keywords

pain; TENS; hyperalgesia; opioid; inflammation; analgesia

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The midbrain periaqueductal grey (PAG) surrounds the midbrain aqueduct (Osborne et al., 1996) and is implicated in a wide variety of functions including opioid-mediated analgesia (Gebhart et al., 1988; Fields et al., 1991; Osborne et al., 1996; Vaughan and Christie, 1997). Two separate, and distinct, nociceptive modulatory systems operate in the caudal PAG: a dorsal system which encompasses the dorsomedial, dorsolateral and lateral subdivisions of the PAG; and a ventral system which includes the ventrolateral PAG and dorsal raphe (reviewed by Morgan, 1991).

Opioid administration into the vlPAG in the rat (Jensen and Yaksh, 1989; Krzanowska and Bodnar, 1999; Tershner et al., 2000) and cat (Oliveras et al., 1974), as well as electrical stimulation of the PAG in humans (Hosobuchi et al., 1977) produces antinociception. Interestingly, opioids appear to interact exclusively with the ventral system, as antinociception produced by electrical stimulation of the ventral, but not dorsal, PAG is attenuated by the opioid antagonist naloxone (Cannon et al., 1982). Furthermore, microinjection of the opioid agonist morphine produces antinociception when microinjected into the vlPAG (Yaksh et al., 1976). Although morphine produces explosive motor behavior when injected into the lateral PAG, this behavior is also accompanied by antinociception (Jacquet and Lajtha, 1974; Jensen and Yaksh, 1986; Morgan et al., 1998). This antinociception occurs even when the aversive reactions are blocked (Morgan et al., 1987).

The PAG produces antinociception through a relay in the rostroventral medial medulla (RVM), a region which encompasses the nucleus raphe magnus and the adjacent reticular formation. In rat, as many as 18% of PAG neurons project to the RVM (Osborne et al., 1996) and are distributed throughout the dorsomedial, lateral and ventrolateral PAG divisions, but are absent in the dorsolateral PAG division (Reichling and Basbaum, 1991). Inactivation of the RVM disrupts antinociception mediated by stimulation of the PAG (Prieto et al., 1983; Sandkuhler and Gebhart, 1984). Further, microinjection of morphine into the RVM produces antinociception (Jensen and Yaksh, 1986; Morgan et al., 1998; Morgan and Whitney, 2000). Thus, opioid-mediated analgesia activates a pathway with neurons that project from the vlPAG to the RVM, and subsequently to the spinal cord dorsal horn (Bagley et al., 2005).

Transcutaneous electric nerve stimulation (TENS) is a non-pharmacological treatment for pain that produces antinociception through activation of opioid receptors in the spinal cord and RVM (Sluka et al., 1999; Kalra et al., 2001). Specifically, low (4 Hz) frequency TENS activates μ-opioid receptors and high (100 Hz) frequency TENS activates δ-opioid receptors (Sluka et al., 1999; Kalra et al., 2001). Further, repeated application of TENS, low or high frequency, produces analgesic tolerance and a cross-tolerance to μ- and δ-opioid receptors spinally, respectively (Chandran and Sluka, 2003). As TENS produces an opioid-mediated antinociception that utilizes the RVM (Kalra et al., 2001) and antinociception evoked from the PAG is opioid-mediated and includes a relay in the RVM, we hypothesized that the PAG mediates the antinociception produced by TENS.

EXPERIMENTAL PROCEDURES

All experiments were approved by Animal Care and Use Committee at the University of Iowa (Iowa City, IA, USA) and are in accordance with the guidelines of National Institutes of Health on use of laboratory animals. This study used the minimum number of animals to obtain statistical significance. Adult male Sprague–Dawley rats (*n*=64; 225–350 g, Harlan, Indianapolis, IN, USA) were used for this study. The animals were housed in a 12-h light/dark cycle, and the testing was done only in the light cycle. Food and water were available to the animals *ad libitum*.

Induction of inflammation

Immediately after baseline behavioral measurements that are described below, rats were anesthetized with isoflurane (2–4%) and the left knee joint was injected intra-articularly with a mixture of 3% carrageenan and 3% kaolin (0.1 ml in sterile saline, pH 7.4) (Sluka and Westlund, 1993). The inflammation is considered acute for the first 24 h, when there is neutrophil infiltration. By 1 week, the inflammation converts to chronic, as identified histologically by macrophage infiltration. This model is used to mimic arthritic conditions and shows good predictability for drug effects (Radhakrishnan et al., 2003). After induction of knee inflammation, the rats were returned to their cages and allowed to recover for 24 h. Within 24 h, the animals exhibit signs of inflammation such as edematous and warm knee joints and also behavioral signs such as guarding and decreased weight bearing on the inflamed limb (Sluka and Westlund, 1993).

Cannula implantation and microinjections

Intracerebral guide cannulae were placed in the ventrolateral (vlPAG) or dorsolateral (dlPAG) periaqueductal grey 3 to 5 days before induction of knee joint inflammation. The rats were anesthetized with an i.p. injection of sodium pentobarbital (Nembutal, 50 mg/kg, Ovation Pharmaceucticals, Deerfield, IL, USA) and secured in a stereotaxic head holder to implant the guide cannula (17.5 mm in length, 26 gauge; Plastics One, Roanoke, VA, USA). After the midline incision, the skull was exposed, and a small hole drilled for placement of the guide cannula. The guide cannula was 1 mm above the vlPAG, using the following coordinates: interaural: 1.7 mm; mediolateral: +0.6 mm; and dorsoventral: −5.0 mm below the skull surface. For dlPAG, the guide cannula was 1 mm above the dlPAG, using the following coordinates: interaural: 1.7 mm; mediolateral: +0.6 mm; and dorsoventral: −4.8 mm below the skull surface (Paxinos and Watson, 2005). Cannulae were secured to the skull by stainless-steel screws and dental cement (Urban and Smith, 1994). Cannula was implanted ipsilateral to the inflamed knee joint. A dummy cannula (33 gauge, Plastics One) was inserted into the guide cannula to maintain its patency. All rats were allowed to recover 3 to 5 days after surgery before behavioral testing.

