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Review article

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The proposed mechanisms of action of CBD in epilepsy

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ABSTRACT – Highly purified cannabidiol (CBD) (approved as Epidiolex® in the United States and as EPIDYOLEX from the EU agency) has demonstrated efficacy with an acceptable safety profile in patients with Lennox–Gastaut or Dravet syndrome in four randomized controlled trials. While the mechanism of action of CBD underlying the reduction of seizures in humans is unknown, CBD possesses affinity for multiple targets, across a range of target classes, resulting in functional modulation of neuronal excitability, relevant to the pathophysiology of many disease types, including epilepsy. Here we present the pharmacological data supporting the role of three such targets, namely Transient receptor potential vanilloid-1 (TRPV1), the orphan G protein-coupled receptor-55 (GPR55) and the equilibrative nucleoside transporter 1 (ENT-1).

Key words: cannabidiol, epilepsy, mechanism, GPR55, TRPV1, adenosine

Cannabidiol (CBD) possesses affinity and functional agonist or antagonist activity at multiple 7-transmembrane receptors, ion channels, and neurotransmitter transporters (Ibeas *et al*. 2015). While a diverse pharmacology would be predicted, target engagement and subsequent therapeutic effect is dependent upon relevant systemic exposure to CBD. Thus, several targets are considered implausible based upon the low affinity and/or potency exhibited by CBD when compared to systemic exposures measured in the plasma of patients receiving therapeutic doses of purified CBD.

CBD has been shown to demonstrate positive effects against a wide spectrum of seizures based on animal model data (Klein *et al*., 2017). Of those targets where engagement is plausible, several have been investigated based upon their physiological relevance to maintaining normal neuronal function (e.g. membrane potential, neurotransmitter release and uptake, and postsynaptic calcium mobilization). While the precise mechanism of action of CBD in the control of epileptic seizures in humans remains unknown, recent evidence has focussed attention upon the following effects of CBD: modulation of intracellular Ca^{2+} (including effects on neuronal Ca^{2+} mobilization via GPR55 and influx via TRPV1) and modulation of adenosine-mediated signaling (*figure 1*). Here, we describe the pharmacological data supporting the roles of GPR55, TRPV1, and adenosine transport in the mechanism of action of CBD in the treatment of seizures in humans, as demonstrated by the ameliorations observed with CBD (Epidiolex in the US and Epidyolex in the EU) in Dravet and Lennox Gastaut syndromes (Devinsky *et al*., 2017, 2018; Thiele *et al*., 2018).

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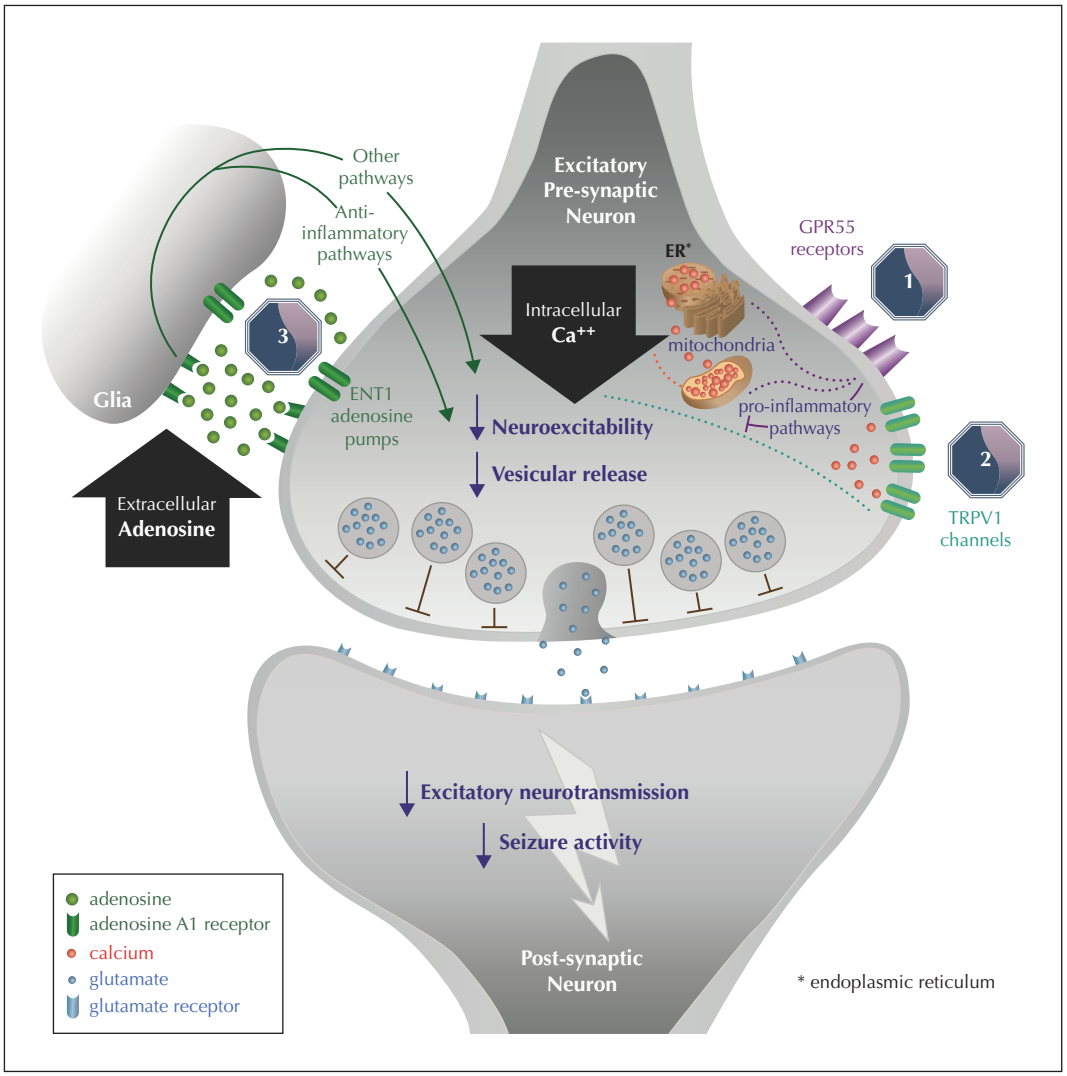

