Seizing Control of Neuronal Activity: Chemogenetic Applications in Epilepsy

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Abstract
The fundamental commonality across pharmacotherapies for the epilepsies is the modulation of neuronal excitability. This poses a clear challenge—patterned neuronal excitation is essential to normal function, thus disrupting this activity leads to side effects. Moreover, the efficacy of current pharmacotherapy remains incomplete despite decades of drug development. Approaches that allow for the selective targeting of critical populations of cells and particular pathways in the brain have the potential to both avoid side effects and improve efficacy. Chemogenetic methods, which combine the selective expression of designer receptors with designer drugs, have rapidly grown in use in the neurosciences, including in epilepsy. This review will briefly highlight the history of chemogenetics, their applications to date in epilepsy, and the potential (and potential hurdles to overcome) for future translation.

Keywords
DREADD, RASSL, PSAM, chemogenetics, hM4D

Much of pharmacology for the last three-quarters of a century has focused on finding, designing, and optimizing increasingly selective ligands for drug targets. Chemogenetic approaches have turned this on its head. Chemogenetic strategies focus on engineering neurotransmitter receptors to decrease or eliminate affinity for endogenous ligands and to produce high-affinity interactions with synthetic ligands or designer drugs. Ideally, these designer drugs avoid interactions with native receptors, providing a means of selectively activating receptor signaling in target cells while avoiding impacts on other physiological processes. As Armbruster and colleagues described in their first paper on Designer Receptors Exclusively Activated by Designer Drugs (DREADDs), it has been a process of “evolving the lock to fit the key”—although, as I discuss below, there has been a fair bit of redesigning of the keys as well.

From the very start, the power of this technology has been evident—the ability to drive or suppress activity in a selective population of cells. Chemogenetic actuators (receptors) are typically delivered to the brain using either transgenic strategies or through viral vector-based approaches. When expressed, these receptors allow for the selective activation or inhibition of brain regions of interest in cell-type and pathway-specific manners. Selective control over discrete populations of cells has been a central quest of neuropharmacology for the better part of a century—the fundamental goal being to modulate only what needs to be modulated while leaving other brain functions intact. In the context of epilepsy, this translates to turning off the cells responsible for a seizure while avoiding the myriad of side effects associated with anti-seizure drugs. The advantages and pitfalls of chemogenetic approaches have been described extensively elsewhere, and...
many tutorials and reviews outline their utility, including epilepsy. Here, I review, in brief, the history of chemogenetic approaches and focus on their application to epilepsy as a potential modality for treatment and some of the challenges and opportunities that lie ahead.

A (Brief) History of Chemogenetics

The history of chemogenetics is reviewed in extensive detail elsewhere, so I summarize it only briefly here. The first chemogenetic approach was described in 1991. While exclusively used in vitro, this approach foreshadowed the development of second, third, and fourth generation constructs, which have been deployed extensively in vivo. The second-generation chemogenetic tools, receptors activated solely by synthetic ligands (RASSLs), were all engineered from native G-protein coupled receptors: the human kappa opioid receptor, the melanocortin-4 receptor, and the serotonin 4B receptor. While the potential for these tools was clear, the most widely deployed of these, the hROi (human RASSL Opioid Gi) induced a range of physiological effects in vivo in the absence of agonist delivery—suggesting constitutive signaling, which limited its utility, and underscoring the need for further tool development.

The third generation tools, DREADDs, were described in 2007 and have since been widely adopted. These receptors were produced by directed molecular evolution, which resulted in variants of the human muscarinic receptor that no longer responded to acetylcholine, and instead responded to clozapine-n-oxide (CNO), a metabolite of the antipsychotic drug clozapine. Muscarinic-receptor-based DREADDs were developed that couple to Gi, Gas, and Gq G-protein signaling, providing a means of suppressing neurons. Together, the constellation of G-protein coupled and ion-channel based chemogenetic actuators offer a wide range of methods for driving intracellular signaling, perturbing ionic homeostasis, and ultimately, activating or silencing select neuronal populations. However, prolonged activation of glycine receptor-based PSAMs has been reported to cause excitatory effects due to a shift in the neuronal chloride gradient. This is a familiar story in epilepsy where dysregulated chloride homeostasis is a mechanism proposed in some cases to underlie ictogenesis and contribute to drug failure; this may suggest caution in using PSAMs in epilepsy.

Pharmacology of Chemogenetic Agonists

In pharmacology, there are 2 types of drug effects—those we know about and those we’ve yet to discover. In the case of chemogenetic actuators, their selectivity is only as good as the selectivity of the drugs we use to activate them. Clozapine-n-oxide, the initial ligand described for muscarinic-based DREADDs, has several unexpected effects. CNO is metabolized to other neuroactive compounds, including clozapine, and as a result, CNO in DREADD-naive animals produces dose-dependent behavioral changes. Moreover, it has been suggested that the in vivo activation of DREADDs after CNO administration is not due to CNO, which is poorly penetrant to the brain, but rather a metabolic production of clozapine. Deciphering which effects are DREADD-mediated, and which are due to off target effects of DREADD agonists is an important consideration.