To examine placement of the cannula into the vlPAG or dlPAG, an equivalent volume of methylene blue dye was injected through the cannula at the end of the experiment. Rats were then euthanized with an overdose of sodium pentobarbital (150 mg/kg i.p.) and transcardially perfused with 4% paraformaldehyde. After this, the brain was removed, stored in 30% sucrose solution, frozen, cross-sectioned at 40 μm on a cryostat and examined under a light microscope for placement of the cannula.

Drug administration

Vehicle (0.5 μl, 0.9% sterile saline) or 5 mM $CoCl_2$ solution (0.5 μl, dissolved in 0.9% sterile saline, Fisher Scientific, NJ, USA) was microinjected into vlPAG or dlPAG through the guide cannula. The dose of $CoCl_2$ was selected from a prior study (Cavun et al., 2004) and through preliminary experiments. Microinjections of $CoCl_2$ in discrete brain areas have been used for reversible functional inactivation (Kretz, 1984; Nuseir et al., 1999; Fisk and Wyss, 2000; Pajolla et al., 2005). Co^{2+} obstructs the ionophore of the voltage-gated Ca^{2+} channel (Hagiwara and Byerly, 1981) and thus induces blockade of Ca^{2+}-dependent release of neurotransmitter from presynaptic terminals (Kretz, 1984). This blockade of neurotransmitter release therefore causes a reversible blockade of neuronal pathways that synapse in the targeted area (Kretz, 1984) and fibers of passage are not affected by $CoCl_2$ (Kretz, 1984).

A 33-gauge injection cannula was connected to a 10-μl Hamilton syringe through PE10 tubing backfilled with sterile saline. The microinjection (0.5 μl) of either $CoCl_2$ or vehicle

was performed over a 2-min period and the travel of the air bubble in the tubing was carefully observed to ensure that the drug solution entered the injection cannula. The needle was left in position for a minute to allow diffusion of drug before the needle was withdrawn. TENS application was performed 1 h after injection of $CoCl_2$, a time when preliminary studies show a maximal effect of $CoCl_2$.

Behavioral assessment

The paw withdrawal threshold and the joint withdrawal threshold were tested for all groups of rats. Paw and joint withdrawal thresholds were assessed before and 24 h after induction of inflammation, and 1 h after TENS application. Rats were tested for PWT with von Frey filaments applied to the paw. Initially, the animals were maintained in their home cages in the behavior room for 30 min to acclimate to the environment. Then, the animals were placed in transparent Lucite cubicles over a wire mesh and acclimated for another 30 min before testing. A series of von Frey filaments with increasing bending forces (9.4–495.8 mN) was applied to the plantar surface of the hind paw until the rat withdrew from the stimulus (Gopalkrishnan and Sluka, 2000). Each filament was applied twice. The lowest force at which the rat withdrew its paw from one of two applications was recorded as the paw withdrawal threshold for mechanical hyperalgesia. A reduction in mechanical withdrawal threshold was interpreted as cutaneous hyperalgesia. This testing method has shown significant statistical test–retest reliability (Sluka et al., 1999).

Rats were also tested for knee joint withdrawal thresholds with a pair of forceps applied to the knee joints as previously described (Vance et al., 2007; DeSantana et al., 2008). Rats were acclimated in a restraining device three times a day 1 h apart for 2 days, each acclimatizing session consisting of 5 min (two days prior to the induction of inflammation). The forceps were equipped with two strain gauges to measure force. To measure the knee joint withdrawal threshold, animals were placed in the restrainer, and the experimenter compressed the knee joint with the tip of the forceps while the hind limb was extended. Compression was continued until the animal withdrew the leg. The maximum force applied at withdrawal was recorded as the joint withdrawal threshold. Three trials 5 min apart at each time period were performed and averaged to obtain one reading per time period. A decrease in withdrawal threshold of the knee joint was interpreted as joint hyperalgesia.

We previously showed that (1) both low and high frequency TENS reduce hyperalgesia induced by kaolin and carrageenan for 12–24 h after administration, (2) application of halothane without TENS has no effect on the paw withdrawal latency to heat induced by joint inflammation, and (3) application of TENS to a non-inflamed knee joint has no effect on the paw withdrawal latency (Sluka et al., 1998).

Administration of TENS

EMPI Select TENS units with an asymmetrical biphasic square wave (EMPI Inc., MN, USA) and half-inch circular electrodes were used. Under isoflurane anesthesia, the left knee joint was shaved and round pre-gelled surface electrodes were applied to the medial and lateral aspects of the inflamed knee joint in the groups receiving active TENS or sham TENS. Animals were observed continuously during TENS to ensure adequate anesthesia and to ensure that the electrodes remained in contact with the skin.

Either high (100 Hz) or low (4 Hz) frequency TENS was administered keeping other parameters constant, i.e. pulse duration (100 μs), sensory intensity and 20 min for stimulation. This strategy allowed a comparison of frequency differences without confounding differences in pulse duration or amplitude. Sensory intensity was determined by increasing the intensity until a muscle contraction was visibly observed and then reducing

the intensity to just below this level. The parameters were selected to model those used clinically (Sluka et al., 1998). The sham TENS group was anesthetized with 1–2% isoflurane and electrodes were placed on their shaved knee joint, but did not receive TENS treatment. Importantly, three rats always were anesthetized with same vaporizer; at least one rat receiving the sham TENS treatment and one rat receiving the active TENS treatment were anesthetized at the same time. This procedure ensured that there always were animals in the sham TENS treatment groups that received the same dose of anesthesia as the active TENS groups.

