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Research report

Electroacupuncture improves gait locomotion, H-reflex and ventral root potentials of spinal compression injured rats

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ABSTRACT

This study explored the effect of electroacupuncture stimulation (EA) on alterations in the Hoffman reflex (H-reflex) response and gait locomotion provoked by spinal cord injury (SCI) in the rat. A compression lesion of the spinal cord was evoked by insufflating a Fogarty balloon located in the epidural space at the T8–9 spinal level of adult Wistar male rats (200–250 gr; n = 60). In different groups of SCI rats, EA (frequencies: 2, 50 and 100 Hz) was applied simultaneously to Huantiao (GB30), Yinmen (BL37), Jizhong (GV6) and Zhiyang (GV9) acupoints from the third post-injury day until the experimental session. At 1, 2, 3 and 4 post-injury weeks, the BBB scores of the SCI group of rats treated with EA at 50 Hz showed a gradual but greater enhancement of locomotor activity than the other groups of rats. Unrestrained gait kinematic analysis of SCI rats treated with EA–50 Hz stimulation showed a significant improvement in stride duration, length and speed ($p < 0.05$), whereas a discrete recovery of gait locomotion was observed in the other groups of animals. After four post-injury weeks, the H-reflex amplitude and H-reflex/M wave amplitude ratio obtained in SCI rats had a noticeable enhancement (217%) compared to sham rats (n = 10). Meanwhile, SCI rats treated with EA at 50 Hz manifested a decreased facilitation of the H-reflex amplitude and H/M amplitude ratio (154%) and a reduced frequency-dependent amplitude depression of the H-reflex (66%). In addition, 50 Hz-EA treatment induced a recovery of the presynaptic depression of the Gs-VRP evoked by PBSt conditioning stimulation in the SCI rat ($63.2 \pm 8.1\%$; n = 9). In concordance with the latter, it could be suggested that 50 Hz-EA stimulation reduced the hyper-excitability of motoneurons and provokes a partial improvement of the locomotive performance and H reflex responses by a possible recovery of presynaptic mechanisms in the spinal cord of experimentally injured rats.

1. Introduction

Spinal cord injury (SCI) is one of the most common impairments of the central nervous system that results in complete or partial loss of both sensory and motor functions due to mechanical spinal damage, which often leads to permanent paralysis (Silverman et al., 2012). Traumatic and non-traumatic lesions represent the two presentations of SCI. The first results from contusion, compression or stretching of the spinal cord, and the second is associated with vertebral spondylosis, tumor compression, vascular ischemia, and inflammatory spinal cord disorders (New et al., 2002). In both humans and animals, SCI induces physiological changes in the motor system which included hyperreflexia and abnormalities of the locomotor behavior (Silverman et al., 2012; Yablon and Stokic, 2004). Several researchers use the Hoffman reflex (H-reflex), to analyze the hyperreflexia following SCI (Milanov, 1994; Yablon and Stokic, 2004). The H-reflex is an electromyographic (EMG) response that results from activation of a synaptic pathway conformed by

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the afferent-motoneuron-muscle circuit (Reyes et al., 2007). In SCI, changes associated with hyperreflexia and spasticity included: alpha motor neuron hyperexcitability (Lin et al., 2007; Milanov, 1994), changes in the intrinsic properties of motoneurons (Bennett et al., 2001) and loss of presynaptic inhibition (Hultborn, 2006). It has been shown that the measurement of H-reflex rate-sensitive depression acquires a particular importance in the assessment of hyperreflexia following SCI (Thompson et al., 1992; Chen et al., 2001).

In general, treatment for functional recovery of SCI included surgery (Bregman et al., 2002), physical therapy (Silverman et al., 2012), drugs (Attal et al., 2009), hormonal treatment (Calderón et al., 2015; Osuna et al., 2016), behavioral therapy (Norrbrink et al., 2006), and supportive treatment (Huston et al., 2011). Because there is still a lack of effective treatment for spinal cord injuries, there has been an increased interest in alternative medical treatments.

Acupuncture is a therapeutic modality that emerged from Traditional Chinese Medicine. The World Health Organization recommends the use of acupuncture for the treatment of a wide variety of diseases (Zhang et al., 2014; Barnes et al., 2008). A relatively novel form of acupuncture is the electrical stimulation of acupuncture points (APs), also known as electroacupuncture (EA), which has been widely used in both clinical and experimental reports (Vickers et al., 2012; Zhao, 2008). In previous studies, it has been shown that application of EA (*Dazhui* (DU14), *Mingmen* (DU4), *Sanyinjiao* (SP6), *Huantiao* (GB30), *Zusanli* (ST36) and *Kunlun* (BL60) as a treatment for SCI contributes to the recovery of several neurologic and functional alterations (Min et al., 2015). It has been found that EA produces an improvement in the locomotor pattern (Peng et al., 2007), which is accompanied by reductions in the process of glial scarring (Yang et al., 2005), oxidative stress (Politis and Korchinski, 1990), laminin expression (Zhu, 2002) and aquaporin transport (AQP-4; Xie et al., 2006). It is thought that EA evokes its effects through the activation of peripheral sensory afferents that in turn synaptically interact with sets of dorsal horn sensory neurons in the spinal cord (Quiroz et al., 2014a,b). However, EA effects on experimental animal models of SCI, particularly at the motoneuron level, are scarcely studied. In this study, we analyzed the effect of EA on locomotor behavior (evaluated with the Open Field Test and gait kinematics analysis), H reflex facilitation and H-reflex frequency-dependent depression evoked in adult rats after a spinal cord compression injury. In addition, to disclose possible presynaptic mechanisms in the effect of EA stimulation, we also analyzed the changes produced by conditioning stimulation of the posterior biceps and semitendinosus (PBSt) on the amplitude of ventral root potentials (VRP) evoked by gastrocnemius nerve (Gs) stimulation, as a test for presynaptic inhibition.

