Endocannabinoids and Retrograde Modulation of Synaptic Transmission

Takako Ohno-Shosaku, Asami Tanimura, Yuki Hashimotodani and Masanobu Kano

Neuroscientist 2012 18: 119 originally published online 29 April 2011
DOI: 10.1177/1073858410397377

Endocannabinoids and Retrograde Modulation of Synaptic Transmission

The Neuroscientist
18(2) 119–132

DOI: 10.1177/1073858410397377
http://nro.sagepub.com
SAGE

Takako Ohno-Shosaku[1], Asami Tanimura[2], Yuki Hashimotodani[2], and Masanobu Kano[2]

Abstract

Since the first reports of endocannabinoid-mediated retrograde signaling in 2001, great advances have been made toward understanding the molecular basis and functions of the endocannabinoid system. Electrophysiological studies have revealed that the endocannabinoid system is functional at various types of synapses throughout the brain. Basic mechanisms have been clarified as to how endocannabinoids are produced and released from postsynaptic neurons and regulate neurotransmitter release through activating presynaptic cannabinoid CB_1 receptors, although there remain unsolved questions and some discrepancies. In addition to this major function, recent studies suggest diverse functions of endocannabinoids, including control of other endocannabinoid-independent forms of synaptic plasticity, regulation of neuronal excitability, stimulation of glia-neuron interaction, and induction of CB_1R-independent plasticity. Using recently developed pharmacological and genetic tools, behavioral studies have elucidated the roles of the endocannabinoid system in various aspects of neural functions. In this review, we make a brief overview of molecular mechanisms underlying the endocannabinoid-mediated synaptic modulation and also summarize recent findings, which shed new light on a diversity of functional roles of endocannabinoids.

Keywords

endocannabinoid, retrograde signaling, CB_1, 2-arachidonylglycerol, diacylglycerol lipase, synapse, marijuana, short-term depression, long-term depression

Historical Overview

Marijuana, a derivative of *Cannabis sativa*, has been used for thousands of years for its therapeutic and mood- or perception-altering properties. In 1964, Δ^9-tetrahydrocannabinol (Δ^9-THC) was identified as the main psychoactive component of marijuana. In the 1990s, two types of receptors for Δ^9-THC, CB_1 (CB_1R) and CB_2 cannabinoid receptors (CB_2R), and two endogenous ligands (endocannabinoids), *N*-arachidonylethanolamide (anandamide) and 2-arachidonylglycerol (2-AG), were found.

In 2001, endocannabinoids were discovered to function as retrograde messengers at synapses in the hippocampus and cerebellum. It is now widely accepted that endocannabinoids are released from postsynaptic neurons upon postsynaptic depolarization and/or receptor activation and act on presynaptic CB_1R to induce transient suppression of transmitter release (endocannabinoid-mediated short-term depression [eCB-STD]). In 2002, endocannabinoid-mediated long-term depression (eCB-LTD) was found in the striatum and nucleus accumbens. Later studies have confirmed that eCB-STD and eCB-LTD can be induced at various types of synapses throughout the brain. Anatomical studies have revealed that the molecular elements of retrograde endocannabinoid signaling are arranged around synapses in functionally relevant manners. Behavioral studies using CB_1R agonists and antagonists or CB_1R knockout mice have demonstrated that the endocannabinoid system is involved in various aspects of neural functions, including learning, drug addiction, feeding behavior, and analgesia.

Recent studies have revealed that the endocannabinoid system has more diverse functional roles than previously thought and is up- or down-regulated in response to

[1]Division of Health Science, Graduate School of Medical Science, Kanazawa University, Kanazawa, Japan
[2]Department of Neurophysiology, Graduate School of Medicine, The University of Tokyo, Tokyo, Japan

Corresponding Author:
Masanobu Kano, Department of Neurophysiology, Graduate School of Medicine, The University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113-0033, Japan
Email: mkano-tky@m.u-tokyo.ac.jp

various factors. In parallel, efficient pharmacological and genetic tools have been developed, facilitating the functional investigation of 2-AG and anandamide signaling pathways. This review provides our current understanding of the endocannabinoid system, with particular emphasis on molecular mechanisms of eCB-STD/LTD and recent topics. Because of limited space, only a limited number of references are included. For more information about original papers, several excellent reviews are available (Alger 2002; Chevaleyre and others 2006; Freund and others 2003; Heifets and Castillo 2009; Kano and others 2009; Piomelli 2003).

Cannabinoid Receptors and Endocannabinoids

CB_1R

The CB_1R, which is responsible for most of the psychotropic actions of Δ^9-THC, is expressed widely throughout the brain. At the cellular level, however, its distribution is heterogeneous. For example, in the cerebral cortex, hippocampus, and amygdala, the expression level of CB_1R is high in cholecystokinin (CCK)–positive inhibitory neurons, low in excitatory neurons, and almost absent in parvalbumin-positive inhibitory neurons. At the subcellular level, CB_1R is preferentially targeted to presynaptic elements. The expression level of CB_1R at presynaptic terminals varies greatly depending on brain regions and synapse types (e.g., Kawamura and others 2006).

Activation of CB_1R triggers multiple signal transduction pathways mainly via the $G_{i/o}$ family of G proteins, leading to a diversity of effects. They include decreases in cyclic AMP (cAMP) level and protein kinase A (PKA) activity, inhibition of voltage-gated Ca^{2+} channels, activation of several types of K^+ channels, and suppression of transmitter release (Turu and Hunyady 2010). Presynaptic mechanisms underlying the suppression of transmitter release involve inhibition of Ca^{2+} channels, activation of K^+ channels, and inhibition of some release mechanisms downstream of Ca^{2+} influx. Relative contributions of these three mechanisms for suppression of transmitter release may be different at different synapses.

Anandamide

Anandamide is a partial agonist for CB_1R and CB_2R and a full agonist for the vanilloid receptor TRPV1. Biochemical pathways for anandamide formation have been investigated but not fully understood. Early studies suggested the significance of *N*-acyltransferase and *N*-acylphosphatidylethanolamine–hydrolyzing phospholipase D (NAPE-PLD). Later, NAPE-PLD was cloned, and the studies using NAPE-PLD knockout mice showed that anandamide can be produced in an NAPE-PLD–independent manner (Okamoto and others 2007). The major enzyme catalyzing anandamide degradation is fatty acid amide hydrolase (FAAH), which was purified and cloned in 1996.

2-AG

2-AG acts as a full agonist at CB_1R and CB_2R and is present in the brain at higher concentrations than anandamide. Biochemical studies have found several pathways for 2-AG generation. The main pathway is the formation from membrane phospholipids through the combined actions of phospholipase C (PLC) and diacylglycerol lipase (DGL) (Fig. 1). Two highly related DGLs were cloned and named DGLα and DGLβ (Bisogno and others 2003).

Monoacylglycerol lipase (MGL), which was first cloned from mouse adipose tissue, is the primary enzyme that catalyzes the hydrolysis of 2-AG (Sugiura and others 2006). A study using a functional proteomic strategy reported that MGL accounted for approximately 85% of 2-AG hydrolysis and that the remaining 15% was catalyzed by other enzymes, such as ABHD6 and ABHD12 (Blankman and others 2007). Different distribution patterns of MGL and ABHD6, the former being presynaptic and the latter being postsynaptic (Fig. 1), suggest that these two enzymes might have nonoverlapping functions (Marrs and others 2010). Interestingly, a recent study reported that mutations in the ABHD12 gene cause a neurodegenerative disease named PHARC (polyneuropathy, hearing loss, ataxia, retinitis pigmentosa, and cataract), indicating that ABHD12 has essential functions in central and peripheral nervous systems and the eye (Fiskerstrand and others 2010). The apparent paradox of its minor contribution to 2-AG hydrolysis versus serious PHARC phenotype remains to be resolved. Oxidation is another pathway for 2-AG metabolism. Among three forms of cyclooxygenase (COX) enzymes (COX-1, -2, -3), only COX-2 preferably recognizes 2-AG as an effective substrate (Vandevoorde and Lambert 2007).

Endocannabinoid-Mediated Short-Term Depression (eCB-STD)

Many electrophysiological studies have shown that endocannabinoids function as retrograde messengers and contribute to short-term and long-term synaptic plasticity. The release of endocannabinoids from postsynaptic neurons is induced by various stimulation protocols that cause Ca^{2+} elevation and/or activation of $G_{q/11}$-coupled receptors. The released endocannabinoids then activate presynaptic CB_1R and suppress transmitter release transiently (eCB-STD) or persistently (eCB-LTD). Various