Several alternate DREADD agonists have been proposed, including clozapine, Compound-21, perlapine, and olanzapine. These compounds also display neuroactive effects. Clozapine and olanzapine are antipsychotic drugs, Compound-21 suppresses the activity of dopamine neurons, and perlapine is a sedative. While no off-target effects of dezchloroclozapine have been reported to date, history suggests that there may well still be effects to be uncovered.

Ion-channel-based chemogenetic tools have a more limited range of agonists: GluCl channels are activated by ivermectin, which carries risks of side effects, and PSAM constructs are potently activated by varenicline, a partial agonist of nicotinic receptors, as well as a handful of varenicline derivatives. From a basic science perspective, selectivity wins the day—the more precise the tools, the more precise the findings. However, from a clinical perspective, several of these compounds are attractive precisely because they have other uses. While clozapine is approved for use in humans, it is limited by severe but rare agranulocytosis and neutropenia. However, olanzapine and varenicline are also approved for use in humans and have well-established safety and tolerability profiles. Ultimately, it becomes a question of how selective is selective enough. For example, varenicline activates PSAMs at concentrations at least 2 orders of magnitude lower than those required to activate nicotinic receptors suggesting that dosing that avoids activating endogenous receptors may be possible.
Olanzapine, by contrast, activates muscarinic DREADDs at concentrations similar to those at which it blocks native receptors.\textsuperscript{27} While this increases the possibility of off-target effects, if these side effects are tolerable (and not counterproductive), olanzapine may remain a target of high translational potential. In both cases, the fact that these compounds are already approved for clinical use certainly would speed up the translational timeline.

**Applications to Epilepsy**

Before DREADDs were used to study epilepsy, epilepsy was used in part to validate DREADDs.\textsuperscript{31} The first transgenic mice expressing Gq-coupled DREADDs displayed limbic seizures and status epilepticus upon administration of CNO, demonstrating the power of this approach to drive neural activity remotely. This approach has recently been revisited using focal silencing of cortical interneurons as a model for focal onset neocortical epilepsy.\textsuperscript{32} While a recent systematic review provided a full account of papers using chemogenetic approaches in epilepsy,\textsuperscript{7} here I highlight some initial findings and subsequent studies that represented either novel targeting approaches in epilepsy or improvement in translational potential.

In 2014, the Kullman laboratory published the first report of DREADDs used to treat rather than trigger seizure activity. They reported that focal chemogenetic inhibition of a neocortical focus potently suppressed seizures.\textsuperscript{33} In 2016, my laboratory extended these findings beyond the seizure focus, demonstrating that chemogenetic silencing of the midline thalamus potently suppressed amygdala-kindled seizures\textsuperscript{34} and the Kokaia laboratory reported DREADD-mediated antiseizure effects in hippocampal organotypic slices.\textsuperscript{35} These initial therapeutic studies demonstrated the efficacy of DREADD-mediated strategies across a range of brain networks and in a range of seizure models.

Subsequent reports extended DREADD-based therapeutic approaches in epilepsy from chemogenetic silencing to chemogenetic activation—selective activation of parvalbumin interneurons in the hippocampus suppresses seizure-like events in hippocampal slices and behavioral seizures in a focally evoked model of temporal lobe epilepsy,\textsuperscript{36} as well as in the acute and chronic intrahippocampal kainate models and the hippocampal kindling model in mice.\textsuperscript{37} The identification of small enhancer regions that can be used to selectively drive virally delivered transgenes selectively within GABAergic neurons\textsuperscript{18} places selective targeting of inhibitory neurons in human gene therapy within grasp.

Designer Receptors Exclusively Activated by Designer Drugs-based approaches have also revealed new insights into local circuit physiology in mice with chronic epilepsy. These findings range from verification of the long-standing hypotheses regarding proconvulsant roles of newborn neurons in the hippocampus after epileptogenic insults\textsuperscript{39,40} to dissection of the role of hippocampal mossy cells in acute and chronic seizures,\textsuperscript{41} to interactions between medial septal input to hippocampal somatostatin neurons.\textsuperscript{42} At the macrocircuit level, DREADDs have been used to identify a feedforward role of parvalbumin neurons within the corticothalamocortical network that generates absence seizures,\textsuperscript{33} uncovered a feedforward pathway from the substantia nigra to the parafascicular thalamus that can be activated to reduce seizures,\textsuperscript{44} and selectively identify an amygdala-midline thalamus-prefrontal cortex circuit necessary for the expression of kindled seizures.\textsuperscript{45}

