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Proceedings of the Epilepsy Foundation's 2017 Cannabinoids in Epilepsy Therapy Workshop

1. Introduction

The Cannabinoids in Epilepsy Therapy workshop took place at Stanford University in October 2017 and brought together researchers, clinicians, and patient advocates to facilitate an open discussion on two key topics. The first was to review the state of current research on the therapeutic value of cannabinoids (CB) and how the endocannabinoid (eCB) signaling system contributes to the control of seizures. The second was to identify where there are gaps in what we know about targeting the CB system to control seizures and disrupt seizure networks. Thus, the workshop provided opportunities for a diverse assembly of scientists, clinicians, and patient representatives to network with each other and share lessons learned about successfully researching CBs in the US. This review summarizes the key topics that were discussed and their conclusions.

Epilepsy is the 4th most prevalent neurological disorder and is characterized by spontaneous, recurrent seizures that can result in a spectrum of consequences, from disrupted quality of life, to developmental delays, injury, or early death. Despite the availability of over 25 antiseizure drugs (ASDs), 1/3 of patients still experience pharmacoresistant seizures that are not fully controlled with current ASD therapies [1]. Therefore, the identification of new effective treatment strategies is imperative. Initial anecdotal observations from patients with epilepsy, early preclinical testing, and recent human clinical trials suggest that cannabidiol (CBD), a constituent of the cannabis plant that does not trigger euphoria, may significantly reduce seizure frequency and incidence [2–4]. However, the efficacy of CBD and other compounds targeting cannabinoid receptors is significantly different depending on preclinical seizure model, including the animal strain and age, as well as the dosage and route of administration of the compounds. Human clinical trial results also suggest that seizures respond to CBD in some individuals and not in others [2, 4]. Thus, this workshop served to discuss the current scientific understanding behind CBD and other CBs for seizure control, and to determine what questions remain about their antiseizure mechanism of action and how those unknown areas can be addressed.

2. Basic science

2.1. Cannabinoid compounds, their receptors, and signaling mechanisms

The endocannabinoid (eCB) signaling system – the body's own collection of cannabinoid ligands and receptors – forms an intricate network comprised of two primary G protein-coupled receptors (GPCRs): CB_1 and CB_2 receptors (CB_1R and CB_2R), their endogenous ligands anandamide (AEA) and 2-arachidonoylglycerol (2-AG), and the enzymes involved in their synthesis and degradation [5]. Importantly, CB1Rs are the most widely expressed and abundant GPCR in the central nervous system, CB1Rs are the most widely expressed on GABAergic presynaptic boutons in the cortex and hippocampus [6, 7]. On the other hand, CB_2Rs are predominantly expressed by hematopoietic cells and to a lesser extent by select neurons [8]. Thus, the major role of the eCB signaling system is to modulate an excitatory and inhibitory balance in the brain by distinctively controlling GABA and glutamate release, as well as by modulating the production of immunomodulators [5, 7, 8]. In fact, unlike most neurotransmitters that are synthesized and stored in presynaptic vesicles for subsequent release, 2-AG and anandamide are synthesized in an on-demand, activity-dependent manner from lipid membrane precursors [9]. This implicates the eCB signaling system as a homeostatic mechanism for regulating neuronal network activity in a state-dependent manner with a high degree of spatial and temporal selectivity. Accordingly, the functional role of the eCB signaling system in the control of the excitatory and inhibitory balance in the brain makes it an ideal target for seizure control by protecting against hyperexcitability only when and where it may occur [10].

2.2. Cannabinoids, eCB signaling, and epilepsy

Just as eCBs are produced in response to synaptic activity, evidence suggests that pathophysiological conditions, such as epilepsy, modify the eCB signaling system [10, 11]. For example, in animal models of seizures, CB_1Rs are downregulated acutely following seizure induction and subsequently upregulated in the chronic phase [10]. In patients with temporal lobe epilepsy, CB_1R expression in the hippocampus is downregulated from glutamatergic axon terminals and upregulated on GABAergic terminals [10]. Therefore, the specific change in CB_1R expression by neuron subtype is thought to contribute to the mechanism underlying the development of seizures in particular neuronal networks.

