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著者	Ohno-Shosaku Takako, Kano Masanobu
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Endocannabinoid-mediated retrograde modulation of synaptic transmission

Takako Ohno-Shosaku¹, Masanobu Kano²

¹Department of Impairment Study, Graduate School of Medical Science, Kanazawa University, Kanazawa 920-0942, Japan, ²Department of Neurophysiology, Graduate School of Medicine, The University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113-0033, Japan

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Correspondence should be addressed to:

Masanobu Kano

Department of Neurophysiology, Graduate School of Medicine, The University of Tokyo

7-3-1 Hongo, Bunkyo-ku, Tokyo 113-0033, Japan

Phone: +81-3-5802-3314

Fax: +81-3-5802-3315

E-mail: mkano-tky@m.u-tokyo.ac.jp

Abstract

One of the two major endocannabinoids, 2-arachidonoylglycerol (2-AG), serves as a retrograde messenger at various types of synapses throughout the brain. Upon postsynaptic activation, 2-AG is released immediately after *de novo* synthesis, activates presynaptic CB₁ cannabinoid receptors, and transiently suppresses neurotransmitter release. When CB₁ receptor activation is combined with some other factors such as presynaptic activity, the suppression is converted to a long-lasting form. Whereas 2-AG primarily transmits a rapid, transient, point-to-point retrograde signal, the other major endocannabinoid, anandamide, may function as a relatively slow retrograde or non-retrograde signal or as an agonist of the vanilloid receptor. The endocannabinoid system can be up- or down-regulated by a variety of physiological and environmental factors including stress, which might be clinically important.

Introduction

Endocannabinoids retrogradely modulate synaptic transmission widely throughout the central nervous system [1-5]. They are released from postsynaptic neurons, activate presynaptic CB₁ cannabinoid receptors, and suppress transmitter release either transiently (endocannabinoid-mediated short-term depression; eCB-STD) or persistently (endocannabinoid-mediated long-term depression; eCB-LTD). The eCB-STD and eCB-LTD are induced at various types of GABAergic and glutamatergic synapses throughout the brain. The ability of each synapse to express eCB-STD/LTD depends primarily on whether the presynaptic terminal expresses CB₁ receptors. In addition to their well-established functions as retrograde messengers, endocannabinoids might also function in non-retrograde manners [4]. In the last few years evidence has also accumulated to suggest that the endocannabinoid signaling system itself is regulated by various factors [6]. In this article, we review recent advances in the molecular mechanisms of endocannabinoid signaling and its plastic changes induced by neuromodulators and environmental factors.

Standard 2-AG model of eCB-STD

The molecular mechanisms of endocannabinoid release involved in eCB-STD have been studied in a variety of preparations. Here we show the standard 2-AG model (Fig. 1), which can explain most, if not all, results of electrophysiological studies [1-5]. The conditions that induce the production and release of 2-AG are mechanistically classified into three types; increase in intracellular Ca²⁺ concentration ([Ca²⁺]_i) (Ca²⁺-driven

endocannabinoid release, CaER), activation of $G_{q/11}$ -coupled receptors (basal receptor-driven endocannabinoid release, basal RER), and the combination of these two (Ca^{2+} -assisted RER) [1,2,6]. After released from postsynaptic neurons, 2-AG activates presynaptic CB_1 receptors and suppresses transmitter release. Termination of the retrograde signal depends on degradation of 2-AG by monoacylglycerol lipase (MGL). Although the expression of MGL is highly heterogeneous [7,8], 2-AG is degraded in a synapse non-specific manner by MGL concentrated in particular cell types [8].

The CaER is responsible for the eCB-STD induced by depolarizing a postsynaptic neuron, which is termed DSI (depolarization-induced suppression of inhibition) or DSE (depolarization-induced suppression of excitation) for inhibitory or excitatory synapses, respectively. When depolarization causes a large, transient increase in $[Ca^{2+}]_i$ to micromolar levels through activation of voltage-gated Ca^{2+} channels, diacylglycerol (DG) is produced through some as yet unidentified mechanism (Fig. 1A, red arrows). DG is then converted to 2-AG by diacylglycerol lipase α (DGL α). The basal RER is responsible for the eCB-STD induced by activation of $G_{q/11}$ -coupled receptors, such as group I metabotropic glutamate receptors (mGluRs) or M_1/M_3 muscarinic acetylcholine receptors (mAChRs), without need of postsynaptic Ca^{2+} elevation. Many other receptors have also been reported to induce RER, which include 5-HT $_2$ -type serotonin receptors, protease-activated receptor 1 (PAR1), and the receptors for orexin, oxytocin and CCK [3]. When $G_{q/11}$ -coupled receptors are activated, DG is produced by PLC β , the subtype of which depends on brain areas (Fig. 1A, blue arrows). DG is then converted to 2-AG by DGL α . The Ca^{2+} -assisted RER accounts for the eCB-STD induced by the combination of a small increase in $[Ca^{2+}]_i$ and weak receptor activation, both of which can be subthreshold for triggering endocannabinoid release. This synergistic effect can be explained by the Ca^{2+} dependency of receptor-driven PLC β stimulation [9-11] (Fig. 1A, green arrow).

