Neuroscience 291 (2015) 260–271

EARLY APPLIED ELECTRIC FIELD STIMULATION ATTENUATES SECONDARY APOPTOTIC RESPONSES AND EXERTS NEUROPROTECTIVE EFFECTS IN ACUTE SPINAL CORD INJURY OF RATS

C. ZHANG,[a] G. ZHANG,[a] W. RONG,[b] A. WANG,[a] C. WU[a] AND X. HUO[a]*

[a] *Beijing Key Laboratory of Bioelectromagnetism, Institute of Electrical Engineering, Chinese Academy of Sciences, No. 6 Beiertiao, Zhongguancun, Haidian District, Beijing 100190, China*

[b] *Department of Orthopedics, Beijing Tsinghua Changgung Hospital Medical Center, Tsinghua University, Li Tang Road No. 168, Dongxiaokou Town, Changping District, Beijing 102218, China*

Abstract—Injury potential, which refers to a direct current voltage between intact and injured nerve ends, is mainly caused by injury-induced Ca^{2+} influx. Our previous studies revealed that injury potential increased with the onset and severity of spinal cord injury (SCI), and an application of applied electric field stimulation (EFS) with the cathode distal to the lesion could delay and attenuate injury potential formation. As Ca^{2+} influx is also considered as a major trigger for secondary injury after SCI, we hypothesize that EFS would protect an injured spinal cord from secondary injury and consequently improve functional and pathological outcomes. In this study, rats were divided into three groups: (1) sham group, laminectomy only; (2) control group, subjected to SCI only; and (3) EFS group, received EFS immediately post-injury with the injury potential modulated to 0 ± 0.5 mV by EFS. Functional recovery of the hind limbs was assessed using the Basso, Beattie, and Bresnahan (BBB) locomotor scale. Results revealed that EFS-treated rats exhibited significantly better locomotor function recovery. Luxol fast blue staining was performed to assess the spared myelin area. Immunofluorescence was used to observe the number of myelinated nerve fibers. Ultrastructural analysis was performed to evaluate the size of myelinated nerve fibers. Findings showed that the EFS group rats exhibited significantly less myelin loss and had larger and more myelinated nerve fibers than the control group rats in dorsal corticospinal tract (dCST) 8 weeks after SCI. Furthermore, we found that EFS inhibited the activation of calpain and caspase-3, as well as the expression of Bax, as detected by Western blot analysis. Moreover, EFS decreased cellular apoptosis, as measured by TUNEL, within 4 weeks post-injury. Results suggest that early EFS could significantly reduce spinal cord degeneration and improve functional and historical recovery. Furthermore, these neuroprotective effects may be related to the inhibition of secondary apoptotic responses after SCI. These findings support further investigation of the future clinical application of EFS after SCI.

Key words: electric field stimulation, spinal cord injury, secondary injury, apoptosis, injury potential.

INTRODUCTION

Spinal cord injury (SCI), which involves primary and secondary injury mechanisms, is the most devastating injury to the spinal cord. The initial impact directly results in immediate hemorrhage and rapid tissue injury at the injury site (Oyinbo, 2011). Structural damage, such as plasma membrane integrity impairment and sodium–calcium exchanger dysfunction, causes a marked increase in Ca^{2+} influx within injured axons at the injury site after acute injury (Mikkelsen et al., 2004). Injury potential, which refers to a direct current potential gradient between intact and injured nerve ends, is mainly caused by injury-induced Ca^{2+} influx (Geddes and Hoff, 1971; Goodman et al., 1985; Zuberi et al., 2008). Our previous papers accordingly revealed that injury potential increased with the onset and severity of SCI (Pan et al., 2011). Injury potential increased dramatically within 30-min post-injury and then decreased gradually to a normal value of 0 mV after several hours post-injury. The initial amplitude of injury potential positively correlated with the severity of SCI.

The injury-induced influx of excessive Ca^{2+} into cells is also considered, among others, as a major mechanism for secondary injury (Imaizumi et al., 1997; Xiong et al., 2007). Ca^{2+} influx not only diffuses to adjacent regions, which results in further tissue breakdown (Ray et al., 2003; Beirowski et al., 2005), but also inappropriately stimulates a variety of apoptosis-related proteins to activate the apoptotic pathway, thereby aggravating irreversible tissue loss and dysfunction to worsen the primary lesion (Beattie et al., 2000; Huff et al., 2011).

Previous investigations indicated that early Ca^{2+} blocking is a viable therapeutic strategy that could reduce the degree of secondary injury and prevent

*Corresponding author. Tel: +86-10-82547242; fax: +86-10-82547164.
E-mail address: huoxl@mail.iee.ac.cn (X. Huo).

