Experimental Neurology 265 (2015) 180–192

Contents lists available at ScienceDirect

Experimental Neurology

journal homepage: www.elsevier.com/locate/yexnr

Regular Article

Treatment with direct-current stimulation against cingulate seizure-like activity induced by 4-aminopyridine and bicuculline in an in vitro mouse model

Wei-Pang Chang [a], Hsiang-Chin Lu [b], Bai-Chuang Shyu [a,b,*]

[a] Graduate Institute of Life Sciences, National Defense Medical Center, Taipei, Taiwan, ROC
[b] Institute of Biomedical Sciences, Academia Sinica, Taipei, Taiwan, ROC

ARTICLE INFO

Article history:
Received 26 February 2014
Revised 13 January 2015
Accepted 5 February 2015
Available online 12 February 2015

Keywords:
Anterior cingulate cortex
Seizure
Synaptic plasticity
Transcranial direct-current stimulation
Thalamocortical system

ABSTRACT

Clinical studies have shown that cathodal transcranial direct-current stimulation (tDCS) application can produce long-term suppressive effects on drug-resistant seizures. Whether this long-term effect produced by cathodal tDCS can counterbalance the enhancement of synaptic transmission during seizures requires further investigation. Our hypothesis was that the long-term effects of DCS on seizure suppression by the application of cathodal DCS occur through a long-term depression (LTD)-like mechanism. We used a thalamocingulate brain slice preparation combined with a multielectrode array and patch recording to investigate the underlying mechanism of the suppressive effect of DCS on anterior cingulate cortex (ACC) seizures. Patch-clamp recordings showed that cathodal DCS significantly decreased spontaneous excitatory postsynaptic currents (EPSCs) and epileptic EPSCs caused by the 4-aminopyridine. Fifteen minutes of DCS application reliably induced LTD, and the synaptic activation frequency was an important factor in LTD formation. The application of DCS alone without continuous synaptic activation did not induce LTD. Direct-current stimulation-induced LTD appeared to be *N*-methyl-D-aspartate (NMDA)-dependent, in which the application of the NMDA receptor antagonist D-1-2-amino-5-phosphonopentanoic acid (APV) abolished DCS-induced LTD, and the immediate effect remained. Direct-current stimulation-induced LTD and the long-term effects of DCS on seizure-like activities were also abolished by okadaic acid, a protein phosphatase 1 inhibitor. The long-term effects of DCS on seizures were not influenced by the depotentiation blocker FK-506. Therefore, we conclude that the long-term effects of DCS on seizure-like activities in brain slice occur through an LTD-like mechanism.

Introduction

Epilepsy is a common neurological disorder. Approximately 1% of the population worldwide suffers from this disease. Thirty percent of patients with epilepsy suffer from drug-resistant seizures (Schiller and Najjar, 2008). Alternative treatment strategies, such as transcranial magnetic stimulation (TMS) and transcranial direct-current stimulation (tDCS), provide non-invasive approaches for controlling pharmacoresistant seizures. Previous studies showed that tDCS is effective for treating Alzheimer's disease related symptoms (Hansen, 2012), for Parkinson disease therapy (Benninger et al., 2010), for increasing motor learning ability (Karok and Witney, 2013), for lessening poststroke motor deficits (Ayache et al., 2012) and for treating intractable seizures (Yook et al., 2011). Clinical studies reveal the complex nature of the effects of tDCS, in which it can produce both short- and long-term suppressive effects on seizures (Ghai et al., 2000; Warren and Durand, 1998; Auvichayapat et al., 2013). However, the underlying mechanism of the long-term effect of tDCS has remained elusive. Additionally, the stimulation parameters, including orientation, field strength, and stimulation duration, must be tested in animal models to achieve its optimal effects.

The immediate effects of DCS on seizure-like activity have mostly been evaluated in the hippocampus and motor cortex (Bikson et al., 2001; Ghai et al., 2000; Gluckman et al., 2001). The immediate effect of DCS involves the passing of currents from the extracellular space (ECS), resulting in the polarization of cell membranes and modulation

