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Cannabidiol Keeps Hippocampal Hyperactivity in Check

Cannabidiol Modulates Excitatory-Inhibitory Ratio to Counter Hippocampal Hyperactivity

Rosenberg EC, Chamberland S, Bazelot M, Nebet ER, Wang X, McKenzie S, Jain S, Greenhill S, Wilson M, Marley N, Salah A, Bailey S, Patra PH, Rose R, Chenouard N, Sun SD, Jones D, Buzsaki G, Devinsky O, Woodhall G, Scharfman HE, Whalley BJ, Tsein RW. *Neuron.* 2023;111:1-19. doi:10.1016/j.neuron.2023.01.018

Cannabidiol (CBD), a non-euphoric component of cannabis, reduces seizures in multiple forms of pediatric epilepsies, but the mechanism(s) of anti-seizure action remain unclear. In one leading model, CBD acts at glutamatergic axon terminals, blocking the pro-excitatory actions of an endogenous membrane phospholipid, lysophosphatidylinositol (LPI), at the G-protein-coupled receptor GPR55. However, the impact of LPI-GPR55 signaling at inhibitory synapses and in epileptogenesis remains under-explored. We found that LPI transiently increased hippocampal CA3-CA1 excitatory presynaptic release probability and evoked synaptic strength in WT mice, while attenuating inhibitory postsynaptic strength by decreasing GABAARg2 and gephyrin puncta. LPI effects at excitatory and inhibitory synapses were eliminated by CBD pre-treatment and absent after GPR55 deletion. Acute pentylenetrazole-induced seizures elevated GPR55 and LPI levels, and chronic lithium-pilocarpine-induced epileptogenesis potentiated LPI's pro-excitatory effects. We propose that CBD exerts potential anti-seizure effects by blocking LPI's synaptic effects and dampening hyperexcitability.

Commentary

Cannabidiol (CBD) is the primary nonpsychoactive component of cannabis and has been shown to be therapeutic in patients with severe early childhood epilepsy. In fact, CBD (Epidiolex) has received Federal Drug Administration approval for the treatment of Dravet syndrome (DS), Lennox-Gastaut syndrome, and tuberous sclerosis complex. Although CBD has been shown to reduce seizure burden and normalize neuronal activity in preclinical rodent models, its mechanism of action remains incompletely understood and warrants further investigation.

There are multiple endogenous endocannabinoid receptors, including CB_1R, CB_2R, and GPR55. Multiple studies have shown that CBD can act as a GPR55 antagonist by blocking the proexcitatory effects of the endogenous GPR55 agonist, lipid lysophosphatidylinositol (LPI), but the mechanisms by which CBD modulates the excitation inhibition (E:I) ratio are unclear. In the current study by Rosenberg and colleagues,[1] the authors hypothesize that CBD modulates the LPI-GPR55 signaling pathway to restore the balance between hippocampal excitation and inhibition, which results in an anti-seizure effect. First, the authors observed an increase in hippocampal GPR55 expression and LPI levels in the lithium–pilocarpine rat model, a model of chronic epilepsy, and following the acute administration of the proconvulsants pentylenetetrazole (PTZ) and kainic acid. They also found that chronic epilepsy potentiated the proexcitatory effects of LPI.[1] Moreover, the authors demonstrated that LPI binds and activates GPR55, which in turn, increases presynaptic glutamate release and simultaneously attenuates inhibitory synaptic strength by reducing $GABA_AR_{\gamma 2}$ and gephyrin puncta. The authors propose that endocannabinoids and lysophosphatidic acid, a lipid related to LPI, reduce excitation and inhibition to counteract the excess neuronal excitability driven by LPI. Furthermore, they speculate that when these lipid modulators are altered (e.g., after a seizure), the balance between neuronal excitation and inhibition can no longer be maintained in the brain.

Consistent with previous reports of its anti-seizure effects, CBD administration (200 mg/kg) reduced behavioral seizures, increased the latency to electrographic seizures, and decreased total EEG power following PTZ administration. Importantly, pretreatment with CBD was able to prevent the seizure-induced increase in hippocampal GPR55 expression and LPI levels. Cannabidiol pretreatment also blocked LPI-mediated effects at excitatory and inhibitory synapses, and the benefits of CBD were not observed after deletion of GPR55 (using homozygous GPR55 knockout mice). These observations were consistent with the authors' hypothesis that CBD

regulates neuronal excitability by the involvement of the LPI-GPR55 pathway.