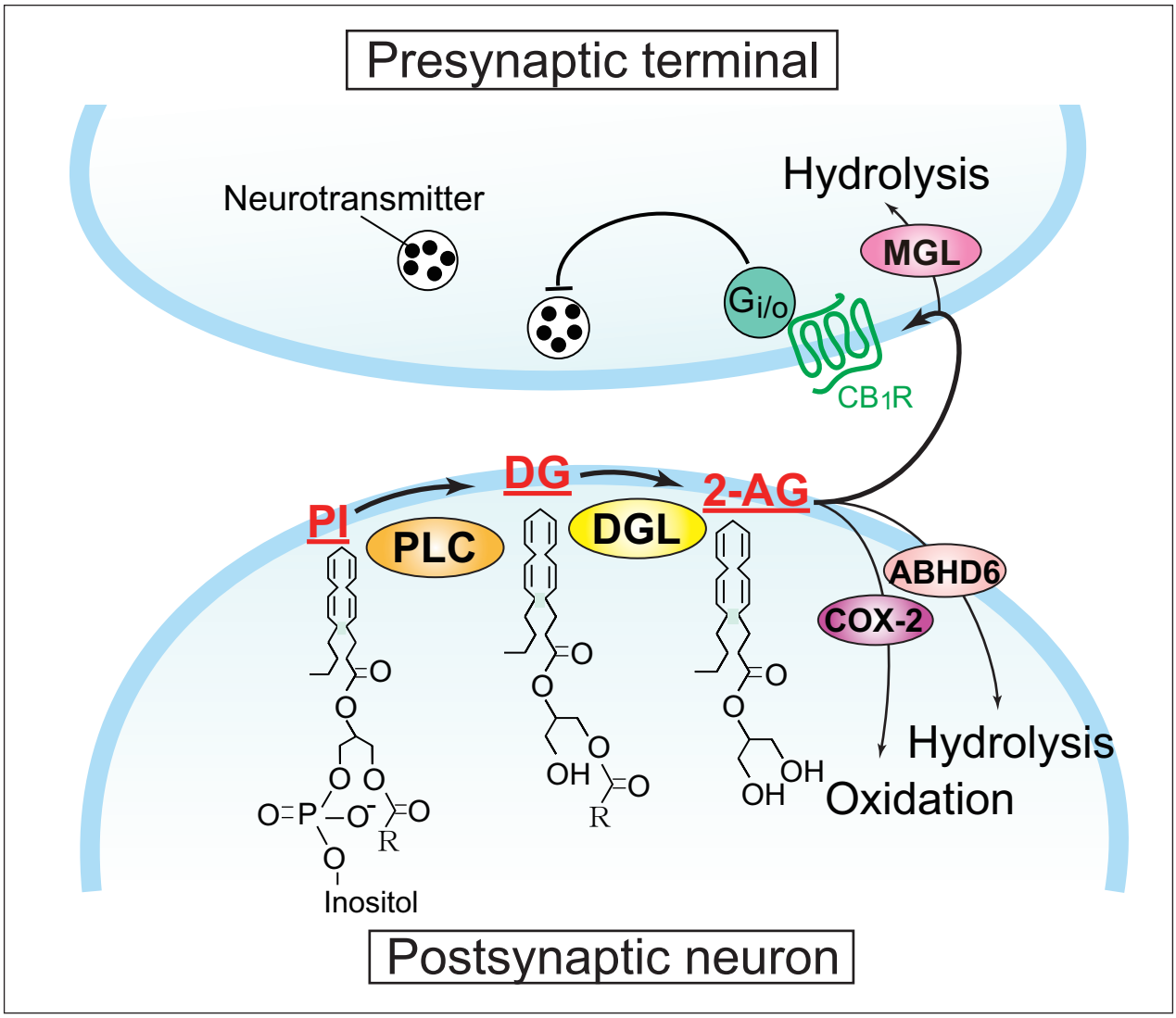

Figure 1. Key features of 2-AG signaling. Phospholipase C (PLC) hydrolyzes the arachidonic acid–containing membrane phospholipid such as phosphatidylinositol (PI) and generates diacylglycerol (DG), which is then converted to 2-AG by diacylglycerol lipase (DGL). The 2-AG released from the postsynaptic neuron enters into the presynaptic membrane, activates CB_1R, and is degraded by presynaptic monoacylglycerol lipase (MGL). In the postsynaptic neuron, 2-AG is degraded by ABHD6 or oxygenated by COX-2. Activation of presynaptic CB_1R causes suppression of neurotransmitter release through inhibition of Ca^{2+} channels or some other mechanisms.

forms of eCB-STD/LTD have been reported throughout the brain. In this section, we discuss the molecular mechanisms that explain how endocannabinoids are produced and released in response to each stimulation protocol and induce eCB-STD.

STD Depending on Ca^{2+}-Driven Endocannabinoid Release (CaER)

In several forms of eCB-STD, endocannabinoid release is induced by a large postsynaptic Ca^{2+} elevation alone, which we call Ca^{2+}-driven endocannabinoid release (CaER). These include DSI (depolarization-induced suppression of inhibition), DSE (depolarization-induced suppression of excitation), and presynaptic suppression caused by Ca^{2+} influx through NMDA-type glutamate receptors (NMDARs).

The DSI was discovered in the early 1990s first in the cerebellum and then in the hippocampus. In 1991, depolarization of cerebellar Purkinje cells was found to induce transient suppression of inhibitory inputs to the depolarized cells (Llano and others 1991). In 1992, the same phenomenon was found in hippocampal CA1 neurons and was termed DSI (Pitler and Alger 1992). Because DSI

was induced by postsynaptic Ca^{2+} elevation and expressed presynaptically, retrograde signaling was thought to be involved. It took 10 years to identify the retrograde messenger that mediates DSI. In 2001, endocannabinoids were shown to mediate retrograde signaling for hippocampal (Ohno-Shosaku and others 2001; Wilson and Nicoll 2001) and cerebellar DSI (Kreitzer and Regehr 2001a). Since then, endocannabinoid-mediated DSI has been reported in various brain regions including the striatum, globus pallidus, substantia nigra, cerebral cortex, amygdala, and hypothalamus (Alger 2002; Chevaleyre and others 2006; Kano and others 2009).

At the same time of the discovery that DSI is mediated by endocannabinoids, depolarization of cerebellar Purkinje cells was found to induce transient suppression of excitatory transmission (Kreitzer and Regehr 2001b), which was termed DSE. Similarly to DSI, DSE was induced by postsynaptic Ca^{2+} elevation, expressed presynaptically, and was dependent on retrograde endocannabinoid signaling (Kreitzer and Regehr 2001b). The DSE has also been reported in many other brain regions including the hippocampus, cerebral cortex, hypothalamus, ventral tegmental area, and dorsal cochlear nucleus (Alger 2002; Chevaleyre and others 2006; Kano and others 2009).

Using cultured hippocampal neurons, stimulation of NMDARs was demonstrated to induce a transient suppression of inhibitory transmission (Ohno-Shosaku and others 2007). This suppression required postsynaptic Ca^{2+} elevation and endocannabinoid signaling. The NMDAR-driven eCB-STD was not affected by the blockade of postsynaptic voltage-gated Ca^{2+} channels, indicating that Ca^{2+} influx through NMDAR directly induces endocannabinoid release.

A current model for the mechanisms underlying DSI/DSE and NMDAR-driven eCB-STD is illustrated in Figure 2(a). When the activation of voltage-gated Ca^{2+} channels or NMDARs causes a large Ca^{2+} elevation, 2-AG is synthesized in a DGLα-dependent manner. 2-AG is then released from postsynaptic neurons and suppresses the transmitter release through activation of presynaptic CB_1R. The Ca^{2+} concentration required for CaER is estimated to be at the micromolar level, but it might be dependent on the duration of Ca^{2+} elevation. Evidence for the contribution of 2-AG rather than anandamide is provided by the results showing that the time course of DSI/DSE is prolonged by the inhibition of 2-AG–hydrolyzing enzyme (MGL) but not anandamide-hydrolyzing enzyme (FAAH). Studies on mice lacking DGLα have revealed that 2-AG produced by DGLα mediates DSI/DSE (Gao and others 2010; Tanimura and others 2010).

How Ca^{2+} elevation induces 2-AG release in a DGLα-dependent manner is, however, unclear. A simple model is that Ca^{2+} elevation stimulates an unidentified enzyme that produces diacylglycerol, and the produced diacylglycerol is then converted to 2-AG by DGLα, as illustrated in Figure 2(a). In agreement with this "on-demand synthesis model," DGL inhibitor tetrahydrolipstatin (THL) was reported to suppress DSI/DSE (Hashimotodani and others 2008; Uchigashima and others 2007). Some other studies, however, reported that the THL treatment failed to suppress DSI/DSE (Min and others 2010b; Safo and Regehr 2005). The reason for this discrepancy in experimental results is unclear. On the assumption that DSI/DSE is blocked by genetic deletion of DGLα but not by acute pharmacological blockade of DGL, another model ("preformed model") was recently proposed (Min and others 2010a), which hypothesizes that 2-AG is preformed by DGLα, stored within cells, and released upon Ca^{2+} elevation. In either model, the enzyme that produces diacylglycerol contributing to CaER remains to be determined.

STD Depending on Receptor-Driven Endocannabinoid Release (RER)

Several types of $G_{q/11}$-coupled receptors are capable of driving eCB-STD in a Ca^{2+}-independent manner. They include group I metabotropic glutamate (mGluR), M_1/M_3 muscarinic, 5-HT_2–type serotonin (5-HT_2R), orexin, and oxytocin receptors. In these forms of eCB-STD, the endocannabinoid release is induced by strong activation of the receptors without need of postsynaptic Ca^{2+} elevation, which we call receptor-driven endocannabinoid release (RER).

The mGluRs include eight subtypes (mGluR1-8) and are divided into three groups. Group I mGluRs (mGluR1 and mGluR5) are coupled to the $G_{q/11}$ type of G proteins. In 2001, the Ca^{2+}-independent form of eCB-STD, which was induced by activation of group I mGluRs, was found in the cerebellum (Maejima and others 2001). Application of the group I mGluR agonist DHPG caused a transient suppression of the excitatory transmission to Purkinje cells. This suppression was demonstrated to be mediated by retrograde endocannabinoid signaling. Unlike DSI/DSE, the mGluR-driven eCB-STD was not blocked by the treatments that suppress postsynaptic Ca^{2+} elevation. The mGluR-driven eCB-STD is now known to occur in various regions of the brain, including the hippocampus, striatum, nucleus accumbens, medial nucleus of the trapezoid body, and periaqueductal gray (Kano and others 2009).