Designer Receptors Exclusively Activated by Designer Drugs-based approaches have shown utility in genetic epilepsies—chemogenetic activation of prefrontal cortex pyramidal cells normalized seizure threshold in the Cacna1a haploinsufficient mouse.\textsuperscript{46} Similarly, mice with the autism candidate gene, Ash1L, deleted in the frontal cortex, display seizures that are rescued by a combination of inhibitory DREADDs in the frontal cortex and systemic diazepam, but not by either treatment alone.\textsuperscript{47} This underscores the potential of chemogenetics for the treatment of genetic epilepsies and highlights the potential for synergistic therapy with standard pharmacological approaches. Along these lines, chemogenetic inhibition of subicular pyramidal neurons ameliorated phenytoin resistance in the phenytoin-resistant kindled rat.\textsuperscript{38}

Beyond seizures, chemogenetics has also been deployed in preclinical studies as a method to ameliorate cognitive and emotional dysfunction in epilepsy. For example, DREADD-based silencing of corticotropin-releasing factor neurons both suppressed seizures and normalized depression-like comorbidities in the pilocarpine model of temporal lobe epilepsy in mice.\textsuperscript{49} In the same model, silencing dentate gyrus granule cells reduced cognitive dysfunction,\textsuperscript{50} while in the intrahippocampal kainic acid model, hippocampal DREADD-mediated inhibition normalized risk avoidance behavior.\textsuperscript{51} Chemogenetic silencing of the amygdala has also been reported to reduce comorbid anxiety in GABRG2 mutant mice with seizures.\textsuperscript{52} Given that comorbidities of epilepsy are highly prevalent,\textsuperscript{53} often challenging to treat, and complicated by polypharmacy, chemogenetic approaches may provide a compelling option.

**Advancing Toward Translation**

As has been noted by others, epilepsy may well be the first indication to reach a clinical trial for DREADDs.\textsuperscript{54} Individuals scheduled for surgical resection as a treatment for epilepsy present a rare opportunity to test the efficacy of chemogenetics—if therapy is ineffective or side effects arise, tissue resection could proceed as planned. Clinical translation of chemogenetics likely depends on the ability to perform gene therapy. In the past 5 years, the approval of adeno-associated virus (AAV)-mediated gene therapy for inherited retinal dystrophy and spinal muscular atrophy has significantly raised the prospects of further AAV-based gene therapies. Adeno-associated virus vectors are far and away the most common methods of delivery for chemogenetic actuators. Viral delivery to the nervous system is, of course, not without its challenges. The use of chemogenetic technology in nonhuman primates has proven more difficult than in rodents. Still, a range of studies have
now reported promising effects—although none to date have done so in the context of epilepsy. Neutralizing antibodies against AAV vectors, impaired intracellular trafficking of chemogenetic constructs to the cell membrane in primates, and the cost and challenges associated with developing clinical-grade vectors all pose additional hurdles to translating chemogenetics to epilepsy patients.

One hurdle that preclinical studies have, however, begun to address is the efficacy of a range of DREADD agonists and the durability of DREADD-mediated anti-seizure effects. A series of studies from Raedt and colleagues in the “4Brain” group in Ghent have demonstrated sustained suppression of seizures in the intrahippocampal kainate model through DREADDs targeting hippocampal pyramidal cells, the long-term suppression of seizures in the rat kainate model using alternate DREADD agonists clozapine and olanzapine, and efficacy of the DREADD agonists clozapine and JHU37160 in the mouse intrahippocampal kainate model. Interestingly, in the latter study, both drugs significantly outperformed levetiracetam at reducing seizures. Moreover, a recent report demonstrated high oral bioavailability, blood–brain barrier penetrance, and functional impact of DREADD activation by deschloroclozapine in macaques, providing perhaps another viable pharmacological strategy.

Conclusions
As multiple groups work to move this technology closer to translation, several intermediate steps may further boost confidence in this approach. This includes tests of efficacy in epilepsy for new-generation tools such as PSAMs, and proof of concept in nonrodent species. Extension of chemogenetic methods in epilepsy to nonhuman primates or a “clinical” trial in canine epilepsy might provide additional support for this bridge to translation. The continued application of this technology in rodent models will, in parallel, lead to new insights into the basic mechanisms of the epilepsies. From 3 citations using chemogenetics in 2008 to a rate of more than a hundred a year a decade later, the rapid rise of chemogenetics has helped to usher in (along with optogenetic approaches) a renaissance in systems-level neuroscience. The potential for chemogenetics in epilepsy is clear—it provides a method for selective and “remote” control of neuronal activity. It is primed for continued impact both at the bench and, in the not-too-distant future, at the bedside.

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