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EFFECTS OF TRANSCRANIAL DIRECT CURRENT STIMULATION ON HEMICHANNEL PANNEXIN-1 AND NEURAL PLASTICITY IN RAT MODEL OF CEREBRAL INFARCTION

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Abstract—The aim of this study was to investigate the effects of transcranial direct current stimulation (TDCS) on hemichannel pannexin-1 (PX1) in cortical neurons and neural plasticity, and explore the optimal time window of TDCS therapy after stroke. Adult male Sprague–Dawley rats ($n = 90$) were randomly assigned to sham operation, middle cerebral artery occlusion (MCAO), and TDCS groups, and underwent sham operation, unilateral middle cerebral artery (MCA) electrocoagulation, and unilateral MCA electrocoagulation plus TDCS (daily anodal and cathodal 10 Hz, 0.1 mA TDCS for 30 min beginning day 1 after stroke), respectively. Motor function was assessed using the beam walking test (BWT), and density of dendritic spines (DS) and PX1 mRNA expression were compared among groups on days 3, 7, and 14 after stroke. Effects of PX1 blockage on DS in hippocampal neurons after hypoxia–ischemia were observed. TDCS significantly improved motor function on days 7 and 14 after stroke as indicated by reduced BWT scores compared with the MCAO group. The density of DS was decreased after stroke; the TDCS group had increased DS density compared with the MCAO group on days 3, 7, and 14 (all $P < 0.0001$). Cerebral infarction induced increased PX1 mRNA expression on days 3, 7, and 14 ($P < 0.0001$), and the peak PX1 mRNA expression was observed on day 7. TDCS did not decrease the up-regulated PX1 mRNA expression after stroke on day 3, but did reduce the increased post-stroke PX1 mRNA expression on days 7 and 14 ($P < 0.0001$). TDCS increased the DS density after stroke, indicating that it may promote neural plasticity after stroke. TDCS intervention from day 7 to day 14 after stroke demonstrated motor function improvement and can down-regulate the elevated PX1 mRNA expression after stroke. © **2012 IBRO. Published by Elsevier Ltd. All rights reserved.**

Key words: transcranial direct current stimulation, hemichannel pannexin-1, stroke, density of dendritic spines.

INTRODUCTION

According to the World Health Report 2002, 15 million people suffer stroke worldwide each year. One third of them die and another one third are permanently disabled (WHO, 2002). Over the past four decades, there was more than a 40% decrease in stroke incidence in developed countries and greater than a 100% increase in stroke incidence in developing countries. The overall stroke incidence rates in developing countries exceeded those in developed countries by 20% from 2000 to 2008 (Feigin et al., 2009). Although in developed countries, the incidence of stroke is declining due to better control of blood pressure and a reduced smoking population, the overall rate of stroke remains high due to the aging of the population (WHO, 2002). Stroke is the fourth leading cause of death in America and a leading cause of adult disability (National Center for Health Statistics, 2012).

The basic goal of cerebral infarction treatment is to promote the recovery of neurological function. The recovery of neural function after cerebral infarction relies on neural plasticity and regional neural functional reorganization (i.e., integration of the neurological function of the damaged areas to the surrounding undamaged areas or the contralateral cerebral hemisphere) (Pascual-Leone et al., 2005). From the electrophysiological point of view, enhancement of ipsilateral cortical excitability and reduction of excitability of the contralateral cortex is the basic starting point for the neurological function recovery (Talelli and Rothwell, 2006). Transcranial direct current stimulation (TDCS) is a noninvasive, safe, and inexpensive technique that has been studied as a therapeutic approach for different neurologic disorders (Arul-Anandam et al., 2009; Williams et al., 2009). In stroke patients, the contralesional motor region exerts an undue inhibitory influence on the lesional motor region, which might hinder recovery. Simultaneous anodal TDCS of the affected hemisphere and cathodal TDCS of the unaffected

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Abbreviations: BWT, beam walking test; ET, electroacupuncture therapy; MCAO, middle cerebral artery occlusion; NMDA, *N*-methyl-D-aspartate; PCR, polymerase chain reaction; PX1, hemichannel pannexin-1; SO, sham operation; TDCS, transcranial direct current stimulation.

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hemisphere may increase the cortical excitability of one hemisphere while causing decrease of cortical excitability in the contralateral hemisphere, making TDCS an especially useful tool for the rehabilitation of patients with stroke (Boggio et al., 2007). Transcranial direct current stimulation may be used alone or combined with standard physical therapies to induce changes in cortical excitability and improve motor function in stroke patients (Boggio et al., 2007; Bolognini et al., 2009). These effects may be affected by polarity, duration of therapy and adopted current intensity (Bolognini et al., 2009).

