Review

Control of synaptic function by endocannabinoid-mediated retrograde signaling

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Abstract: Since the first reports in 2001, great advances have been made towards the understanding of endocannabinoid-mediated synaptic modulation. Electrophysiological studies have revealed that one of the two major endocannabinoids, 2-arachidonoylglycerol (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 then acts retrogradely onto presynaptic cannabinoid CB_1 receptors and induces suppression of neurotransmitter release either transiently or persistently. These forms of 2-AG-mediated retrograde synaptic modulation are functional throughout the brain. The other major endocannabinoid, anandamide, mediates a certain form of endocannabinoid-mediated long-term depression (LTD). Anandamide also functions as an agonist for transient receptor potential vanilloid receptor type 1 (TRPV1) and mediates endocannabinoid-independent and TRPV1-dependent forms of LTD. 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. In this review, I will make an overview of the mechanisms underlying endocannabinoid-mediated synaptic modulation.

Keywords: endocannabinoid, retrograde signaling, synapse, cannabinoid receptor, 2-arachidonylglycerol, anandamide

1. Introduction

Marijuana, a derivative of the plant *Cannabis sativa*, has been used for recreational and therapeutic purposes for a thousands of years. Marijuana inhalation causes variety of psychomotor effects including euphoria and relaxation, feelings of well-being, grandiosity, altered perception of passage of time, visual distorsions, drowsiness, diminished coordination and memory impairment. Marijuana can also increase appetite, attenuate nausea, and relieve chronic pain, which led to the use of cannabinoids for therapeutic purposes.[1),2)] However, how marijuana modifies various brain functions had been a long-standing mystery. The first breakthrough of cannabinoid research is the identification of Δ^9-tetrahydrocannabinol (Δ^9-THC) as the main psychoactive component of marijuana in 1964.[3)] The second breakthrough is the identification of receptors for Δ^9-THC. The first receptor, termed cannabinoid CB_1 receptor (CB_1R), was found in 1990,[4)] and the second

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Abbreviations: Δ^9-THC: Δ^9-Tetrahydrocannabinol; 2-AG: 2-arachidonoylglycerol; ABHD6 and ABHD12: α/β hydrolase domain 6 and 12; CCK: cholecystokinin; COX-2: cyclooxygenase-2; CaER: Ca^{2+}-driven endocannabinoid release; DGL: diacylglycerol lipase; DHPG: 3,5-dihydroxyphenylglycine; DSE: depolarization-induced suppression of excitation; DSI: depolarization-induced suppression of inhibition; FAAH: fatty acid amide hydrolase; LTD: long-term depression; MGL: monoacylglycerol lipase; NAPE-PLD: *N*-arachidonoyl phosphatidylethanolamine-hydrolyzing phospholipase D; PHARC: polyneuropathy, hearing loss, ataxia, retinitis pigmentosa, and cataract; PLC: phospholipase C; RER: receptor-driven endocannabinoid release; RIM1α: Rab3-interacting molecule 1α; STD: short-term depression; THL: tetrahydrolipstatin; TRPC6: transient receptor potential cation channel subfamily C member 6; TRPV1: transient receptor potential vanilloid type 1 receptor; eCB-LTD: endocannabinoid-mediated long-term depression; eCB-STD: endocannabinoid-mediated short-term depression.

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one, termed cannabinoid CB_2 receptor (CB_2R), was reported in 1993.[5] These are seven-transmembrane receptors coupled to $G_{i/o}$ protein and display distinct expression patterns in animal's body. CB_1Rs are richly expressed in the brain, whereas CB_2Rs are mainly expressed in the immune system of the periphery. The third breakthrough is the identification of endogenous ligands for cannabinoid receptors (endocannabinoid). The first endocannabinoid, *N*-arachidonoylethanolamine, was found in 1992 and termed "anandamide" after the Sanscrit word that means "bliss".[6] In 1995, two groups identified another endocannabinoid, 2-arachidonoylglycerol (2-AG).[7,8] Anandamide and 2-AG have been regarded as two major endocannabinoids in animal's tissues.

The fourth breakthrough, which is functionally most important, is the discovery that endocannabinoids mediate retrograde signaling at synapses. In 2001, my group and two other laboratories independently reported that depolarization of postsynaptic neurons causes endocannabinoid-mediated transient suppression of transmitter release from presynaptic terminals in the hippocampus and cerebellum.[9–11] 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) (for review, see refs. 12–15). Moreover, endocannabinoid-mediated long-term depression (eCB-LTD) was reported in 2002 in the striatum[16] and nucleus accumbens.[17] Later studies have confirmed that eCB-STD and eCB-LTD can be induced at variety of synapses throughout the central and peripheral nervous systems (for review, see refs. 12–15). In support of electrophysiological data, immunohistochemical studies demonstrate that the molecules for retrograde endocannabinoid signaling are arranged around synapses in functionally relevant manners.[18–23] These endocannabinoid signaling molecules are richly expressed in various brain areas including the hippocampus, cerebral cortex, amygdale, dorsal and ventral striatum, hypothalamus, cerebellum, and spinal cord.[13,24] These brain regions are considered to be responsible for the neural functions that have been shown to depend on endocannabinoid signaling. Behavioral studies with pharmacological and genetic manipulation of retrograde endocannabinoid signaling have demonstrated that it is involved in various aspects of neural functions, including learning and memory, mood and anxiety, drug addiction, feeding behavior, motor learning and analgesia (for review, see refs. 13, 25).