Figure 1. Proposed multimodal mechanism of action of CBD in epilepsy.

GPR55 was first identified as an orphan Class A G protein-coupled receptor (GPCR) enriched in brain (Sawzdargo *et al.*, 1999) and was originally suggested as a novel cannabinoid receptor (Ryberg *et al.*, 2007) and the subject of patent claims (Brown and Wise, 2001). However, poor sequence homology of GPR55 relative to CB_1 and CB_2 receptors, divergent pharmacology, and signal transduction suggest an alternative classification is appropriate, although at the time of writing, GPR55 remains an orphan receptor. GPR55 has been shown to utilize G_q, G_{12}, or G_{13} for signal transduction and the subsequent increased intracellular Ca^{2+} concentration through release of inositol triphosphate (IP3)-gated intracellular Ca^{2+} stores and activation of RhoA and phospholipase C.

In 2007, Ryberg *et al.* (2007) identified endogenous 2-arachidonylglycerol (2-AG), virodhamine (O-arachidonoyl ethanolamine), noladin ether (2-arachidonoyl glyceryl ether), oleoylethanolamide and palmitoylethanolamide (PEA), exogenous Δ^9-tetrahydrocannabinol (Δ^9-THC), and CP55940 as GPR55 agonists and first described CBD's antagonism of the GPR55 receptor. While the putative endogenous GPR55 receptor agonist, l-α-lysophosphatidylinositol (LPI), has been consistently described as a micromolar potency agonist of GPR55 (Kapur *et al.*, 2009), up to, and since this finding, the pharmacology of GPR55 has been the subject of significant investigation and has revealed emerging complexity.

As described by Sharir & Abood (2010), the molecular pharmacology of CBD at GPR55 is dependent upon the recombinant or endogenously expressing system and on the signalling system examined. The example pertinent to the present review is the

observation that while CBD can antagonize LPI-induced, GPR55-mediated stimulation of GTPγS binding and ERK phosphorylation, no such effect on LPI-induced β-arrestin recruitment is observed. These data are suggestive of biased antagonism where the ligand, in this case CBD, possesses functional selectivity for G-protein-mediated cellular events over another. In the absence of convincing pharmacological data describing competition of LPI and CBD for the same binding site at the GPR55 receptor, this remains plausible. Furthermore, in native systems where LPI interacts with multiple molecular targets, it is possible that the apparent antagonism of LPI-mediated physiological effects by CBD may be through interaction with one or more of such targets, including GPR55.

Moreover, coupling the role of GPR55 in modulation of neuronal excitability to its involvement in the pathophysiology of seizure was bolstered by the observation that GPR55 receptor expression is increased in the epileptic hippocampus (Rosenberg *et al.*, 2018).