Experimental design

Baseline paw and knee joint withdrawal thresholds were measured bilaterally prior to the induction of the knee joint inflammation. Twenty-four hours after induction of knee joint inflammation, paw and knee joint withdrawal thresholds were reassessed and then, the animals were microinjected with either $CoCl_2$ or saline. An hour following the microinjection, the rats were lightly anesthetized with 1–2% isoflurane for placement of the electrodes. TENS was then applied for 20 min.

Following baseline and post-inflammation (24 h) withdrawal thresholds, rats (n=64) were randomly divided into 12 groups, six for vlPAG: (1) Sham TENS+vehicle (n=6); (2) Sham TENS+$CoCl_2$ (n=6); (3) High TENS+vehicle (n=6); (4) High TENS+$CoCl_2$ (n=6); (5) Low TENS+vehicle (n=6); (6) Low TENS+$CoCl_2$ (n=6); and six for dlPAG: (7) Sham TENS +vehicle (n=5); (8) Sham TENS+$CoCl_2$ (n=4); (9) High TENS+vehicle (n=6); (10) High TENS+$CoCl_2$ (n=5); (11) Low TENS+vehicle (n=4); (12) Low TENS+$CoCl_2$ (n=4). One hour after application of TENS, animals were retested for withdrawal thresholds. Importantly, all behavioral tests were done by the same experimenter who was blinded to the drug injection and to the TENS group. All experimental design is shown in Fig. 1.

Statistical analysis

Since the data for mechanical withdrawal thresholds of the paw were not evenly distributed, and were on a discontinuous logarithmic scale, non-parametric analysis with the Kruskal–Wallis test examined differences between groups. Joint withdrawal threshold data were evenly distributed and on a continuous scale and were thus examined for differences with a repeated measures ANOVA for differences across time and between groups. Post hoc testing between individual groups was performed with a Tukey's test (parametric) or signed rank test (nonparametric) as appropriate. Parametric t-test and non-parametric Wilcoxon matched pairs test were used to analyze changes in the paw and joint withdrawal thresholds within groups, respectively. P value <0.05 was considered significant.

RESULTS

Effects of $CoCl_2$ microinjection into the vlPAG on withdrawal thresholds

Joint inflammation significantly decreased the withdrawal thresholds of the paw 24 h after injection of kaolin and carrageenan (Fig. 2). In preliminary experiments (n=6), microinjection of 5 mM $CoCl_2$ into the vlPAG ipsilaterally 24 h after induction of inflammation significantly increased the withdrawal threshold to mechanical stimulation of the paw (Fig. 2). The effect of $CoCl_2$ peaked 30 min after microinjection and lasted through 90 min. Withdrawal thresholds returned to pre-$CoCl_2$ values 2 h after microinjection of $CoCl_2$ (Fig. 2). Thus, we applied TENS for 20 min beginning 1 h after $CoCl_2$ to have an adequate block of synaptic transmission during TENS, and to test behavioral responses 1 h after the end of the TENS treatment so that the effects of $CoCl_2$ were no longer present. The analgesia produced by TENS would still be present at this time as the effects of TENS last a minimum of 12 h (Sluka et al., 1998).

Distribution of microinjection sites in the vlPAG and dlPAG

Histological analysis showed that microinjection sites were distributed in the vlPAG or the dlPAG. Fig. 3A displays the injection sites in all groups in the vlPAG, and Fig. 3B shows sites in the dlPAG. Sites outside the vlPAG or dlPAG including the lateral PAG (n=2), superior colliculus (n=6) and aqueduct (n=1) were removed from analysis. The sites plotted show the area of maximum concentration of the dye.

Effects of the blockade of the vlPAG and dlPAG on TENS antinociception

There were no significant differences between groups for mechanical withdrawal threshold of the paw or knee joint either before or 24 h after induction of inflammation. Twentyfour hours after the induction of inflammation there was a significant decrease in both paw and knee joint withdrawal thresholds (P<0.01; Figs. 4A and 5A).

In the group of rats microinjected with saline, either high or low frequency TENS significantly reversed the primary (knee joint) and secondary (paw) hyperalgesia when compared to the withdrawal thresholds prior to TENS treatment (P<0.001) or with sham TENS treatment (P<0.001; Fig. 4A). However, microinjection of $CoCl_2$ into the vlPAG prior to application of either high or low frequency TENS prevented the increases in withdrawal thresholds normally observed by TENS. Withdrawal threshold of the paw and knee joint was significantly lower in the groups treated with TENS and $CoCl_2$ into the vlPAG when compared to the group treated with TENS and vehicle; and was not significantly different from sham TENS groups or from the pre-TENS withdrawal thresholds (Fig. 5A). However, microinjection of $CoCl_2$ into the dlPAG prior to application of either high or low frequency TENS had no effect on the antihyperalgesia produced by TENS. All the TENS groups after treatment were different from sham after treatment with TENS for both the muscle and paw withdrawal threshold. However no difference between cobalt and vehicle for each frequency was observed for the muscle (145±18% HF+$CoCl_2$ vs. 143±18%, HF+Vehicle; 152±20% LF+$CoCl_2$ vs. 132±8%, LF+Vehicle), or the paw (355±66% HF +$CoCl_2$ vs. 606±166%, HF+Vehicle; 439±112% LF+$CoCl_2$ vs. 682±143% LF+Vehicle) (Figs. 4B and 5B).