Recent studies have revealed that the endocannabinoid system has diverse roles in the modulation of neural circuit function over eCB-STD/LTD. Moreover, endocannabinoid signaling itself has been shown to undergo plastic changes in response to various factors. This review provides an up to date understanding of how endocannabinoid signaling modulates synaptic transmission and neural circuit functions. Space limitation allows me to include a limited number of references. For more information about original papers, several excellent reviews are available.[12–15,26–30]

2. Molecules responsible for endocannabinoid-mediated synaptic modulation CB_1R

The CB_1R, the first identified receptor for Δ^9-THC, is expressed widely throughout the brain but its distribution is heterogeneous at the cellular level.[19–23] For example, in the cerebral cortex, hippocampus and amygdala, CB_1R is highly expressed in a subset of GABAergic inhibitory interneurons that express an intestinal peptide hormone, cholecystokinin (CCK), but almost absent in another class of inhibitory neurons that possess a calcium-binding protein, parvalbumin.[31] In contrast, CB_1R is weakly expressed in most excitatory neurons.[18,19] The CB_1R is preferentially targeted to presynaptic terminals and axons. The expression level of the CB_1R at presynaptic terminals varies greatly depending on brain regions and synapse types. For example, in the cerebellum, the CB_1R is highly expressed in parallel fibers (*i.e.*, the axons of granule cells) and their presynaptic terminals, inhibitory presynaptic terminals of interneurons in the molecular layer, but is absent in axons of Purkinje cells.[22] In the striatum, the CB_1R is highly expressed in inhibitory presynaptic terminals of medium spiny neurons and parvalbumin-positive interneurons, whereas its expression is low at excitatory presynaptic terminals from the cortex and is absent at excitatory terminals from the thalamus.[20]

Activation of CB_1R triggers multiple signal transduction pathways mainly through the $G_{i/o}$ family of heterotrimeric G proteins, including 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.[32] Presynaptic mechanisms underlying endocannabinoid-mediated 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, CB_2R and a full agonist for the transient receptor potential vanilloid type 1 (TRPV1) receptor,[33] a cation channel to which vanilloids such as capsaicin bind. Biochemical pathways for the synthesis of anandamide have not yet been fully understood. Early studies suggested that *N*-acyltransferase and *N*-arachidonoyl phosphatidylethanolamine-hydrolyzing phospholipase D (NAPE-PLD) play a major role for anandamide biosynthesis. Later studies using NAPE-PLD-knockout mice, however, revealed that anandamide can be produced in an NAPE-PLD-independent manner.[34] The major enzyme catalyzing anandamide degradation is fatty acid amide hydrolase (FAAH), which was purified and cloned in 1996.[35]

2-AG. 2-AG functions as a full agonist of CB_1R and CB_2R, and its content in the brain is much higher than that of anandamide.[36] Biochemical studies have found several pathways for 2-AG biosynthesis. 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.[37] Detailed immunoelectron microscopic analyses demonstrate that DGLα is expressed postsynaptically, particularly concentrated at the spine head in hippocampal pyramidal cells[18),20] or at spine neck in cerebellar Purkinje cells.[22]

2-AG is hydrolyzed into arachidonic acid and glycerol primarily by monoacylglycerol lipase (MGL), which was first cloned from mouse adipose tissue.[36] A functional proteomic study has demonstrated that MGL accounts for ~85% of 2-AG hydrolysis, and that other enzymes, such as α/β hydrolase domain 6 and 12 (ABHD6 and ABHD12), contributes to the hydrolysis of the remaining 15%.[38] Since MGL and ABHD6 display different distribution patterns, the former being presynaptic and the latter being postsynaptic (Fig. 1), these two enzymes are considered to have non-overlapping functions.[39] Interestingly, a neurodegenerative disease named PHARC (polyneuropathy, hearing loss, ataxia, retinitis pigmentosa, and cataract) is caused by mutations in the ABHD12 gene, indicating that ABHD12 plays important roles in the central and peripheral nervous systems and the eye.[40] The apparent paradox of its minor contribution to 2-AG hydrolysis versus serious PHARC phenotype remains to be resolved. Besides hydrolysis, oxidation by cyclooxygenase (COX) is another pathway for 2-AG degradation. Among three forms of COX (COX-1, -2, -3), only COX-2 preferably recognizes 2-AG as an effective substrate.[41] Since morphological data suggest that Cox-2 is expressed at postsynaptic sites,[42] this enzyme may play complementary roles with MGL in 2-AG degradation.

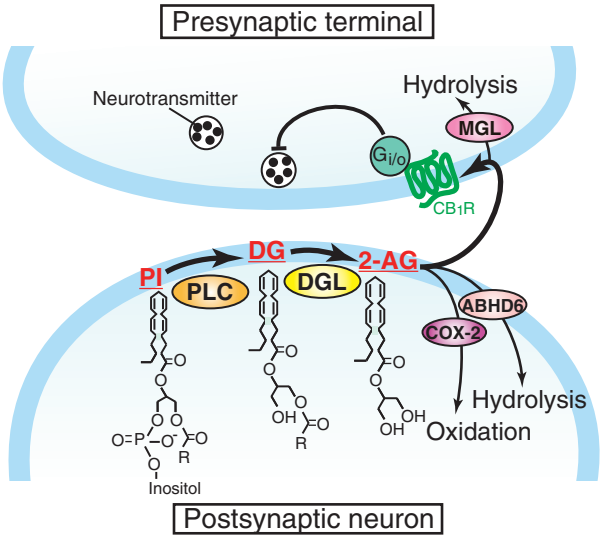

Fig. 1. Key features of 2-AG signaling. PLC hydrolyzes arachidonic acid-containing membrane phospholipid such as phosphatidylinositol (PI) and generates diacylglycerol (DG), which is then converted to 2-AG by DGL. The 2-AG released from the postsynaptic neuron enters into the presynaptic membrane, activates CB_1R and is degraded by presynaptic MGL. In the postsynaptic neuron, 2-AG is degraded by ABHD6 or oxigenated by COX-2. Activation of presynaptic CB_1R causes suppression of neurotransmitter release through inhibition of Ca^{2+} channels or some other mechanisms. (Reproduced from Ohno-Shosaku *et al.*, 2012[15]).