The second form of RER was found in 2002. In hippocampal slices, it was observed that a cholinergic agonist, carbachol, suppressed inhibitory transmission to CA1 pyramidal cells in a CB_1R-dependent manner, and the receptors responsible for this effect were pharmacologically determined to be muscarinic acetylcholine receptors (mAChRs) rather than nicotinic receptors (Kim

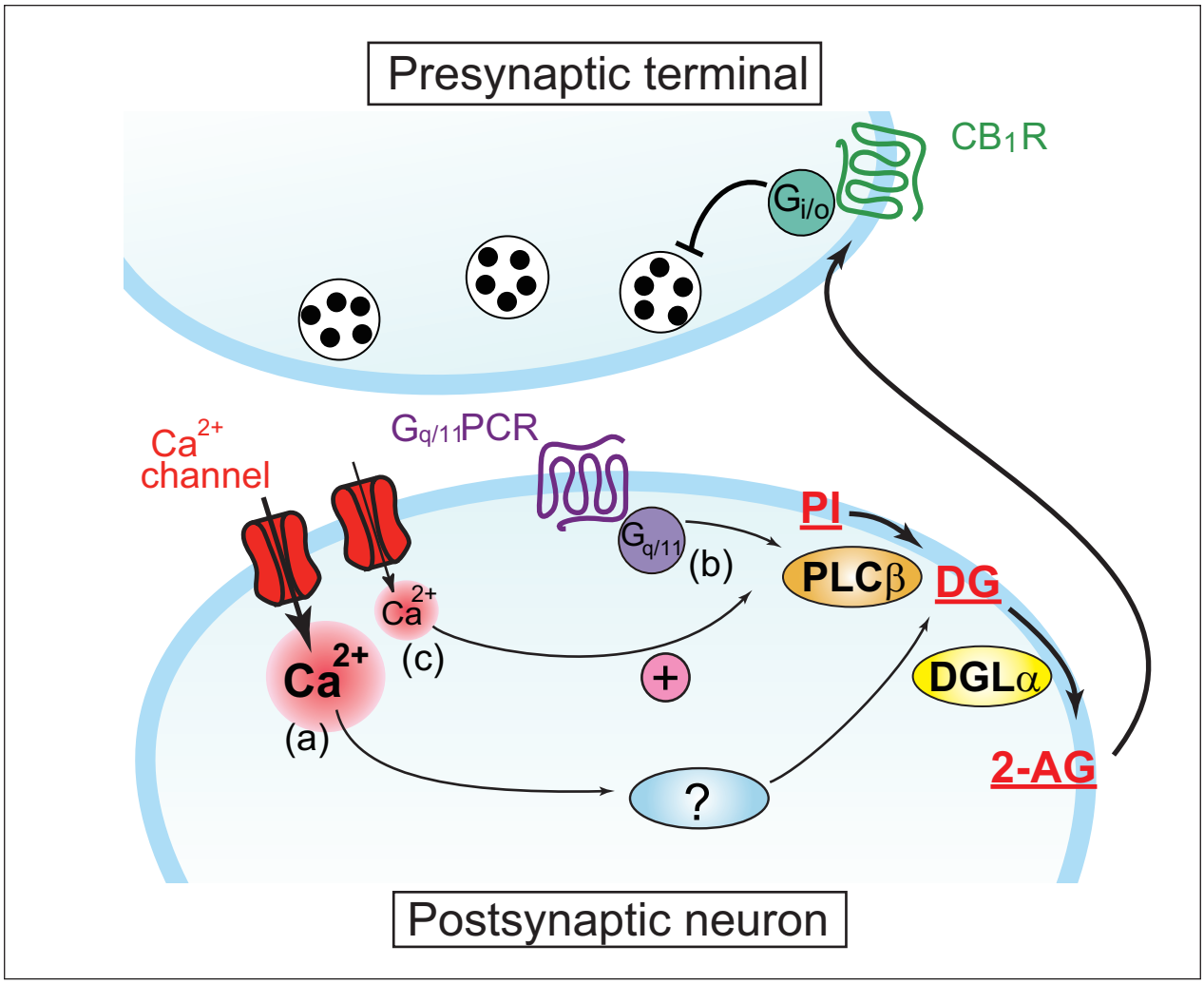

Figure 2. Molecular mechanisms of eCB-STD. When a large Ca^{2+} elevation is caused by activation of voltage-gated Ca^{2+} channels or NMDARs, 2-AG is generated in a DGLα-dependent manner (a). A key enzyme of this pathway, which is expected to be activated by Ca^{2+} elevation and produces diacylglycerol, is not identified. When PLCβ is stimulated by activation of $G_{q/11}$-coupled receptors, diacylglycerol is generated and converted to 2-AG by DGLα (b). When subthreshold activation of $G_{q/11}$-coupled receptors is combined with a small Ca^{2+} elevation, 2-AG is produced through a PLCβ-dependent pathway because the receptor-driven PLCβ stimulation is Ca^{2+} dependent (c). The 2-AG is released from postsynaptic neurons, activates presynaptic CB_1R, and induces transient suppression of transmitter release.

and others 2002). The mAChRs include five subtypes (M_1–M_5). M_1, M_3, and M_5 receptors are coupled to $G_{q/11}$, whereas M_2 and M_4 receptors are coupled to $G_{i/o}$. The subtypes of mAChRs involved in eCB-STD were determined by using genetically engineered mice lacking each subtype of mAChRs. The data clearly showed that M_1 and M_3 receptors are responsible for the induction of eCB-STD (Fukudome and others 2004). The mAChR-driven eCB-STD is also found at hippocampal excitatory synapses (Straiker and Mackie 2007) and striatal inhibitory synapses (Narushima and others 2007).

Following the discoveries of mGluR- and mAChR-driven eCB-STDs, several other $G_{q/11}$-coupled receptors were demonstrated to drive eCB-STD. In the inferior olive in which $G_{q/11}$-coupled 5-HT_2Rs are highly expressed on neuronal dendrites and soma, serotonin receptor agonists were reported to suppress excitatory synaptic transmission. The 5-HT_2R–dependent component of suppression was CB_1R dependent, indicating that 5-HT_2R drives eCB-STD (Best and Regehr 2008). In addition to the $G_{q/11}$-coupled receptors for classical neurotransmitters, neuropeptide receptors are also capable of driving endocannabinoid release. Suppressing effects of orexin-B on the excitatory transmission to serotonergic neurons in the dorsal raphe nucleus and oxytocin on both the excitatory and inhibitory synaptic transmissions to

magnocellular neurons in the supraoptic nucleus have been reported. These forms of suppression were CB_1R dependent and therefore can be classified as receptor-driven eCB-STD.

A current model for the mechanisms underlying $G_{q/11}$-coupled receptor–driven eCB-STD without need of Ca^{2+} elevation is illustrated in Figure 2(b). When postsynaptic $G_{q/11}$-coupled receptors are stimulated, diacylglycerol is produced through activation of PLCβ and converted to 2-AG by DGLα. 2-AG is then released from postsynaptic neurons and suppresses the transmitter release through presynaptic CB_1R. The type of PLCβ is different in different brain areas and neuron types. The mGluR-driven and mAChR-driven eCB-STDs were absent in PLCβ1-deficient hippocampal neurons (Hashimotodani and others 2005), while mGluR-driven eCB-STD was absent in PLCβ4-deficient cerebellar Purkinje cells (Maejima and others 2005). These electrophysiological results are consistent with the in situ hybridization data showing that the main PLCβ isozyme is PLCβ1 in the hippocampus and PLCβ4 in the rostral portion of the cerebellum (Watanabe and others 1998). Involvement of DGLα is supported by the studies using DGL inhibitors and DGLα knockout mice. The experimental results so far reported are consistent with this model (Edwards and others 2006).

STD Depending on Ca^{2+}-Assisted RER

An eCB-STD can be induced when a small Ca^{2+} elevation and weak receptor activation, both of which are the subthreshold for triggering endocannabinoid release, are combined (Hashimotodani and others 2005; Kim and others 2002; Maejima and others 2005; Narushima and others 2007). This phenomenon results from the enhancement of RER by subthreshold Ca^{2+} elevation. In cultured hippocampal neurons, mAChR-driven PLCβ activation was monitored by using the diacylglycerol-activated cation channel TRPC6 as a biosensor for the PLC activity. The muscarinic agonist–induced TRPC6 current, which was confirmed to be PLCβ1 dependent, was shown to be highly sensitive to intracellular Ca^{2+} concentration within a physiological range and greatly enhanced by Ca^{2+} elevation (Hashimotodani and others 2005). Moreover, mGluR-driven and mAChR-driven eCB-STDs were confirmed to depend on intracellular Ca^{2+} levels in the cerebellum and hippocampus (Hashimotodani and others 2005; Maejima and others 2005). Figure 2(c) shows a current model of the mechanisms underlying the endocannabinoid release induced by combined subthreshold Ca^{2+} elevation and receptor activation, which we term Ca^{2+}-assisted RER. When mild activation of postsynaptic $G_{q/11}$-coupled receptors is combined with a small Ca^{2+} elevation (submicromolar range), the endocannabinoid 2-AG is produced through the PLCβ-dependent pathway. In contrast, it remains to be determined whether CaER mechanism itself is enhanced by the activation of $G_{q/11}$-coupled receptors.

