Chapter 33
Cerebellar Circuits: Biochemistry, Neurotransmitters and Neuromodulators

Cannabinoids as Modulators in the Cerebellum

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Abstract Cannabinoid CB_1 receptors (CB_1Rs) are the most widespread G-protein-coupled receptors (GPCRs) in the mammalian CNS. CB_1Rs are present on inhibitory and excitatory presynaptic terminals supplying Purkinje cells (PCs), the sole output of the cerebellar cortex, where CB_1R activation suppresses transmitter release. CB_1Rs are part of the endocannabinoid (eCB) system, activated by the lipid mediator 2-arachidonoyl glycerol (2-AG) via retrograde transmission within the cerebellum. CB_1Rs also mediate synaptic plasticities to modulate cerebellar learning. This review will discuss the latest knowledge regarding CB_1R circuitry and signalling and their potential modulation.

Keywords CB_1 receptor • Endocannabinoid • Purkinje cells

33.1 Introduction

The release of excitatory glutamate, from parallel fibres (PF) and climbing fibres (CF), and inhibitory GABA, predominantly from basket cell interneurons (INs), onto output PCs, is intimately controlled by CB_1Rs in the cerebellar cortex (Stephens 2009). CB_1Rs are activated by eCBs and may be modulated by exogenous synthetic cannabinoids and plant-derived phytocannabinoids (Pertwee et al. 2010; Hill et al. 2012). The major effect of presynaptic CB_1R activation is suppression of neurotransmitter release (Kano et al. 2009). Cannabinoids also activate CB_2 receptors, expressed predominantly in microglia within the CNS, and potentially GPR55 receptors (Pertwee et al. 2010). However, key known effects of cannabinoids on synaptic function are mediated via presynaptic CB_1Rs (see Hoffmann and Lupia 2013).

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D.L. Gruol et al. (eds.), *Essentials of Cerebellum and Cerebellar Disorders*,
DOI 10.1007/978-3-319-24551-5_33

33.2 CB_1R Expression Within the Cerebellum

CB_1Rs are expressed at presynaptic terminals onto PCs, the principal neurons of the cerebellar cortex, which provide inhibitory innervation of deep cerebellar nuclei. Thus, CB_1Rs are uniquely positioned to control cerebellar function and, in particular, modulation of fine motor co-ordination. CB_1Rs are expressed on perisynaptic PF membranes, with lower expression at CF inputs onto dendritic shafts (Kawamura et al. 2006). CB_1Rs are expressed at higher levels on inhibitory IN presynaptic terminals, predominantly on basket cells, which form specialized 'pinceau' regions surrounding the PC axon initial segment (Rodríguez-Cueto et al. 2014). In the cerebellar cortex, CB_1Rs are activated preferentially by 2-AG (Szabo et al. 2006), over the other major brain eCB, arachidonylethanolamide (anandamide). The 2-AG biosynthetic enzyme diacylglycerol lipase-α (DAGLα) is localized to PC somatodendritic regions, with a subcellular distribution at the base of postsynaptic spines (Yoshida et al. 2006). The 2-AG degrading enzyme monoacylglycerol lipase (MGL) is localised to PF terminals (Tanimura et al. 2012). CB_1R expression has also been reported on microglial and astrocytes within the cerebellar cortex (Rodríguez-Cueto et al. 2014); the role of the CB_1R at such tripartite synapses will be of clear future interest.

In PC postsynaptic dendritic spines, 2-AG is synthesised by DAGLα from DAG (produced from phosphatidylinositol 4,5-bisphosphate (PIP2) by phospholipase C (PLC)) in turn activated by $G\alpha_{q/11}$ GPCRs. 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. CB_1Rs are predominantly coupled to $G\alpha_{i/o}$ to inhibit VGCCs (via Gβγ) and/or activate K^+ channels (via AC inhibition and subsequent reduction in cAMP). 2-AG is catabolised by MGL produced by PF terminals.

33.3 CB_1R Signalling in the Cerebellum

During retrograde transmission, 2-AG is synthesised *de novo* from diacylglycerol (DAG) by DAGLα and released 'on-demand' from postsynaptic somatodendritic PC regions to act on presynaptic CB_1Rs (Urbanski et al. 2010; see Fig. 33.1). Release of 2-AG, by not yet fully resolved pathways, is triggered predominantly by increases in postsynaptic Ca^{2+} concentration via depolarization-induced opening of voltage-gated Ca^{2+} channels (VGCCs) and/or synaptically-driven activation of ionotropic AMPA receptors and certain $G\alpha_{q/11}$-coupled GPCRs (Ohno-Shosaku and Kano 2014). Presynaptic CB_1Rs couple predominantly to $G\alpha_{i/o}$ subunits, which inhibit adenylyl cyclase (AC)-mediated generation of cyclic adenosine monophosphate (cAMP) and liberate Gβγ subunits (Pertwee et al. 2010). CB_1Rs activation causes pertussis toxin-sensitive inhibition of VGCCs and activation of inwardly rectifier K^+ channels (Guo and Ikeda 2004), CB_1Rs can also link directly to the vesicular release machinery; these effects are mediated by Gβγ subunits (Stephens 2009).