DISCUSSION

In the current study, we injected $CoCl_2$ into the PAG to investigate if the PAG was involved in the antinociceptive pathway activated by stimulation with TENS. Our data demonstrated a complete blockade of the effects of high and low frequency TENS following microinjection of $CoCl_2$ into the vlPAG, but not the dlPAG. These data are in agreement with prior data from our laboratory showing that TENS produces analgesia through activation of opioid receptors in the RVM (Kalra et al., 2001).

Opioid analgesia in the PAG

There is a growing literature indicating distinct dorsolateral and ventral antinociceptive systems within the PAG (Behbehani, 1995; Cannon et al., 1982; Morgan, 1991; Morgan et al., 1989). Numerous studies show that morphine microinjection into the ventral PAG produces analgesia (Jacquet and Lajtha, 1976; Yaksh et al., 1976; Lewis and Gebhart, 1977; Jensen and Yaksh, 1986; Siuciak and Advokat, 1987; Behbehani, 1995). The behavioral antinociception produced by microinjection of morphine into the vlPAG, but not the dlPAG, decreases with repeated administration (Jacquet and Lajtha, 1976; Lewis and Gebhart, 1977; Siuciak and Advokat, 1987; Tortorici et al., 1999, 2001; Morgan et al., 2005a,b), a phenomenon known as opioid tolerance. This opioid tolerance is restricted to the vlPAG, and does not occur with administration in the dlPAG (Tortorici et al., 1999). As with morphine, repeated application of TENS results in analgesic tolerance by the fourth day with

a cross-tolerance at opioid receptors in the spinal cord (Chandran and Sluka, 2003) further supporting a role for the opioid-analgesic system in TENS analgesia.

Similar to morphine, microinjection of δ-opioid receptor agonists produces antinociception as measured by the hot plate and tail-flick test (Jensen and Yaksh, 1986). Moreover, microinjection of a δ-opioid receptor antagonist into the vlPAG prevented the antinociception produced by electrical stimulation of the amygdala further supporting a role for δ-opioid receptors in the PAG (Tershner and Helmstetter, 2000). These data suggest that activation of the ventral PAG produces an opioid-mediated analgesia that utilizes μ- and δ-opioid receptors.

The PAG does not have a major projection to the spinal cord (Basbaum and Fields, 1984), but produces its effects through a relay in the RVM, i.e. the nucleus raphe magnus (NRM) and adjacent structures (Kuypers and Maisky, 1975; Castiglioni et al., 1978; Mantyh and Peschanski, 1982; Urban and Smith, 1994). The morphine-induced analgesia from the PAG produces antinociception by activation of both μ- and δ-opioid receptors in the RVM (Kiefel et al., 1993; Urban and Smith, 1994). The RVM in turn projects to the spinal cord and reduces activity of nociceptive dorsal horn neurons, to result in an analgesic effect (Zhuo and Gebhart, 1997; Venegas and Schaible, 2004). The antinociception produced by microinjection of morphine in the vlPAG is prevented by blockade of NMDA, and both μ- and δ-opioid receptors in the RVM (Kiefel et al., 1993; Spinella et al., 1996). These effects of activation of the vlPAG inhibitory pathway by morphine modulate neuronal activity in the RVM such that there was an increase in off-cell activity and a decrease in on-cell activity (Cheng et al., 1986). Thus, the PAG projects through the RVM to produce inhibition. The RVM, then projects to the spinal cord using serotoninergic and non-serotoninergic cells to inhibit dorsal horn neurons (Zhuo and Gebhart, 1991; Venegas and Schable, 2004).

The PAG and RVM work synergistically to produce analgesia. Coadministration of μ-opioid agonists into the RVM and the PAG results in a profound synergistic interaction (Rossi et al., 1994). Coadministration of DAMGO into one region with deltorphin in the other also results in a significant synergy, whereas, if DAMGO and deltorphin are coadministered together in the same brain area there is an additive effect. These findings suggest the existence of mu/mu and mu/delta synergy between the PAG and RVM (Rossi et al., 1994).

We therefore hypothesize that TENS utilizes the opioid analgesia system originating in the vlPAG which projects through the RVM to the spinal cord. Both high and low frequency TENS produce their analgesia effects by activation of μ- and δ-opioid receptors in the RVM and the spinal cord (Sluka et al., 1999; Kalra et al., 2001). Further, low frequency TENS releases 5-HT in the spinal cord and activates serotoninergic receptors, 5-HT_2 and 5-HT_3 (Radhakrishnan et al., 2003; Sluka et al., 2006). High frequency TENS does not utilize 5-HT but releases GABA that activates $GABA_A$ receptors in the spinal cord (Radhakrishnan et al., 2003; Maeda et al., 2007). Both high and low frequency TENS reduce dorsal horn neuron sensitization after inflammation (Ma and Sluka, 2001), and the consequent hyperalgesia (Sluka et al., 1999). Thus, TENS, both high and low frequency requires activation of neurons in the vlPAG, the RVM, and spinal cord, and utilizes opioid mechanisms to produce analgesia.

Although the PAG and the RVM are clearly involved in the analgesia produced by both low and high frequency TENS, other mechanisms including peripheral, segmental spinal analgesia, or systemic effects are also possible. Indeed prior studies show that effects of low and/or high frequency TENS can be prevented by blockade of opioid or α-2 noradrenergic receptors at the site of stimulation (King et al., 2005; Resende et al., 2004). Spinally, there is

activation and release of GABA, activation of muscarinic receptors, and activation of opioid receptors, all of which could be related to either spinal segmental inhibition or descending inhibitory pathways (Maeda et al., 2007; Radharkshinan and Sluka, 2003; Sluka et al., 1999). Thus, multiple mechanisms acting in concert or independently could be responsible for the analgesia produced by TENS.