More physiological ways of inducing eCB-STD are via synaptic activity (synaptically-driven eCB-STD) [1] (Fig. 1B). If glutamate is released from excitatory presynaptic terminals in a sufficient amount to induce postsynaptic Ca^{2+} elevation and/or mGluR activation, 2-AG is released through the mechanism for CaER, basal RER or Ca^{2+} -assisted RER. Excitatory synaptic activity is therefore potentially effective in inducing 2-AG release. Synaptically-driven eCB-STD can be either homosynaptic (Fig. 1B, red arrow) or heterosynaptic [1] (Fig. 1B, blue arrow).

The standard 2-AG model described above is supported by a considerable number of studies. DSI, DSE, receptor-driven eCB-STD and synaptically-driven eCB-STD are all inhibited by pharmacological blockade of DGL [12] and genetic

deletion of DGL α [13-15]. The mGluR- or mAChR-driven eCB-STD is blocked by genetic deletion of PLC β 1 for hippocampal neurons [9] or PLC β 4 for cerebellar Purkinje cells [10]. The termination of DSI/DSE is prolonged by genetic deletion [8,16] and pharmacological blockade of 2-AG hydrolyzing enzyme (MGL), but not anandamide (another major endocannabinoid) hydrolyzing enzyme (fatty acid amide hydrolase, FAAH) [17].

On-demand vs. pre-formed

It is generally thought that 2-AG is not stored in neurons, but synthesized on demand upon stimulation. However, this “on-demand synthesis model” was challenged by an alternative model that 2-AG is pre-formed by DGL α , pooled within cells, and mobilized from this hypothetical pre-formed 2-AG pools upon stimulation without the contribution of DGL α [18,19]. This model was developed to reconcile the apparent discrepancy in the experimental results between genetic and pharmacological blockade of DGL. DGL α knockout mice were generated independently by three groups, and they all exhibit complete loss of eCB-STD [13-15]. In contrast, the reported effects of acute pharmacological blockade of DGL were highly controversial [19]. A classical DGL inhibitor, tetrahydrolipstatin (THL), inhibited eCB-STD in some studies, but not in others. Inconsistent results were also observed with a novel potent DGL inhibitor, OMDM-188. OMDM-188 failed to inhibit DSI in one study [20], whereas it inhibited DSI but not mGluR-driven STD in another study [21]. More recently, however, effects of OMDM-188 on multiple types of eCB-STD including CaER, basal RER, Ca²⁺-assisted RER and synaptically-driven eCB release were systematically reexamined in acute slices of the hippocampus, cerebellum and striatum and in cultured hippocampal neurons [12]. The data clearly demonstrate that DSI, DSE, receptor-driven eCB-STD and synaptically-driven eCB-STD in these preparations are consistently blocked by the treatment with OMDM-188 [12]. Importantly, application of OMDM-188 as short as 2 min was sufficient to block DSI in cultured hippocampal neurons [12]. This study has confirmed that there is no discrepancy in the results between genetic and pharmacological blockade of DGL, supporting the “on-demand synthesis model” rather than the “pre-formed pool model”.

Given that the “on-demand synthesis model” is correct, why were DGL inhibitors ineffective in blocking eCB-STD in some studies? One possibility is insufficient penetration of the inhibitors. Since THL and OMDM-188 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. The effectiveness of THL and OMDM-188 also depends on whether or not DGL activity is rate limiting for 2-AG production. 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, as previously reported [21]. Whether the suppression of synaptic transmission by 2-AG is maximal or submaximal is also a key factor determining the effectiveness of DGL inhibitors. The inconsistent results of pharmacological studies might result from these differences.

Standard 2-AG model of eCB-LTD

eCB-LTD is induced mostly by repetitive afferent stimulation with or without postsynaptic depolarization, but also by postsynaptic firing [1,4,22,23]. Most types of eCB-LTD are mediated by 2-AG. It is generally thought that eCB-LTD and eCB-STD share the same molecular mechanisms for 2-AG release, that is, CaER, basal RER and Ca^{2+} -assisted RER. Typically, afferent stimulation causes glutamate release from excitatory presynaptic terminals, activates AMPA receptors and mGluRs on the postsynaptic membrane, and induces 2-AG release. 2-AG then activates CB_1 receptors on the same presynaptic terminals releasing glutamate (homosynaptic) (Fig. 2, red arrow) or neighboring presynaptic terminals (heterosynaptic) (Fig. 2, blue arrow).

Although presynaptic mechanisms of eCB-LTD have not been fully elucidated, inhibition of the cAMP/PKA pathway seems to be crucial [4]. Involvement of calcineurin, RIM1 α and Rab3 has also been suggested [4]. In most if not all cases, activation of CB_1 receptors is not enough to induce LTD, and an additional presynaptic mechanism(s) is required [24], such as presynaptic electrical activity [25,26], Ca^{2+} elevation [25,26], NMDA receptor activation [27], M_2 receptor activation [28] and/or D_2 receptor activation [29,30] (Fig. 2). This associative feature may ensure the selective induction of eCB-LTD at the active synapses.