Interestingly, homozygous loss-of-function variants in *MBOAT7*, which encodes for the protein lysophosphatidylinositol acyltransferase I (LPIAT1), have been previously reported in patients with intellectual disability, epilepsy, and autism.[2] LPIAT1 is an enzyme that transfers arachidonic acid to LPI to generate phosphatidylinositol containing arachidonic acid, an important lipid in the human brain. These observations demonstrate that there may be genetic forms of epilepsy associated with direct modulation of LPI. In the future, it would be valuable to explore whether mutations in other genes that directly or indirectly modulate LPI are also responsible for some cases of epilepsy.

The mechanisms for the anti-seizure effects of CBD have also been explored in a mouse model of DS (*Scn1a*$^{+/-}$ mutants). Kaplan and colleagues demonstrated that CBD (100-200 mg/kg) reduced the severity and duration of hyperthermia-induced seizures and the frequency of spontaneous seizures in *Scn1a*$^{+/-}$ mutant mice.[3] Furthermore, lower doses of CBD were able to restore more normal social interaction in the mutants. Kaplan et. al, also found that CBD was able to reestablish more normal interneuron excitability in the *Scn1a*$^{+/-}$ mutants and that antagonism of GPR55 blocked this CBD-induced increase in inhibitory neurotransmission. These observations suggest that CBD-mediated restoration of neuronal excitability in *Scn1a*-derived epilepsy also requires GPR55. To explore whether GPR55 may be a genetic modifier for *Scn1a*-derived epilepsy, Anderson et. al, evaluated GPR55 expression in *Scn1a*$^{+/-}$ mutants. Interestingly, they found that *Scn1a*$^{+/-}$ mutants on a mixed 129 × B6 genetic background, which results in a more severe seizure phenotype, had higher *Gpr55* mRNA expression in the cortex and hippocampus compared to *Scn1a*$^{+/-}$ mutants on a pure 129 background.[4] To test whether GPR55 modulates seizure susceptibility in *Scn1a* epilepsy, Anderson et. al, genetically reduced (using heterozygous *Gpr55* knockout mice) and pharmacologically blocked GPR55 (using the GPR55 antagonist CID2921524) in *Scn1a*$^{+/-}$ mutants and surprisingly found no effect on hyperthermia-induced seizures, spontaneous seizure frequency, or survival.[4] While Kaplan et. al, showed that GPR55 is necessary for CBD-mediated seizure protection in *Scn1a*-derived epilepsy,[3] the observations by Anderson et. al, demonstrate that 50% knockdown of GPR55 is not sufficient to have an anti-seizure effect in *Scn1a*$^{+/-}$ mutants.[4]

While the current study focuses on the anti-seizure effects of CBD mediated by LPI-GPR55 signaling, it would also be valuable to explore whether CBD-related improvements in behavior might also work through similar mechanisms. GPR55 mRNA is expressed in the hippocampus,[5,6] which is a brain region that plays a critical role in synaptic plasticity and learning and memory. Hurst and colleagues found that LPI enhances long-term potentiation in the CA1 of the hippocampus via activation of GPR55; however, LPI did not have any effect on long-term depression.[6] Marichal-Cancino et. al, found that LPI is important for developing learning strategies and that administration of the GPR55 antagonist CID16020046 decreased spatial memory.[7] Contrary to these observations, GPR55 knockout mice exhibit normal synaptic transmission, including short- and long-term plasticity, and normal associative learning.[5]

Given the opposing effects of the LPI-GPR55 signaling pathway on seizure susceptibility, synaptic plasticity, and learning and memory, the therapeutic potential and possible side effects of direct modulation of LPI, GPR55, or the LPI-GPR55 pathway are unclear and additional research is warranted. Furthermore, the LPI-GPR55 pathway has also been implicated in other diseases, including obesity and cancer. The current study by Rosenberg and colleagues demonstrates that CBD modulates the LPI-GPR55 pathway to restore the balance between hippocampal excitation and inhibition, providing a mechanistic basis for the anti-seizure effects of CBD. It would be worthwhile to explore whether CBD-mediated improvements in behavior are driven by the same mechanisms. A greater understanding of the mechanisms underlying the ability of CBD to reduce seizure frequency and improve behavior might result in the identification of additional patient populations that could benefit from CBD treatment.

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Declaration of Conflicting Interests

The author declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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