Facilitation from the PAG

The current study showed that microinjection of $CoCl_2$ into the vlPAG reversed the hyperalgesia induced by knee joint inflammation, suggesting a role for the PAG in facilitating nociception after injury. Recent studies support a role for the PAG in facilitation of nociception (Heinricher et al., 2004; Guo et al., 2006). This facilitatory effect from the PAG involves prostaglandin E_2 and BDNF (Heinricher et al., 2004; Guo et al., 2006). Microinjection of prostaglandin E_2 decreases the paw withdrawal latency to heat, and activates facilitatory cells in the RVM, i.e. on-cells (Heinricher et al., 2004). Further there are increases in BDNF in the PAG, the BDNF receptor TrkB in the RVM after inflammation, and blockade of TrkB receptors in the RVM reverses hyperalgesia (Guo et al., 2006). There is substantial evidence that the RVM mediates facilitation of nociception after injury in numerous animal models including joint inflammation (Urban et al., 1996), pancreatitis (Vera-Portocarrero et al., 2006), neuropathic pain (Burgess et al., 2002), visceral pain (Zhuo and Gebhart, 2002), and inflammatory pain (Guan et al., 2004; Sugiyo et al., 2005). Thus, there is emerging evidence that the vlPAG facilitates nociception, and this facilitation is mediated through the RVM.

Clinical significance

The use of TENS can therefore be thought of as a non-pharmacological tool to engage our endogenous analgesic system. It utilizes endogenous opioids acting on their receptors to produce analgesia without side effects normally observed with exogenous opioids. Early clinical studies show that low frequency TENS utilizes opioid receptors to produce analgesia (Sjolund and Eriksonn, 1979). Further high frequency TENS increases the concentration of β-endorphins increase in the bloodstream and cerebrospinal fluid, and methionine–enkephalin in the cerebrospinal fluid, in human subjects (Han et al., 1991; Salar et al., 1981). Together, these clinical studies in human subjects, along with animals studies on mechanisms support a role for activation of opioid-mediated analgesia utilizing the PAG–RVM pathway for both high and low frequency TENS. Since TENS utilizes known pharmacological pathways, particularly opioids, TENS should be administered with similar principles. Clinicians should be aware of the potential for the development of tolerance, as well as potential interactions of TENS with the patient's pharmacological therapy. For example, if subjects have been taking opioids long enough to develop tolerance then low frequency TENS, which utilizes μ-opioid receptors, should be avoided. Preclinical studies in rats support this since low frequency TENS is ineffective in rats that were made previously tolerant to opioids (Sluka et al., 2000). Further, combining pharmacological agents with TENS could enhance the effectiveness of the treatment, and reduce side effects of the drug. For example combining morphine or clonidine with TENS enhances the analgesic effect so that a lower dose of the drug produces a similar degree of analgesia (Sluka, 2000; Sluka and Chandran, 2002). Thus, future studies should examine the effects of combining common pharmaceutical agents for treatment of pain with TENS in both animal and human subjects, and should be aimed at developing mechanisms to prevent tolerance (Hingne and Sluka, 2008; DeSantana et al., 2008).

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Abbreviations

dlPAG	dorsolateral periaqueductal grey
PAG	periaqueductal grey
RVM	rostroventral medial medulla
TENS	transcutaneous electric nerve stimulation
vlPAG	ventrolateral periaqueductal grey

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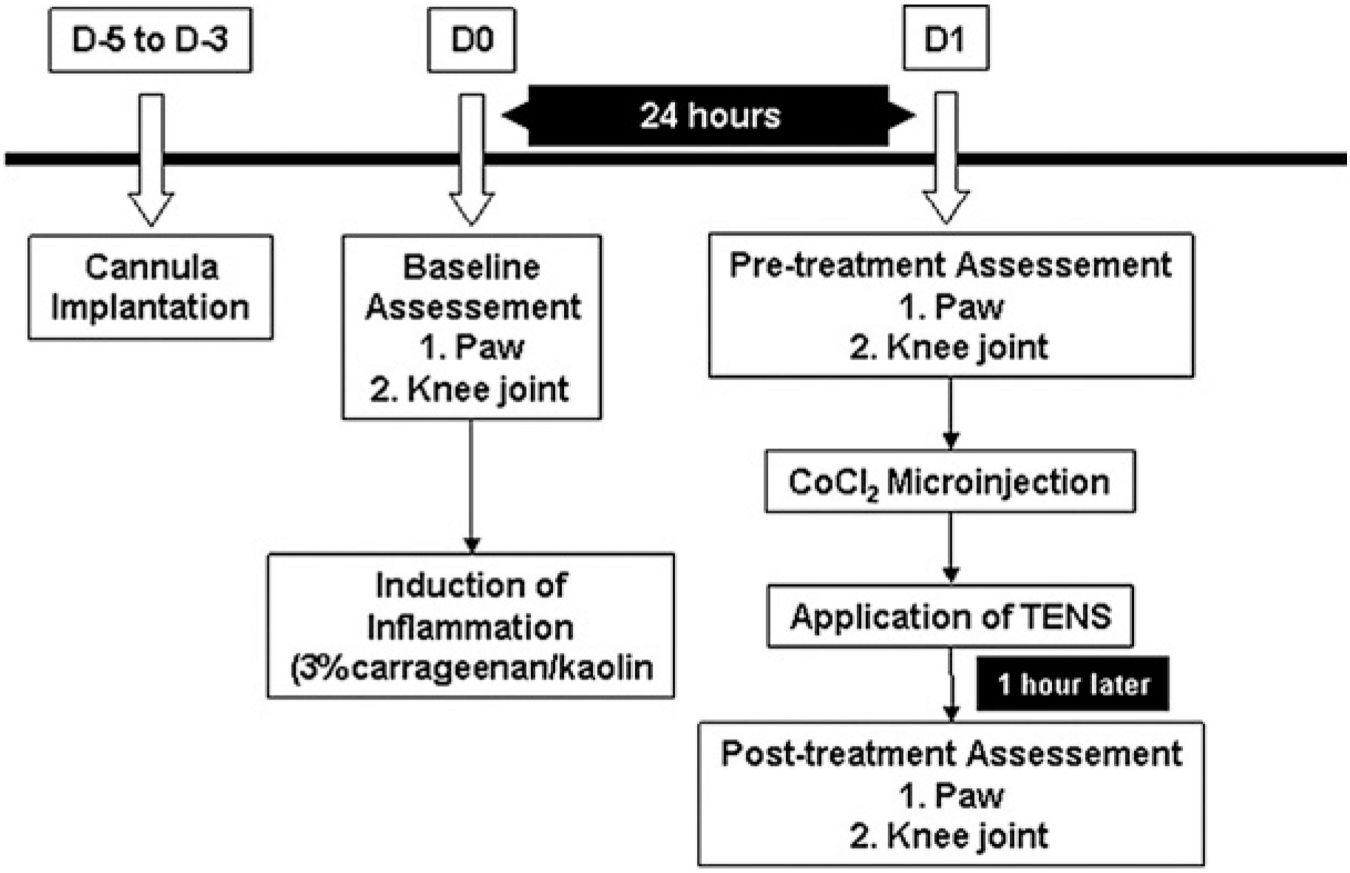

