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Chemogenetic Seizure Control: Keeping the Horses in the BARN(I)

Acetylcholine Receptor Based Chemogenetics Engineered for Neuronal Inhibition and Seizure Control Assessed in Mice

Nguyen QA, Klein PM, Xie C, Benthall KN, Iafrati J, Homidan J, Bendor JT, Dudok B, Farrell JS, Gschwind T, Porter CL, Keravala A, Dodson GS, Soltesz I. *Nat Commun*. 2024;15(1):601. doi:10.1038/s41467-024-44853-8

Epilepsy is a prevalent disorder involving neuronal network hyperexcitability, yet existing therapeutic strategies often fail to provide optimal patient outcomes. Chemogenetic approaches, where exogenous receptors are expressed in defined brain areas and specifically activated by selective agonists, are appealing methods to constrain overactive neuronal activity. We developed BARNI (Bradanicline- and Acetylcholine-activated Receptor for Neuronal Inhibition), an engineered channel comprised of the α7 nicotinic acetylcholine receptor ligand-binding domain coupled to an α1 glycine receptor anion pore domain. Here we demonstrate that BARNI activation by the clinical stage α7 nicotinic acetylcholine receptor-selective agonist bradanicline effectively suppressed targeted neuronal activity, and controlled both acute and chronic seizures in male mice. Our results provide evidence for the use of an inhibitory acetylcholine-based engineered channel activatable by both exogenous and endogenous agonists as a potential therapeutic approach to treating epilepsy.

Commentary

Despite the development of dozens of anti-seizure medications over the last 100+ years, epilepsy remains intractable for roughly a third of people suffering from the disease.[1] The failure of new drugs to move the needle on intractable epilepsy suggests there may be an inherent limit to traditional pharmacological approaches, in which success or failure may simply reflect whether an effective dose can be achieved before intolerable side effects occur. In this light, preclinical strategies to directly target the seizure focus are being vigorously pursued. Such approaches could dramatically expand the potential range of neuronal control—up to and including complete neuronal silencing—to a degree that is simply not possible with systemically administered drugs. While surgical and neurostimulation techniques are being continually refined, approaches to express newly developed genetically encoded tools to control neuronal activity hold tremendous promise. The ability to target these tools toward specific neuronal classes could enable greater efficacy while mitigating off-target effects.

The most well-developed of these tools are optogenetic molecules, which use modified light-activated receptors to control activity, and chemogenetic molecules, which control activity following treatment with an exogenous ligand. The former option holds the potential advantage of having excellent temporal control,[2] although the need to place fiber optic light guides into the brain to activate the molecules is considered a significant limitation for translation. Chemogenetic approaches, on the other hand, avoid the need for fiber optics while still maintaining selectivity by utilizing ligands designed to be otherwise biologically inert. Chemogenetic approaches are also better suited for long-term treatment, although this comes at the expense of temporal precision.[3]

Perhaps the most widely utilized forms of chemogenetic manipulation are designer receptors exclusively activated by designer drugs (DREADDs). In brief, DREADDs are G protein-coupled receptors that bind a specific ligand to facilitate either activation or silencing of target cells. The primary ligand, clozapine-N-oxide (CNO), was originally considered to have no off-target effects, although there is now mounting evidence that CNO induces subtle changes.[4] Notably, CNO can be metabolized to clozapine, which is used as an atypical antipsychotic and has a host of adverse effects. An additional challenge for chemogenetic therapies—particularly given their slower temporal dynamics—is their sole reliance upon exogenous activation. Taking a drug to block an impending seizure may not be feasible for patients with particularly rapid and unpredictable seizure onset.