3. Endocannabinoid-mediated short-term depression (eCB-STD)

Since the first reports on endocannabinoid as synaptic retrograde messengers in 2001,[9)–11] numerous electrophysiological studies have been performed. It is now widely accepted that endocannabinoids function as retrograde messengers and contribute to short-term and long-term synaptic plasticity (for review, see refs. 12–15). 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.

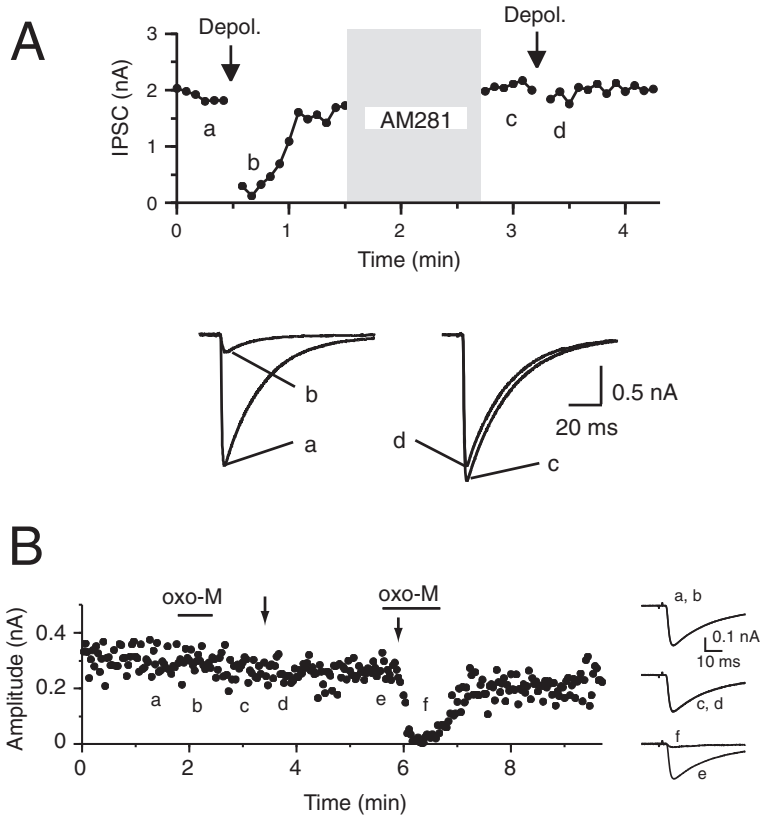

Fig. 2. Representative cases for Ca^{2+}-driven endocannabinoid release (CaER) and Ca^{2+}-assisted Receptor-driven endocannabinoid release (RER). **A.** Representative data showing DSI, a form of CaER, in a neuron pair from a hippocampal culture. The presynaptic neuron was stimulated and inhibitory postsynaptic currents (IPSCs) were recorded from the postsynaptic neuron. Amplitudes of IPSCs were plotted as a function of time (upper panel) and IPSCs recorded at the indicated time points were shown in the lower panels. Depolarizing pulses (to 0 mV for 5 sec) to the postsynaptic neuron (downward arrow) induced a transient suppression of IPSCs (DSI). After treatment of the CB_1 antagonist AM281, DSI was abolished. (Reproduced from Hashimotodani *et al.*, 2007[28]). **B.** Representative data showing Ca^{2+}-assisted RER in a neuron pair from a hippocampal culture. Time course of IPSC amplitude (left panel) and IPSC recorded at the indicated time points (right panels) were shown. Application of a low concentration of the muscarinic agonist oxotremorine-M (oxo-M, 0.3 µM) or a weak depolarizing pulse (downward arrow) caused no detectable suppression of IPSCs when applied alone. However, combined application of these two caused a robust suppression of IPSCs. (Reproduced from Hashimotodani *et al.*, 2005[60]).

After release from postsynaptic neurons, endocannabinoids activate presynaptic CB_1R, and suppress transmitter release transiently (eCB-STD) or persistently (eCB-LTD). Various forms of eCB-STD/LTD have been reported to occur at inhibitory and excitatory synapses in various regions of the brain. In this section, I will 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). Endocannabinoid release can be induced by a large postsynaptic Ca^{2+} elevation alone, which has been termed Ca^{2+}-driven endocannabinoid release (CaER).[13),15)] This form of eCB-STD includes DSI (depolarization-induced suppression of inhibition)[10),11)] (Fig. 2A), DSE (depolarization-induced suppression of excitation),[9)] and presynaptic suppression caused by Ca^{2+} influx through NMDA-type glutamate receptors (NMDARs).[43)]

DSI was reported in early 1990s first in the cerebellum and then in the hippocampus. In 1991, Llano *et al.* discovered that depolarization of cerebellar Purkinje cells induces transient suppression of inhibitory inputs to the depolarized cells.[44)] In 1992, Pitler and Alger reported the same phenomenon in hippocampal CA1 neurons and termed it "DSI".[45)] Because DSI requires postsynaptic Ca^{2+} elevation and causes suppression of transmitter release from