2. Materials and methods

2.1. Animals

Male Wistar rats (n = 60) weighing 200–250 g (8–10 weeks old) obtained from our institution were used. All animals had free access to food and water and were housed under identical environmental conditions of light and dark cycles (12:12 h) and temperature (22–24 °C). All experiments were performed in accordance with the guidelines contained in NIH publications No. 80-23 (revised 1996) and the Mexican Official Norm (NOM-062-ZOO-1999) on the Principles of Laboratory Animal Care. The study protocol was approved by the institutional bioethics committee for the Care and Handling of Laboratory Animals (Protocol 0267-05, CINVESTAV).

2.2. Surgical procedures and animals groups

Initially the rats were randomly assigned into five groups by using a random number table: (1) sham control group (n = 10), (2) compression injury without EA treatment group (SCI-UT n = 12), (3) compression injury with EA treatment at 2 Hz (SCI-EA 2 Hz group; n = 11), (4) 50 Hz (SCI-EA 50 Hz group; n = 14) and (5) 100 Hz (SCI-EA 100 Hz group; n = 13). The method used for producing a compression injury was similar to that described previously (Lonjon et al., 2010). Animals were anesthetized with an intraperitoneal injection of a mixed solution of ketamine (100 mg/kg) and xylazine (2 mg/kg). To provoke the compression injury in the rat spinal cord, a Fogarty catheter (2 French x 60 cm, size: 0.67 mm, Ethimed) was inserted into the epidural space through a hole drilled in the posterior arch of the T-10 vertebra, and a groove was drilled on T-11 to guide the insertion of the catheter. The balloon was positioned at the T8–9 level, inflated with sterilized water (10 μl) using a Hamilton syringe, and left in place for 5 min. The sham group of rats (n = 10) underwent the same protocol, except for balloon inflation.

After surgery, the back skin and muscles were sutured with a sterile thread and stainless wound clips, and the animals were allowed to recover from surgery and anesthesia in a clean, heated cage. All rats received an intramuscular injection of penicillin and procaine (5,000,000 U/ml/day), and Furazolidone was spread onto the lesion to prevent infection. The animals were housed in individual cages and individually received intensive care, which included manual expression of urine and excrement until the day of the experiment. Because nine rats died after SCI, and six died during the electrophysiological recording session, the final number of animals included in each group was the following: SCI-UT (n = 10); SCI-EA 2 Hz group (n = 8), SCI-EA 50 Hz group (n = 9); and SCI-EA 100 Hz group (n = 8).

2.3. Electroacupuncture treatment

EA was applied simultaneously at two pairs of acupoints. One pair was situated at the Jizhong (GV6, anode) and Zhiyang (GV9 cathode) acupoints and the other pair were Huantiao (GB30 anode) and Yinmen (BL37 cathode), bilaterally (Fig. 1). These acupoints were selected because they are needling points in acupuncture treatments following SCI in humans (Heo et al., 2013a,b) and contributed to neuroplasticity in the spinal cord after injury (Min et al., 2015; Ding et al., 2011). The *Huantiao* (GB30) acupoint was located at the posterior upper border of the hip joint of the hindlimbs. The *Yinmen* (BL37) acupoint was situated where the long head of the biceps femoris muscle and the semitendinosus muscle converged (upper to the middle part of the popliteal crease), whereas the *Jizhong* (GV6) acupoint was located posterior to the midline, below the spinous process of the eleventh thoracic vertebra (maintaining the animal in the prone position). The *Zhiyang* (GV9) acupoint was situated on the posterior midline and in the depression below the spinous process of the seventh thoracic vertebra in the prone position. EA was applied daily (resting on weekends) by means of an electroacupuncture stimulator (AWQ-104L, China), and it consisted of a sequence of biphasic pulse trains of 2.5 s in duration followed by periods of 2.5 s with no stimulation (Fig. 2). The biphasic pulse stimuli was chosen in this study to avoid the polarization of each needle because of electrolysis and break off in tissue (Pomeranz, 1995). Another advantage of biphasic stimulus is that each pair of needles receives symmetrical current pulses which appears to be very useful to neuromodulate the physiological state of the spinal cord in humans (Gerasimenko et al., 2015). The intensity of the EA current pulses was adjusted to induce slight twitches in hind limbs (1 mA).

Because it has been showed that EA stimulation induced neuroprotective, remyelination, antioxidative and anti-inflammatory