Abbreviations: BBB, Basso, Beattie, and Bresnahan; dCST, dorsal corticospinal tract; EFS, electric field stimulation; IF, immunofluorescent; LFB, Luxol fast blue; OFS, oscillating electrical fields; SBDP, spectrin breakdown product; SCI, spinal cord injury; WB, Western blot; TEM, transmission electron microscopy.

http://dx.doi.org/10.1016/j.neuroscience.2015.02.020
0306-4522/

further damage to the spinal cord axons (Liverman et al., 2005). An approach for reducing Ca^{2+} influx by applied electric field stimulation (EFS) was reported by Strautman et al. (1990). The study showed that the movement of Ca^{2+} was greatly reduced by the applied field with the cathode distal to the lesion, and the movement of Ca^{2+} increased upon application of a field of the opposite polarity. However, no proper indexes have been established to determine the optimal EFS parameters, such as the stimulating voltage and duration.

Based on previous approaches, we further investigated the optimal parameters of EFS for therapy in our laboratory (Zhang et al., 2013). In a rat model of SCI, stimulating EFS voltages were established to offset the rostral and caudal injury potential (injury potential compensation). After 30 min of stimulation, results showed that the injury potential was significantly delayed during EFS and was attenuated after EFS. Therefore, this study further determined the efficacy of early EFS in terms of protecting the spinal cord from degeneration and accelerating regeneration after SCI. We also examined the expression and activation of calcium-activated apoptotic proteins to determine the underlying therapy mechanism of EFS following SCI.

EXPERIMENTAL PROCEDURES

Animal uses and groups

Adult female Sprague–Dawley (SD) rats weighting 200–250 g were purchased from Beijing HFK Bio-Technology Co., Ltd. (Beijing, China). Animal experimental procedures were performed according to the National Guidelines for Experimental Animal Welfare (Ministry of Science and Technology of People's Republic of China, 2006) and were approved by the Animal Welfare Committee of the Beijing Key Laboratory of Bioelectromagnetism. The rats were randomly assigned to three groups: sham group rats, (n = 53) which underwent laminectomy only; control group rats (n = 53), which underwent SCI only; and EF group rats (n = 53), which received EFS after SCI. The rats were housed in a temperature-controlled room (23 ± 1 °C) with a 12:12 dark/light cycle and were given free access to food and ultrapure water. All attempts were made to minimize the number of animals used and to avoid undue animal suffering.

Surgical protocol and applied electric field procedure

Surgical protocol. The spinal cords of all three groups of rats were exposed by three small laminectomies located at T8, T10, and T12. For EFS group rats, two electric field stimulators were applied for each rat (Fig. 1). Two anodes were sutured at both sides of the paravertebral muscle of T10, whereas the two cathodes of one stimulator were sutured at the paravertebral muscle of T8. Two cathodes of another stimulator were sutured beside T12. The distances between adjacent electrodes were approximately 1 cm. SCI was induced by dropping a weight of 10 g from a height of 50 mm onto an impounder (with a diameter of 0.2 cm), which was gently placed on the spinal cord. The sham group rats underwent laminectomy only. The control group rats underwent SCI only.

Applied electric field procedure. The injury potentials were measured by glass electrodes immediately after SCI (Fig. 2). The glass electrodes, which consisted of upper and lower glass tubes, were described in our previous article (Zhang et al., 2013). The upper tube was filled with 3 M KCl solution and contained a calomel electrode that was connected to voltmeters via conducting wires. The lower tube was filled with 0.9% saline and plugged by a small bulk of porous ceramic. The two solutions in the upper and lower tubes were separated by agar.

The tip of one glass electrode, which connected the negative input of the voltmeter, was gently placed on

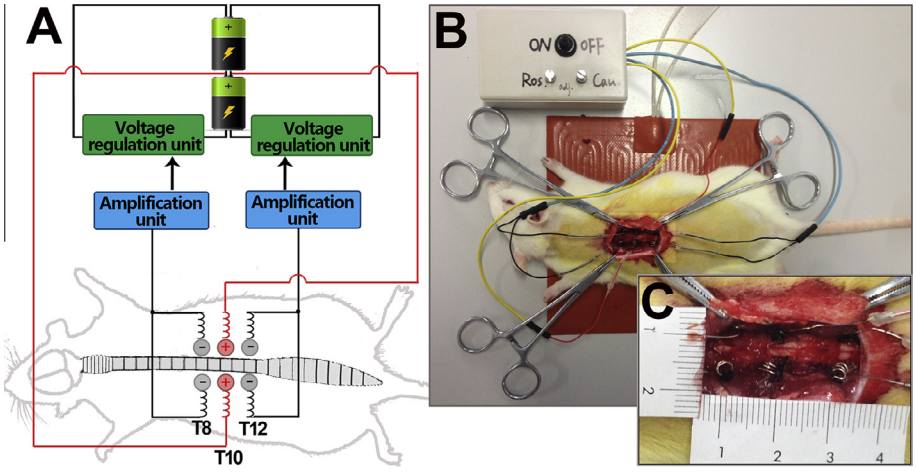

Fig. 1. Experimental setup for applied electric field stimulation in rats SCI. (A) Schematic diagram of the applied electric field stimulation. (B) Image of the electric field stimulation procedure. The electric field stimulator was packaged in a plastic box, and the rostral and caudal stimulating voltages were regulated through the knobs on the box. (C) Enlarged image of electrode suture. The anodes were sutured at the paravertebral muscle of T10, whereas the cathodes were sutured beside T8 and T12. The distances between the adjacent electrodes were approximately 1 cm.