Accordingly, ample evidence shows that targeting CB_1R controls seizure incidence and severity [12, 13]. In brief, activation of CB_1R with either the synthetic cannabinoid agonists, WIN55,212-2 and arachidonyl-2′-chloroethylamide (ACEA), or the phytocannabinoid, Δ^9-tetrahydrocannabinol (THC), produces pronounced antiseizure effects in multiple preclinical seizure models with subtle differences between seizure type and induction method, the dose of the compound, and animal age [13]. By contrast, CB_1R antagonists elicit proconvulsant effects across most, if not all, preclinical models [13]. This may not be unexpected because most cannabinoid agonists are quite multimodal compared with cannabinoid antagonists, often acting at both CB_1R/

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CB_2R and transient receptor potential (TRP) channels [14, 15]. More importantly, the potent activity of THC at CB_1R mediates its euphoric effects and limits its therapeutic potential [15].

By sharp contrast, CBD does not produce euphoric effects perhaps partly because of its different mechanism of action. The mechanism of action of CBD is polymodal and includes: minimal potency at the orthosteric binding sites of CB1/CB2Rs, allosteric modulation of CB1R, antagonism of GPR55, and activation of the transient receptor potential vanilloid (TRPV) receptor family [16]. Furthermore, unlike THC, CBD reduces seizure burden in all but one of the preclinical models tested thus far [13]. Interestingly, CBD reduces seizure frequency in most, but not all, patients with Dravet syndrome and other treatment-resistant epilepsies [2, 4]. The significant efficacy of CBD treatment shown in recent human clinical trials emphasize the need to continue preclinical testing to better understand the underlying molecular mechanisms mediating this therapeutic benefit and how to optimize it. For example, in a genetically defined mouse model of Dravet syndrome that phenocopies the human condition, CBD not only reduces seizure frequency as observed in the recent human clinical trial, but also reduces seizure duration and severity [17]. It should be emphasized that acute CBD treatment also provided significant benefit for the behavioral comorbidities associated with the disease by rescuing impaired social behaviors that mirror autism-like deficits [17]. Finally, mechanistic studies show that these therapeutic effects correlate with increased GABAergic signaling via antagonism of GPR55 and decreased firing of excitatory neurons [17]. This work illustrates how continued preclinical evaluation of the mechanism of action of CBD will solidify and extend the scope of CBD treatment indications by providing critical molecular insights into the mechanisms mediating therapeutic benefits.

Recent evidence suggests that newly developed medicines designed to modulate eCB signaling may also be used as a distinct therapeutic modality to treat seizures. Levels of the endogenous ligands, AEA and 2-AG, are increased acutely after seizures in rodent models [18, 19]. The activity-dependent synthesis of eCBs and their agonism at cannabinoid receptors suggests that this increase acts as a negative feedback mechanism to inhibit glutamate release and dampen hyperexcitatory circuits [10, 20]. In line with this view, increases in eCB levels triggered by small molecule inhibitors of eCB reuptake and hydrolysis also yield primarily antiseizure effects [13]. A clear advantage linked to the inhibition of select eCB-hydrolyzing enzymes is to selectively control eCB signaling where these endogenous ligands are produced by highly active neurons without broadly activating the CB_1R throughout the brain and producing euphoric and cognitive impairing effects [21]. A search for novel eCB hydrolyzing enzymes has identified the serine hydrolase, ABHD6, as a factor controlling the level of 2-AG at CB receptors [21]. In fact, ABHD6 inhibition decreases pentylenetetrazole (PTZ)-induced seizure incidence and severity, an effect that was sustained in CB_1R and CB_2R knockout mice [22]. These remarkable CB receptor-independent effects were blocked by the application of the $GABA_A$ antagonist picrotoxin, which agrees with reports showing that 2-AG exerts a positive allosteric modulatory effect on the $GABA_A$ receptor. This mechanism associated with ABHD6 inhibition is advantageous in that it may preclude chronic treatment-induced tolerance associated with targeting the orthostatic binding site of GPCRs [22, 23]. Collectively, this evidence suggests that ABHD6 inhibition could be a novel therapeutic strategy for epilepsy, although requiring further characterization of responsive seizure types.