Contribution of anandamide to endocannabinoid signaling

The other major endocannabinoid, anandamide, also contributes to the modulation of synaptic transmission (Fig. 3). Several recent studies reported anandamide-mediated LTD [31-35] (Fig. 3A). Lerner and Kreitzer reported two types of eCB-LTD in medium spiny neurons (MSNs) driving the indirect pathway in the dorsolateral striatum [31]. Low frequency stimulation induces 2-AG-mediated LTD, whereas high frequency stimulation induces CB_1 -dependent, anandamide-mediated LTD [31] (Fig. 3A, eCB-LTD). Anandamide may contribute to LTD not only as a cannabinoid agonist but also as an agonist for transient receptor potential vanilloid receptor type 1 (TRPV1). In

indirect pathway MSNs of the nucleus accumbens, Grueter et al. suggested that low frequency stimulation induces anandamide-mediated LTD, partly by activating presynaptic CB₁ receptors (Fig. 3A, eCB-LTD) and partly by activating postsynaptic TRPV1 channels (Fig. 3A, TRPV1-LTD) [32]. In the dentate gyrus, Chavez et al. reported that pairing stimulation induces CB₁-independent, TRPV1-dependent LTD, which is expressed postsynaptically and mediated by anandamide [33]. On the other hand, 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-LTD, respectively, in the extended amygdala [34].

Anandamide may also generate the endocannabinoid tone (Fig. 3B) which contributes to homeostatic plasticity in the hippocampus [36] and the regulation of hypothalamic-pituitary-adrenal (HPA) axis activity in the amygdala [37] (Fig. 4A). Of note, chronic MGL blockade (continuous elevation of 2-AG) causes down-regulation of CB₁ receptors and functional antagonism of the endocannabinoid system, whereas blockade of FAAH (continuous elevation of anandamide) causes sustained agonism without down-regulation of CB₁ [38]. This finding implies that anandamide has an advantage over 2-AG for functioning as a tonic signal. Taking all these findings into account, it is conceivable that the two major endocannabinoids, 2-AG and anandamide, mediate different types of signals for synaptic modulation [5,38]. 2-AG primarily transmits a rapid, transient, point-to-point retrograde signal, whereas anandamide may function as a relatively slow, retrograde or non-retrograde signal or as a vanilloid agonist.

As described above, there are many studies of the functional roles of anandamide. It should be noted, however, that the evidence for the involvement of anandamide in synaptic plasticity is largely indirect, because enzymatic pathways for anandamide production are complex and not fully understood. In some studies, the contribution of anandamide was suggested principally by the ineffectiveness of DGL inhibitors. Considering the fact that pharmacological results with DGL inhibitors have been highly controversial and are not as reliable as genetic manipulations, experimental data should be interpreted with caution. More direct, convincing evidence will be required in future studies.

Other possible mechanisms

Several additional mechanisms have been suggested to contribute to endocannabinoid signaling, including (1) *N*-arachidonoyldopamine (NADA) [39], (2) glial [40-42] and (3) mitochondrial [43] CB₁ receptors, (4) FAAH-like anandamide transporter (FLAT)

[44] and (5) PLA₂ [45,46]. (1) NADA, like anandamide, is an agonist at both cannabinoid receptors and TRPV1 channels. Recently, it was reported that NADA is tonically released from dopamine neurons, but not neighboring GABAergic neurons, to suppress their inhibitory inputs in the Substantia Nigra pars compacta [39]. (2) Timing-dependent LTD at cortical excitatory synapses has been shown to require the activation of CB₁ receptors, which is believed to be located on presynaptic terminals (Fig. 2). This hypothesis was challenged by a recent study, which suggested that the CB₁ receptors mediating LTD are not located on presynaptic terminals, but on perisynaptic astrocyte processes [41]. (3) Using mitochondrial fractions and anti-CB₁ polyclonal antibodies, it was shown that CB₁ receptors are expressed on mitochondrial membranes and are involved in the regulation of neuronal energy metabolism [43]. However, these findings were questioned by a more recent study [47], which has demonstrated that the previously used anti-CB₁ antibodies can bind to the mitochondrial protein stomatin-like protein 2, and the previously reported effects of cannabinoid agonist on mitochondrial respiration can be replicated in synaptosome-enriched, but not pure mitochondrial preparations. (4) FLAT was reported as a critical molecular component of anandamide transport in neural cells [44]. The study showed that FLAT is a catalytically silent splice variant of FAAH, is expressed robustly throughout the brain, and facilitates the translocation of anandamide into cells. A more recent study, however, suggested that FLAT does not serve as a global intracellular anandamide carrier [48]. The study showed that the expression of FLAT is very low in the brain, and that overexpressed FLAT displays residual catalytic activity, suggesting that its capability to potentiate anandamide uptake may stem from its enzymatic rather than transport activity. (5) A recent study using PLA₂-knockout mice proposed that Ca²⁺ elevation activates PLA₂ to liberate arachidonic acid which is then converted to 2-AG and induces DSE in cerebellar Purkinje cells [45]. However, since this result cannot be reconciled with the standard 2-AG model (Fig. 1A) that is supported by a large number of previous studies, further careful studies are required to judge the validity of the result.

Regulation and plasticity of the endocannabinoid system

The endocannabinoid system depends on many molecular elements, including Ca²⁺ channels, various types of G_{q/11}-coupled receptors, PLCβ, DGLα, MGL and CB₁ receptor. Thus, any changes in their functional activities or expression levels influence the endocannabinoid system. Evidence has accumulated to suggest that the endocannabinoid system is regulated by various postsynaptic and presynaptic receptors, and also influenced by environmental and pathological factors [6].

