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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.

Key words: pain, TENS, hyperalgesia, opioid, inflammation, analgesia.

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

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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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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