Cannabinoids

34

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Abstract

The endocannabinoid system (eCBS) consists principally of (i) endogenous transmitters, including the lipid mediator 2-arachidonoyl glycerol (2-AG) and also arachidonoyl ethanolamide (anandamide), (ii) the metabolic enzymes that control endocannabinoid (eCB) production and degradation, and (iii) the cannabinoid CB_1 and CB_2 receptors (CB_1Rs and CB_2Rs) upon which eCBs exert their action. Acting in concert, these elements of the eCBS coordinate the endocannabinergic tone in the cerebellum and throughout the CNS. This tone mediates short- and long-term plasticities to control cerebellar functions, including fine motor control and associative learning paradigms. Deficits in eCBS cerebellar circuitry are associated not only with disease phenotypes, most notably spinocerebellar ataxias (SCAs), but also with increasing evidence for roles in other psychopathologies and cognitive disorders. The eCBS is also the target of exogenous cannabis, principally due to the actions of Δ^9-tetrahydrocannabidol (Δ^9-THC). Δ^9-THC mediates the "high" associated with illicit cannabis use, but some advocate that Δ^9-THC also has medicinal benefits. Less controversial is the recent use of cannabidiol (CBD) as the main cannabis constituent with reported medicinal benefits; here, CBD in isolation from the plant is the preferred option. A previous review focused on CB_1R signaling in the cerebellum and its association with cerebellar dysfunction. This updated review will consolidate the description of the eCBS bringing new findings into light and will explore potential new therapeutic targets and consider associated strategies that target the eCBS.

Keywords

Endocannabinoid system · Cannabinoid CB_1 receptors
Cannabinoid CB_2 receptors · 2-arachidonoyl glycerol
Diacylglycerol lipase · Monoacylglycerol lipase
Δ^9-tetrahydrocannabidol · Cannabidiol

34.1 Introduction to Cannabinergic Cerebellar Circuitry

It is well known that eCBs, principally 2-AG (Szabo et al. 2006), are released "on-demand" from Purkinje cells (PCs), the neuronal element which represents the sole controlling output of the cerebellar cortex. 2-AG is released predominantly from PC dendrites to act retrogradely on CB_1Rs expressed on presynaptic axons, including excitatory parallel fibers (PF), climbing fibers, and inhibitory basket cell interneurons (INs), and stellate cells (Kawamura et al. 2006; Rodríguez-Cueto et al. 2014a; Stephens 2016a; Fig. 34.1). CB_1Rs are the most prominently expressed G protein-coupled receptors in the cerebellum, and indeed in the CNS (Herkenham et al. 1991). These features combine to afford the cannabinergic circuitry a unique and privileged role in controlling cerebellar function. CB_1Rs are known to play a major role in long-term plasticity at PF-PC synapses (Carey et al. 2011), proposed to be critical for cerebellar learning (Ito 1972). The role of presynaptic CB_1R signaling in short-term plasticity in the cerebellum is well studied using protocols such as depolarization-induced suppression of inhibition (DSI) or depolarization-induced suppression of excitation (DSE) (Kreitzer and Regehr 2001). It has been shown that presynaptic CB_1R expression can be regulated by physiological synaptic activity patterns and that such activity is linked to the regulation of eCB levels by degradative enzymes (Yang et al. 2019); this plasticity is proposed to be important for associative learning paradigms. Genetic deletion of CB_1R causes impairment of fine motor control, rather than gross changes in motor function, and also impairs cerebellar devel-