Abbreviations: 4-AP, 4-aminopyridine; ACC, anterior cingulate cortex; aCSF, artificial cerebral spinal fluid; ANOVA, analysis of variance; APV, D-1-2-amino-5-phosphonopentanoic acid; ATP, adenosine triphosphate; BDNF, brain-derived neurotrophic factor; CSD, current-source density; DBS, deep brain stimulation; DCS, direct-current stimulation; DMSO, dimethyl sulfoxide; ECS, extracellular space; EEG, electroencephalogram; EGTA, ethylene glycol tetraacetic acid; FLE, frontal lobe epilepsy; fEPSP, field excitatory postsynaptic potential; GTP, guanosine-5′-triphosphate; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; LTD, long-term depression; LTP, long-term potentiation; MEA, multielectrode array; MLA, methyllycaconitine; MT, medial thalamus; NKCC, Na–K–Cl cotransporter; NMDA, *N*-methyl-D-aspartate; PP1, protein phosphatase 1; sEPSC, spontaneous excitatory postsynaptic current; sEPSP, spontaneous excitatory postsynaptic potential; SPSS, Statistical Product and Service Solutions; tDCS, transcranial direct-current stimulation; Th, thalamus; TMS, transcranial magnetic stimulation

* Corresponding author at: Institute of Biomedical Sciences, Academia Sinica, Taipei, Taiwan, ROC. Fax: +886 2 2782 9224.
E-mail address: bmbai@gate.sinica.edu.tw (B.-C. Shyu).

http://dx.doi.org/10.1016/j.expneurol.2015.02.002
0014-4886/

of neuronal activity (Andreasen and Nedergaard, 1996; Bikson et al., 2004; Jefferys, 1981). The orientation of pyramidal neurons is crucial for the effect of the electric field. The direction of the electric field must be parallel to the somatic–dendritic axis of pyramidal neurons to achieve a maximal effect (Gluckman et al., 2001).

Polarizing field can also exert long-lasting effect. It is known to influence long-term synaptic plasticity in the human motor cortex (Grundey et al., 2012; Kuo et al., 2007). These studies indicate that the long-term effect of tDCS likely occurs though a long-term potentiation (LTP)-like mechanism (Cheeran et al., 2008; Nitsche et al., 2003, 2004). A recent brain slice study confirmed that DCS-mediated potentiation is brain-derived neurotrophic factor (BDNF) — and *N*-methyl-D-aspartate (NMDA)-dependent (Fritsch et al., 2010). The LTP/long-term depression (LTD) mechanism is important in memory and learning, and the induction of LTD might be helpful in suppressing seizure. Previous studies used deep brain stimulation (DBS) and found that DBS could alter synaptic plasticity and change seizure thresholds (Gaito, 1980; Weiss et al., 1995). A recent study of DBS showed that an LTD stimulation protocol (0.1 Hz stimulation) could delay basolateral amygdala kindling (Velisek et al., 2002). Low-frequency stimulation-induced LTD also effectively lowers the frequency and amplitude of seizure-like activity in hippocampal slices (Albensi et al., 2004). These results indicate that DBS can induce LTD and might lead to the suppression of seizures. Although DCS-LTP has been characterized (Fritsch et al., 2010), unknown is whether DCS produces similar LTD-like effects. Moreover, whether changes in synaptic transmission mediated by DCS exert long-term effects on seizures needs further investigation.

Frontal lobe epilepsy (FLE) is the second most prevalent focal epilepsy syndrome. Epilepsy in the anterior cingulate cortex (ACC) is included as part of epileptic syndromes of frontal lobe origin, which often manifest with simple partial seizures (Nadkarni and Devinsky, 2009). Focal ACC epilepsies are often non-lesional with the cause unknown. Most focal ACC epilepsies are believed to be idiopathic and cryptogenic. Frontal lobe epilepsy and ACC seizures are often drug-resistant (Biraben et al., 2001; Zaatreh et al., 2002) and the evaluation of alternative treatments, such as tDCS, is needed. Seizures that arise from the ACC are difficult to study because this region lies deep within the brain, and the proximity between the right and left ACC increases the difficulty in identifying where seizures actually start (Geier et al., 1977; Mazars, 1970; Nadkarni and Devinsky, 2009). There are some reports of the application of the tDCS to the cingulate cortex (Nelson et al., 2014; Karim et al., 2010; Keeser et al., 2011). tDCS is shown to affect vigilance, decision making and emotion through alteration of ACC activities. tDCS usually affects large brain regions, and it is difficult to exclude non-specific effects. However, this tDCS feature may provide the potential to control or alter network activity across large brain areas. Whether tDCS modulates neuronal excitability and seizure activity in this brain region has not yet been characterized. Therefore, determining whether tDCS has suppressive effects on FLE or ACC seizures would be helpful for clinical treatment.