Nguyen and colleagues[5] have taken full advantage of the ability to design optimized chemogenetic molecules, reporting on an approach to improve therapeutic efficacy by utilizing

a molecule that can be activated by an exogenous ligand *and* endogenous changes in neurotransmitter levels associated with seizures. The approach utilizes a chimeric engineered ligand-gated ion channel that incorporates an altered α7 nicotinic acetylcholine receptor joined to an α1 glycine pore domain. This novel receptor has affinity for both bradanicline and acetylcholine and is called Bradanicline- and Acetylcholine-activated Receptor for Neuronal Inhibition (BARNI). Bradanicline has a safer pharmacological profile compared to other chemogenetic ligands, such as CNO, while acetylcholine sensitivity provides a mechanism for "closed-loop" seizure control. The closed-loop component holds considerable promise and is an active area of research.[6,7]

Through both *in vitro* and *ex vivo* experiments, they demonstrated that BARNI activation reduces excitability in a dose-dependent manner in mouse hippocampal neurons. *In vivo* experiments confirmed that BARNI activation increased thresholds for evoked seizures. To extend these results to epileptic seizures, they utilized the mouse intrahippocampal kainic acid (IHKA) model of temporal lobe epilepsy. Unilateral kainic acid injection was combined with bilateral injection of an adeno-associated viral vector to induce BARNI expression in hippocampal neurons. Bradanicline treatment given 6 weeks after IHKA reduced spontaneous seizures for several hours, an effect which could then be extended to over 10 hours with a second dose.

The authors demonstrated that BARNI is an effective tool to modulate hippocampal excitability and, in so doing, reduce seizure frequency. Perhaps most striking, they noticed that mice expressing BARNI had a lower seizure burden regardless of whether bradanicline was administered. They postulated that this effect was due to the binding of endogenous acetylcholine to BARNI following neurotransmitter release during protracted seizures. Through dual calcium and acetylcholine imaging, they were able to demonstrate that acetylcholine levels increase significantly during both evoked and spontaneous seizures. This raises the exciting prospect that new approaches, such as BARNI, may be able to provide both on-demand and closed-loop seizure control.

While the findings are an exciting advance in the development of chemogenetic therapies for epilepsy, additional work is needed. Elucidating the molecular and circuit alterations mediating seizure reduction with BARNI (in the absence bradanicline) will be important. Identifying these changes will instruct the development of the next generation of chemogenetic tools. Additionally, BARNI-expression in this study included both excitatory and inhibitory neurons and, as acknowledged by the authors, future studies selectively targeting just excitatory neurons are needed to determine how best to utilize these tools. While no overt behavioral changes were observed with this study, continued monitoring of potential impacts on cognition will help to optimize genetic tools and ensure their safety. Finally, although the present study centered on temporal lobe epilepsy and hippocampal expression, similar approaches could be evaluated for extra-temporal lobe epilepsies. Given the diversity of epilepsy syndromes, demonstrating efficacy in a range of animal models is critical to facilitate successful clinical translation.

BARNI is an exciting addition to the growing list of chemogenetic compounds that have been tested for seizure control in animal models over the last decade.[8] Such approaches are appealing for their potential translatability, and initial clinical trials could focus on patients eligible for surgical resection to treat their seizures. If chemogenetic therapy proves effective, then further invasive procedures would be unnecessary. However, the option to resect both the seizure focus and the genetically altered cells would still be available should chemogenetics prove ineffective or lead to intolerable side effects. While the translational potential is compelling, several challenges remain that need to be overcome before these therapeutics can be brought to the clinic. First, there is a scale problem when considering moving from rodent models to humans. Epileptic foci in humans greatly exceed the size of an entire rodent brain, so titrating this therapy to transfect a sufficient number of cells may prove challenging, though recent work in primates is promising.[9] Secondly, there is still a need to address the long-term efficacy of these approaches through longitudinal monitoring studies. Acute seizure control in rodents does not necessarily imply that the treatment will remain effective over decades. A third consideration is that these treatments will need to be optimized for patients with chronic and intractable epilepsy, since initial trials will likely focus on these populations. Given that the pathology of epilepsy evolves considerably over years, there can be significant differences between the pathology found in humans with long-standing intractable epilepsy and in animal models following initial seizures. Cell loss can be substantial in chronic epilepsy (i.e., hippocampal sclerosis), and careful consideration of which surviving cells can, and should, be targeted for chemogenetics will be important. Nonetheless, one hundred years on from the first true epilepsy drug, chemogenetic seizure control may offer the best hope for patients with intractable epilepsy.

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Declaration of Conflicting Interests

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