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Electroacupuncture combined with MK-801 prolongs anti-hyperalgesia in rats with peripheral inflammation

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Abstract

Our previous study showed that electroacupuncture (EA), an adjuvant to conventional medicine, significantly attenuated hyperalgesia in a rat model of inflammatory pain. In the present study, we evaluated the potential additive and/or synergism of EA and a sub-effective dose of dizocilpine maleate (MK-801), a non-competitive *N*-methyl-D-aspartate (NMDA) receptor antagonist, on hyperalgesia in the same rat model of inflammatory pain.

Hyperalgesia, manifesting as decreased paw withdrawal latency (PWL) to a noxious stimulus, was induced by injecting complete Freund's adjuvant (CFA) into the plantar surface of one hind paw of each rat. EA treatments were given at acupoint GB30 immediately after and 2 h after CFA. MK-801 at 0.001 mg/rat was given (i.t.) 10 min before each of the two EA treatments. PWL was measured prior to and 2.5 and 5 h post-CFA.

Ten and 100 Hz EA significantly inhibited CFA-induced hind paw hyperalgesia. Both 10 and 100 Hz EA combined with the sub-effective dose of 0.001 mg/rat MK-801 showed prolonged anti-hyperalgesia with no side effects. These results demonstrate that EA combined with a sub-effective dose of this NMDA receptor antagonist enhances anti-hyperalgesia, and this combination may provide an effective strategy for pain management.

Keywords: Hyperalgesia; Acupuncture; NMDA receptor; MK-801; Complete Freund's adjuvant; Intrathecal injection

1. Introduction

Various chronic inflammatory diseases affect a large population of patients. Conventional medicine such as non-steroidal anti-inflammatory drugs (NSAIDs) and recently developed COX-2 inhibitors may be associated with significant adverse effects such as gastrointestinal disturbances (Stiel, 2000; Scheiman, 2001; Davies and Jamali, 2004). It is documented that as many as 36–62% of patients use complementary and alternative medicine, including acupuncture, as an adjunct to conventional medicine (Eisenberg et al., 1998; Barnes et al., 2004).

Our study with an animal model of inflammatory pain showed that electroacupuncture (EA) at acupoint Huantiao (GB30) significantly attenuated complete Freund's adjuvant (CFA)-induced hyperalgesia (Lao et al., 2004). It has also been demonstrated that the combination of a sub-effective dose of indomethacin, a classic NSAID drug, or morphine and EA treatment produces greater anti-hyperalgesia than either agent alone and than the sum effects of the individual agents (Zhang et al., 2004a,b).

Additionally, considerable evidence suggests that excessive activation of the *N*-methyl-D-aspartate (NMDA) receptor, an ionotropic glutamate receptor, plays a key role in the development of hyperalgesia and central hyperexcitability (Dubner and Ruda, 1992; Kolhekar et al., 1993; Ren and Dubner, 1993; Sluka and Westlund, 1993), and that

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NMDA antagonists can be used in the management of chronic pain (Dickenson et al., 1997; Ren et al., 1992). However, high dose NMDA antagonists have been shown to cause such side effects as disruption of motor coordination (Hama et al., 2003). Clearly, alternatives or adjunctives to conventional medicine would be clinically useful in the treatment of persistent pain. We hypothesize that the combination of a sub-effective dose of dizocilpine maleate (MK-801), a classic NMDA antagonist, and EA treatment produces greater anti-hyperalgesia than either agent alone in a rat model of inflammatory pain.

2. Methods

2.1. Animal preparation

Male Sprague–Dawley rats weighing 280–350 g (Harlan) were kept under controlled environmental conditions (22 °C ± 0.5 °C, relative humidity 40–60%, 7 a.m. to 7 p.m. alternate light–dark cycles, food and water ad libitum). Animal protocols were approved by the Institutional Animal Care and Use Committee (IACUC) at the University of Maryland School of Medicine. The ethical guidelines for the treatment of animals of the International Association for the Study of Pain were followed in these experiments.