The Ca^{2+}-assisted RER appears to be physiologically more relevant for induction of eCB-STD/LTD as compared to CaER and RER because a major component of the synaptically driven eCB-STD is caused by Ca^{2+}-assisted RER (see below). Another important feature of Ca^{2+}-assisted RER is that PLCβ functions as a coincidence detector of $G_{q/11}$-coupled receptor activation and Ca^{2+} elevation, leading to the production of 2-AG (Hashimotodani and others 2005). In timing-dependent eCB-LTD, PLCβ may play a crucial role as a coincident detector (Nevian and Sakmann 2006).

Recently, BDNF was reported to suppress the inhibitory transmission in a CB_1R-dependent manner in the neocortex (Lemtiri-Chlieh and Levine 2010). This effect was suppressed by inhibition of postsynaptic Ca^{2+} rise. Although molecular mechanisms underlying BDNF-induced endocannabinoid release are not determined, a likely candidate is Ca^{2+}-assisted RER involving PLCγ instead of PLCβ.

Synaptically Driven eCB-STD

Besides postsynaptic depolarization and/or pharmacological activation of $G_{q/11}$-coupled receptors, synaptic activity can induce eCB-STD. Repetitive stimulation of excitatory synaptic inputs has been shown to induce eCB-STD, which we call synaptically driven eCB-STD. When sufficient glutamate is released from excitatory presynaptic terminals by repetitive stimulation, postsynaptic depolarization, Ca^{2+} elevation, and mGluR activation can be induced. These are all potentially effective in triggering endocannabinoid release. Therefore, synaptically driven eCB-STD may involve CaER, RER, or Ca^{2+}-assisted RER, depending on stimulation and recording conditions. For example, eCB-STD at parallel fiber (PF) to Purkinje cell synapses in the cerebellum was induced by a brief burst of PF stimulation (5–10 pulses at 50–100 Hz) (Brown and others 2003; Maejima and others 2005). This synaptically driven eCB-STD required both Ca^{2+} elevation and mGluR1 activation, indicating a predominant contribution of Ca^{2+}-assisted RER. The synaptically driven eCB-STD induced by intense PF stimulation (100 pulses at 100 Hz) at the same synapses was resistant to mGluR1 antagonist, indicating an additional contribution of CaER. The PF to Purkinje cell synapses undergo eCB-STD when subthreshold PF stimulation was combined with stimulation of climbing fiber, the other excitatory input to Purkinje cells (Brenowitz and Regehr 2005). This associative eCB-STD is thought to involve Ca^{2+}-assisted RER. When a large amount of endocannabinoids is released upon synaptic stimulation, endocannabinoids can spread and induce eCB-STD heterosynaptically. At inhibitory synapses on Purkinje cells, for example, eCB-STD

was induced by intense climbing fiber stimulation (50 pulses at 5 Hz) (Urbanski and others 2010).

Endocannabinoid-Mediated Long-Term Depression (eCB-LTD)

Several forms of long-term depression (LTD) of synaptic transmission are known to be dependent on retrograde endocannabinoid signaling (eCB-LTD). Because there is an excellent review focusing on eCB-LTD (Heifets and Castillo 2009), we briefly summarize its main features. The eCB-LTD can be induced at excitatory synapses in the dorsal striatum, nucleus accumbens, cerebral cortex, dorsal cochlear nucleus, cerebellum, and hippocampus and at inhibitory synapses in the hippocampus, amygdala, and ventral tegmental area (Heifets and Castillo 2009). Stimulation protocols used for the induction of eCB-LTD include repetitive stimulations with low frequency (e.g., 1 Hz, 100 pulses), medium frequency (e.g., 10 Hz, 5 minutes), and high frequency (e.g., 100 Hz, 1 second); theta burst stimulation; and pairing protocols.

It is generally thought that molecular mechanisms underlying endocannabinoid release for the induction of eCB-LTD are the same as those for eCB-STD. The dominant pathway may be different at different brain regions and also depend on experimental conditions. Pharmacological data strongly suggest that Ca^{2+}-assisted RER dominantly contributes to the induction of eCB-LTD in most brain regions, including the dorsal striatum, nucleus accumbens, cerebellum, prefrontal cortex, and sensory cortex. Contribution of RER is also suggested in some cases.

As downstream signaling of CB_1R activation, involvement of the cAMP/PKA cascade has been suggested in certain forms of eCB-LTD (Heifets and Castillo 2009; Fig. 3). Evidence is accumulating that CB_1R activation can induce eCB-LTD only when combined with presynaptic activity. One mechanism explaining the requirement of presynaptic activity was proposed for hippocampal eCB-LTD at interneuron-pyramidal cell synapses (Heifets and others 2008). The study suggests that presynaptic activity causes Ca^{2+} elevation, which in turn activates a Ca^{2+}-activated phosphatase, calcineurin (Fig. 3). In concert with reduced PKA activity by CB_1R activation, calcineurin shifts the phosphorylation/dephosphorylation balance toward dephosphorylation. The resulting decrease in phosphorylation of target proteins causes suppression of transmitter release. In this model, presynaptic terminals are thought to integrate two different signals, one reflecting postsynaptic activity (endocannabinoid) and the other reflecting presynaptic activity, to induce LTD. This associative nature of eCB-LTD may ensure that only active presynaptic terminals are specifically depressed among those facing endocannabinoids released from postsynaptic neurons. The target proteins required for eCB-LTD expression remain to be determined. The finding that eCB-LTD is absent in the hippocampus and amygdala of RIM1α knockout mice suggested RIM1α as the target protein (Chevaleyre and others 2007). However, a subsequent study using knockin mice demonstrated RIM1α phosphorylation at serine-413, which was expected to be essential for presynaptic plasticity, was not required for PKA-dependent long-term presynaptic plasticity including hippocampal eCB-LTD (Kaeser and others 2008). Further studies are required to obtain a clear understanding of presynaptic expression mechanisms for eCB-LTD.

Activities of Endocannabinoid Signaling

Basal Activity of Endocannabinoid Signaling

Whether CB_1R is constitutively active even in the absence of agonists is still debated (Turu and Hunyady 2010). However, it is evident that CB_1R shows basal activity through tonically released endocannabinoids under certain conditions. The basal activity of presynaptic CB_1R is primarily determined by the activity of postsynaptic neurons. For example, application of the CB_1R antagonist AM251 augmented the inhibitory transmission from CCK-positive basket cells to CA1 pyramidal cells in the hippocampus. This augmenting effect of AM251 was abolished by injecting the Ca^{2+} chelator BAPTA to postsynaptic neurons, indicating that endocannabinoids are tonically released in a Ca^{2+}-dependent manner. A similar augmentation of synaptic transmission by AM251 and its dependence on postsynaptic Ca^{2+} level are reported in the hypothalamus.

Whether the tonic activity of CB_1R is dependent on either or both 2-AG and anandamide is not fully understood. In cultured hippocampal neurons, basal inhibitory transmission was sensitive to inhibition of MGL, but not of FAAH, indicating the presence of tonically released 2-AG (Hashimotodani and others 2007). By contrast, a recent study using hippocampal slice cultures reported the importance of anandamide tone in homeostatic plasticity (Kim and Alger 2010). In this study, changes in inhibitory synaptic properties after chronic blockade of neural activity were examined. Whereas a large population of inhibitory synapses was homeostatically scaled down, CB_1R-positive inhibitory synapses were selectively augmented. This augmentation was attributed to the reduced tonic action of anandamide rather than 2-AG. The authors suggest that unique properties of anandamide, such as partial agonism and a weak desensitizing effect on CB_1R, seem appropriate for exerting tonic action on CB_1R. The postsynaptic localization of the degradation enzyme FAAH and the presence of intracellular stores (adiposomes) and binding proteins for anandamide