Postsynaptic and presynaptic receptors can regulate endocannabinoid release and CB₁ signaling, respectively. In the striatum, postsynaptic D₂ and A_{2A} receptors regulate endocannabinoid release and eCB-LTD positively and negatively, respectively, by modulating mGluR signaling through regulator of G protein signaling 4 (RGS4) [31]. Enzymatic activity of DGL α is reduced after phosphorylation by CaMKII [49], suggesting the possibility that DGL α might be regulated by CaMKII-involving signals under physiological conditions. Presynaptic CB₁ receptor signaling is influenced by the activity of colocalized presynaptic receptors. In the hippocampus, tonic activation of A₁ receptors by endogenous adenosine inhibits presynaptic CB₁ signaling [50]. Because CB₁ and A₁ receptors are both G_{i/o}-coupled, it is conceivable that they use overlapping sets of G α subunits and interact with each other.

Considering that the endocannabinoid system contributes to many brain functions including learning, memory, stress regulation and analgesia, its plastic changes induced by environmental factors are clinically important. One such factor is stress. There is growing evidence for functional interaction between the endocannabinoid system and activity along the HPA axis. The endocannabinoid system plays a key role in regulating the HPA axis under both basal and stressful conditions. It was proposed that a tonic level of anandamide within the basolateral amygdala provides a steady-state inhibition of HPA axis activity, and that glucocorticoid-induced 2-AG release from paraventricular nucleus neurons mediates rapid negative feedback effects of glucocorticoids on the HPA axis [37]. Conversely, stress-induced HPA axis activity alters 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 [6,37,51]. For example, repetitive immobilization stress impaired DSI/DSE by down-regulating CB₁ receptors in the paraventricular nucleus of the hypothalamus [52], whereas chronic stress enhanced DSI partially by down-regulating MGL in the basolateral amygdala [53]. Interestingly, *in vivo* recordings of medial prefrontal cortex (mPFC)-evoked spike probability from the bed nucleus of the stria terminalis showed that acute restraint stress switched CB₁-dependent plasticity from LTD to LTP, and that the stress-elicited shift in plasticity was controlled by CB₁ receptors on excitatory terminals [54]. Mechanisms of these stress-induced plastic changes in the endocannabinoid system remain to be elucidated.

A possible relationship between the endocannabinoid system and psychiatric disorders has also been suggested, by exploring genetic morphisms in the human genes for the CB₁ receptor and FAAH [55], and by examining eCB-STD/LTD in animal

models of psychiatric disorders including autism, schizophrenia and Fragile X syndrome [56-60]. In these animal models, alteration in DSI, mGluR-driven eCB-STD, eCB-LTD or the endocannabinoid tone has been reported. Further studies are required to understand the mechanisms that cause these alterations.

Conclusions

The endocannabinoid 2-AG plays an important role as a retrograde messenger at both excitatory and inhibitory synapses. It is synthesized and released from postsynaptic neurons in an activity-dependent manner, and suppresses transmitter release transiently (eCB-STD) or persistently (eCB-LTD). Although considerable progress has been made in recent years with regard to the molecular mechanisms of eCB-STD and eCB-LTD, several questions remain unsolved. First, the enzyme that produces DG upon Ca^{2+} elevation has not been identified. Second, it is not determined whether 2-AG can move across postsynaptic and presynaptic membranes, as well as the synaptic cleft, freely or requires some special machinery. Third, presynaptic mechanisms of eCB-LTD are not completely clear. Fourth, anandamide synthetic pathways are not fully understood. Although accumulating evidence suggests that the endocannabinoid system plays a role in many brain functions, and can be up- or down-regulated by a variety of factors, the precise mechanisms are poorly understood. Of note, the endocannabinoid system contributes to the regulation of the HPA axis and stress response and, conversely, undergoes plastic changes in response to stress condition. Understanding these features of the endocannabinoid system may be clinically important for the development of the methods of treating patients with stress-related disorders.

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Figure legends

Figure 1.

Molecular mechanisms of endocannabinoid-mediated short-term depression (eCB-STD). **A:** The production of diacylglycerol (DG) is induced by either a large Ca^{2+} elevation, which is caused by depolarization-induced activation of voltage-gated Ca^{2+} channels (VGCC), through unidentified mechanisms (red arrows), or strong activation of $\text{G}_{q/11}$ -coupled receptors such as mGluRs and mAChRs through $\text{PLC}\beta$ (blue arrows). If weak activation of $\text{G}_{q/11}$ -coupled receptors is combined with a small Ca^{2+} elevation, both of which are subthreshold for DG production when given alone, receptor-driven $\text{PLC}\beta$ stimulation is enhanced by Ca^{2+} to produce DG (green arrow). DG is then converted to 2-AG by diacylglycerol lipase α ($\text{DGL}\alpha$). 2-AG is released from the postsynaptic neuron, and activates presynaptic CB_1 receptors to suppress transmitter release (STD). 2-AG is hydrolysed mostly by presynaptic monoacylglycerol lipase (MGL) and arachidonic acid (AA) is produced. **B:** Excitatory synaptic activity releases glutamate, which activates postsynaptic AMPA-type glutamate receptors (AMPA) and mGluRs. Activation of AMPARs causes Ca^{2+} elevation through activating VGCCs. The resulting postsynaptic Ca^{2+} elevation and/or mGluR activation causes the release of 2-AG through the mechanisms illustrated in A. 2-AG activates CB_1 receptors on the same presynaptic terminals releasing glutamate (red arrow, homosynaptic) or neighboring terminals (blue arrow, heterosynaptic), and suppresses transmitter release (STD).