Under pentobarbital sodium anesthesia (50 mg/kg i.p.), the rats were prepared for intrathecal injection. The atlanto-occipital membrane at the level between the head and neck (i.e., approximately the obex level) was exposed and a 7.5-cm length of PE-10 tubing was inserted into the subarachnoid space through a slit made in the membrane. The catheter was advanced to the level of the lumbar spinal cord and filled with saline (approximately 7–10 μl), and the outer end was plugged. The rats were allowed to recover for 7 days after the operation prior to induction of hyperalgesia. Animals with gross signs of motor impairment were excluded from the study. Evans blue was injected via the catheter and the location of the distal end of the catheter was verified.

Inflammatory hyperalgesia was induced by injecting 0.08 ml of CFA, which was suspended in an 1:1 oil/saline emulsion and contained 40 μg *Mycobacterium tuberculosis* (Sigma), subcutaneously into the plantar surface of one hind paw of each rat using a 25 gauge hypodermal needle. Hyperalgesia was determined by a decrease in PWL to a noxious thermal stimulus (Hargreaves et al., 1988). The effect of the hyperalgesia on the normal behavior of the CFA-inflamed rats appeared to be minimal, and they showed normal grooming behavior and levels of activity (Lao et al., 2001, 2004).

2.2. Experimental design

Rats were randomly divided into the following groups (n = 7–9 per group): (1) MK-801 (Sigma), 0.001 (n = 8), 0.005 (n = 9) and 0.01 (n = 8) mg/rat (10 μl i.t.); (2) saline control (10 μl i.t., n = 9); (3) MK-801 (0.001 mg/rat, n = 9) or saline (n = 7) plus 10 Hz EA; (4) MK-801 (0.001 mg/rat, n = 8) or saline (n = 8) plus 100 Hz EA; and (5) sham EA (n = 9). The MK-801 was dissolved in saline and administered (i.t.) 10 min before each of two EA treatments.

2.3. Acupuncture treatment

Detailed EA procedure has been described previously (Lao et al., 2004). To maximize the anti-hyperalgesia, animals were given two 20-min EA treatments, one immediately after CFA administration and the second 2 h post-CFA. Our previous study tested a single EA treatment, administered at the time of injection, but it produced no anti-hyperalgesia in this animal model (Lao et al., 2004). Previously determined EA parameters of low frequency (10 Hz) and high frequency (100 Hz) at 3 mA and 0.1 ms pulse width, each of which showed significant anti-hyperalgesic effects in the rat inflammation model (Lao et al., 2004), were used in the present study. The equivalent of human acupoint GB30 on the rat's hind limbs was treated bilaterally. In humans, GB30 is located at the junction of the lateral 1/3 and medial 2/3 of the distance between the greater trochanter and the sacral hiatus; underneath are the sciatic nerve, inferior gluteal nerve and gluteal muscles (Cheng, 1999). Anatomically comparable landmarks were used to locate GB30.

GB30 was chosen based on traditional Chinese medicine (TCM) meridian theory (O'Connor and Bensky, 1981) and its successful use in previous studies (Xu et al., 1993; Lao et al., 2004; Zhang et al., 2004a,b). Our previous study (Lao et al., 2004) showed that EA at acupoint GB30, but not at TE5 (the 5th acupoint on the Triple Energizer Meridian) or at sham points, including the point at the opposite aspect of GB30 and an unnamed abdominal point, showed significant anti-hyperalgesia.

The animals were gently handled for 30 min each day for 2–3 days to habituate them before acupuncture treatment. After cleaning the skin with alcohol swabs, one investigator swiftly inserted disposable acupuncture needles (gauge #32, 0.5 in. in length) with electrodes soldered to their handles bilaterally, approximately 0.5 in. into GB30 while another held the animal gently. The needles and the electrodes were stabilized with adhesive tape (Lao et al., 2004). The procedure typically lasted less than 20 s and caused little apparent distress to the animal.

During EA treatment, each rat was placed under an inverted clear plastic chamber (approximately 5 in.×8 in.×11 in.) but was neither restrained nor given any anesthetic. EA was delivered by an electrical stimulator (A300 Pulsemaster, World Precision Instruments) via an isolator (A360D Stimulus Isolator, World Precision Instruments), which converts electrical voltage into electrical current. While EA frequency was held constant, intensity was adjusted slowly over the period of approximately 2 min