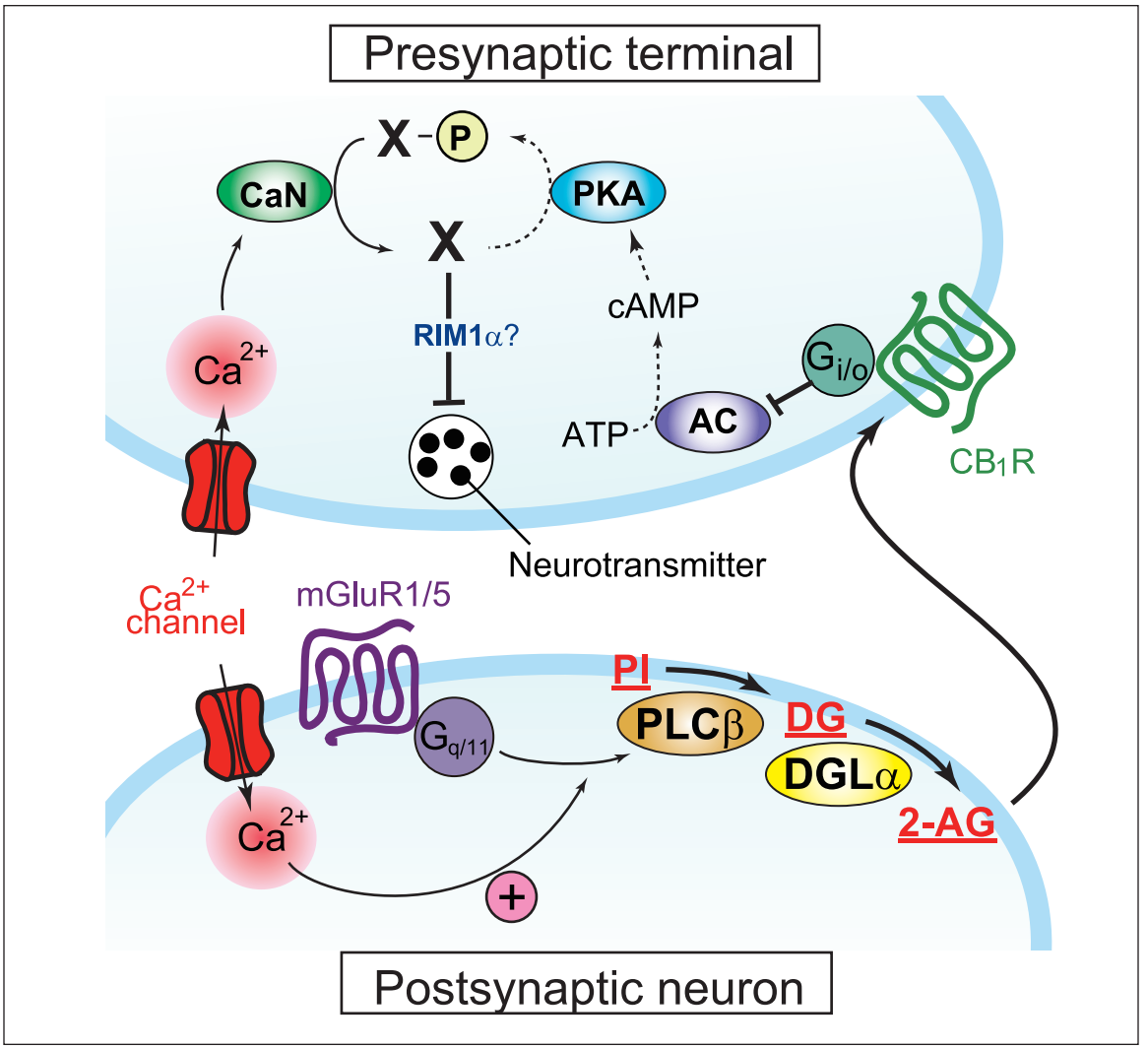

Figure 3. Molecular mechanisms of eCB-LTD. Excitatory synaptic inputs activate group I mGluR, which facilitates 2-AG synthesis through the PLCβ-DGLα pathway. This mGluR-driven 2-AG synthesis is enhanced by concomitant Ca^{2+} elevation, which is not necessary for eCB-LTD induction at some synapses. Activation of presynaptic CB_1R by 2-AG inhibits adenylyl cyclase (AC), decreases cAMP levels, and reduces protein kinase A (PKA) activity. Presynaptic activity causes Ca^{2+} elevation, which activates a phosphatase, calcineurin (CaN). In concert with reduced PKA activity, calcineurin facilitates dephosphorylation of target proteins (X), and induces persistent suppression of transmitter release through RIM1α-dependent and/or -independent processes.

(Maccarrone and others 2010) may provide additional advantages.

Regulation of Presynaptic CB_1R Signaling

The presynaptic CB_1R signaling process leading to suppression of transmitter release is influenced by several presynaptic factors. At inhibitory synapses on hippocampal CA1 neurons, the suppression of synaptic transmission by the CB_1R agonist was overcome by increasing the firing rate of presynaptic inhibitory neurons to 20 Hz or more (Fig. 4A). Consistent with this finding, DSI, a typical form of eCB-STD at these synapses, was also influenced by the activity levels of presynaptic neurons (Foldy and others 2006). The authors suggest that the ability of presynaptic activity to overcome CB_1R signaling likely involves the voltage-dependent removal of G protein–mediated inhibition of Ca^{2+} channels and summation of subthreshold presynaptic Ca^{2+} elevations.

Another factor influencing presynaptic CB_1R signaling is the activity of colocalized presynaptic receptors. At hippocampal excitatory synapses on CA1 neurons, CB_1R-dependent suppression of glutamate release was affected by the endogenous adenosine level through the presynaptic A_1 adenosine receptor (A_1R). Tonic activation of A_1R inhibited the CB_1R function (Fig. 4C), and its

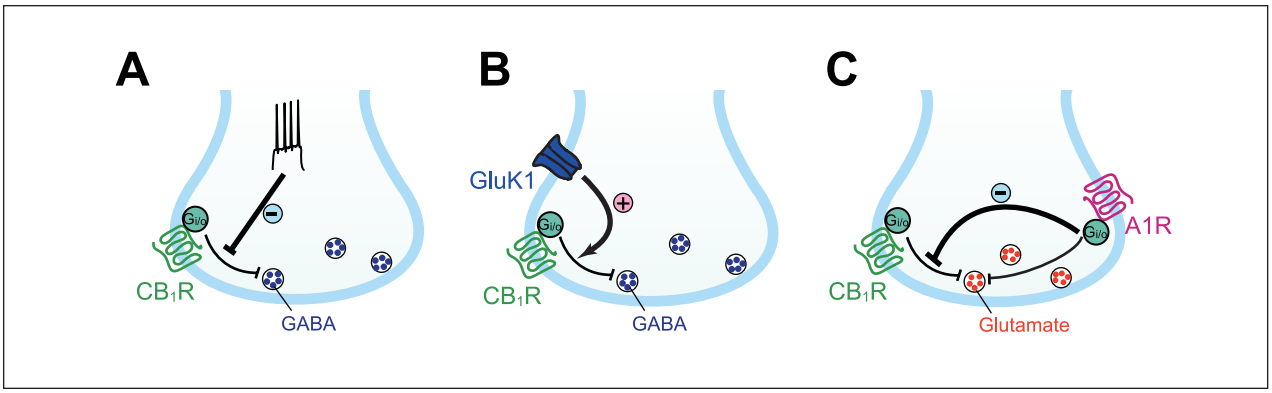

Figure 4. Modulation of presynaptic CB_1R signaling. (A) Suppression of transmitter release by activation of presynaptic CB_1R is overcome by increasing the firing rate of presynaptic fibers, presumably through voltage-dependent removal of G protein–mediated inhibition of Ca^{2+} channels and summation of subthreshold presynaptic Ca^{2+} elevations. (B) Activation of presynaptic GluK1 kainate receptor facilitates CB_1R signaling by an undetermined mechanism and suppresses transmitter release. (C) Tonic activation of presynaptic A_1 adenosine receptor (A_1R) inhibits the CB_1R function. Note that CB_1R and A_1R are both $G_{i/o}$-coupled receptors and may use overlapping sets of Gαsubunits.

blockade with caffeine reversed the effect (Hoffman and others 2010). This interaction between CB_1R and A_1R is reasonable because they are both $G_{i/o}$-coupled receptors and may use overlapping sets of Gα subunits. Another example is the presynaptic GluK1 kainate receptor, which plays dual roles in the modulation of transmitter release at hippocampal inhibitory synapses (Lourenco and others 2010). The GluK1 activation enhanced transmitter release at CB_1R-negative synapses but suppressed the release at CB_1R-positive synapses through augmentation of presynaptic CB_1R signaling (Fig. 4B). How GluK1 facilitates CB_1R signaling has not been determined.

Regulation of Postsynaptic Endocannabinoid Release

Likewise, endocannabinoid release is influenced by several postsynaptic factors, although the mechanisms are largely unknown. At hippocampal inhibitory synapses, mutual priming effects were found between DSI and mGluR-driven eCB-STD (Edwards and others 2008). The DSI was enhanced by prior activation of mGluR, while mGluR-driven eCB-STD was enhanced by prior depolarization. Experimental data indicated that this effect cannot be explained by Ca^{2+}-assisted RER, and therefore, it was recognized as a priming effect. In the striatum, dopamine D_2 and adenosine A_{2A} receptors contribute to positive and negative regulations of endocannabinoid release, respectively. These receptors are both expressed in indirect pathway medium spiny neurons and have opposing effects on cAMP accumulation. The endocannabinoid release and eCB-LTD were promoted by the activation of D_2 receptors and inhibition of A_{2A} receptors, whereas they were suppressed by the inhibition of D_2 receptors (Kreitzer and Malenka 2005; Kreitzer and Malenka 2007; Lerner and others 2010).

Regulation of the Endocannabinoid System

The endocannabinoid system consists of several molecular elements, such as $G_{q/11}$-coupled receptors, PLCβ, DGLα, CB_1R, MGL, and FAAH. Thus, any changes in the functional expression or activity of these elements influence the endocannabinoid system. Evidence is accumulating for its regulation by developmental and environmental factors. Hippocampal DSI is modest at the early stage of development and becomes robust at a later stage. This developmental change in DSI is attributed primarily to postsynaptic mechanisms (Zhu and Lovinger 2010). Experimental manipulations also influence the endocannabinoid system. Experimental seizures, tetanic stimulation, and repetitive low-frequency stimulation upregulate hippocampal DSI. This potentiation of DSI was attributed to an increase in the number of CB_1R on CCK-positive inhibitory terminals. Diet-induced obese mice exhibited many features of the enhanced endocannabinoid system, including elevated levels of 2-AG, anandamide, an increase of DGL activity, and enhancement of hippocampal DSI and eCB-LTD (Massa and others 2010). In contrast, chronic or single exposure to Δ^9-THC decreased cannabinoid sensitivity and blocked eCB-LTD in the nucleus accumbens and hippocampus (Mato and others 2004). Stress down-regulated endocannabinoid sensitivity and blocked DSI/DSE in the paraventricular nucleus of the hypothalamus (Wamsteeker and others 2010). In contrast to these total up- or down-regulation of the endocannabinoid system, selective regulation was achieved by homer1a, which differently regulated different

forms of eCB-STD. In hippocampal neurons, overexpression of homer1a enhanced DSE but inhibited mGluR-driven eCB-STD at the same synapses. BDNF treatment, which increases transcription of homer1a, enhanced DSE and inhibited mGluR-driven eCB-STD (Roloff and others 2010). The mechanisms and physiological significance of these regulations of the endocannabinoid system remain to be elucidated.