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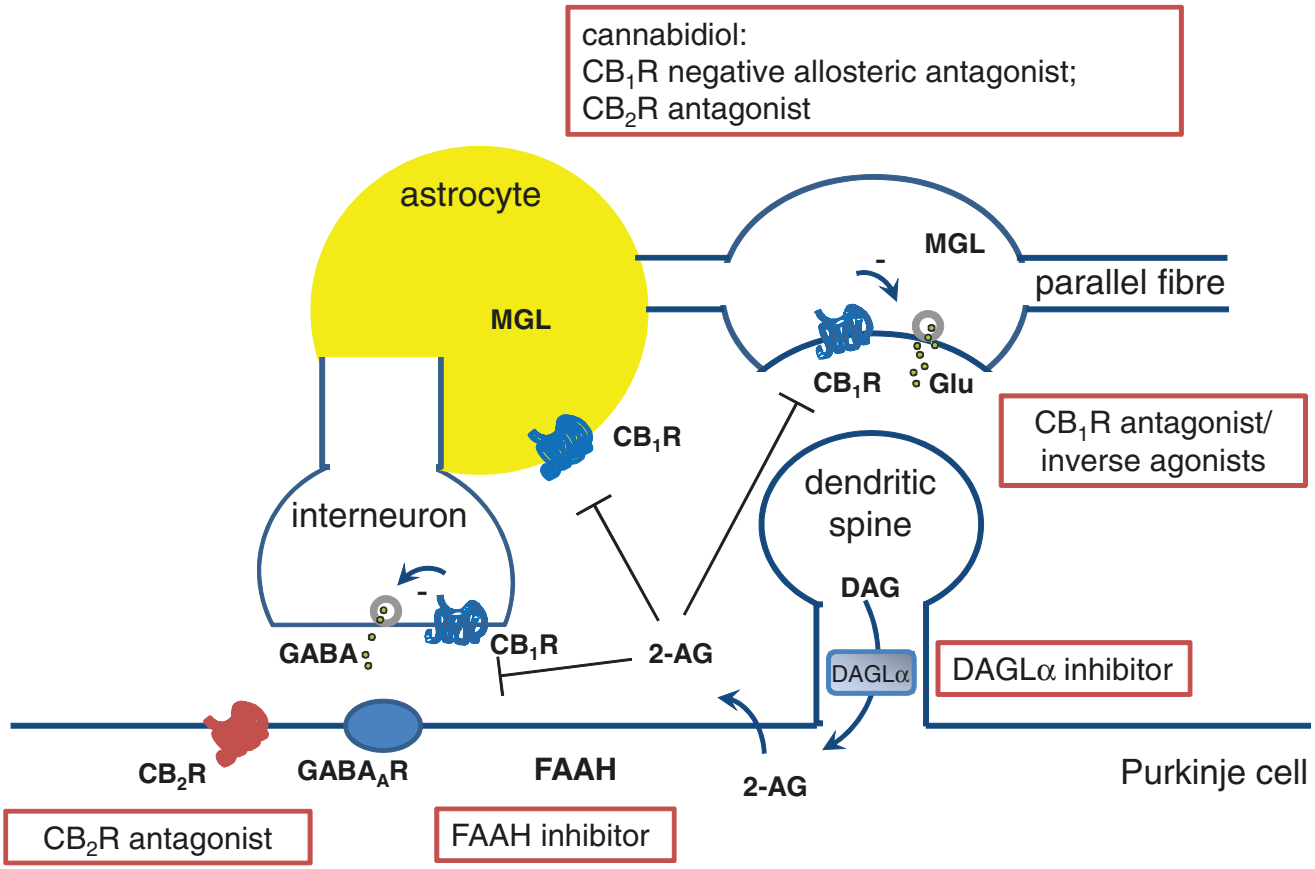

Fig. 34.1 Cerebellar endocannabinoid system and potential pharmacological targets. In Purkinje cell postsynaptic dendritic spines, 2-AG is synthesized by DAGLα from DAG. 2-AG is released retrogradely to act on presynaptic CB_1Rs at excitatory PF and inhibitory IN terminals to suppress release of glutamate (Glu) or GABA, respectively. Astrocytes and microglial also express CB_1Rs. PCs also express CB_2Rs, predominantly in soma. 2-AG is degraded by MGL, produced in presynaptic PF terminals, and also in astrocytes. FAAH is expressed in PC soma. Red boxes indicate potential pharmacological intervention strategies