Fig. 1.
Time line for the experiment.

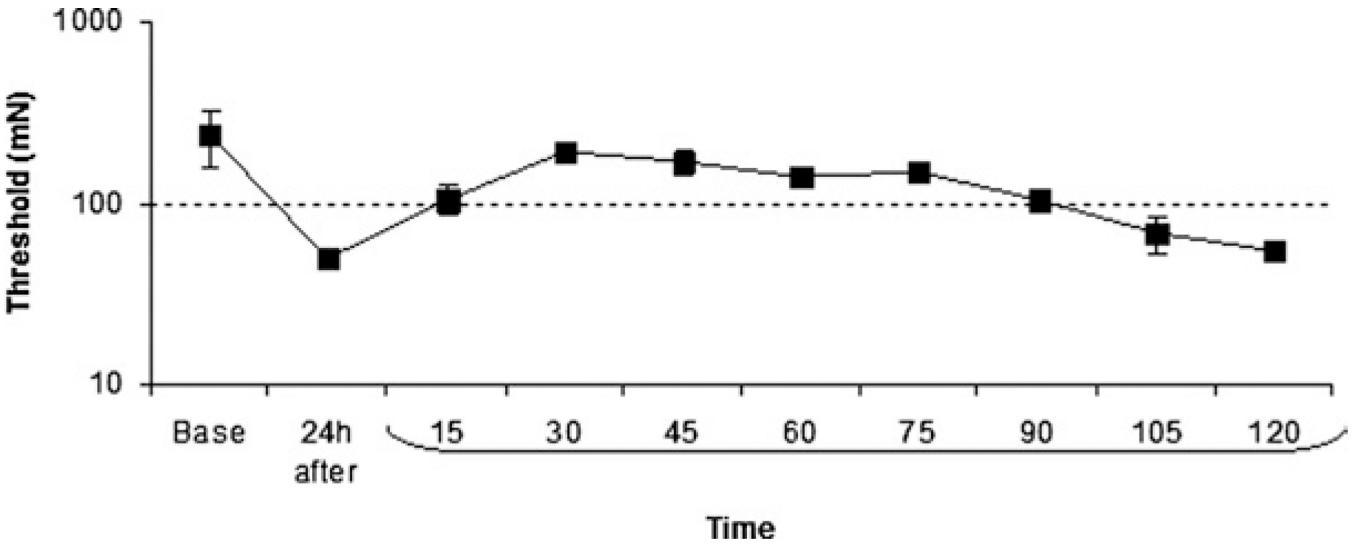

Fig. 2.

Microinjection of cobalt chloride ($CoCl_2$) into the vlPAG 24 h after induction of inflammation increased the mechanical withdrawal threshold of the paw. The effect of $CoCl_2$ peaked 30 min after microinjection and lasted through 90 min.