Other Functions of Endocannabinoids

In addition to the aforementioned well-defined functions as retrograde messengers in eCB-STD/LTD, endocannabinoids may have other roles in the regulation of neuronal functions. In this section, we describe recent findings on the roles of endocannabinoids in control of other endocannabinoid-independent forms of synaptic plasticity, regulation of neuronal excitability, stimulation of glia-neuron interaction, and induction of CB_1R-independent plasticity. In these functions, endocannabinoids behave not only as retrograde messengers but also as paracrine or autocrine regulators or even as intracellular mediators (Fig. 5). Although these studies have shed light on a diversity of functional roles of endocannabinoids, further studies are necessary to elucidate whether and how they contribute to endocannabinoid-related neural functions.

Modulation of Endocannabinoid-Independent Synaptic Plasticity

Endocannabinoids contribute to synaptic plasticity not only by inducing eCB-STD/LTD but also by modulating other endocannabinoid-independent synaptic plasticity (Shin and others 2010; van Beugen and others 2006) (Fig. 5a). In cerebellar Purkinje cells, parallel fiber (PF) stimulation with a short duration and high frequency induces a presynaptic form of long-term potentiation (LTP), which depends on Ca^{2+}-sensitive adenylyl cyclase and PKA. This PF-LTP was suppressed by coactivation of climbing fibers (CFs) in a CB_1R-dependent manner (van Beugen and others 2006). In the lateral nucleus of the amygdala, low-frequency stimulation of thalamic input induced postsynaptic LTP when it was combined with postsynaptic depolarization but induced presynaptic LTP without postsynaptic depolarization. This result indicates that the combined stimulation not only induces postsynaptic LTP but also suppresses presynaptic LTP. This suppression of presynaptic LTP was shown to involve endocannabinoids, which are released by postsynaptic mGluR1 activation (Shin and others 2010). It is unknown how presynaptic CB_1R activation suppresses presynaptic LTP induction. One possibility is that CB_1R activation suppresses up-regulation of the cAMP/PKA pathway and RIM1α-dependent modifications of the release machinery, which may be required for the induction of presynaptic LTP.

Regulation of Neuronal Excitability

Although the primary function of endocannabinoid signaling is to regulate the synaptic transmission through presynaptic CB_1R, endocannabinoids also modulate excitability of neurons through somatodendritic CB_1R. In the cerebellum, depolarization of Purkinje cells induced a transient decrease in the firing rate of nearby interneurons in a CB_1R-dependent manner (Kreitzer and others 2002) (Fig. 5b). The study suggests that endocannabinoids are released from depolarized Purkinje cells and activate somatodendritic K^+ channels of nearby interneurons through activation of CB_1R. Because interneurons project their axons at some distance, the decrease in the firing rate influences the activities of the target neurons (Fig. 5b'). Thus, the effect of endocannabinoids released from one neuron can spread beyond the limits of endocannabinoid diffusion. In addition to this paracrine action, endocannabinoids may act as autocrine regulators (Fig. 5c). In layer 5 of the cerebral cortex, a subpopulation of GABAergic interneurons, classified as low-threshold spiking (LTS) neurons, underwent a long-lasting hyperpolarization following their own repetitive firing, which was termed slow self-inhibition (SSI) (Bacci and others 2004). This SSI required Ca^{2+} elevation and CB_1R activation for induction and was expressed as a long-lasting increase in somatodendritic K^+ conductance. A further study demonstrated that SSI is dependent on PLC and DGL (Marinelli and others 2008). These results suggest that SSI is mediated by autocrine 2-AG that is produced in a Ca^{2+}-dependent manner through PLC and DGL. The same research group also found that a similar endocannabinoid-mediated SSI can be induced in 31% of cortical layer 2/3 pyramidal neurons (Marinelli and others 2009).

Glia-Neuron Interaction

There is evidence that glial cells contribute to endocannabinoid signaling (Stella 2004). Glial cells express CB_1R and are able to produce and degrade endocannabinoids, indicating that they have the ability to communicate with neighboring neurons and glial cells through endocannabinoid signaling. A recent study demonstrated the presence of such an endocannabinoid-mediated interaction between neurons and astrocytes in the hippocampus (Navarrete and Araque 2010). This study showed that endocannabinoids released from a depolarized pyramidal neuron potentiated the excitatory transmission to neighboring neurons through the mechanisms involving astrocytic CB_1R-driven Ca^{2+}

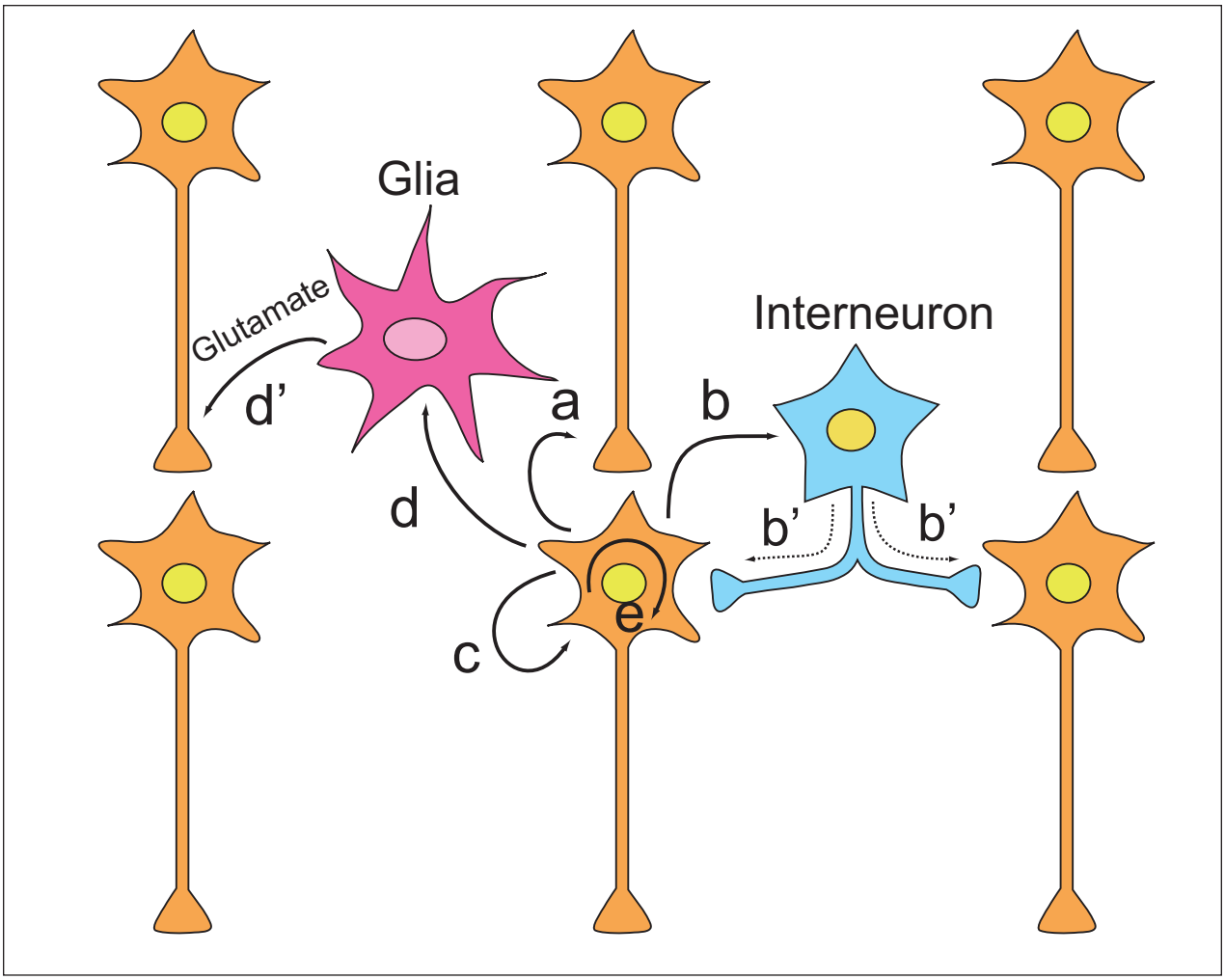

Figure 5. A diversity of functional roles of endocannabinoids. (a) Endocannabinoids released from postsynaptic neurons activate presynaptic CB_1R and suppress a CB_1R-independent form of presynaptic long-term potentiation (LTP). (b) Endocannabinoids released from depolarized neurons act on somatodendritic CB_1R of nearby interneurons and activate K^+ channels. The resultant decrease in the firing rate influences the activities of target neurons (b'). (c) Certain types of neurons undergo a long-lasting hyperpolarization following their own repetitive firing. This hyperpolarization is mediated by autocrine 2-AG. (d) Endocannabinoids released from depolarized neurons activate astrocytic CB_1R and cause Ca^{2+} elevation, which triggers glutamate release from astrocytes. The released glutamate then potentiates the excitatory transmission by acting on presynaptic group I mGluRs (d'). (e) At certain types of synapses, synaptic stimulation induces a novel type of postsynaptic long-term depression (LTD), which is CB_1R independent, TRPV1 dependent, and anandamide dependent. In its induction process, anandamide behaves as an intracellular mediator. See text for details.

elevation (Fig. 5d). According to the proposed model, Ca^{2+} elevation triggers glutamate release from astrocytes, and the released glutamate then potentiates the excitatory transmission through the activation of presynaptic group I mGluRs (Fig. 5d'). The results of this study suggest that endocannabinoids might exert opposite neuromodulatory effects, that is, DSE and the astrocyte-mediated potentiation, in certain experimental conditions. Because spread of endocannabinoids in the extracellular space is limited, DSE should be limited to the synapses close to the endocannabinoid release site. In contrast, astrocyte-mediated potentiation might spread more widely because astrocytes can release glutamate relatively distant from the endocannabinoid release site through CB_1R-mediated release of Ca^{2+} from internal stores.

