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Research report

Influence of the epileptiform discharge microenvironment on the differentiation of oligodendrocyte precursor cells

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ABSTRACT

The use of related cell cultured models to investigate the genesis and development of epilepsy is beneficial for exploring the mechanism of epileptogenesis. Recent reports have described the myelination damage caused by epilepsy in animal models; however, limited reports have focused on the influence of epilepsy on remyelination in vitro. The current study was designed to investigate the effect of the epileptiform discharge microenvironment on the differentiation of oligodendrocyte precursor cells (OPCs). Neurons with epileptiform discharge released more glutamic acid than normal neurons, which was detected by HPLC. The RT-qPCR, immunofluorescence and Western Blot results showed myelin basic protein (MBP) loss in the epileptiform discharge neuron microenvironment, with increased GluR2 subunit expression. In addition, an α-amino-3-hydroxy-5-methyl-4-isoazolepropionic acid (AMPA) receptor antagonist altered the influence of epilepsy on OPC differentiation. This study confirmed the positive effect of epilepsy on the differentiation of OPCs and verified the critical role of glutamic acid and the AMPA receptor in this process, which provides a potential treatment strategy for epilepsy.

1. Introduction

Epilepsy is a type of chronic nervous disease, and the pathogenesis is complex and unclear. Stereotype, paroxysm, and chronic are the main clinical symptoms. Repeat seizures would destroy the normal neural circuit of the brain, which results in brain injury and serious sequelae for patients (Holopainen, 2008). To date, most research has focused on the structure and function of neurons to investigate the pathogenesis and drug targets of epilepsy; however, refractory epilepsy continues to occur (Kwan and Brodie, 2000). Consequently, it is necessary to survey the relationship between other neural cells and epilepsy.

OPC, a type of glia cell in the CNS, can differentiate into mature oligodendrocyte cells (OLs) to form a myelin sheath (Nishiyama et al., 2009). During the development of OPCs, they differentiate into late OPCs, immature oligodendrocyte cells, and final mature oligodendrocyte cells. Oligodendrocyte lineage cells exhibit different markers during the developmental period. In brief, platelet-derived growth factor receptor alpha (PDGFRα) is a special marker of OPCs; moreover, cyclic nucleotide phosphodiesterase (CNPase) appears and advances during differentiation, and MBP positive cells are considered to be mature oligodendrocyte cells (Guardiola-Diaz et al., 2012).

To the best of our knowledge, OPCs in the subventricular zone (SVZ) would proliferate, migrate to lesion, and differentiate into OLs when demyelination occurs in the brain (Kaneko et al., 2013). Because of the synaptic and non-synaptic connections between OPCs and neurons, neurons can regulate the development of OPCs via neurotransmitter secretion. In addition, the α-amino-3-hydroxy-5-methyl-4-isoazolepropionic acid (AMPA) receptor, one type of ionic glutamic acid receptor, can mediate the influence of glutamic acid on OPC differentiation (Fannon et al., 2015; Gudz et al., 2006). The myelin sheath has the ability to accelerate neural

Abbreviations: AMPA, α-amino-3-hydroxy-5-methyl-4-isoazolepropionic acid; OPC, oligodendrocyte precursor cell; MBP, myelin basic protein; PDGFRα, platelet-derived growth factor receptor alpha; CNPase, cyclic nucleotide phosphodiesterase; olig2, oligodendrocyte transcription factor-2; ODM, OPC differentiation medium; NNM, medium of normal neurons; INM, medium of epileptiform discharge neurons induced by magnesium-free extracellular fluid; PDSs, paroxysmal depolarizing shifts; GluCEST, glutamate chemical exchange saturation transfer; NMDA, N-methyl-D-aspartate; PLP, protein lipid protein.

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signal transduction, protect neurons and provide them with nutrition (White and Kramer Albers, 2014; Yang et al., 2013). As a result, neurons and oligodendrocyte lineage cells may influence each other. Seizures may occur in demyelinating diseases (such as multiple sclerosis). Our research team has previously shown that the susceptibility to seizures has a positive association with the degree of demyelination (Ye et al., 2013), which indicated that demyelination participates in epileptogenesis and epilepsy progression. Moreover, white matter abnormalities in the brains of epilepsy patients were detected by diffusion tensor imaging (Anderson and Rodriguez, 2011). Myelin basic protein (MBP) expression declined in lithium-pilocarpine-induced epileptogenesis animals, the myelin size and thickness decreased, and the myelinated nerve fibre length shortened (Ye et al., 2013). MBP expression in the hippocampus and corpus callosum decreased after a status epilepticus acute period and sustained impairments until a later stage (Li et al., 2013). A recent report by our group showed that the MBP expression and the number of oligodendrocyte cells decreased in the acute phase of seizures, and the loss aggravated gradually during the process of epilepsy (Luo et al., 2015). These findings signified that seizures affected remyelination.