opment (Kishimoto and Kano 2006; Martinez et al. 2020). Endogenous or exogenous activation of CB_1Rs, the latter for example by Δ^9-THC in cannabis, developmentally regulates synaptic strength and network activity (Barnes et al. 2020). In particular, CB_1R expression has been reported to undergo a development switch, with pronounced but transient expression at presumptive mossy fiber afferent terminals in the cerebellum of newborn rodents, prior to the establishment of the well-described dominant CB_1R expression on excitatory or inhibitory afferents to PCs in the adult cerebellum (Barnes et al. 2020). Glial cell elements, including astrocytes and microglial, are also reported to express CB_1R within the cerebellar cortex (Rodríguez-Cueto et al. 2014a). Such studies point to the critical importance not only of cannabinergic pathways in the cerebellum but also to the potential of illicit cannabis use to disrupt cerebellar circuitry and cause deficits in function. At a functional level, we have shown that du^{2J} "ducky" ataxic mice have deficits in CB_1R-mediated signaling that could contribute to disease phenotype (Wang et al. 2013). Disruptions in eCBS signaling have been implicated in cerebellar disease states including SCA2 and SCA3 (Kasumu and Bezprozvanny 2012; Rodríguez-Cueto et al. 2016). In a similar manner, a significant reduction in CB_1R protein expression within the cerebellum has also been reported in a mouse model of Dravet syndrome (DS) carrying a knock-in missense mutation in the *Scn1a* gene (which is defective in DS) (Satta et al. 2021); these deficits are correlated with altered, potentially cerebellar-related, behaviors. At a human level, the partial CB_1R agonist Δ^9-THC is well known to mediate the "high" associated with illegal cannabis use. While there are some proponents that advocate the benefits of medicinal cannabis use, it is much less clear if Δ^9-THC is the cannabis component that mediates any proposed benefit(s). The general medical consensus is, rather, that Δ^9-THC is deleterious for CNS and cerebellar function. For example, neuroimaging studies examining effects of exogenous cannabis reported reductions in cerebellar volume, activity patterns, and deficits in cerebellar-dependent work-

ing memory and learning (Blithikioti et al. 2019); moreover, these deficits were correlated positively with heavy cannabis use in adolescents.

CB_2Rs may also contribute to eCBS effects in the cerebellum. In general, the roles of CB_2Rs in other CNS regions have come under recent research focus. Within the cerebellum, studies have reported some contradictory findings regarding CB_2R expression; however, functional electrophysiological data support the role of CB_2R in cerebellar circuitry. Thus, Sadanandan et al. (2020) reported a variable expression of CB_2R in PC soma from juvenile mice and, moreover, that exogenous CB_2R agonists can reduce evoked inhibitory (but not excitatory) transmission at PCs. Of further interest was that CB_2R-mediated responses were postsynaptic in origin and that DSI was entirely dependent on CB_1Rs, and not CB_2Rs. Hence, it appears that CB_2R signaling differs from that of CB_1R, in that it is not mediated by retrograde signaling by PC-derived eCBs. The authors suggest that CB_2R signaling may be more relevant under conditions of sustained eCB release or, potentially, when activated by exogenous agents such as Δ^9-THC (Sadanandan et al. 2020). Some further support for a potential pathophysiological role of CB_2Rs in the cerebellum is the report of elevated CB_2R expression postmortem in patients with SCAs (Rodríguez-Cueto et al. 2014a); of further interest, this increase in CB_2R expression was reported to be co-incident with that of CB_1R, suggesting a potential symbiotic change in eCBS signaling during cerebellar disease.

