EPILEPSY CURRENTS

Current Literature

In Basic Science

Finding Your Inner Light: Using Bioluminescence to Control Seizures

Chemically Activated Luminopsins Allow Optogenetic Inhibition of Distributed Nodes in an Epileptic Network for Non-invasive and Multi-site Suppression of Seizure Activity.

Tung JK, Shiu FH, Ding K, Gross RE. *Neurobiol Dis* 2018;109(Pt A):1–10.

Although optogenetic techniques have proven to be invaluable for manipulating and understanding complex neural dynamics over the past decade, they still face practical and translational challenges in targeting networks involving multiple, large, or difficult-to-illuminate areas of the brain. We utilized inhibitory luminopsins to simultaneously inhibit the dentate gyrus and anterior nucleus of the thalamus of the rat brain in a hardware-independent and cell-type specific manner. This approach was more effective at suppressing behavioral seizures than inhibition of the individual structures in a rat model of epilepsy. In addition to elucidating mechanisms of seizure suppression never directly demonstrated before, this work also illustrates how precise multi-focal control of pathological circuits can be advantageous for the treatment and understanding of disorders involving broad neural circuits such as epilepsy.

Commentary

Over the last 100 years, scientists have developed dozens of drugs to control seizures in patients with epilepsy, and these drugs serve as the mainstay for the clinical treatment of the disease (1). Somewhat depressingly, however, the most recently developed drugs perform little better— failing in about 1/3 of patients— than phenobarbital, which was put into clinical practice before World War I (2). Although new drugs have improved safety and tolerability, the problem of intractability remains. The inability of traditional pharmacologic approaches to make significant inroads in treating intractable epilepsy suggests that researchers may have reached the limit of what these agents can do. One natural limitation is off-target effects of pharmacologic agents, in which the drug interacts with molecules other than the intended target, producing unwanted side effects. However, even a perfect drug—meaning one that interacts specifically and only with the intended binding site—is likely to produce unwanted effects. This is a natural consequence of evolutionary mechanisms that have led to the expression of target molecules, such as ion channels, in many distinct cell and tissue types. Drugs given systemically, therefore, will interact with targets in brain regions that influence seizures, with targets outside these brain regions not directly involved in seizures, and even with targets outside the CNS that express evolutionarily related molecules that may contain identical binding sites. While it is likely that a dose can be found to control seizures in most patients with epilepsy, all too often intolerable side-effects develop before effective seizure control can be achieved. Biology, therefore, may exert a fundamental limit on the specificity that can be achieved with traditional pharmacology.

Promisingly, numerous approaches are being developed in animal models that have the potential to overcome this specificity problem. One such approach is optogenetics (3). Optogenetic approaches use light-activated ion channels and pumps to either inhibit or excite target neurons. Because the proteins are exogenous and can be targeted to distinct neuronal populations, a level of spatial specificity can be achieved that far exceeds that of traditional pharmacology. In addition, because the proteins are light activated, an unprecedented level of temporal control can be achieved, allowing neurons to be switched on or off with millisecond-level resolution. Indeed, these strengths have been used to develop "closed loop" systems in rodents, in which EEG acquisition and seizure detection software are combined with optogenetic cell inhibition to block seizures seconds after onset (4).

A key limitation of optogenetic approaches, however, is the need to implant fiber optic light guides directly into the brain. Moreover, emitted light is quickly scattered and absorbed by brain tissue, limiting the volume and depth of tissue that can be manipulated. Finally, preclinical studies have focused on rodents, whose brains are a fraction of the size of human adults. If one assumes that the volume of tissue that will need to be inhibited in a patient with epilepsy is similar to the volume typically removed to control seizures during epilepsy surgery, then it is clear that providing sufficient light to an epileptic focus in humans will be a challenge with fiber optics.

Epilepsy Currents, Vol. 18, No. 3 (May/June) 2018 pp. 182–183

Earlier work by Tung and colleagues provides a potential solution to this problem. Rather than relying on an exogenous light source to activate an inhibitory opsin, they developed bioluminescent inhibitory opsins that can self-activate after treatment with coelenterazine (5). Coelenterazine is a luciferin expressed by a number of bioluminescent jellyfish. The molecule emits light after oxidation by a luciferase encoded into the luminopsin. By generating their own light, these inhibitory luminopsins can be activated in large targets deep in the brain without the need for invasive fiber optic implants.

In their most recent publication, discussed here, Tung and colleagues used an adeno-associated viral vector to express an inhibitory luminopsin in target neuronal populations and then demonstrated that it could be used to suppress acute, focal seizures induced by direct infusion of the GABA antagonist bicuculline into rat brain. They also found that inhibiting hippocampal granule cells reduced the duration of seizures evoked by systemic injection of pentylenetetrazole. Inhibiting the anterior nucleus of the thalamus decreased seizure duration and increased the latency to seizure onset. The investigators then took full advantage of their approach to simultaneously inhibit dentate gyrus and thalamus, leading to reductions in seizure duration and seizure severity—a greater effect than targeting either region alone. The ability to selectively target multiple brain regions is a key advantage of their approach and has both clinical and research implications.

Understanding how different brain regions act together to produce and regulate seizures remains a key unresolved question in the epilepsy field. While seizures may begin focally, they spread through the brain along anatomical and functional routes, and these interactions likely regulate key components of the seizure, such as threshold, severity. and duration. Indeed, Tung and colleagues' finding that dentate versus thalamic inhibition regulated different features of the seizure implies that these brain regions make distinct contributions to seizure phenomics.

Luminopsins add to a growing toolkit for increasingly precise in vivo manipulation of neuronal activity. They possess some of the same advantages of DREADDs (designer receptors exclusively activated by designer drugs) (6), but with greater potential versatility, as many different luciferase variants exist (7). Luminopsins are also directly coupled to ion channels, while DREADDs alter activity via G proteins, giving the former faster kinetics and reduced chance for off-target effects. Variants on the approach are also being developed. Chen and colleagues (8), for example, published a conceptually similar approach, in which they developed nanoparticles that convert near-infrared light—which can penetrate deep into brain—into visible light of sufficient intensity to activate or inhibit nearby neurons expressing channelrhodopsins. This range of available techniques creates even greater opportunity for combinatorial approaches to simultaneously target multiple brain regions.

Whether or not luminopsins can be translated to the clinic to control seizures remains an open question. Adeno-associated viral vectors are in clinical trials for a wide range of disorders, the first of which recently received FDA approval (9). The activating agent, coelenterazine, appears to be well tolerated in rodents, although toxicology data are limited and the effects of chronic exposure are unknown. Nonetheless, these increasingly powerful approaches are likely to yield fundamental new insights into the mechanisms regulating epileptogenesis and seizure spread in the brain, making it an exciting time to be in the epilepsy field. As these techniques evolve, they also hold promise for transforming the treatment of epilepsy.

by Steve C. Danzer, PhD

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