TRPV1-Dependent Postsynaptic LTD

Two recent studies reported a novel form of LTD, which is anandamide dependent but CB_1R independent. In D_2-positive medium spiny neurons of the nucleus accumbens, low-frequency stimulation induced robust LTD.

This LTD included two components, one being presynaptic and CB_1R dependent and the other being postsynaptic and TRPV1 dependent. The latter component was induced by TRPV1 activation through mGluR5-driven anandamide production and expressed postsynaptically through endocytosis of AMPA receptors (Grueter and others 2010). The TRPV1-dependent postsynaptic LTD in dentate gyrus granule cells, which was induced by paring stimulation of medial perforant path input, exhibited similar properties. This LTD was independent of CB_1R and dependent on postsynaptic mGluR5, PLC, TRPV1, and Ca^{2+} (Chavez and others 2010). During the induction of these postsynaptic forms of LTD, anandamide is thought to behave as an intracellular mediator (Fig. 5e).

Conclusion

In the last two decades, great advances have been made in cannabinoid research. In the 1990s, biochemical studies had identified cannabinoid receptors and endocannabinoids and characterized the enzymes involved in the generation and degradation of endocannabinoids, some of which have been cloned successfully. In the next decade, electrophysiological studies had revealed how endocannabinoids are released from neurons and induce short-term and long-term forms of synaptic plasticity. The endocannabinoid 2-AG is produced from membrane lipids upon postsynaptic Ca^{2+} elevation and/or activation of $G_{q/11}$-coupled receptors and released from postsynaptic neurons. The released 2-AG acts retrogradely onto presynaptic CB_1R and induces suppression of neurotransmitter release. The 2-AG signal is inactivated mostly by presynaptic MGL and to some extent by postsynaptic COX-2 and ABHD6. In parallel with these electrophysiological studies, behavioral studies using pharmacological and genetic tools have revealed that the endocannabinoid system is involved in various aspects of brain functions. Recent studies have revealed more diverse functions of endocannabinoids than previously thought. Endocannabinoids function as not only retrograde messengers but also paracrine or autocrine regulators or even intracellular mediators. It has also been demonstrated that the endocannabinoid system itself is plastic, which can be either up- or down-regulated by experimental or environmental conditions. Of clinical interest is the stress-induced down-regulation of the endocannabinoid system. Because the endocannabinoid system is involved in anxiety, depression, addiction, appetite, and feeding behavior, its down-regulation by stress might influence these neural functions. How is the endocannabinoid system up- or down-regulated by environmental conditions? What is the physiological significance of the plasticity of the endocannabinoid system itself? In the next decade, considerable efforts will be made to address these unsolved issues.

Declaration of Conflicting Interests

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

Funding

The author(s) disclosed receipt of the following financial support for the research and/or authorship of this article: This work has been supported in part by Grants-in-Aid for Scientific Research 21220006 (M.K.), 22-5681 (A.T.), 20021014 (T.O.-S.), and 20500357 (T.O.-S.); the Strategic Research Program for Brain Sciences (Development of Biomarker Candidates for Social Behavior); and the Global COE Program (Integrative Life Science Based on the Study of Biosignaling Mechanisms) from MEXT, Japan.

References

Alger BE. 2002. Retrograde signaling in the regulation of synaptic transmission: focus on endocannabinoids. Prog Neurobiol 68(4):247–86.

Bacci A, Huguenard JR, Prince DA. 2004. Long-lasting self-inhibition of neocortical interneurons mediated by endocannabinoids. Nature 431(7006):312–6.

Best AR, Regehr WG. 2008. Serotonin evokes endocannabinoid release and retrogradely suppresses excitatory synapses. J Neurosci 28(25):6508–15.

Bisogno T, Howell F, Williams G, Minassi A, Cascio MG, Ligresti A, and others. 2003. Cloning of the first sn1-DAG lipases points to the spatial and temporal regulation of endocannabinoid signaling in the brain. J Cell Biol 163(3):463–8.

Blankman JL, Simon GM, Cravatt BF. 2007. A comprehensive profile of brain enzymes that hydrolyze the endocannabinoid 2-arachidonoylglycerol. Chem Biol 14(12):1347–56.

Brenowitz SD, Regehr WG. 2005. Associative short-term synaptic plasticity mediated by endocannabinoids. Neuron 45(3):419–31.

Brown SP, Brenowitz SD, Regehr WG. 2003. Brief presynaptic bursts evoke synapse-specific retrograde inhibition mediated by endogenous cannabinoids. Nat Neurosci 6(10):1048–57.

Chavez AE, Chiu CQ, Castillo PE. 2010. TRPV1 activation by endogenous anandamide triggers postsynaptic long-term depression in dentate gyrus. Nat Neurosci 13(12):1511–8.

Chevaleyre V, Heifets BD, Kaeser PS, Sudhof TC, Castillo PE. 2007. Endocannabinoid-mediated long-term plasticity requires cAMP/PKA signaling and RIM1α. Neuron 54(5):801-12.

Chevaleyre V, Takahashi KA, Castillo PE. 2006. Endocannabinoid-mediated synaptic plasticity in the CNS. Annu Rev Neurosci 29:37–76.

Edwards DA, Kim J, Alger BE. 2006. Multiple mechanisms of endocannabinoid response initiation in hippocampus. J Neurophysiol 95(1):67–75.

Edwards DA, Zhang L, Alger BE. 2008. Metaplastic control of the endocannabinoid system at inhibitory synapses in hippocampus. Proc Natl Acad Sci U S A 105(23):8142–7.

Fiskerstrand T, H'Mida-Ben Brahim D, Johansson S, M'Zahem A, Haukanes BI, Drouot N, and others. 2010. Mutations in ABHD12 cause the neurodegenerative disease PHARC: an inborn error of endocannabinoid metabolism. Am J Hum Genet 87(3):410–7.

Foldy C, Neu A, Jones MV, Soltesz I. 2006. Presynaptic, activity-dependent modulation of cannabinoid type 1 receptor-mediated inhibition of GABA release. J Neurosci 26(5):1465–9.

Freund TF, Katona I, Piomelli D. 2003. Role of endogenous cannabinoids in synaptic signaling. Physiol Rev 83(3):1017–66.

Fukudome Y, Ohno-Shosaku T, Matsui M, Omori Y, Fukaya M, Tsubokawa H, and others. 2004. Two distinct classes of muscarinic action on hippocampal inhibitory synapses: M_2-mediated direct suppression and M_1/M_3-mediated indirect suppression through endocannabinoid signalling. Eur J Neurosci 19(10):2682–92.

Gao Y, Vasilyev DV, Goncalves MB, Howell FV, Hobbs C, Reisenberg M, and others. 2010. Loss of retrograde endocannabinoid signaling and reduced adult neurogenesis in diacylglycerol lipase knock-out mice. J Neurosci 30(6):2017–24.

Grueter BA, Brasnjo G, Malenka RC. 2010. Postsynaptic TRPV1 triggers cell type-specific long-term depression in the nucleus accumbens. Nat Neurosci 13(12):1519–25.

Hashimotodani Y, Ohno-Shosaku T, Kano M. 2007. Presynaptic monoacylglycerol lipase activity determines basal endocannabinoid tone and terminates retrograde endocannabinoid signaling in the hippocampus. J Neurosci 27(5):1211–9.

Hashimotodani Y, Ohno-Shosaku T, Maejima T, Fukami K, Kano M. 2008. Pharmacological evidence for the involvement of diacylglycerol lipase in depolarization-induced endocanabinoid release. Neuropharmacology 54(1):58–67.

Hashimotodani Y, Ohno-Shosaku T, Tsubokawa H, Ogata H, Emoto K, Maejima T, and others. 2005. Phospholipase Cβ serves as a coincidence detector through its Ca^{2+} dependency for triggering retrograde endocannabinoid signal. Neuron 45(2):257–68.

Heifets BD, Castillo PE. 2009. Endocannabinoid signaling and long-term synaptic plasticity. Annu Rev Physiol 71:283–306.

Heifets BD, Chevaleyre V, Castillo PE. 2008. Interneuron activity controls endocannabinoid-mediated presynaptic plasticity through calcineurin. Proc Natl Acad Sci U S A 105(29):10250–5.

Hoffman AF, Laaris N, Kawamura M, Masino SA, Lupica CR. 2010. Control of cannabinoid CB_1 receptor function on glutamate axon terminals by endogenous adenosine acting at A_1 receptors. J Neurosci 30(2):545–55.

Kaeser PS, Kwon HB, Blundell J, Chevaleyre V, Morishita W, Malenka RC and others. 2008. RIM1α phosphorylation at serine-413 by protein kinase A is not required for presynaptic long-term plasticity or learning. Proc Natl Acad Sci U S A 105(38):14680-5.

Kano M, Ohno-Shosaku T, Hashimotodani Y, Uchigashima M, Watanabe M. 2009. Endocannabinoid-mediated control of synaptic transmission. Physiol Rev 89(1):309–80.

Kawamura Y, Fukaya M, Maejima T, Yoshida T, Miura E, Watanabe M, and others. 2006. The CB1 cannabinoid receptor is the major cannabinoid receptor at excitatory presynaptic sites in the hippocampus and cerebellum. J Neurosci 26(11):2991–3001.