Figure 2.

Molecular mechanisms of endocannabinoid-mediated long-term depression (eCB-LTD). Afferent stimulation and/or postsynaptic depolarization induces 2-AG release by activating postsynaptic AMPARs, mGluRs and VGCCs. Mechanistically, this process is the same as that for eCB-STD, and includes CaER, basal RER and Ca^{2+} -assisted RER. The released 2-AG then activates CB_1 receptors on the same presynaptic terminals releasing glutamate (homosynaptic, red arrow) or neighboring terminals (blue arrow, heterosynaptic). LTD is induced when the activation of CB_1 receptors is combined with some other factors in the presynaptic terminal, such as presynaptic electrical activity, Ca^{2+} influx through VGCCs or NMDA-type glutamate receptors (NMDARs) and the activation of D_2 or M_2 receptors.

Figure 3.

Functions of anandamide in the regulation of synaptic transmission. **A:** Anandamide-mediated LTD. Glutamate released by afferent stimulation activates postsynaptic mGluRs to induce anandamide production. Anandamide activates presynaptic CB₁ receptors to induce presynaptic LTD (eCB-LTD), and activates postsynaptic TRPV1 channels to induce postsynaptic LTD that is expressed as a change in the function or number of postsynaptic glutamate receptors (TRPV1-LTD). **B:** Endocannabinoid tone generated by anandamide. At basal Ca²⁺ levels, anandamide is continuously produced and released. This anandamide-mediated endocannabinoid tone controls synaptic transmission in a sustained fashion.

References

- [1] Kano M, Ohno-Shosaku T, Hashimotodani Y, Uchigashima M, Watanabe M: **Endocannabinoid-mediated control of synaptic transmission.** *Physiol Rev* 2009, **89**:309-380.
 - [2] Ohno-Shosaku T, Tanimura A, Hashimotodani Y, Kano M: **Endocannabinoids and retrograde modulation of synaptic transmission.** *Neuroscientist* 2012, **18**:119-132.
 - [3] Katona I, Freund TF: **Multiple functions of endocannabinoid signaling in the brain.** *Annual review of neuroscience* 2012, **35**:529-558.
 - [4] Castillo PE, Younts TJ, Chavez AE, Hashimotodani Y: **Endocannabinoid signaling and synaptic function.** *Neuron* 2012, **76**:70-81.
 - [5] Piomelli D: **More surprises lying ahead. The endocannabinoids keep us guessing.** *Neuropharmacology* 2014, **76 Pt B**:228-234.
 - *[6] Iremonger KJ, Wamsteeker Cusulin JI, Bains JS: **Changing the tune: Plasticity and adaptation of retrograde signals.** *Trends Neurosci* 2013, **36**:471-479.
- This review summarizes recent findings that endocannabinoid signaling is modulated by various neuromodulators and neural activity, and also exhibits plastic changes in response to in vivo experience.
- [7] Uchigashima M, Yamazaki M, Yamasaki M, Tanimura A, Sakimura K, Kano M, Watanabe M: **Molecular and morphological configuration for 2-arachidonoylglycerol-mediated retrograde signaling at mossy cell-granule cell synapses in the dentate gyrus.** *J Neurosci* 2011, **31**:7700-7714.
 - ** [8] Tanimura A, Uchigashima M, Yamazaki M, Uesaka N, Mikuni T, Abe M, Hashimoto K, Watanabe M, Sakimura K, Kano M: **Synapse type-independent degradation of the endocannabinoid 2-arachidonoylglycerol after retrograde synaptic suppression.** *Proc Natl Acad Sci U S A* 2012, **109**:12195-12200.

This study demonstrates that MGL is present only in particular types of presynaptic terminals, yet eCB-STD is prolonged rather non-selectively at both MGL-positive and MGL-negative synapses in the cerebellum of MGL knockout mice. This study also shows that MGL expressed in astroglia significantly contributes to 2-AG degradation, because eCB-STD is prolonged in the cerebellum of conditional MGL knockout mice in which MGL is expressed only in astroglia.

- [9] Hashimotodani Y, Ohno-Shosaku T, Tsubokawa H, Ogata H, Emoto K, Maejima T, Araishi K, Shin HS, Kano M: **Phospholipase C β serves as a coincidence detector through its Ca²⁺ dependency for triggering retrograde endocannabinoid signal.** *Neuron* 2005, **45**:257-268.
- [10] Maejima T, Oka S, Hashimotodani Y, Ohno-Shosaku T, Aiba A, Wu D, Waku K, Sugiura T, Kano M: **Synaptically driven endocannabinoid release requires Ca²⁺-assisted metabotropic glutamate receptor subtype 1 to phospholipase C β 4 signaling cascade in the cerebellum.** *J Neurosci* 2005, **25**:6826-6835.
- [11] Sugawara Y, Echigo R, Kashima K, Minami H, Watanabe M, Nishikawa Y, Muranishi M, Yoneda M, Ohno-Shosaku T: **Intracellular calcium level is an important factor influencing ion channel modulations by PLC-coupled metabotropic receptors in hippocampal neurons.** *Brain Res* 2013, **1512**:9-21.
- **[12] Hashimotodani Y, Ohno-Shosaku T, Tanimura A, Kita Y, Sano Y, Shimizu T, Di Marzo V, Kano M: **Acute inhibition of diacylglycerol lipase blocks endocannabinoid-mediated retrograde signalling: Evidence for on-demand biosynthesis of 2-arachidonoylglycerol.** *J Physiol* 2013, **591**:4765-4776.