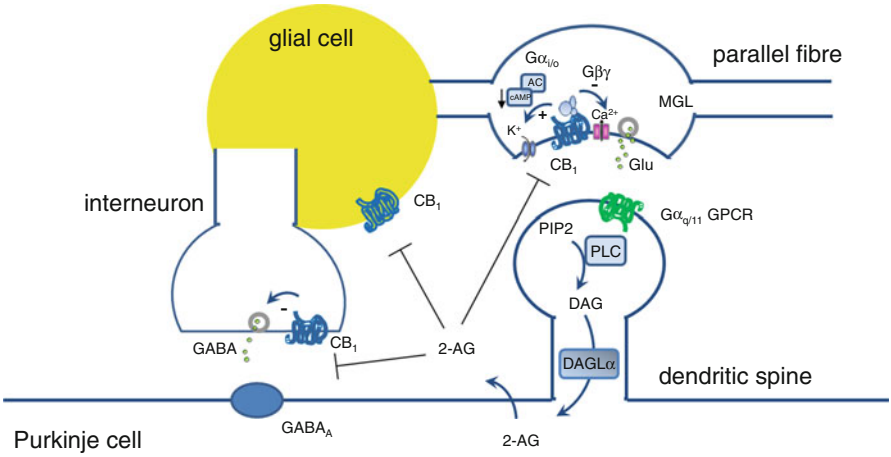

Fig. 33.1 2-AG is fundamental to cannabinoid signalling in the cerebellar cortex

CB_1Rs activation by synthetic agonists such as WIN55212-2 (WIN55) and CP55940, inhibits action potential-evoked, and spontaneous, inhibitory postsynaptic currents (IPSCs) at IN-PC synapses or excitatory postsynaptic currents (EPSCs) at PF-PC and CF-PC synapses; CB_1Rs activation also reduces frequency of 'miniature' IPSCs (mIPSCs) at IN-PC synapses (Takahashi and Linden 2000). CB_1R antagonists/inverse agonists increase mIPSCs (Ma et al. 2008); such effects are consistent with the presence of a strong, modulatable eCB tone in the cerebellum (Kreitzer et al. 2002).

Release of 2-AG causes short-term or long-term modulation of neurotransmitter release, offering the potential to fine-tune motor learning in the cerebellum. A prominent form of short-term modulation is the suppression of inhibitory GABA release from IN terminals (depolarization-induced suppression of inhibition, DSI) or suppression of excitatory glutamate release (depolarization-induced suppression of excitation, DSE) (Kreitzer and Regehr 2001). A major presynaptic effect in DSE is inhibition of VGCCs (Brown et al. 2004). eCBs also mediate long-term synaptic plasticity, due to repeated stimulation of synaptic inputs, but also in response to prolonged postsynaptic activity, and requiring additional activity to augment CB_1R effects (Ohno-Shosaku and Kano 2014). Both long-term depression and potentiation are modulated by CB_1R at PF-PC synapses, the balance of effects is proposed to underlie cerebellar learning (Vogt and Canepari 2010).

33.4 Association of CB_1Rs with Cerebellar Dysfunction

Disruption of cerebellar circuitry is commonly associated with ataxia, a spectrum of diseases associated with motor co-ordination deficits. Whilst animals lacking CB_1R have no gross deformities, they have deficits in eyeblink conditioning, suggesting that CB_1Rs control discrete cerebellar-dependent, motor learning processes

(Kishimoto and Kano 2006). In fact, CB_1R agonists cause severe motor incoordination and can be used to induce ataxia (Patel and Hillard 2001). We have shown that du^{2J} 'ducky' ataxic mouse mutants exhibit irregular PC firing and disrupted CB_1R-mediated signalling that could contribute to disease phenotype (Wang et al. 2013). In post-mortem spinocerebellar ataxia brain tissue, CB_1R expression was increased in glial and PCs (Rodríguez-Cueto et al. 2014); therefore, up-regulated CB_1R could be useful disease marker and/or may serve a neuroprotective function (Stephens 2016).

CB_1R antagonists/inverse agonists have been shown to increase inhibitory neurotransmission at IN-PC synapses (Ma et al. 2008). Such agents have potential to dampen cerebellar excitability. These agents included rimonabant; however, rimonabant withdrawal as a therapeutic anti-obesity agent, due to fears of increased suicide and depression, has curtailed several related drug development programmes. Potential alternatives are CB_1R negative allosteric antagonists, such as Org-27569 and PSNCBAM-1 (Ross 2007). These agents, somewhat paradoxically, increase binding of orthosteric ligands, but decrease their efficacy; this occurs in a ligand-dependent fashion (Baillie et al. 2013). We have shown such 'functional selectivity' for PSNCBAM-1 at IN-PC synapses (Wang et al. 2011). Moreover, unlike CB_1R antagonists/inverse agonists, PSNCBAM-1 had no intrinsic effects on inhibitory transmission. These studies indicate that negative allosteric antagonists offer potential advantages including reduced side effects and toxicity and present future potential for selective manipulation of the eCB system within the cerebellar cortex.

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