34.2 Metabolic Control of the eCBS

eCBS function is intimately controlled by a series of metabolic enzymes. 2-AG is synthesized from diacylglycerol (DAG) by the lipase DAGLα in PC postsynaptic dendritic spines and is degraded by serine hydrolases, predominantly monoacylglycerol lipase (MGL), produced in presynaptic terminals and also in astrocytes (Yoshida and Fukaya 2006; Tanimura et al. 2012; Viader et al. 2015; Stephens 2016a; Fig. 34.1). Work using conditional MGL knockout mice has demonstrated that neuronal and astrocytic cells act cooperatively to regulate eCB-mediated retrograde synaptic depression in the cerebellum (Viader et al. 2015). Moreover, Chen et al. (2016) have shown that neurons and astrocytes combine effectively to regulate spatial 2-AG levels, limiting distribution and, hence, synapse-specific signaling within the cerebellum. While 2-AG is recognized as the most prominent eCB in the cerebellum, there is evidence that the enzyme fatty acid amide hydrolase (FAAH), which acts to degrade the eCB anandamide, is expressed throughout the cerebellum, in particular in PCs, cerebellar nuclei and the molecular layer (Suárez et al. 2008). Thus, there is potential to target a series of enzymes, including DAGLα, MGL, and FAAH to modulate eCB tone (see Fig. 34.1). In this regard, levels of degrative MGL and FAAH have both been reported to be increased in postmortem cerebellar tissue in patients with SCAs (Rodríguez-Cueto et al. 2014b).

34.3 Therapeutic Targeting of the eCBS in the Cerebellum

The cerebellum frequently overcomes its "little brain" status and is now recognized for its importance in cognitive and emotional learning and neurodevelopment. By extension, deficits in cerebellar circuitry can lead to a range of psychopathologies and cognitive disorders, including SCAs, autism, schizophrenia, and attention deficit and hyperactivity disorders (Stephens 2016a; Stoodley 2016; Hariri 2019). Knowledge of the different elements within the eCBS may be exploited to develop therapeutic agents. General pharmacological strategies are also summarized in Fig. 34.1. These strategies include the use of CB_1R antagonist/inverse agonists such as prototypic rimonabant; such agents most likely work by reducing constitutive endocannabinergic tone via an inverse agonism action. However, rimonabant, introduced as an anti-obesity agent, was subsequently withdrawn amid post-marketing identification of potential adverse psychiatric effects. The therapeutic targeting of different eCB enzymes has also been explored. For example, the DAGLα inhibitor, orlistat, is an anti-obesity agent that targets the gastrointestinal tract. However, the FAAH inhibitor, BIA 10–247, under investigations for various central indications including anxiety and Parkinson's disease as well as for anti-obesity potential, was another high-profile case where serious adverse events, including the death of one volunteer, resulted in termination of human trials. Overall, reports of adverse central effects have somewhat curtailed drug discovery in this area, although peripherally acting drugs are still under investigation and such avenues may lead to improved safety profiles and re-ignite this area.

A compound of on-going therapeutic interest is CBD. CBD is licensed to treat severe childhood epilepsies (Williams and Stephens 2020) and has potential to treat cerebellar diseases including SCAs (Stephens 2016b). Although an exact mechanism of action is still under debate, CBD has potential to modulate the eCBS via different proposed mechanisms, including a negative allosteric antagonism of CB_1Rs and antagonism of CB_2Rs. In general, there is now good evidence that CBD, rather than Δ^9-THC, mediates many of the proposed beneficial effects of medicinal cannabis. Indeed, CBD is reported to ameliorate the effects of the CB_1R partial agonist Δ^9-THC, including in cerebellar tissue (Whalley et al. 2019), and may act to limit effects of endogenous can-

nabinoid agonists in a similar manner (e.g., Hohmann et al. 2019). Functional magnetic resonance imaging studies have shown that CBD decreases blood oxygen level-dependent signaling in the mammalian cerebellum (Sadaka et al. 2021), consistent with a general inhibition of activity; such a mechanism may support CBD positive therapeutic effects on disease states linked to over activity of the cerebellar circuitry.

Overall, diseases of the cerebellum continue to be ripe for therapeutic invention involving the eCBS and there are clear opportunities to exploit the critical contribution of the eCBS to cerebellar circuitry, in particular output of PCs, using different pharmacological strategies. However, lessons will need to be learned from the identification of different adverse effects associated with some high-profile therapeutic failures in order to progress this area over the next few years.

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