It is important to note that the strategy of modulating eCB signaling through the inhibition of eCB inactivating enzymes for therapeutic benefit is complicated by significant interactions with presynaptic proteins connecting neurons at the synapse, such as neurexins. Indeed, an additional layer of regulation recently discovered for eCBs comes from presynaptic β-neurexins which might contribute to the modulation of postsynaptic eCB synthesis via a transsynaptic regulatory loop that alters tonic postsynaptic synthesis [24, 25]. Thus, selective targeting of eCB signaling may come from a more in-depth molecular understanding of the steady state of eCB signaling as an intrinsic and constitutive activator of CB_1R signaling that will then be adjusted by other cell signaling events [26]. Together, the work that was dedicated to better understand CB, eCB signaling, and epilepsy led to discovering how the signaling and function of the eCB system under physiological and pathophysiological conditions are changed, and provided a better understanding of how to target this signaling network with novel candidate compounds that are likely to move into more translational studies.

3. Translational

3.1. The work of the Epilepsy Therapy Screening Program on CBs

The Epilepsy Therapy Screening Program (ETSP) [27] has been instrumental in the identification and evaluation of new potential drug therapies for the treatment of epilepsy. As mentioned above, in spite of a wealth of preclinical data and the availability of over 25 ASDs, 30% of patients continue to experience seizures not fully controlled with current ASDs [1]. To address this unmet need, the ETSP refined its drug screening approach to focus on preclinical models characterized by pharmacoresistant seizures. The ETSP prioritized two acute seizure models: the maximal electroshock and 6-Hz 44-mA stimulation tests that represent generalized tonic–clonic seizures and focal seizures, respectively [28, 29]. While these models exhibit electrographic signatures similar to that of human patients with epilepsy, both occur in neurologically intact animals devoid of the neuronal network remodeling resulting in behavioral alterations that are common in human patients with epilepsy. Therefore, corneal kindling and spontaneous bursting hippocampal slice models were included to identify compounds that may only be effective in a hyperexcitable network and are resistant to many traditional ASDs [28]. Additionally, screening in the Theiler's murine encephalomyelitis virus (TMEV) model of viral encephalitis-acquired epilepsy is characterized by handling induced seizures, hippocampal sclerosis, and neuroinflammation. This model is currently employed to help identify ASDs with potential disease-modifying properties [28].

Based on the preclinical evidence that CBD exhibits antiseizure effects, the ETSP investigated in greater detail the translational potential of CBD as an antiseizure drug by subjecting this compound to their refocused screening paradigm. In the acute seizure models, CBD displayed dose-dependent seizure protection at doses that did not impair motor function [30]. Similarly, CBD exerts dose-dependent protection against the chronic corneal kindling model, suggesting that CBD treatments may suppress seizure spread and focal activity [30]. Importantly, in the lamotrigine-resistant amygdala kindling model, where evoked seizures are resistant to lamotrigine and other select ASDs, CBD did not modify behavioral or electrographic seizure events, indicating that this compound does not have broad nonspecific effects on seizures and instead reduces seizures in a subset of models [30, 31]. Preliminary evidence suggests that CBD produces antiseizure effects in the TMEV model of viral encephalitis-induced epilepsy, as indicated by its ability to reduce the total number of seizures and cumulative seizure burden without halting hippocampal atrophy [32]. This work suggests that CBD may be preferentially efficacious against generalized tonic–clonic seizures than against clonic seizures [30]. While broadly exerting antiseizure effects in most models, the potency of CBD differs among the models tested. A possible explanation of this difference is that the therapeutic response of CBD could be due to its action at distinct targets depending on the model. This work further emphasizes the need for a better understanding of CBD's effects on its several molecular target candidates in each epileptic conditions, as well as if and how its therapeutic effect is affected when used in combination with additional ASDs and other drug therapies, as recently reviewed [33].

The ETSP analyzed combinatorial effects of CBD and other ASDs using drug combination analysis with isobolograms to determine drug synergy in preclinical models of motor seizures. Preliminary data suggest a synergistic relationship between CBD and levetiracetam in a 1-to-1 ratio [34].

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