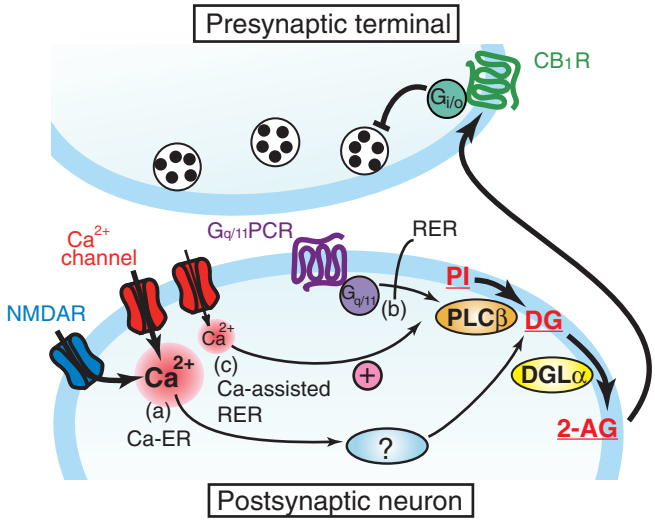

Fig. 3. Molecular mechanisms of eCB-STD. When a large Ca^{2+} elevation is caused by activation of voltage-gated Ca^{2+} channels or NMDARs, 2-AG is generated in a DGLα-dependent manner (**a**, CaER). A key enzyme of this pathway, which is expected to be activated by Ca^{2+} elevation and produce diacylglycerol, 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**, RER). When subthreshold activation of $G_{q/11}$-coupled receptors is combined with a small Ca^{2+} elevation, 2-AG is produced through PLCβ-dependent pathway because the receptor-driven PLCβ stimulation is Ca^{2+}-dependent (**c**, Ca^{2+}-assisted RER). 2-AG is released from postsynaptic neurons, activates presynaptic CB_1R, and induces transient suppression of transmitter release. (Reproduced with modification from Ohno-Shosaku *et al.*, 2012[15]).

presynaptic terminals, some mechanisms of "retrograde signaling" were thought to exist. Candidates of such retrograde messengers were sought, but it took 10 years to identify the mechanism that mediates DSI. In 2001, Ohno-Shosaku *et al.* and Wilson and Nicoll reported independently that endocannabinoids mediate retrograde signaling for DSI in the hippocampus[10),11)] (see Fig. 2A). Since then, endocannabinoid-mediated DSI has been reported in various brain regions including the striatum, globus pallidus, substantia nigra, cerebral cortex, amygdala, and hypothalamus (for review, see refs. 13, 26, 46).

At the same time of the discovery that endocannabinoids mediate DSI, Kreitzer and Regehr discovered that depolarization of cerebellar Purkinje cells induces transient suppression of excitatory transmission,[9)] which was termed DSE. They found that, similarly to DSI, DSE was induced by postsynaptic Ca^{2+} elevation, expressed presynaptically, and mediated by retrograde endocannabinoid signaling.[9)] DSE has also been reported in many other brain regions including the hippocampus, cerebral cortex, hypothalamus, ventral tegmental area, and dorsal cochlear nucleus (for review, see refs. 13, 26, 46).

DSI and DSE are dependent on Ca^{2+} influx to postsynaptic neurons through voltage-gated Ca^{2+} channels. It was unclear whether the NMDA-type glutamate receptor (NMDAR), another major route of Ca^{2+} influx into neurons, can contribute to endocannabinoid release. In 2007, Ohno-Shosaku *et al.* demonstrated that stimulation of NMDARs induces a transient suppression of inhibitory transmission in cultured hippocampal neurons, which requires postsynaptic Ca^{2+} elevation and endocannabinoid signaling.[43)] Blockade of postsynaptic voltage-gated Ca^{2+} channels had no effect on NMDAR-driven eCB-STD, indicating that Ca^{2+} influx through NMDAR directly induces endocannabinoid release.[43)]

A current model for the mechanisms of eCB-STD depending on CaER (DSI/DSE and NMDAR-driven eCB-STD) is illustrated in Fig. 3(**a**). Activation of voltage-gated Ca^{2+} channels or NMDARs causes a large Ca^{2+} elevation in postsynaptic neurons, and produces 2-AG in a DGLα-dependent manner. 2-AG is then released from postsynaptic neurons, binds to presynaptic CB_1R and suppresses transmitter release by inhibiting presynaptic voltage-gated Ca^{2+} channels. CaER requires elevation of intracellular Ca^{2+} concentration to micromolar range, but the Ca^{2+} level might be dependent on the duration of Ca^{2+} elevation. Several lines of evidence strongly suggest that 2-AG rather than anandamide mediates eCB-STD depending on CaER. Inhibition of 2-AG hydrolyzing enzyme (MGL), but not anandamide hydrolyzing enzyme

(FAAH) has been shown to prolong DSI/DSE.[47] Most importantly, Tanimura *et al.* (2010) and Gao *et al.* (2010) demonstrated independently that DSI and/or DSE are totally absent in the hippocampus, striatum or cerebellum of mice lacking DGLα.[48),49] Later, Yoshino *et al.* confirmed the lack of DSI in the prefrontal cortex of DGLα deficient mice.[50]

How Ca^{2+} elevation in neurons leads to 2-AG synthesis and release in a DGLα-dependent manner remains unclear. A simple model is that Ca^{2+} activates an unidentified enzyme that produces diacylglycerol which is then converted to 2-AG by DGLα, as illustrated in Fig. 3**(a)**. There is, however, a controversy as to this "on-demand 2-AG synthesis model" which I will discuss later.

STD depending on receptor-driven endocannabinoid release (RER). Soon after the reports on the roles of endocannabinoid signaling in DSI/DSE, Maejima *et al.* reported that activation of group I metabotropic glutamate receptor (mGluR) causes eCB-STD in cerebellar Purkinje cells in a Ca^{2+}-independent manner.[51] After this report, various $G_{q/11}$-coupled receptors including, M_1/M_3 muscarinic, 5-HT_2-type serotonin (5-HT_2R), orexin and oxytocin receptors have been shown to be capable of driving eCB-STD (for review, see refs. 13–15). In these forms of eCB-STD, the endocannabinoid release is induced by strong activation of the receptors without need of postsynaptic Ca^{2+} elevation,[51] which is termed receptor-driven endocannabinoid release (RER).[13),15]

The mGluRs include eight subtypes (mGluR1-8), and are divided into three groups, namely group I (mGluR1 and 5), group II (mGluR2, 3) and group III (mGluR4, 6, 7 and 8). Group I mGluRs are coupled to $G_{q/11}$-type of G proteins. In 2001, Maejima *et al.* reported that application of the group I mGluR agonist 3,5-dihydroxyphenylglycine (DHPG) caused a transient suppression of the excitatory transmission to Purkinje cells.[51] This suppression was abolished when the G protein signaling in postsynaptic neurons is blocked by inclusion of GTPγS or GDPβS in the recording pipette, indicating that some retrograde signaling from postsynaptic neurons to presynaptic terminals is required. This phenomenon occurred with a high concentration of the Ca^{2+} chelator BAPTA in the recording pipette, indicating that group I mGluR activation can trigger eCB-STD in a Ca^{2+}-independent manner. 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 (for review, see refs. 13–15).