This study demonstrates that eCB-STD is consistently blocked by acute pharmacological inhibition of DGL, supporting the “on-demand synthesis model” rather than the “pre-formed pool model”. A novel potent DGL inhibitor, OMDM-188, effectively blocks hippocampal DSI, striatal DSI, cerebellar DSI, cerebellar DSE, hippocampal mGluR-driven eCB-STD and cerebellar synaptically-driven eCB-STD after the treatment of slices or cultured neurons with OMDM-188 for 1 hr (slices) or 2-8 minutes (cultured neurons).

- [13] Tanimura A, Yamazaki M, Hashimotodani Y, Uchigashima M, Kawata S, Abe M, Kita Y, Hashimoto K, Shimizu T, Watanabe M, Sakimura K *et al*: **The**

endocannabinoid 2-arachidonoylglycerol produced by diacylglycerol lipase α mediates retrograde suppression of synaptic transmission. *Neuron* 2010, **65**:320-327.

- [14] Gao Y, Vasilyev DV, Goncalves MB, Howell FV, Hobbs C, Reisenberg M, Shen R, Zhang MY, Strassle BW, Lu P, Mark L *et al*: **Loss of retrograde endocannabinoid signaling and reduced adult neurogenesis in diacylglycerol lipase knock-out mice.** *J Neurosci* 2010, **30**:2017-2024.
- [15] Yoshino H, Miyamae T, Hansen G, Zambrowicz B, Flynn M, Pedicord D, Blat Y, Westphal RS, Zaczek R, Lewis DA, Gonzalez-Burgos G: **Postsynaptic diacylglycerol lipase mediates retrograde endocannabinoid suppression of inhibition in mouse prefrontal cortex.** *J Physiol* 2011, **589**:4857-4884.
- [16] Zhong P, Pan B, Gao XP, Blankman JL, Cravatt BF, Liu QS: **Genetic deletion of monoacylglycerol lipase alters endocannabinoid-mediated retrograde synaptic depression in the cerebellum.** *J Physiol* 2011, **589**:4847-4855.
- [17] Hashimotodani Y, Ohno-Shosaku T, Kano M: **Presynaptic monoacylglycerol lipase activity determines basal endocannabinoid tone and terminates retrograde endocannabinoid signaling in the hippocampus.** *J Neurosci* 2007, **27**:1211-1219.
- [18] Min R, Di Marzo V, Mansvelder HD: **DAG lipase involvement in depolarization-induced suppression of inhibition: Does endocannabinoid biosynthesis always meet the demand?** *Neuroscientist* 2010, **16**:608-613.
- [19] Alger BE, Kim J: **Supply and demand for endocannabinoids.** *Trends Neurosci* 2011, **34**:304-315.
- [20] Min R, Testa-Silva G, Heistek TS, Canto CB, Lodder JC, Bisogno T, Di Marzo V, Brussaard AB, Burnashev N, Mansvelder HD: **Diacylglycerol lipase is not involved in depolarization-induced suppression of inhibition at unitary inhibitory connections in mouse hippocampus.** *J Neurosci* 2010, **30**:2710-2715.

- [21] Zhang L, Wang M, Bisogno T, Di Marzo V, Alger BE: **Endocannabinoids generated by Ca^{2+} or by metabotropic glutamate receptors appear to arise from different pools of diacylglycerol lipase.** *PLoS One* 2011, **6**:e16305.
 - [22] Gerdeman GL, Ronesi J, Lovinger DM: **Postsynaptic endocannabinoid release is critical to long-term depression in the striatum.** *Nat Neurosci* 2002, **5**:446-451.
 - *[23] Younts TJ, Chevalleyre V, Castillo PE: **CA1 pyramidal cell theta-burst firing triggers endocannabinoid-mediated long-term depression at both somatic and dendritic inhibitory synapses.** *J Neurosci* 2013, **33**:13743-13757.
- For the induction of eCB-LTD, afferent stimulation is generally used. In contrast, this study demonstrates that eCB-LTD can be induced by theta-burst firing (TBF) of postsynaptic neurons. Moreover, TBF-induced eCB-LTD is expressed at both somatic and dendritic inhibitory synapses.
- [24] Castillo PE: **Presynaptic LTP and LTD of excitatory and inhibitory synapses.** *Cold Spring Harbor perspectives in biology* 2012, **4**:a005728.
 - [25] Singla S, Kreitzer AC, Malenka RC: **Mechanisms for synapse specificity during striatal long-term depression.** *J Neurosci* 2007, **27**:5260-5264.
 - [26] Heifets BD, Chevalleyre V, Castillo PE: **Interneuron activity controls endocannabinoid-mediated presynaptic plasticity through calcineurin.** *Proc Natl Acad Sci U S A* 2008, **105**:10250-10255.
 - [27] Sjostrom PJ, Turrigiano GG, Nelson SB: **Neocortical LTD via coincident activation of presynaptic NMDA and cannabinoid receptors.** *Neuron* 2003, **39**:641-654.
 - [28] Ahumada J, Fernandez de Sevilla D, Couve A, Buno W, Fuenzalida M: **Long-term depression of inhibitory synaptic transmission induced by spike-timing dependent plasticity requires coactivation of endocannabinoid and muscarinic receptors.** *Hippocampus* 2013, **23**:1439-1452.
 - [29] Pan B, Hillard CJ, Liu QS: **D₂ dopamine receptor activation facilitates**