Functional antagonism of GPR55 by purified CBD was investigated by examination of LPI-stimulated ERK1/2 phosphorylation in human GPR55-expressing HEK 293 cells. Purified CBD (1 μM) produced a parallel, rightward and marginally downward shift in the LPI concentration response curve, indicating functional antagonism of the GPR55 receptor. The primary evidence for the role of GPR55 in CBD's mechanism of action comes from studies in which the effect of LPI on neuronal excitability was assessed in acute hippocampal slice preparations. Here, the effect of purified CBD on LPI-induced GPR55-mediated modulation of mEPSC was assessed by whole-cell patch clamp recording in hippocampal slices from epileptic rats, sacrificed two weeks following a 60-minute episode of sustained pilocarpine-induced temporal lobe seizure and littermate, vehicle-treated controls. Activation of GPR55 by LPI increased mEPSC frequency in hippocampal slices, the magnitude of which was significantly greater in epileptic than non-epileptic rats. Since LPI effects on mEPSC frequency are transient, CBD was applied 20 minutes before LPI and functionally antagonized GPR55-mediated increases in mEPSC frequency in both non-epileptic and epileptic conditions.

Adenosine has been described as the endogenous modulator of neuronal excitability (Dunwiddie, 1980); and the brain's endogenous anticonvulsant and neuroprotectant (Weltha *et al.*, 2018). The endogenous nucleoside adenosine is released locally upon cellular insult and mediates its physiological effects via interaction with four 7-transmenbrane G-protein-coupled receptors whose classification (A_1, A_{2a}, A_{2b}, and A_3) is based upon sequence homology and pharmacology. Each adenosine receptor subtype possesses a well-defined tissue distribution and second messenger coupling, reflecting their role in the modulation of neuronal excitability, inflammation, and cardiovascular function.

Given their expression in the CNS, in consideration of the role of adenosine as a negative modulator of excitatory transmission and therefore seizure termination, the key receptor subtypes of interest are A_1, A_{2a}, and A_3. The anticonvulsive effects of adenosine are largely attributable to the activation of pre- and postsynaptic Gi/o protein-coupled adenosine A_1 receptors, which upon activation by locally released adenosine, mediate the inhibition of presynaptic calcium influx, and postsynaptic hyperpolarisation through enhancement of inwardly rectifying potassium channels (Fredholm *et al.*, 2005). In addition to the global inhibitory tone conferred by A_1 receptor activation, adenosine further fine tunes neuromodulation, in part, by heterodimerization with other G-protein-coupled receptors and affects all major neurotransmitter and neurotrophin systems (Sebastiao and Ribeiro, 2009).

Adenosine is a well-characterized endogenous anticonvulsant and seizure terminator of the brain through agonism of A_1 and A_{2A} receptors, respectively. While an anti-inflammatory mechanism for seizure control through agonism of A_{2a} receptors has been proposed (Sesbastiao *et al.*, 2000; Ribeiro *et al.*, 2012; Amorim, 2016), a causal link between regulation of neuroinflammation and seizure has not been demonstrated. Therefore, it is yet to be proven that agonism of A_{2a} receptors is implicated in the control of seizure, preventing instigation of neuroinflammatory processes or visa-versa. Endogenous adenosine is uniquely able to control neuronal excitability on multiple levels, and, consequently, any pathological disruption of adenosine homeostasis is likely to affect network excitability. Evidence for the role of adenosine in seizure can be categorised in terms of effects of maladaptive changes in adenosine metabolism observed in epilepsy and the effect of selective pharmacological tools. Maladaptive changes in adenosine metabolism due to increased expression of the astroglial enzyme, adenosine kinase (ADK), play a major role in epileptogenesis (Bioson, 2016). Increased expression of ADK has dual roles in both reducing the inhibitory tone of adenosine in the brain, which consequently reduces the threshold for seizure generation. This process also drives an increased flux of methyl groups through the transmethylation pathway, thereby increasing global DNA methylation (Weltha *et al.*, 2018). Through these mechanisms, adenosine is uniquely positioned to link metabolism with epigenetic outcome. Therapeutic adenosine augmentation therefore is not only central to suppression of seizures in epilepsy, but possibly also to the prevention of epilepsy and its progression overall.