Subsequently, Kim *et al.* reported in hippocampal slices that a cholinergic agonist, carbachol, suppressed inhibitory synaptic transmission to CA1 pyramidal cells in a CB_1R-dependent manner.[52] They showed pharmacologically that the receptors responsible for this effect were muscarinic acetylcholine receptors (mAChRs) rather than nicotinic receptors.[52] Among the five subtypes of mAChRs (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 $G_{i/o}$. Fukudome *et al.* determined the subtypes of mAChRs involved in eCB-STD by using genetically engineered mice lacking each subtype of mAChRs. The data clearly demonstrate that M_1 and M_3 receptors are responsible for the induction of eCB-STD.[53] The mAChR-driven eCB-STD is also found at hippocampal excitatory synapses[54] and striatal inhibitory synapses.[55]

After the discoveries of mGluR- and mAChR-driven eCB-STDs, several groups demonstrated that other $G_{q/11}$-coupled receptors also can 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 suppressed excitatory synaptic transmission. This 5-HT_2R-dependent component of suppression was abolished by blockade of CB_1R, indicating that 5-HT_2R drives eCB-STD.[56] In addition, $G_{q/11}$-coupled receptors for neuropeptide are also capable of driving endocannabinoid release. Suppressing effects of orexin-B on the excitatory transmission to serotonergic neurons in the dorsal raphe nucleus,[57] and oxytocin on both the excitatory and inhibitory synaptic transmissions to magnocellular neurons in the supraoptic nucleus[58] have been reported. These forms of synaptic suppression were CB_1R-dependent, and therefore can be classified as receptor-driven eCB-STD.

Figure 3**(b)** illustrates a current model for the mechanisms underlying $G_{q/11}$-coupled receptor-driven eCB-STD without need of Ca^{2+} elevation. When postsynaptic $G_{q/11}$-coupled receptors are stimulated, diacylglycerol is produced through PLCβ, and converted to 2-AG by DGLα. 2-AG is then released from postsynaptic neurons, binds to presynaptic CB_1R and suppresses transmitter release. PLCβ consists of 4 isoforms (PLCβ1-4) and the type of PLCβ is different in different brain areas.[59] Hashimotodani *et al.* demonstrated that eCB-STD driven by mGluR or mAChR activation is absent in PLCβ1-deficient hippocampal neurons,[60] while Maejima *et al.* found that mGluR-driven eCB-STD is absent in PLCβ4-deficient cerebellar Purkinje cells.[61] These electro-

physiological data are consistent with those of *in situ* hybridization showing that PLCβ1 and PLCβ4 are the major PLCβ isozymes in the hippocampus and the rostral portion of the cerebellum, respectively.[59] Studies using DGL inhibitors[20),47),57),62),63] and DGLα-KO mice[49] support the involvement of DGLα in $G_{q/11}$-coupled receptor-driven eCB-STD. The experimental results so far reported are largely consistent with this model. There is, however, also a controversy as to this "on-demand 2-AG synthesis model" and I will discuss this issue later.

STD depending on Ca^{2+}-assisted RER. The third form of eCB-STD can be induced when small Ca^{2+} elevation and weak $G_{q/11}$-coupled-receptor activation, both of which are subthreshold for triggering endocannabinoid release, are applied simultaneously[52),55),60),61),64)–66] (see Fig. 2B). This phenomenon results from the enhancement of RER by subthreshold Ca^{2+} elevation. In cultured hippocampal neurons, Hashimotodani *et al.* monitored the degree of PLCβ activation by mAChR using the diacylglycerol-activated cation channel TRPC6 (transient receptor potential cation channel subfamily C member 6) as a biosensor for the PLC activity.[60] They confirmed that the TRPC6-mediated inward current induced by muscarinic agonist was dependent on PLCβ1. Then they demonstrated that the current was highly sensitive to intracellular Ca^{2+} concentration within a physiological range, and was greatly enhanced by a Ca^{2+} elevation.[60] Moreover, they confirmed that mGluR-driven and mAChR-driven eCB-STDs depended on intracellular Ca^{2+} levels in the hippocampus.[60] In the same year, Maejima *et al.* reported that mGluR-driven eCB-STD was dependent on intracellular Ca^{2+} levels in cerebellar Purkinje cells.[61] Figure 3**(c)** shows a current model of the mechanisms underlying the endocannabinoid release induced by combined subthreshold Ca^{2+} elevation and receptor activation, which is termed Ca^{2+}-assisted RER.[64] 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 PLCβ-dependent pathway. Another possible candidate mediating Ca^{2+}-assisted RER is PLCγ. Lemtiri-Chlieh and Levine reported that BDNF suppresses inhibitory transmission in a CB_1R-dependent manner in the neocortex, which is suppressed by inhibition of postsynaptic Ca^{2+} rise.[67] Although molecular mechanisms underlying this eCB-STD are not determined, a likely candidate is Ca^{2+}-assisted RER involving PLCγ instead of PLCβ.