endocannabinoid-mediated long-term synaptic depression of GABAergic synaptic transmission in midbrain dopamine neurons via cAMP-protein kinase A signaling. *J Neurosci* 2008, **28**:14018-14030.

- [30] Chiu CQ, Puente N, Grandes P, Castillo PE: **Dopaminergic modulation of endocannabinoid-mediated plasticity at GABAergic synapses in the prefrontal cortex.** *J Neurosci* 2010, **30**:7236-7248.

- **[31] Lerner TN, Kreitzer AC: **RGS4 is required for dopaminergic control of striatal LTD and susceptibility to Parkinsonian motor deficits.** *Neuron* 2012, **73**:347-359.

This study demonstrates that striatal eCB-LTD is modulated by D₂ and A_{2A} receptors through RGS4. According to the model presented, D₂ and A_{2A} receptors oppositely modulate cAMP/PKA and high PKA activity inhibits mGluR signaling via RGS4. Inhibition of mGluR signaling prevents the production of 2-AG and anandamide.

- [32] Grueter BA, Brasnjo G, Malenka RC: **Postsynaptic TRPV1 triggers cell type-specific long-term depression in the nucleus accumbens.** *Nat Neurosci* 2010, **13**:1519-1525.

- [33] Chavez AE, Chiu CQ, Castillo PE: **TRPV1 activation by endogenous anandamide triggers postsynaptic long-term depression in dentate gyrus.** *Nat Neurosci* 2010, **13**:1511-1518.

- [34] Puente N, Cui Y, Lassalle O, Lafourcade M, Georges F, Venance L, Grandes P, Manzoni OJ: **Polymodal activation of the endocannabinoid system in the extended amygdala.** *Nat Neurosci* 2011, **14**:1542-1547.

- [35] Khlaifia A, Farah H, Gackiere F, Tell F: **Anandamide, cannabinoid type 1 receptor, and NMDA receptor activation mediate non-hebbian presynaptically expressed long-term depression at the first central synapse for visceral afferent fibers.** *J Neurosci* 2013, **33**:12627-12637.

- [36] Kim J, Alger BE: **Reduction in endocannabinoid tone is a homeostatic mechanism for specific inhibitory synapses.** *Nat Neurosci* 2010, **13**:592-600.

- [37] Hill MN, Patel S, Campolongo P, Tasker JG, Wotjak CT, Bains JS: **Functional interactions between stress and the endocannabinoid system: From synaptic signaling to behavioral output.** *J Neurosci* 2010, **30**:14980-14986.
- [38] Schlosburg JE, Blankman JL, Long JZ, Nomura DK, Pan B, Kinsey SG, Nguyen PT, Ramesh D, Booker L, Burston JJ, Thomas EA *et al*: **Chronic monoacylglycerol lipase blockade causes functional antagonism of the endocannabinoid system.** *Nat Neurosci* 2010, **13**:1113-1119.
- *[39] Freestone PS, Guatteo E, Piscitelli F, di Marzo V, Lipski J, Mercuri NB: **Glutamate spillover drives endocannabinoid production and inhibits GABAergic transmission in the Substantia Nigra pars compacta.** *Neuropharmacology* 2013, **79C**:467-475.
- This is the first report suggesting the endocannabinoid tone mediated by *N*-arachidonoyldopamine (NADA), which is produced in a mGluR-dependent manner, suppresses GABAergic transmission in nigral dopamine neurons but not in adjacent non-dopaminergic neurons. The data show that DSI and the endocannabinoid tone are mediated by distinct endocannabinoids, that is, 2-AG and NADA, respectively.
- [40] Navarrete M, Araque A: **Endocannabinoids potentiate synaptic transmission through stimulation of astrocytes.** *Neuron* 2010, **68**:113-126.
- [41] Min R, Nevian T: **Astrocyte signaling controls spike timing-dependent depression at neocortical synapses.** *Nat Neurosci* 2012, **15**:746-753.
- [42] Han J, Kesner P, Metna-Laurent M, Duan T, Xu L, Georges F, Koehl M, Abrous DN, Mendizabal-Zubiaga J, Grandes P, Liu Q *et al*: **Acute cannabinoids impair working memory through astroglial CB1 receptor modulation of hippocampal LTD.** *Cell* 2012, **148**:1039-1050.
- [43] Benard G, Massa F, Puente N, Lourenco J, Bellocchio L, Soria-Gomez E, Matias I, Delamarre A, Metna-Laurent M, Cannich A, Hebert-Chatelain E *et al*: **Mitochondrial CB(1) receptors regulate neuronal energy metabolism.** *Nat Neurosci* 2012, **15**:558-564.
- [44] Fu J, Bottegoni G, Sasso O, Bertorelli R, Rocchia W, Masetti M, Guijarro A,