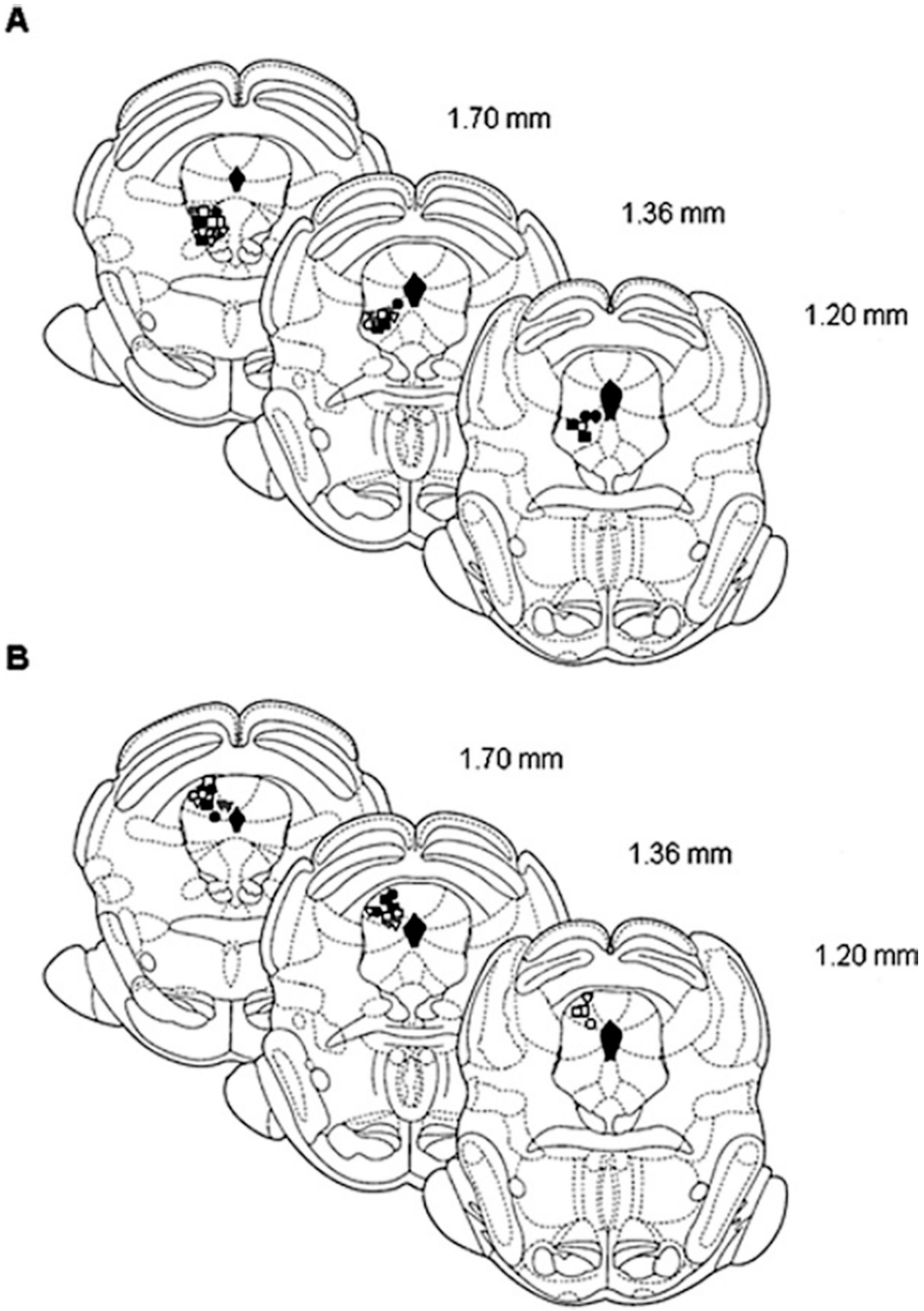

Fig. 3.

Schematic coronal sections of the rat brain adapted from Paxinos and Watson (2005) illustrating approximate sites of microinjections into the vlPAG (A) and dlPAG (B). Numbers indicate that distance from the interaural in millimeters. Only rats with injection sites in or immediately adjacent to the vlPAG or dlPAG were included in data analysis. Symbols represent the microinjection sites; filled symbols indicate animals microinjected with $CoCl_2$ and open symbols, vehicle. Animals were stimulated with high frequency TENS (squares), low frequency TENS (circles) or sham TENS (triangles).