Since weak depolarization of postsynaptic membrane and activation of postsynaptic $G_{q/11}$-coupled receptors can be achieved by synaptic activity, Ca^{2+}-assisted RER appears to be most relevant for synaptically-driven eCB-STD (see below) among CaER, RER and Ca^{2+}-assisted RER. Another important feature of Ca^{2+}-assisted RER is that this mechanism is suitable for detecting coincidence between the activity of presynaptic terminals and that of postsynaptic neurons. More specifically, PLCβ functions as a coincidence detector of $G_{q/11}$-coupled receptor activation and Ca^{2+} elevation, leading to the production of 2-AG.[60),64] In line with this notion, PLCβ may play a crucial role as a coincident detector in timing-dependent eCB-LTD.[68]

Synaptically-driven eCB-STD. In several brain areas, synaptic activity has been demonstrated to induce eCB-STD, which is termed synaptically-driven eCB-STD.[13),66] Repetitive stimulation of excitatory synaptic inputs is often an effective way of inducing robust eCB-STD. When sufficient glutamate is released from excitatory presynaptic terminals by repetitive stimulation, it can induce postsynaptic depolarization mediated by AMPA receptors and resultant Ca^{2+} elevation, and simultaneous activation of mGluR. Each of these three events may be capable of triggering endocannabinoid release by itself. Synaptically-driven eCB-STD may thus involve CaER, RER or Ca^{2+}-assisted RER, depending on stimulation and recording conditions. At parallel fiber to Purkinje cell synapses in the cerebellum, eCB-STD can be induced by a brief burst of parallel fiber stimulation (5–10 pulses at 50–100 Hz).[61),69] This synaptically-driven eCB-STD required both Ca^{2+} elevation and mGluR1 activation in postsynaptic Purkinje cells, indicating predominant contribution of Ca^{2+}-assisted RER.[61] In contrast, eCB-STD induced by intense parallel fiber stimulation (100 pulses at 100 Hz) was resistant to an mGluR1 antagonist, indicating that contribution of CaER is predominant.[61] Furthermore, Brenowitz and Regehr reported that parallel fiber to Purkinje cell synapses undergo eCB-STD when subthreshold parallel fiber stimulation was combined with stimulation of climbing fiber, the other excitatory input to Purkinje cells.[70] Climbing fiber inputs induce depolarization and resultant Ca^{2+} elevation in Purkinje cells, whereas parallel fiber stimulation activates mGluR1 concentrated postsynaptically, which fulfills the requirements of Ca^{2+}-assisted RER. On the other hand, at inhibitory synapses on Purkinje cells, eCB-STD can be induced by intense

climbing fiber stimulation (50 pulses at 5 Hz).[71] This heterosynaptic eCB-STD is thought to be dependent on endocannabinoids released from Purkinje cells by climbing fiber stimulation presumably through CaER.

Degradation of 2-AG after eCB-STD. Duration of eCB-STD is dependent on the speed of degradation and removal of 2-AG around synaptic terminals. Since about 85% of 2-AG is hydrolyzed into arachidonic acid and glycerol by MGL,[38] the localization of this enzyme was thought to be crucial for determining the duration of eCB-STD. Immunohistochemical data indicate that the expression of MGL is highly heterogeneous, being very rich at particular types of presynaptic terminals but totally absent at others.[21),72] MGL is also present in astrocypes.[21),72] Tanimura *et al.* recently reported that eCB-STD is prolonged rather non-selectively at both MGL-positive and MGL-negative synapses in the cerebellum of MGL knockout mice.[72] This study also shows that eCB-STD is prolonged in the cerebellum of conditional MGL knockout mice in which MGL is expressed only in astroglia.[72] This study clearly indicates that 2-AG is degraded in a synapse non-specific manner by MGL concentrated in particular cell types including astroglia.[72]

4. On-demand vs. pre-formed

2-AG is generally thought to be synthesized "on demand" upon cellular stimulation. However, this "on-demand synthesis model" was recently challenged and an alternative model was proposed.[73),74] According to this model, 2-AG is constitutively synthesized by DGLα and pooled within cells and mobilized from this hypothetical "pre-formed 2-AG pools" upon stimulation without the contribution of DGLα.[73),74] The reason for developing this new model was to reconcile the apparent discrepancy between the results from DGLα knockout mice and those from pharmacological blockade of DGL. Three groups independently generated DGLα knockout mice and they consistently exhibit complete loss of eCB-STD.[48)–50] In contrast, the reported effects of acute pharmacological blockade of DGL on eCB-STD were highly controversial.[73] A classical DGL inhibitor, tetrahydrolipstatin (THL), inhibited eCB-STD in some studies,[28),75)–77] but not in others.[78),79] Inconsistent results were also reported regarding a novel potent DGL inhibitor, OMDM-188. This compound failed to inhibit DSI in one study,[80] whereas it inhibited DSI but not mGluR-driven STD in another study.[77] Recently, Hashimotodani *et al.*, systematically reexamined the effects of OMDM-188 on eCB-STD induced by CaER, basal RER, Ca^{2+}-assisted RER and synaptically-driven eCB release in acute slices of the hippocampus, cerebellum and striatum as well as in cultured hippocampal neurons.[81] The data clearly demonstrate that all forms of eCB-STD examined, including DSI, DSE, receptor-driven eCB-STD and synaptically-driven eCB-STD, are consistently blocked by the treatment with OMDM-188.[81] Importantly, application of OMDM-188 as short as 2 min was sufficient to block DSI in cultured hippocampal neurons.[81] This study has confirmed that there is no discrepancy in the results between genetic and pharmacological blockade of DGL, and strongly support the "on-demand synthesis model".