- Lodola A, Armirotti A, Garau G, Bandiera T *et al*: **A catalytically silent FAAH-1 variant drives anandamide transport in neurons.** *Nat Neurosci* 2012, **15**:64-69.
- [45] Wang DJ, Yang D, Su LD, Xie YJ, Zhou L, Sun CL, Wang Y, Wang XX, Shen Y: **Cytosolic phospholipase A₂ alpha/arachidonic acid signaling mediates depolarization-induced suppression of excitation in the cerebellum.** *PLoS One* 2012, **7**:e41499.
- [46] Wang DJ, Su LD, Wang YN, Yang D, Sun CL, Zhou L, Wang XX, Shen Y: **Long-term potentiation at cerebellar parallel fiber-purkinje cell synapses requires presynaptic and postsynaptic signaling cascades.** *J Neurosci* 2014, **34**:2355-2364.
- [47] Morozov YM, Dominguez MH, Varela L, Shanabrough M, Koch M, Horvath TL, Rakic P: **Antibodies to cannabinoid type 1 receptor co-react with stomatin-like protein 2 in mouse brain mitochondria.** *Eur J Neurosci* 2013, **38**:2341-2348.
- [48] Leung K, Elmes MW, Glaser ST, Deutsch DG, Kaczocha M: **Role of FAAH-like anandamide transporter in anandamide inactivation.** *PLoS One* 2013, **8**:e79355.
- [49] Shonesy BC, Wang X, Rose KL, Ramikie TS, Cavener VS, Rentz T, Baucum AJ, 2nd, Jalan-Sakriar N, Mackie K, Winder DG, Patel S *et al*: **CAMKII regulates diacylglycerol lipase- α and striatal endocannabinoid signaling.** *Nat Neurosci* 2013, **16**:456-463.
- [50] Hoffman AF, Laaris N, Kawamura M, Masino SA, Lupica CR: **Control of cannabinoid CB₁ receptor function on glutamate axon terminals by endogenous adenosine acting at A₁ receptors.** *J Neurosci* 2010, **30**:545-555.
- *[51] Senst L, Bains J: **Neuromodulators, stress and plasticity: A role for endocannabinoid signalling.** *J Exp Biol* 2014, **217**:102-108.

This review summarizes the studies demonstrating the roles of the endocannabinoid signaling in the stress response, and the stress-induced plastic changes of the

endocannabinoid system.

- [52] Wamsteeker JI, Kuzmiski JB, Bains JS: **Repeated stress impairs endocannabinoid signaling in the paraventricular nucleus of the hypothalamus.** *J Neurosci* 2010, **30**:11188-11196.
 - [53] Patel S, Kingsley PJ, Mackie K, Marnett LJ, Winder DG: **Repeated homotypic stress elevates 2-arachidonoylglycerol levels and enhances short-term endocannabinoid signaling at inhibitory synapses in basolateral amygdala.** *Neuropsychopharmacology* 2009, **34**:2699-2709.
 - *[54] Glangetas C, Girard D, Groc L, Marsicano G, Chaouloff F, Georges F: **Stress switches cannabinoid type-1 (CB₁) receptor-dependent plasticity from LTD to LTP in the bed nucleus of the stria terminalis.** *J Neurosci* 2013, **33**:19657-19663.
- The bed nucleus of the stria terminalis (BNST) is a key target of stress, and regulates the HPA axis. This study shows that acute stress reverses the CB₁-dependent plasticity from LTD to LTP in the BNST, and that the stress-elicited LTP involves CB₁ receptors located on excitatory terminals.
- [55] Hillard CJ, Weinlander KM, Stuhr KL: **Contributions of endocannabinoid signaling to psychiatric disorders in humans: Genetic and biochemical evidence.** *Neuroscience* 2012, **204**:207-229.
 - [56] Du H, Kwon IK, Kim J: **Neuregulin-1 impairs the long-term depression of hippocampal inhibitory synapses by facilitating the degradation of endocannabinoid 2-AG.** *J Neurosci* 2013, **33**:15022-15031.
 - [57] Foldy C, Malenka RC, Sudhof TC: **Autism-associated neuroligin-3 mutations commonly disrupt tonic endocannabinoid signaling.** *Neuron* 2013, **78**:498-509.
 - [58] Maccarrone M, Rossi S, Bari M, De Chiara V, Rapino C, Musella A, Bernardi G, Bagni C, Centonze D: **Abnormal mGlu 5 receptor/endocannabinoid coupling in mice lacking FMRP and BC1 RNA.** *Neuropsychopharmacology* 2010, **35**:1500-1509.

- [59] Zhang L, Alger BE: **Enhanced endocannabinoid signaling elevates neuronal excitability in fragile X syndrome.** *J Neurosci* 2010, **30**:5724-5729.
- [60] Jung KM, Sepers M, Henstridge CM, Lassalle O, Neuhofer D, Martin H, Ginger M, Frick A, DiPatrizio NV, Mackie K, Katona I *et al*: **Uncoupling of the endocannabinoid signalling complex in a mouse model of fragile X syndrome.** *Nat Commun* 2012, **3**:1080.

Figure 1.

Figure 2_Ohno-Shosaku & Kano

Figure 2.

Figure 3_Ohno-Shosaku & Kano

Figure 3.