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Review

Optogenetic control of astrocytes: Is it possible to treat astrocyte-related epilepsy?

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ABSTRACT

Epilepsy is a neurological disorder that affects around 1% of the population worldwide. The two main therapies, pharmacology and the electrical stimulation, both have some shortcomings. For instance, pharmacological therapy is frequently accompanied by side effects, and current anticonvulsive drugs fail to be effective to around a third of patients. These patients could suffer astrocyte-related epilepsy, as increasing evidence indicates that dysfunctions of astrocytes can result in epilepsy. However, epilepsy drugs that affect astrocytes are not available currently. Although electrical stimulation has benefited many patients, the electrode stimulates unselective neurons or circuits. All these need to develop new strategies for improving the life of the patients. As channelrhodopsins (ChRs) were discovered, a novel method referred to as "optogenetics" was developed. It has advantages over electrical stimulation of being less-invasiveness and allowing spatiotemporally stimulation. Recently, a number of experiments have explored the treatments for epilepsy with optogenetic control of neurons. Here, we discuss the possibility that an optogenetic approach could be used to control the release of gliotransmitters and improve astrocyte function such as glutamate and K^+ uptake, and thereby offer a potential strategy to investigate and treat astrocyte-related epilepsy.

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1. Introduction

Epilepsy is a neurological disorder that affects around 1% of the population worldwide. Although the cause of epilepsy is not yet well known, the balance between excitatory and inhibitory neurons is lost in patients with epilepsy. This imbalance could be caused by dysfunction of excitatory neurons or/and inhibitory neurons. To date, pharmacological therapy and electrical stimulation are the main approaches to treat patients. However, pharmacological therapy is frequently accompanied by side effects (Eadie, 2012), and current anticonvulsive drugs fail to be effective to around a third of patients. Recent studies have indicated that astrocytes could play important roles in epilepsy since astrocytes regulate

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the concentrations of substances such as potassium and glutamate in extracellular space. In the brain, neural activity increases extracellular K^+ concentration, which is balanced by several mechanisms such as K^+ spatial buffering mainly mediated by Kir channels in astrocytes (Kofuji and Newman, 2009). It has been reported that impairing astrocytes' potassium buffering results in seizures (Haj-Yasein et al., 2011; Rangroo et al., 2013). Higher extracellular glutamate concentration resulting from dysfunction of astrocytes also contributes to epilepsy. For example, Tanaka et al. reported that mice lacking GLT1 (one of glutamate transporters in astrocytes' membranes) suffer epileptic seizure (Tanaka et al., 1997). This might suggest that patients resistant to current anticonvulsive drugs have astrocyte-related epilepsy. However, epilepsy drugs that affect astrocytes are not available currently. Although electrical stimulation has benefited many patients, the electrode stimulates unselective neurons or circuits. All these call for developing new strategies to overcome these shortcomings for improving the life of the patients. As channelrhodopsins (ChRs) were discovered, a novel method referred to as "optogenetics" was developed (Deisseroth et al., 2006). Optogenetics has been widely used to study neural and non-neural functions (Miesenböck, 2011; Yawo et al., 2013). Recently, it was also used to investigate and treat neurological diseases such as blindness and spinal cord injury (Ji et al., 2013). Numerous experiments have indicated that an optogenetic approach could overcome the above shortcomings and provide a promising strategy to treat epilepsy. In this review, we first describe the working principle of optogenetic actuators. We then present the growing data on optogenetic control of specific neurons, such as principal neurons and interneurons, involved in epilepsy and highlight the potential of treating astrocyte-related epilepsy with an optogenetic approach. Finally, we discuss the challenges and future perspectives of optogenetic treatment of epilepsy.

2. Overview of optogenetics

In 2003, Hegemann's group cloned channelrodopsin-2 (ChR2) and demonstrated that it opens in response to blue light (Nagel et al., 2003). Soon the possibility that neural activity can be manipulated by introducing the ChR2 gene was demonstrated independently by three groups (Boyden et al., 2005; Li et al., 2005; Ishizuka et al., 2006). In 2006, the term, optogenetics was introduced by Karl Deisseroth (Deisseroth et al., 2006). As the term implies, optogenetics is an approach that integrates optical (opto-) and genetic (genetics) methods to control the activities of specific excitable cells or neuronal circuits. After the emergence of ChR2, the first optogenetic actuator, a growing number of new rhodopsins were introduced into optogenetics. In general, optogenetic actuators can be divided into two types: one depolarizes cell membrane, the other hyperpolarizes. Each is referred to as the depolarizing and hyperpolarizing actuator or rhodopsin, respectively.