The reasons for the inconsistent results in previous studies regarding the effect of DGL inhibitors are unclear. One possibility is insufficient penetration of these compounds into brain tissues. Since OMDM-188 and THL are highly lipophilic, they are difficult to penetrate into brain slices. The effectiveness of these DGL inhibitors therefore depends on whether their targets (*e.g.*, soma or dendrites) are located near the surface or in the depth of slices. Another possibility is related to whether DGL activity is rate limiting for 2-AG production or not. For example, if DGL activity is rate limiting for CaER, but not for RER, these two types of eCB-STD can have different sensitivity to the DGL inhibitors.[77]

5. Endocannabinoid-mediated long-term depression (eCB-LTD)

Long-term depression (LTD) of synaptic transmission dependent on retrograde endocannabinoid signaling (eCB-LTD) has been found in several areas of the brain. Because an excellent review focusing on eCB-LTD is available,[29] its main features will be briefly summarized here. eCB-LTD occurs 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.[29] eCB-LTD is induced mostly by repetitive afferent stimulation with or without postsynaptic depolarization, and also by postsynaptic firing[16),82] (for review, see refs. 12, 13). Evidence suggests that, eCB-LTD is mediated by 2-AG in most cases and that eCB-LTD and eCB-STD share the same molecular mechanisms for 2-AG release (*i.e.*, CaER, basal RER and Ca^{2+}-assisted RER) (Fig. 4). Typically, afferent stimulation causes glu-

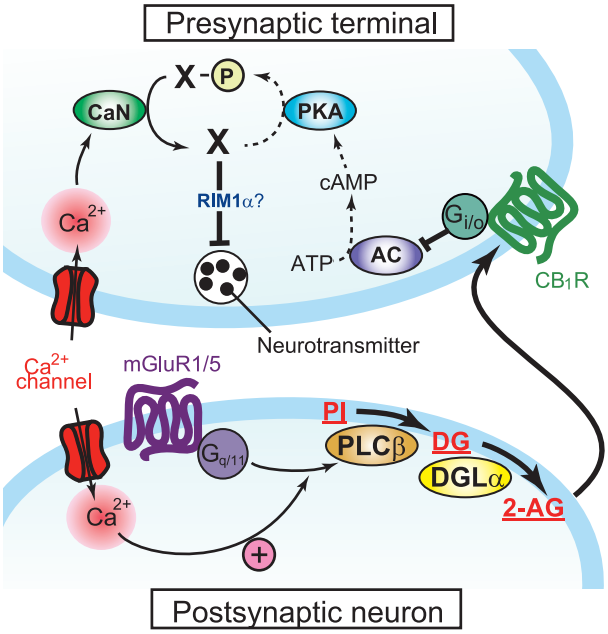

Fig. 4. Molecular mechanisms of eCB-LTD. Excitatory synaptic inputs activate group I mGluR, which facilitates 2-AG synthesis through PLCβ-DGLα pathway. This mGluR-driven 2-AG synthesis is enhanced by concomitant Ca^{2+} elevation, which is not necessary for eCB-LTD induction at some synapses. Activation of presynaptic CB_1R by 2-AG inhibits adenylyl cyclase (AC), decreases cAMP levels and reduces 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. (Reproduced from Ohno-Shosaku *et al.*, 2012[15]).

tamate release from excitatory presynaptic terminals, activates AMPA receptors and mGluRs on the postsynaptic membrane, and induces 2-AG release. 2-AG then activates CB_1Rs on the same presynaptic terminals releasing glutamate (homosynaptic) or neighboring presynaptic terminals (heterosynaptic). eCB-STD can be induced by a brief burst of afferent stimulation and resultant release of 2-AG that causes transient activation of presynaptic CB_1Rs. In contrast, eCB-LTD requires a long-lasting afferent stimulation for several minutes (5 to 10 minutes) and resultant release of 2-AG that causes persistent activation of CB_1Rs. Importantly, LTD induced by spike timing stimulation in the rodent somatosensory cortex has been reported to involve endocannabinoid signaling.[83] This form of spike-timing dependent plasticity may underlie development and critical period plasticity of the whisker map in the rodent somatosensory cortex.[84]

Although presynaptic mechanisms of eCB-LTD have not been fully elucidated, inhibition of the cAMP/PKA pathway seems to be crucial.[12),15] Involvement of calcineurin, the small GTP-binding protein Rab3, and Rab3-interacting molecule 1α (RIM1α), has also been suggested[12),15] (Fig. 4). In most, if not all cases, activation of CB_1R is not enough to induce LTD, and an additional presynaptic mechanism(s) is required,[85] such as presynaptic electrical activity,[86),87] Ca^{2+} elevation,[86),87] NMDAR activation,[88] M_2 receptor activation[89] and/or D_2 receptor activation.[90),91] This associative feature may ensure the selective induction of eCB-LTD at active synapses. For example, presynaptic activity induces Ca^{2+} elevation and leads to activation of calcineurin, which shifts the phosphorylation/dephosphorylation balance towards dephosphorylation in concert with reduced PKA activity by CB_1R activation. The resulting decrease in phosphorylation of target proteins causes suppression of transmitter release selectively from presynaptic terminals that have been active during CB_1R activation (Fig. 4).

6. Roles of anandamide in synaptic modulation

Several recent studies demonstrate that the other major endocannabinoid, anandamide, also contributes to the modulation of synaptic transmission. In particular, anandamide-mediated LTD has been reported in several brain areas.[92)–96] Two distinct types of eCB-LTD has been reported in medium spiny neurons (MSNs) of the indirect pathway in the dorsolateral striatum.[95] Low frequency stimulation induces LTD mediated by 2-AG, whereas high frequency stimulation induces CB_1-dependent LTD that is mediated by anandamide.[95] Since anandamide is a partial agonist for CB_1R and a full agonist for TRPV1, it may contribute to LTD through two distinct pathways involving CB_1R and TRPV1. In line with this notion, Grueter *et al.* suggested that low frequency stimulation induces anandamide-mediated LTD, partly by activating presynaptic CB_1Rs and partly by activating postsynaptic TRPV1 channels in indirect pathway MSNs of the nucleus accumbens.[93] In the dentate gyrus, Chavez *et al.* reported that pairing stimulation induces LTD that is CB_1-independent and TRPV1-dependent.[92] They showed this LTD was expressed postsynaptically and mediated by anandamide.[92] In contrast, Puente *et al.* suggested that 2-AG mediates both eCB-STD and eCB-LTD in the striatum, whereas 2-AG and anandamide mediate eCB-STD and TRPV1-dependent LTD, respectively, in the

extended amygdala.[96] Besides contribution to LTD induction, anandamide has been shown to generate the endocannabinoid tone. Experimental evidence suggests that it contributes to homeostatic plasticity in the hippocampus[97] and the regulation of activity along hypothalamic-pituitary-adrenal (HPA) axis in the amygdala.[98] Taking together, the two major endocannabinoids, 2-AG and anandamide, are considered to mediate different types of signals for synaptic modulation.[99] 2-AG primarily transmits a rapid, transient, point-to-point retrograde endocannabinoid signal. In contrast, anandamide may mediate a relatively slow, retrograde or non-retrograde endocannabinoid signal and/or may function as a vanilloid agonist.

