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Is On-Demand Dynorphin Destined to Be in Demand to Decrease Seizures?

Dynorphin-Based "Release on Demand" Gene Therapy for Drug-Resistant Temporal Lobe Epilepsy

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Focal epilepsy represents one of the most common chronic central nervous system diseases. The high incidence of drug resistance, devastating comorbidities, and insufficient responsiveness to surgery pose unmet medical challenges. In the quest of novel, disease-modifying treatment strategies of neuropeptides represent promising candidates. Here, we provide the "proof of concept" that gene therapy by adeno-associated virus vector transduction of preprodynorphin into the epileptogenic focus of well-accepted mouse and rat models for temporal lobe epilepsy leads to suppression of seizures over months. The debilitating long-term decline of spatial learning and memory is prevented. In human hippocampal slices obtained from epilepsy surgery, dynorphins suppressed seizure-like activity, suggestive of a high potential for clinical translation. Adeno-associated virus delivered preprodynorphin expression is focally and neuronally restricted and release is dependent on high-frequency stimulation, as it occurs at the onset of seizures. The novel format of "release on demand" dynorphin delivery is viewed as a key to prevent habituation and to minimize the risk of adverse effects, leading to long-term suppression of seizures and of their devastating sequel.

Commentary

Despite continued advances in pharmaceutical development and a plethora of anti-seizure drugs (ASDs) available to clinicians, many patients with epilepsy cannot achieve effective seizure control. Patients with temporal lobe epilepsy (TLE), the most common focal epilepsy, unfortunately show high rates of pharmacoresistance.[1] Furthermore, systemic ASD administration affects areas of the brain outside the seizure focus as well as other organ systems, producing cognitive, emotional, hormonal, and other side effects that can be so intolerable that patients are reluctant to continue with the prescribed course of drug treatment.[2] Another difficult aspect is the need for chronic administration of ASDs in accordance with the episodic and unpredictable nature of seizures. That is, some ASD dose must always be "on board" in order to effectively prevent seizure occurrence, but drug tolerance developed in response to sustained exposure can subsequently reduce ASD efficacy. Therefore, a treatment that is: (1) specifically *targeted and localized* to only the seizure focus; and (2) produced acutely *only when specifically needed* to stop a seizure would be a major advance in reducing deleterious side effects, promoting patient adherence to treatment, and potentially improving the overall efficacy of seizure control.

The release of small-molecule neurotransmitters (eg, glutamate and - γ-aminobutyric acid) can occur stochastically in an action potential-independent manner and/or in response to action potential firing in presynaptic neurons. The release of larger neuropeptides, by contrast, typically requires higher firing frequencies. Therefore, one possibility is that the high firing activity characteristic of a seizure could be harnessed as a trigger to promote the release of neuropeptides.[3] If the effects of receptor activation in response to neuropeptide binding produce an overall decrease in neuronal excitability and population activity in the seizure focus, this scheme could potentially constitute an endogenous system to stop seizures "on-demand." An early example of this strategy demonstrated that adeno-associated virus (AAV)–mediated elevation of neuropeptide Y levels reduced seizures in rats for 2 weeks after AAV injection.[4] In this work, Agostinho et al explored this prospect using the dynorphin system as a test case in preclinical models of TLE.[5]

Dynorphins are endogenous opioid neuropeptides that exert neuromodulatory effects via binding to kappa opioid receptors (KORs) and triggering, for example, downstream activation of G_i-coupled and mitogen-activated protein kinase intracellular signaling pathways.[6,7] The resulting effects produce relatively long-lasting suppression of neural activity. Dynorphin levels can be reduced in seizure foci, but KOR expression is maintained,[8] indicating a mechanism to respond to replacement of dynorphin stores and release remains in situ. In this study, the

authors constructed an AAV to produce the dynorphin precursor peptide, preprodynorphin (AAV-pDyn), in infected cells and hypothesized that seizure activity would trigger dynorphin release from neurons transduced with AAV-pDyn; dynorphin binding to KORs would subsequently reduce neuronal excitability in the epileptogenic focus, and thus reduce seizure activity.