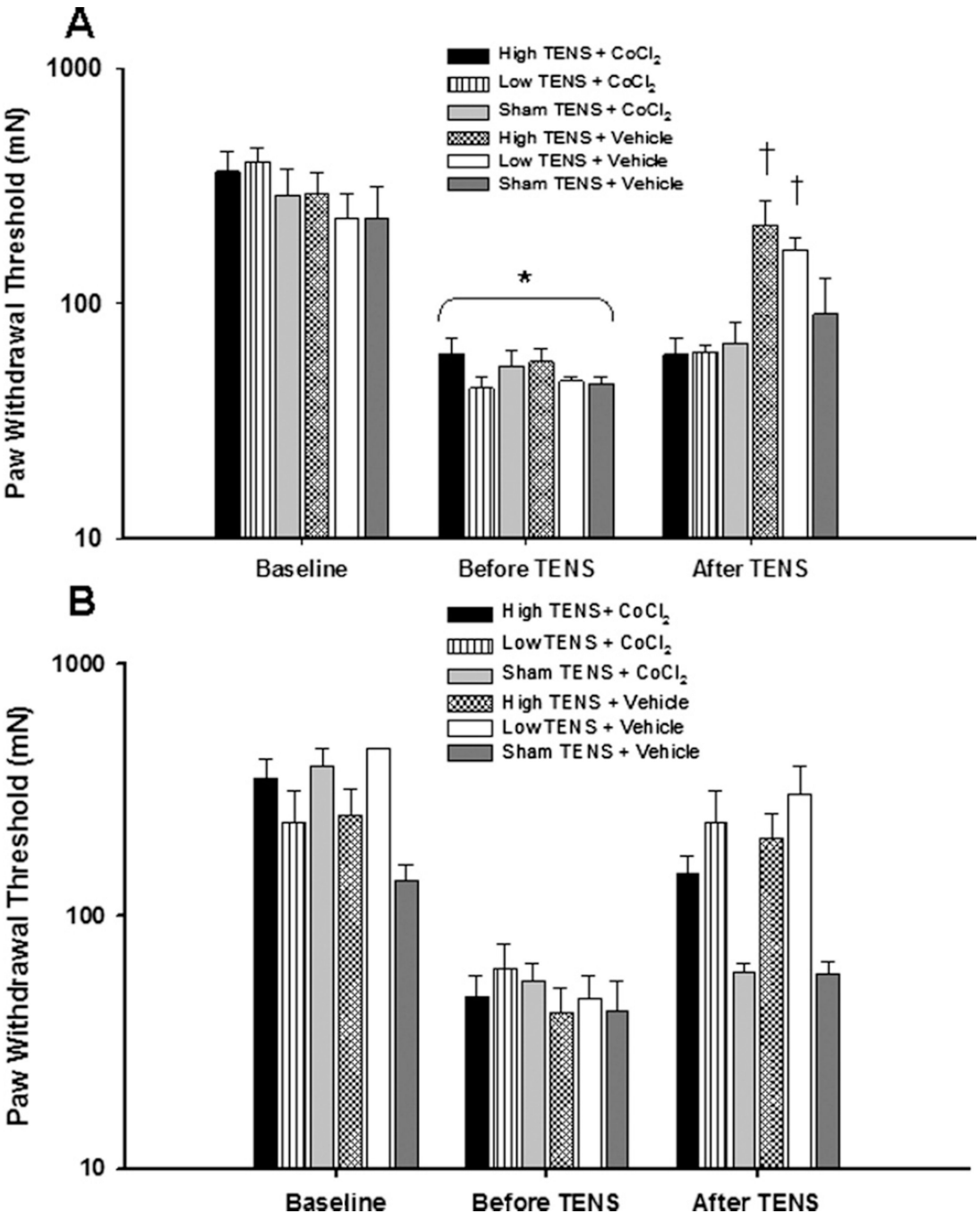

Fig. 4.

Bar graph representing mechanical withdrawal threshold of the paw from animals microinjected with either $CoCl_2$ or vehicle into the (A) vlPAG and (B) dlPAG. Mechanical withdrawal thresholds are illustrated prior to induction of inflammation (Baseline), before application of TENS, and after microinjection of the vlPAG or dlPAG. Data are represented as mean±SEM. *P* value 0.05 was considered statistically significant. * Significantly different from baseline time; † significantly different from vehicle control groups.

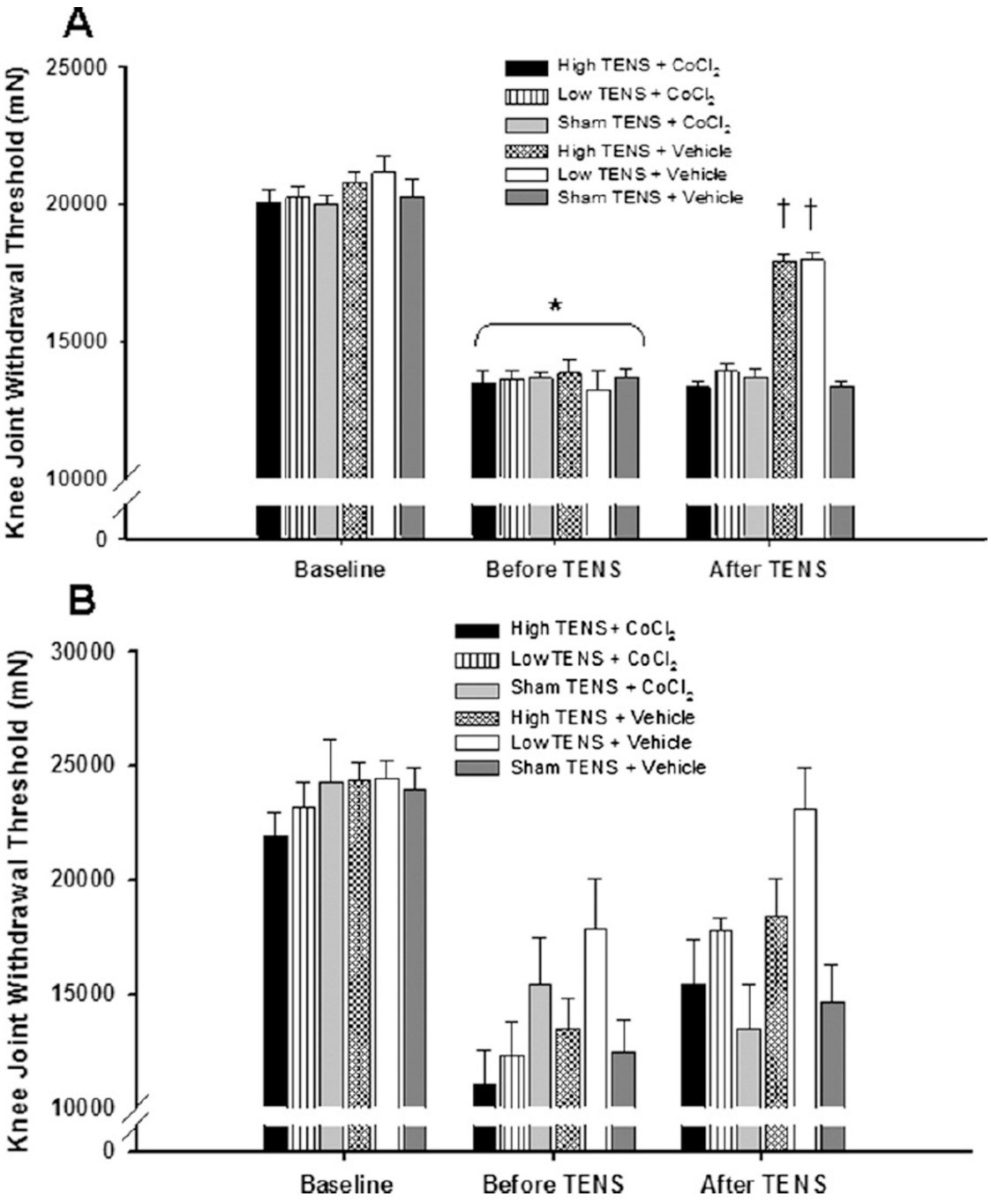

Fig. 5.

Bar graph representing mechanical withdrawal threshold of the knee from animals microinjected with either $CoCl_2$ or vehicle into the (A) vlPAG or (B) dlPAG. Mechanical withdrawal thresholds are illustrated prior to induction of inflammation (Baseline), before application of TENS, and after microinjection of the vlPAG or dlPAG. Data are represented as mean±SEM. *P* value <0.05 was considered statistically significant. * Significantly different from baseline time; † significantly different from vehicle control groups.