Kim J, Alger BE. 2010. Reduction in endocannabinoid tone is a homeostatic mechanism for specific inhibitory synapses. Nat Neurosci 13(5):592–600.

Kim J, Isokawa M, Ledent C, Alger BE. 2002. Activation of muscarinic acetylcholine receptors enhances the release of endogenous cannabinoids in the hippocampus. J Neurosci 22(23):10182–91.

Kreitzer AC, Carter AG, Regehr WG. 2002. Inhibition of interneuron firing extends the spread of endocannabinoid signaling in the cerebellum. Neuron 34(5):787–96.

Kreitzer AC, Malenka RC. 2005. Dopamine modulation of state-dependent endocannabinoid release and long-term depression in the striatum. J Neurosci 25(45):10537–45.

Kreitzer AC, Malenka RC. 2007. Endocannabinoid-mediated rescue of striatal LTD and motor deficits in Parkinson's disease models. Nature 445(7128):643–7.

Kreitzer AC, Regehr WG. 2001a. Cerebellar depolarization-induced suppression of inhibition is mediated by endogenous cannabinoids. J Neurosci 21(20):RC174.

Kreitzer AC, Regehr WG. 2001b. Retrograde inhibition of presynaptic calcium influx by endogenous cannabinoids at excitatory synapses onto Purkinje cells. Neuron 29:717–27.

Lemtiri-Chlieh F, Levine ES. 2010. BDNF evokes release of endogenous cannabinoids at layer 2/3 inhibitory synapses in the neocortex. J Neurophysiol 104(4):1923–32.

Lerner TN, Horne EA, Stella N, Kreitzer AC. 2010. Endocannabinoid signaling mediates psychomotor activation by adenosine A_{2A} antagonists. J Neurosci 30(6):2160–4.

Llano I, Leresche N, Marty A. 1991. Calcium entry increases the sensitivity of cerebellar Purkinje cells to applied GABA and decreases inhibitory synaptic currents. Neuron 6:565–74.

Lourenco J, Cannich A, Carta M, Coussen F, Mulle C, Marsicano G. 2010. Synaptic activation of kainate receptors gates presynaptic CB_1 signaling at GABAergic synapses. Nat Neurosci 13(2):197–204.

Maccarrone M, Dainese E, Oddi S. 2010. Intracellular trafficking of anandamide: new concepts for signaling. Trends Biochem Sci 35(11):601–8.

Maejima T, Hashimoto K, Yoshida T, Aiba A, Kano M. 2001. Presynaptic inhibition caused by retrograde signal from metabotropic glutamate to cannabinoid receptors. Neuron 31:463–75.

Maejima T, Oka S, Hashimotodani Y, Ohno-Shosaku T, Aiba A, Wu D, and others. 2005. Synaptically driven endocannabinoid release requires Ca^{2+}-assisted metabotropic glutamate

receptor subtype 1 to phospholipase Cβ4 signaling cascade in the cerebellum. J Neurosci 25(29):6826–35.

Marinelli S, Pacioni S, Bisogno T, Di Marzo V, Prince DA, Huguenard JR, and others. 2008. The endocannabinoid 2-arachidonoylglycerol is responsible for the slow self-inhibition in neocortical interneurons. J Neurosci 28(50):13532–41.

Marinelli S, Pacioni S, Cannich A, Marsicano G, Bacci A. 2009. Self-modulation of neocortical pyramidal neurons by endocannabinoids. Nat Neurosci 12(12):1488–90.

Marrs WR, Blankman JL, Horne EA, Thomazeau A, Lin YH, Coy J, and others. 2010. The serine hydrolase ABHD6 controls the accumulation and efficacy of 2-AG at cannabinoid receptors. Nat Neurosci 13(8):951–7.

Massa F, Mancini G, Schmidt H, Steindel F, Mackie K, Angioni C, and others. 2010. Alterations in the hippocampal endocannabinoid system in diet-induced obese mice. J Neurosci 30(18):6273–81.

Mato S, Chevaleyre V, Robbe D, Pazos A, Castillo PE, Manzoni OJ. 2004. A single in-vivo exposure to Δ9THC blocks endocannabinoid-mediated synaptic plasticity. Nat Neurosci 7(6):585–6.

Min R, Di Marzo V, Mansvelder HD. 2010a. DAG lipase involvement in depolarization-induced suppression of inhibition: does endocannabinoid biosynthesis always meet the demand? Neuroscientist 16(6):608–13.

Min R, Testa-Silva G, Heistek TS, Canto CB, Lodder JC, Bisogno T, and others. 2010b. Diacylglycerol lipase is not involved in depolarization-induced suppression of inhibition at unitary inhibitory connections in mouse hippocampus. J Neurosci 30(7):2710–5.

Narushima M, Uchigashima M, Fukaya M, Matsui M, Manabe T, Hashimoto K, and others. 2007. Tonic enhancement of endocannabinoid-mediated retrograde suppression of inhibition by cholinergic interneuron activity in the striatum. J Neurosci 27(3):496–506.

Navarrete M, Araque A. 2010. Endocannabinoids potentiate synaptic transmission through stimulation of astrocytes. Neuron 68(1):113–26.

Nevian T, Sakmann B. 2006. Spine Ca^{2+} signaling in spike-timing-dependent plasticity. J Neurosci 26(43):11001–13.

Ohno-Shosaku T, Hashimotodani Y, Ano M, Takeda S, Tsubokawa H, Kano M. 2007. Endocannabinoid signalling triggered by NMDA receptor-mediated calcium entry into rat hippocampal neurons. J Physiol 584(Pt 2):407–18.

Ohno-Shosaku T, Maejima T, Kano M. 2001. Endogenous cannabinoids mediate retrograde signals from depolarized postsynaptic neurons to presynaptic terminals. Neuron 29:729–38.

Okamoto Y, Wang J, Morishita J, Ueda N. 2007. Biosynthetic pathways of the endocannabinoid anandamide. Chem Biodivers 4(8):1842–57.

Piomelli D. 2003. The molecular logic of endocannabinoid signalling. Nat Rev Neurosci 4(11):873–84.

Pitler TA, Alger BE. 1992. Postsynaptic spike firing reduces synaptic $GABA_A$ responses in hippocampal pyramidal cells. J Neurosci 12:4122–32.

Roloff AM, Anderson GR, Martemyanov KA, Thayer SA. 2010. Homer 1a gates the induction mechanism for endocannabinoid-mediated synaptic plasticity. J Neurosci 30(8):3072–81.

Safo PK, Regehr WG. 2005. Endocannabinoids control the induction of cerebellar LTD. Neuron 48(4):647–59.

Shin RM, Tully K, Li Y, Cho JH, Higuchi M, Suhara T, and others. 2010. Hierarchical order of coexisting pre- and postsynaptic forms of long-term potentiation at synapses in amygdala. Proc Natl Acad Sci U S A 107(44):19073–8.

Stella N. 2004. Cannabinoid signaling in glial cells. Glia 48(4):267–77.

Straiker A, Mackie K. 2007. Metabotropic suppression of excitation in murine autaptic hippocampal neurons. J Physiol 578(Pt 3):773–85.

Sugiura T, Kishimoto S, Oka S, Gokoh M. 2006. Biochemistry, pharmacology and physiology of 2-arachidonoylglycerol, an endogenous cannabinoid receptor ligand. Prog Lipid Res 45(5):405–46.

Tanimura A, Yamazaki M, Hashimotodani Y, Uchigashima M, Kawata S, Abe M, and others. 2010. The endocannabinoid 2-arachidonoylglycerol produced by diacylglycerol lipase α mediates retrograde suppression of synaptic transmission. Neuron 65(3):320–7.

Turu G, Hunyady L. 2010. Signal transduction of the CB_1 cannabinoid receptor. J Mol Endocrinol 44(2):75–85.

Uchigashima M, Narushima M, Fukaya M, Katona I, Kano M, Watanabe M. 2007. Subcellular arrangement of molecules for 2-arachidonoyl-glycerol-mediated retrograde signaling and its physiological contribution to synaptic modulation in the striatum. J Neurosci 27(14):3663–76.

Urbanski MJ, Kovacs FE, Szabo B. 2010. Endocannabinoid-mediated synaptically evoked suppression of GABAergic transmission in the cerebellar cortex. Neuroscience 169(3):1268–78.

van Beugen BJ, Nagaraja RY, Hansel C. 2006. Climbing fiber-evoked endocannabinoid signaling heterosynaptically suppresses presynaptic cerebellar long-term potentiation. J Neurosci 26(32):8289–94.

Vandevoorde S, Lambert DM. 2007. The multiple pathways of endocannabinoid metabolism: a zoom out. Chem Biodivers 4(8):1858–81.

Wamsteeker JI, Kuzmiski JB, Bains JS. 2010. Repeated stress impairs endocannabinoid signaling in the paraventricular nucleus of the hypothalamus. J Neurosci 30(33): 11188–96.

Watanabe M, Nakamura M, Sato K, Kano M, Simon MI, Inoue Y. 1998. Patterns of expression for the mRNA corresponding to the four isoforms of phospholipase Cβ in mouse brain. Eur J Neurosci 10(6):2016–25.

Wilson RI, Nicoll RA. 2001. Endogenous cannabinoids mediate retrograde signalling at hippocampal synapses. Nature 410:588–92.

Zhu PJ, Lovinger DM. 2010. Developmental alteration of endocannabinoid retrograde signaling in the hippocampus. J Neurophysiol 103(2):1123–9.