However, the mechanism underlying such neuroplastic changes after TDCS still remains unclear (Venkatakrishnan and Sandrini, 2012). Currently, it is believed that TDCS can introduce enough current to the cerebral cortex without inducing action potentials. It only regulates the membrane resting potential of neurons, which can reduce the spontaneous discharge rate (Liebetanz et al., 2002). Therefore, it only regulates the excitability of neurons in the active state, and will not cause spontaneous discharge of dormant neurons (Wagner et al., 2007). Additionally, TDCS is associated with augmentation or weakening of *N*-methyl-D-aspartate (NMDA) receptor activity (Kim et al., 2010). A clinical study observed that the NMDA receptor antagonist dextromethorphan can block the effects of anodal and cathodal TDCS on nerve cells (Liebetanz et al., 2002) and it was speculated that NMDA receptors are involved in TDCS-induced modulation of neural plasticity. Activation of NMDA receptors results in the opening of nonselective ion channels. Calcium flux through NMDA receptors is thought be critical in synaptic plasticity, a cellular mechanism for learning and memory. These findings suggest a relationship between ion channels and TDCS. Another study observed that carbamazepine selectively eliminated the effects of anodal TDCS without affecting the effects of cathodal TDCS (Nitsche et al., 2004). Carbamazepine stabilizes the inactivated state of sodium channels, suggesting that the effects of anodal TDCS require the participation of ion channels; membrane potential depolarization and cell–cell interactions may be one of its main mechanisms. Previously, many experiments proved that peripheral electroacupuncture therapy (ET) had neural protective function after cerebral ischemia (Huo et al., 2004). Electroacupuncture therapy is a kind of therapy that delivers an electrical current pulse into body through a milli-needle or skin electrode. Our research group carried out a series of studies to explore the mechanisms related to rehabilitation after cerebral ischemia. In a previous study, we found that ET (frequency 10 Hz; intensity 1 mA; 30 min per day) at four acupuncture points 'NEIGUAN' (PC6), 'WAIGUAN' (SJ5), 'SANYINJIAO' (SP6), and 'ZUSANLI' (ST36) significantly suppressed upregulated Na(v)1.1 and Na(v)1.6 expression after cerebral ischemia (Ren et al., 2010).

The role of neuronal gap junction hemichannels has recently attracted increasing attention. The pannexins family is a recently identified protein family that forms large-pore nonselective channels in the plasma membrane of cells. Studies suggest that ischemia may induce opening of the hemichannel pannexin-1, resulting in increased membrane permeability and ionic dysregulation (Bargiotas et al., 2009). Although ASIC1a channels, voltage-dependent $Na+$ channels, NMDA receptors, and transient receptor potential channels participate in this process, the hemichannels are thought to play a key role (Bruzzone et al., 2003). Current oscillation caused by the opening of the hemichannel pannexin-1 keeps the neurons at the membrane resting potential (-60 mV), suggesting that the hemichannel current is the main cause of hypoxic depolarization. At that same time, the opening of hemichannel pannexin-1 leads to exosmosis of glucose and ATP, indicating that the opening of hemichannel pannexin-1 is a key link leading to changes in neuron excitability and intercellular communication after ischemic injury (Thompson et al., 2006). An interesting finding is that pannexin-1 and postsynaptic density protein 95 are present in the postsynaptic membrane, and participate in modulation of synaptic plasticity (Zoidl et al., 2007). In the current study, we aimed to investigate the effects of TDCS on hemichannel pannexin-1 in cortical neurons and neural plasticity in the early stage of cerebral ischemia, and explore the optimal time window of TDCS therapy after cerebral infarction.

EXPERIMENTAL PROCEDURES

Animals and experimental grouping

Ninety adult male Sprague–Dawley rats aged 4–5 months were included in this study. The rats were randomly assigned to the following three groups: sham operation (SO) group, middle cerebral artery occlusion (MCAO) group and TDCS group. In the MCAO and TDCS groups, the cerebral infarction model was constructed with unilateral middle cerebral artery electrocoagulation contralateral to the reaching forelimb (Bederson et al., 1986). In the SO group, the middle cerebral arteries of the rats were not coagulated, but the remaining operations were the same as that in the cerebral infarction model. Postoperative benzylpenicillin (100,000 unit/kg) was used to prevent infection. Bilateral pericranium electrode implantation was performed in each group, but only the TDCS group received TDCS therapy. This study was approved by the Institutional Animal Care and Use Committee of our hospital and was carried out in accordance with the Declaration of Helsinki and with the Institute of Laboratory Animal Resources (1996).

TDCS therapy

To use anodal TDCS to upregulate excitability of the ipsilesional motor cortex and cathodal TDCS to downregulate excitability of the contralesional motor cortex, anodal and cathodal TDCS (Type G6805-2B; Medical Electronic Apparatus Company, Shanghai, China) was given for $\approx$30 min each day starting on day 1 after surgery. Rats received TDCS daily until sacrifice. The TDCS parameters were set as follows: frequency, 10 Hz; intensity, 0.1 mA (Kim et al., 2010). The active electrode was positioned 5 mm to the left and 2 mm in front of the interaural line. Rats were killed on the 3rd, 7th and 14th days after TDCS.