Regarding the mechanism of action of CBD and interaction with the purinergic system, although

lacking appreciable affinity for, and agonist activity at either A_1 or A_{2a} receptors, CBD increases extracellular adenosine as exemplified by the effects observed in the rat nucleus accumbens for two hours post-intrahippocampal injection (Mijangos-Moreno *et al.*, 2014). Furthermore, CBD inhibits adenosine uptake into macrophages and microglia by the equilibrative nucleoside transporter and enhances suppression of tumour necrosis factor alpha (TNFα) (Liou *et al.*, 2008). The most compelling direct evidence for CBD's inhibition of adenosine reuptake comes from the ability of CBD to inhibit adenosine transport in rat synaptosomes. Here, CBD maximally inhibited [^{3}H] adenosine uptake into rat synaptosomes in a concentration-dependent manner (IC_{50} 1.1 μM) and the prototypic positive control equilibrative nucleoside transporter-1 (ENT-1) inhibitor dipyridamole exhibited the expected potency (Nichol *et al.*, 2018). The role of adenosine in neuromodulation (Boison *et al.*, 2016) in addition to CBD's effect on adenosine reuptake, suggests that a component of CBD's mechanism of action in seizure control in Dravet and Lennox-Gastaut syndromes is enhancement of adenosine-mediated signalling through increased availability of extracellular adenosine for agonism of A_1 and possibly other centrally-expressed adenosine receptors.

TRPV1 is expressed widely throughout the central nervous system and peripheral afferent fibres (Caterina *et al.*, 1997; Chung *et al.*, 1985; Roberts *et al.*, 2004; Tóth *et al.*, 2005). TRPV1 promotes neuronal depolarization, increasing their firing rate and synaptic activity (Xing and Li, 2007). TRPV1 can be activated by a number of endogenous and exogenous stimuli including heat, N-acyl amides, arachidonic acid (AA) derivatives, vanilloids, protons, and cannabinoids (De Petrocellis *et al.*, 2017). Initial observations by Bisogno and colleagues (2001) describing CBD as a TRPV1 receptor agonist were confirmed by De Petrocellis *et al.* (2011) who also observed rapid desensitization of the TRPV1 channel following CBD application.

TRPV1 expression is increased in human epilepsy (Sun *et al.*, 2013) and unsurprisingly plays role in regulation of cortical excitability (Mori *et al.*, 2012). The role of TRPV1 in the mechanism of antiepileptic activity of CBD is based upon the observations that CBD can activate and rapidly desensitize TRPV1 receptors at low micromolar concentrations in recombinant systems and in *in vitro* experimental models of epileptiform activity (Iannotti *et al.*, 2014). Here, patch clamp analysis in human TRPV1-transfected HEK293 cells demonstrated that CBD activates and rapidly desensitizes TRPV1 in a concentration-dependent manner and the TRPV1 receptor specificity of the CBD effect confirmed by sensitivity of effect the TRPV1 receptor antagonist, capsazepine.

Moreover, in a recent pivotal study (Gray *et al.*, 2019), the efficacy and potency of CBD on seizure threshold in an acute model of generalized seizure was examined in the TRPV1 knock-out mouse and compared to wild-type litter mates. While CBD dose-dependently increased the current required to induce seizures in 50% of animals, deletion of the *TPRV1* gene resulted in a blunted response to CBD, identifying TRPV1 as a key target implicated in the mechanism of anticonvulsive action of CBD.

In addition to the description of proposed mechanisms of action responsible for the efficacy of CBD against seizures, it is equally important to understand those mechanisms that lack plausibility given the concentrations at which pharmacological engagement is observed. The aetiology of a significant proportion of patients diagnosed with Dravet is a loss of function polymorphism in the *SCN1A* gene. Voltage-gated sodium channel (Na_v) blocking agents used in the treatment of seizures in Dravet Syndrome lack the potential to ameliorate and may exacerbate seizures. While a single report described the modulation of resurgent Na_v current by CBD (Patel *et al.*, 2016) and another described the inhibition of Na_v channel function at concentrations higher than clinically relevant (Ghovanloo *et al.*, 2018), the lack of effect of purified CBD on peak transient current and lack of use-dependent block has been reported (Gray *et al.*, 2017). Similarly, while the positive allosteric modulation of $GABA_A$ chloride current by CBD has been described in recombinant systems (Bakas *et al.*, 2017), this observation has not been independently confirmed and is observed at CBD concentrations in excess of those observed in patients.

While the precise mechanism of action of CBD in humans remains unknown, and there exist several plausible targets engaged by CBD beyond those described here, the preclinical evidence presented strongly implicates three molecular targets in the anticonvulsive properties of CBD. Thus, CBD reduces neuronal excitability through functional antagonism of GPR55 receptors, desensitization of TRPV1 receptors and inhibition of adenosine transport. □

Disclosures.

Full-time employees of GW Pharmaceutical.

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