Channelrhodopsin-2 ChR2 is a typical depolarizing optogenetic actuator. It is a light-sensitive ion channel from the eyespot of *Chlamydomonas reinhardtii* and is involved in the phototaxis of the microorganism. ChR2 consists of a seven-pass transmembrane apoprotein which covalently binds to a retinal molecule. When illuminated by blue light, the photoisomerization of all-*trans*-retinal to 13-*cis* configuration is coupled to conformational changes in the protein and causes the permeation of ions such as H^+, Na^+ and Ca^{2+}. And the light energy is thus converted into an electrical signal by the single ChR2 molecule (Hegemann, 2008). When ChR2 is expressed in neurons, blue light evokes inward currents, which depolarize the membrane, leading to the opening of voltage-gated sodium and calcium channels, and finally to the generation of an action potential (Boyden et al., 2005; Ishizuka et al., 2006). Therefore, one can activate the neurons by light. Recently, researchers have created various ChR variants such as ChRWR, ChEF/ChIEF, ChRGR, and ChR2-T159C that are optimized to specific aims (Wang et al., 2009; Lin et al., 2009; Wen et al., 2010; Mattis et al., 2012). In addition, the other depolarizing optogenetic actuators, VChR1 and MChR1, were derived from *Volvox carteri* and *Mesostigma viride*, respectively (Zhang et al., 2008; Govorunova et al., 2011). Both VChR1 and MChR1 are red-shifted, that is, they absorb longer wavelength light with peaks at 520 and 528 nm, respectively. One advantage of longer wavelengths is that they are less hazardous to cells or tissues.

Halorhodopsin Halorhodopsin (NpHR) is a representative of hyperpolarizing optogenetic rhodopsins isolated from *Natronomonas pharaonis*. It is a light-activated Cl^- pump that has peak absorption at 570 nm (Schobert and Lanyi, 1982). When illuminated by yellow light, NpHR allows chloride ions to be transported into the cell so as to hyperpolarize the membrane. Therefore one can use NpHR to inhibit the activities of neurons. New hyperpolarizing actuators, such as archaerhodopsin-3 (Arch/aR-3) from *Halorubrum sodomense* and archaerhodopsin-T (ArchT) from *Halorubrum* strain TP009, were reported (Chow et al., 2010; Han et al., 2011). More recently, Jaws derived from *Haloarcula salinarum* strain Shark, was demonstrated to be activated by the most red-shifted spectrum of any hyperpolarizing rhodopsin so far (Chuong et al., 2014). Many functions of neurons or neural circuits have been revealed by hyperpolarizing optogenetic rhodopsins (Tønnesen et al., 2009; Arrenberg et al., 2009; Leifer et al., 2011; Tye et al., 2011; Gentet et al., 2012).

To apply optogenetics, three requirements are necessary to be fulfilled; the selection of optogenetic actuators, the targeted expression of optogenetic actuators in the neurons or regions of interest and the light delivery system (Yawo et al., 2013). The latter two issues have been extensively reviewed previously (Kokaia et al., 2012; Yawo et al., 2013). Here, we focus on the selection of optogenetic actuators and the potential of treating epilepsy, especially astrocyte-evoked epilepsy, with optogenetics.

3. Optogenetic control of neurons for the treatment of epilepsy

In epilepsy, the balance between excitatory and inhibitory neurons is lost due to dysfunction of principle or interneurons. Based on the working principle of optogenetics mentioned above, it is possible to control individual neurons to stop epilepsy. In 2009, employing lentiviral (LV) vector Tønnesen et al. targeted NpHR, specifically to the principal neurons of hippocampus under the control of a CaMKIIα promoter and they showed that light-evoked NpHR activity hyperpolarized the targeted neurons and suppressed epileptiform activity in vitro (Tønnesen et al., 2009). This pioneering study paved the way for treating epilepsy with optogenetics. There could be two ways to stop hyperexcitation in epilepsy optogeneticly.

First, as Tønnesen and colleagues did, one can directly inhibit the hyperactivation of targeted neurons with hyperpolarizing actuators (Fig. 1a). For example, Paz and colleagues expressed enhanced halorhodopsin (eNpHR3.0) in the neurons in the ventrobasal thalamus under the regulation of a CaMKIIα promoter (Paz et al., 2013). Meanwhile, Krook-Magnuson et al. employed Cre-loxp system to express eNpHR3.0 specifically in principal cells (Krook-Magnuson et al., 2013). Both of these two groups designed closed-loop devices and could stop the seizures in real time with light lumination. Thereafter, with adeno-associated virus (AAV) vector Sukhotinsky et al. targeted eNpHR3.0 to hippocampal pyramidal cells under control of a CaMKIIα promoter. In their experiments, both the continuous and intermittent illumination delayed the electrographic