Approximately 1 month after intrahippocampal injection of kainic acid (IHKA) in mice,[9] AAV-pDyn or a control AAV expressing only green fluorescent protein (AAV-GFP) was injected into the hippocampal seizure focus. When compared with the baseline before AAV injection, IHKA mice with AAV-pDyn, but not AAV-GFP, displayed reduced hippocampal paroxysmal discharges and generalized seizures within days, and this effect persisted for at least 3 months. Treatment of AAV-pDyn-injected mice with a KOR antagonist transiently increased seizure activity, indicating that the anti-seizure effects of AAV-pDyn resulted from KOR activation. To confirm that this effect was not species- and/or model-specific, the authors also tested the effect of AAV-pDyn in rats subjected to amygdala kindling, and once again observed fewer seizures.

As noted above, cognitive impairments are common side effects of ASDs, and spatial memory loss is a prominent comorbidity in TLE.[10] To determine whether the anti-seizure effects of AAV-pDyn also extended to amelioration of spatial learning deficits, the mice were tested in the Barnes maze. In this commonly used assay of spatial cognition and memory in rodents, a food reward is hidden in one of a series of holes around the perimeter of a circular platform, and the rodent must use environmental cues to learn the location of the food-containing target. When AAV-pDyn was injected within 2 weeks after KA, IHKA mice retained performance values similar to those of nonepileptic control mice, whereas AAV-GFP-injected IHKA mice displayed a learning deficit. Intriguingly, however, when IHKA mice displayed spatial learning deficits at 1 month after KA injection and were subsequently injected with AAV-pDyn, the behavioral performance improved to the levels exhibited by the control mice. These experiments thus suggest that AAV-pDyn treatment may not only improve seizure control but also reverse at least some comorbid declines in learning and memory. This result is perhaps somewhat surprising, given that prodynorphin knockout mice can show reduced aging-associated impairment in spatial learning and memory.[11,12] The authors speculate that the beneficial effect of AAV-pDyn in the context of epilepsy may reflect rescue of function in the contralateral, nonsclerotic hippocampus, although this postulate remains to be tested.

The proposed efficacy of AAV-pDyn as an "on-demand" therapy assumes that high-frequency firing characteristic of a seizure would precipitate release of dynorphin into the surrounding extracellular space, thus rendering it available to bind to KORs on nearby cells. To test whether this is the case, the authors injected AAV-pDyn into the hippocampus of pDyn knockout mice and tested the response to electrical stimulation of perforant path inputs from the entorhinal cortex. Measurement of extracellular dynorphin in the ipsilateral hippocampus confirmed that high-frequency (50 Hz) stimulation, but not low-frequency (0.1 Hz) stimulation, produced an increase in dynorphin content. It should be noted, however, that the concentrations achieved were in the pM range, but the EC50 for KOR activation is in the nM range.[13] Although the authors demonstrated that 600 nM dynorphin can counteract pharmacologically-induced epileptiform events recorded in hippocampal tissue resected from patients with epilepsy, a lower concentration mimicking that detected in microdialysis was not tested. It thus remains unclear whether nM concentrations are achieved when the source of dynorphin is AAV-pDyn. Concentrations measured in microdialysis may represent diluted contents compared with concentrations confined to the synaptic cleft, but whether this is the case remains to be determined.

Overall, these studies provide an interesting further proof of concept for the use of "on-demand" neuropeptide release to achieve localized, acute seizure suppression. It will be worthwhile to identify other neuropeptides that produce similar on-demand anti-seizure effects. In recent years, closed-loop optogenetic approaches have enabled on-demand seizure suppression in preclinical studies, including in the IHKA mouse model used here[14] and have also proven effective at ameliorating comorbid spatial memory impairments.[15] Optogenetics, however, currently requires implantation of a light fiber targeting the seizure focus, which may not be amenable to clinical application. Chemogenetic approaches, which are less invasive, have also shown efficacy in enabling location-selective suppression of seizures in IHKA mice,[16] but these tools lack the temporal specificity of optogenetics. The AAV-pDyn paradigm may thus combine the noninvasive benefits of chemogenetics with the temporal specificity of optogenetics, providing yet another weapon in the preclinical armament against seizures, with good potential for clinical translation.

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