Therefore, epilepsy has a clear connection with remyelination obstacles. To test the hypothesis, we adopted an epileptiform discharge cell model that reproduced the process of epilepsy development. The effects of epilepsy, glutamic acid and AMPA receptor on myelination, as assessed *via* HPLP, RT-qPCR, Western-blot and immunofluorescence assays, would be helpful to determine the drug target of epilepsy treatment.

2. Results

2.1. The establishment of epilepsy cell culture model

To establish the epilepsy cell culture model, the neuron medium was changed to a magnesium-free extracellular fluid on the 11th culture day, and neurons with magnesium-free liquid were cultured in an incubator at 37 °C and 5% CO_2 for 3 h. After 3 h, the magnesium-free liquid was changed to neuron medium. Before and after the neurons were incubated in magnesium-free extracellular fluid, the neuronal action potentials were detected using patch-clamp technology. As shown in Fig. 1A, the discharge frequency of normal neurons that had not been incubated in magnesium-free extracellular fluid was less than 3 Hz (1.32 ± 0.8 8 Hz). However, after treatment with magnesium-free extracellular fluid, more than 90% of the neurons exhibited a paroxysmal sustained spike discharge within 3–10 Hz discharge frequency (5.29 ± 1.85 Hz) (Fig. 1B). This result proved that the cell culture model of epilepsy activity was successfully established.

2.2. Primary cultured OPCs and oligodendrocyte cells identified by immunofluorescence

Oligodendrocyte lineage cells have different types of shapes and markers during their differentiation phase. Primary OPCs expressed two or three neurites in the proliferation phase (Fig. 2A), and during differentiation, gradually more neurites appeared in oligodendrocyte cells (Fig. 2C). PDGFRα (green) was expressed on OPCs (Fig. 2B), but not on mature oligodendrocyte cells. Oligodendrocyte transcription factor-2 (olig2) was expressed throughout development and manifested as green fluorescence (Fig. 2D). On the 3rd day of OPC differentiation, a single small part of cells, neurites of them similar to spider mesh, expressed MBP (red) detected by immunofluorescence (Fig. 2D).

2.3. Difference in glutamic acid concentration between normal and epileptiform discharge neuron microenvironments

2.3.1. Chromatographic separation of glutamic acid

To confirm the resolution of the mixture by HPLC, three groups were assessed: the derivating agent group, the derivating agent + Glu group and the derivating agent + neuron medium group. By comparing the data shown in Fig. 3B, the retention time of glutamic acid was 8 min, and the derivating agent did not interfere in the measurement of glutamic acid.

2.3.2. Difference in glutamic acid concentration between normal and epileptiform discharge groups

Fig. 3A summarizes the general schemes employed to subdivide objects into two groups: the normal group and the epileptiform discharge group. On the 11th day of cultivation, neurons of the epileptiform discharge group were induced by magnesium-free extracellular fluid for 3 h, and the liquid was subsequently changed to neuron medium. The normal group neurons were cultured in normal extracellular fluid for 3 h. The media of normal neurons (NNM) and epileptiform discharge neurons induced by magnesium-free extracellular fluid (INM) 1 h, 2 h, 4 h, 6 h, 12 h, 24 h, 48 h, and 72 h after having been changed into normal medium were collected, and their glutamic acid concentration was

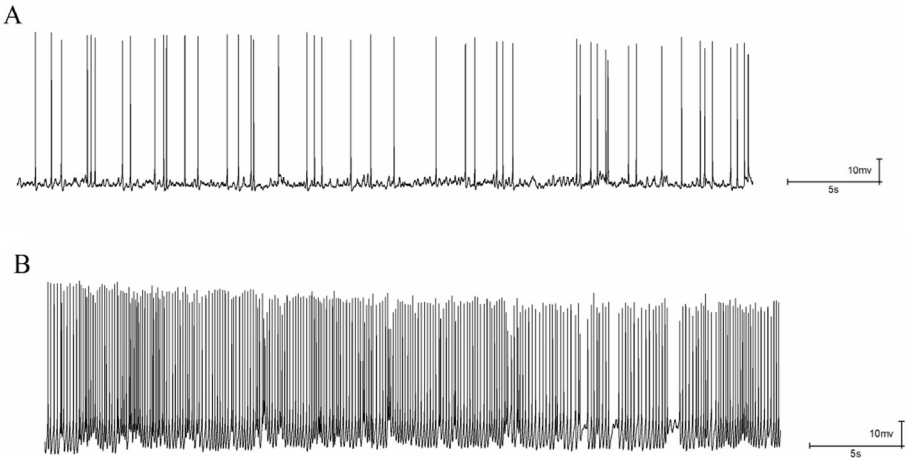

Fig. 1. Patch-clamp whole-cell recording of neurons. (A) Action potentials of normal neurons detected by patch-clamp; the discharge frequency was less than 3 Hz. (B) Spontaneous epileptiform discharge neurons induced by magnesium-free extracellular fluid detected by patch-clamp; the discharge frequency was greater than 3 Hz. n = 10 per group.