7. Plasticity of the endocannabinoid system

As described in previous sections, the endocannabinoid system involves various molecules including voltage-gated Ca^{2+} channels, $G_{q/11}$-coupled receptors, PLCβs, DGLα, MGL and CB_1R. Therefore any changes in their functional activities, expression levels or subcellular distribution can influence the performance of the endocannabinoid system. Recent studies suggest that the endocannabinoid system is regulated by various postsynaptic and presynaptic receptors, and also influenced by environmental and pathological factors.[100] For example, in human and rodents are known to cause behavioral tolerance to cannabinoids,[25),101] which is considered to result from down-regulation of CB_1R. Furthermore, MGL knockout mice and mice with chronic administration of an MGL inhibitor demonstrate cannabinoid tolerance including reduced antinociceptive and hypothermic responses to Δ^9-THC.[102] These symptoms are attributed to chronic activation of CB_1Rs by excess 2-AG and resultant down-regulation of these receptors.

Receptors on the postsynaptic membrane can influence endocannabinoid release. Lerner and Kreitzer showed that postsynaptic D_2 and A_{2A} receptors on MSNs of the striatum regulated endocannabinoid release and eCB-LTD positively and negatively, respectively, by modulating mGluR signaling through regulator of G protein signaling 4 (RGS4).[95] On the other hand, Shonesy *et al.* reported that enzymatic activity of DGLα was reduced after phosphorylation by CaMKII,[103] suggesting the possibility that DGLα might be regulated by CaMKII-involving signals. On the presynaptic side, CB_1R signaling has been shown to be influenced by the activity of colocalized presynaptic receptors. In the hippocampus, Hoffman *et al.* reported that tonic activation of A_1 receptors by endogenous adenosine inhibits presynaptic CB_1 signaling.[104] Because CB_1 and A_1 receptors are both $G_{i/o}$-coupled, they may use overlapping sets of Gα subunits and interact with each other.

Endocannabinoid signaling can also be influenced by environmental factors. For example, stress can alter the endocannabinoid system through HPA axis. Conversely, the endocannabinoid system is known to regulate the HPA axis under both basal and stressful conditions. Several studies indicate that stress-induced HPA axis activity can alter the endocannabinoid system, either positively or negatively depending on the brain region, age and conditions of stress, in several brain regions including the hippocampus, amygdala, hypothalamus, nucleus accumbens and prefrontal cortex.[98),100),105] For example, repetitive immobilization stress is reported to cause down-regulation of CB_1R and impair DSI/DSE in the paraventricular nucleus of the hypothalamus.[106] In contrast, chronic stress caused a partial down-regulation of MGL and enhanced DSI in the basolateral amygdala.[107] Interestingly, acute restraint stress switched CB_1-dependent plasticity from LTD to LTP in the bed nucleus of the stria terminalis, and this stress-elicited shift in plasticity was controlled by CB_1-Rs on excitatory terminals.[108] Molecular and neural circuit mechanisms of these stress-induced plastic changes in the endocannabinoid system remain to be elucidated.

8. Conclusion

In the last two decades, great advances have been made in the cannabinoid research. In 1990s, biochemical studies have identified cannabinoid receptors (CB_1R and CB_2R) and endocannabinoids (anandamide, 2-AG and others), and characterized the enzymes involved in generation and degradation of endocannabinoids, some of which have been cloned successfully. In the next decade, electrophysiological studies 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. After causing synaptic suppression, 2-AG is degraded in a synapse nonspecific manner by MGL concentrated in particular

cell types including astroglia. Postsynaptic COX-2 and ABHD6 may also contributes to inactivation of 2-AG. Besides 2-AG, anandamide has been shown to mediate a certain form of eCB-LTD as well as eCB-independent and TRPV1-dependent form of LTD. 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. Although endocannabinoid signaling is involved in various forms of short-term and long-term synaptic modulation, it remains largely unknown how each form contributes to particular aspects of neural functions. Future studies will elucidate the molecular, cellular and neural circuit mechanisms as to how endocannabinoid-mediated synaptic modulation contributes to various brain functions and the pathophysiology of neuropsychiatric disorders.

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Profile

Masanobu Kano was born at Shizuoka in 1957. He received his medical degree from Tokyo Medical and Dental University in 1982. He completed his PhD at the University of Tokyo, Faculty of Medicine in 1986, where he worked on identification of glutamate receptor subtype involved in cerebellar long-term depression. He became a research associate at Jichi Medical School (Tochigi, Japan) in 1986 and studied cerebellar control of optokinetic eye movements. In 1990, he became independent as an assistant professor at Jichi Medical School, and then joined Max-Planck Institute for biophysical chemistry in Goettingen, Germany, as a visiting researcher. In Goettingen, he learned patch clamping and Ca^{2+} imaging from neurons in brain slices, and discovered long-term potentiation of inhibitory synapse in the cerebellum. He returned to Jichi Medical School in 1992, and started to examine postnatal development and plasticity of cerebellar synapses. He moved to the RIKEN Institute (Wako, Japan) in 1995, and then he became Professor of Physiology at Kanazawa University School of Medicine (Kanazawa, Japan) in 1998. In Kanazawa, his group discovered that endogenous cannabinoids function as a retrograde messenger at synapses. In 2005, he became Professor of Cellular Neuroscience at Osaka University, Graduate School of Medicine. In September 2007, he became Professor of Neurophysiology at the University Tokyo, Graduate School of Medicine.