Previous studies showed that the orientation of the electric field must be parallel to axodendritic fibers of the cortical column to achieve a maximal effect, but obtaining such an orientation in an in vivo preparation is difficult. Additionally, the pharmacological manipulation is difficult in whole-animal preparations. In in vivo studies, the amounts of current passing through a particular brain region are difficult to measure, and also have some non-specific effects such as alteration on vascular activities. Therefore, in the present study, we used an in vitro brain slice preparation that was developed previously (Lee et al., 2007; Chang et al., 2011, 2013) to uncover the underlying mechanism of the effect of DCS on synaptic plasticity and seizure-like activity threshold. Thus, the present study investigated the long-term effects of polarizing field on ACC seizure-like activities. We tested the hypotheses that applied cathodal DCS is highly effective in controlling and modulating seizure-like activity and the long-term effects of cathodal DCS on the suppression of seizure-like activity occurs through an LTD-like mechanism.

Materials and methods

Slice preparation

Four to 8-week-old male C57BL/6J mice were used. The research protocol conformed to National Institutes of Health guidelines in accordance with the Institutional Animal Care and Utilization Committee, Academia Sinica (Taipei, Taiwan). After decapitation, the brain was quickly transferred to cooled oxygenated artificial cerebral spinal fluid (aCSF; 124 mM NaCl, 4.4 mM KCl, 1 mM NaH_2PO_3, 2 mM $MgSO_4$, 2 mM $CaCl_2$, 25 mM $NaHCO_3$, and 10 mM glucose, bubbled with 95% O_2 and 5% CO_2) for 3 min. Slices that contained the pathway from the medial thalamus (MT) to ACC were prepared according to a previously developed method (Lee et al., 2007). Slices (500 μm thickness) were made and then incubated in oxygenated aCSF at room temperature for 1 h. A single slice was then transferred to the recording chamber and kept at 32 °C under continuous perfusion (12 ml/min) with oxygenated aCSF.

Multielectrode array recording

Two types of multielectrode array (MEA) probes were used: 6 × 10 planar MEA (electrode diameter, 30 μm; electrode spacing, 500 μm; impedance, 50 kΩ at 200 Hz; Multi Channel Systems, Reutlingen, Germany) and 8 × 8 MEA (pyramidal-shaped electrode; diameter, 40 μm; tip height, 50 μm; electrode spacing, 200 μm; impedance, 1000 kΩ at 200 Hz; Ayanda Biosystems, Lausanne, Switzerland). A 60-channel amplifier was used with a band-pass filter set between 0.1 Hz and 3 kHz at 1200× amplification (MEA-1060-BC, Multi Channel Systems, Reutlingen, Germany). Data were acquired using MC Rack software at a 10 kHz sampling rate (Multi Channel Systems, Reutlingen, Germany).

Patch-clamp recording

Borosilicate glass pipettes (PC-10, 3–7 MΩ; Narishige, Japan) were used in whole-cell patch-clamp recordings. The pipette solution contained the following: 131 mM K-gluconate, 10 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES), 2 mM ethylene glycol tetraacetic acid (EGTA), 20 mM KCl, 8 mM NaCl, and 2 mM Mg-adenosine triphosphate (ATP) and Na_3-guanosine-5′-triphosphate (GTP; Tocris Bioscience, Ellisville, MO, USA). The pipette solution also contained 6.7 mM biocytin. Recordings were made using an Axon Multiclamp 700B microelectrode amplifier (Molecular Devices, Sunnyvale, CA, USA) with 2× amplification. The amplified signals were digitized by a Power 1401 converter (CED, Cambridge, UK) using spike2 and Signal software.

Seizure-like activity induction and generation of electric fields

Seizure-like activity was induced by the application of 4-aminopyridine (4-AP; 250 μM) and bicuculline (5 μM). Our previous time-control studies showed that maximal and stable responses appeared 2–3 h after drug application (Chang and Shyu, 2013). All of the comparisons were made 2–3 h after 4-AP and bicuculline application. Uniform electric fields were generated by passing currents between two parallel Ag–Cl-coated silver wires placed inside the MEA chamber. Currents were generated by an isolated stimulator (A-M Systems, Carlsborg, WA, USA) under the control of a pulse generator (STG 1002, Multi Channel Systems). The definition of the electric field orientation was based on the direction of the axodendritic axis in the ACC. The orientations of dendrite and soma compartments were demonstrated using Golgi staining (Fig. 1A). The Ag–Cl electrode placed proximal to the ACC was defined as the anode, and the other electrode placed distal to the ACC was defined as the cathode. The field strength generated by the two field orientations (parallel and perpendicular to