

# Ca<sup>2+</sup>-assisted receptor-driven endocannabinoid release: mechanisms that associate presynaptic and postsynaptic activities

著者	Hashimotodani Yuki, Ohno-Shosaku Takako, Kano Masanobu
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**Ca<sup>2+</sup>-assisted receptor-driven endocannabinoid release:  
mechanism relevant to associate pre- and postsynaptic  
activities**

**Yuki Hashimotodani<sup>1</sup>, Ohno-Shosaku Takako<sup>2</sup>, Masanobu Kano<sup>3</sup>**

<sup>1</sup>Department of Neurophysiology, Graduate School of Medicine, Osaka University, Suita  
565-0871, Japan, <sup>2</sup>Department of Impairment Study, Graduate School of Medical Science,

Kanazawa University, Kanazawa 920-0942, Japan, <sup>3</sup>Department of Cellular  
Neuroscience, Graduate School of Medicine, Osaka University, Suita 565-0871, Japan

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**Correspondence should be addressed to:**

**Masanobu Kano**

Department of Cellular Neuroscience, Graduate School of Medicine, Osaka  
University, Suita 565-0871, Japan. Phone: +81-6-6879-3580; Fax:  
81-6-6879-3589; E-mail: [mkano@cns.med.osaka-u.ac.jp](mailto:mkano@cns.med.osaka-u.ac.jp)

Yuki Hashimotodani

[yhashimo@cns.med.osaka-u.ac.jp](mailto:yhashimo@cns.med.osaka-u.ac.jp)

Takako Ohno-Shosaku

[shosaku@med.kanazawa-u.ac.jp](mailto:shosaku@med.kanazawa-u.ac.jp)

## **Summary**

Endogenous cannabinoids (endocannabinoids) serve as retrograde messengers at synapses in various regions of the brain. They are released from postsynaptic neurons and cause transient and long-lasting reduction of neurotransmitter release through activation of presynaptic cannabinoid receptors. Endocannabinoid release is induced by either postsynaptic  $\text{Ca}^{2+}$  elevation or activation of  $\text{G}_{q/11}$ -coupled receptors. When these two stimuli coincide, endocannabinoid release is markedly enhanced, which is attributed to the  $\text{Ca}^{2+}$  dependency of phospholipase  $\text{C}\beta$  ( $\text{PLC}\beta$ ). This  $\text{Ca}^{2+}$ -assisted receptor-driven endocannabinoid release is suggested to participate in various forms of synaptic plasticity, including short-term associative plasticity in the cerebellum and spike-timing dependent long-term depression in the somatosensory cortex. In these forms of plasticity,  $\text{PLC}\beta$  appears to function as a coincident detector of pre- and postsynaptic activities.

## **Introduction**

At chemical synapses in the central nervous system, the signal is transmitted from presynaptic to postsynaptic neurons. The efficacy of this ‘anterograde’ transmission is modulated by a number of factors and also undergoes plastic change, which is thought to be important for brain functions including learning and memory [1]. In certain forms of synaptic plasticity, retrograde messengers, which are released from postsynaptic neurons and act on presynaptic terminals, have been postulated [2,3]. Recently, endogenous cannabinoids (endocannabinoids) have been found to function as retrograde messengers and contribute to synaptic modulation and plasticity [4-6]. Endocannabinoid-mediated retrograde modulation is now known to function at various types of synapses throughout the brain [7,8]. Our knowledge is rapidly expanding, concerning how the endocannabinoid signal is generated and contributes to short-term or long-term synaptic plasticity [7,8]. In this review, we highlight the significance of phospholipase  $\text{C}\beta$  ( $\text{PLC}\beta$ ) in generation of the endocannabinoid signal and discuss its roles in synaptic plasticity.

## **Mechanisms underlying generation of endocannabinoid signal**

### **Endocannabinoid release triggered by postsynaptic depolarization**

In early 1990s, it was found that depolarization of a principal neuron transiently suppressed its inhibitory synaptic inputs in the cerebellum and hippocampus [2,3], and

this phenomenon was termed depolarization-induced suppression of inhibition (DSI). Further studies have revealed that DSI is mediated by retrograde messengers, which were finally identified as endocannabinoids in 2001 [4,5]. Concurrently, the counterpart of DSI at excitatory synapses, termed depolarization-induced suppression of excitation (DSE), was also confirmed to be mediated by endocannabinoids in the cerebellum [6]. Since then, a number of studies reported similar endocannabinoid-mediated DSI or DSE in various brain regions [7]. A current model of DSI/DSE is as follows (Fig. 1, red and black arrows). Postsynaptic depolarization causes a large intracellular  $\text{Ca}^{2+}$  elevation through voltage-gated  $\text{Ca}^{2+}$  channels [9] and triggers the biosynthesis of endocannabinoids. The endocannabinoids then diffuse out and activate presynaptic  $\text{G}_{i/o}$ -coupled type1 cannabinoid receptors (CB1Rs). It is postulated that cannabinoid receptor ligands incorporate into plasma membranes and interact with CB1Rs through fast lateral diffusion across the membrane lipid bilayer because of their lipophilic nature [10]. The CB1R activation causes a suppression of transmitter release in a reversible manner, through multiple mechanisms including modifications of  $\text{Ca}^{2+}$  channels,  $\text{K}^+$  channels and release machinery [11-13]. The  $\text{Ca}^{2+}$ -induced  $\text{Ca}^{2+}$  release in postsynaptic neuron might be additionally involved at some synapses [14]. The peak value of  $\text{Ca}^{2+}$  elevation required for DSI/DSE is in a micromolar range [15,16] but can be lowered by sustaining  $\text{Ca}^{2+}$  elevation [17]. 2-Arachidonoylglycerol (2-AG), one of the two major endocannabinoids, is suggested to mediate DSI/DSE [18-21]. Biochemical studies indicate that 2-AG is produced from membrane lipids through two enzymatic reactions by PLC and diacylglycerol lipase (DGL), although some additional pathways are also available [22]. The involvement of DGL in DSI/DSE is supported by several studies [19-21], but not by some other studies [23-25]. There is no conclusive evidence showing the involvement of PLC in DSI/DSE.

### **Endocannabinoid release triggered by $\text{G}_{q/11}$ -coupled receptor activation**

Endocannabinoid-mediated retrograde modulation driven by  $\text{G}_{q/11}$ -coupled receptors was first found in the cerebellum [26]. The study demonstrated that activation of metabotropic glutamate receptor type 1 (mGluR1) in Purkinje cells (PCs) induced endocannabinoid release and caused suppression of excitatory transmission from climbing fibers (CFs) to PCs in cerebellar slices. Importantly, this modulation did not require  $\text{Ca}^{2+}$  elevation, indicating that the underlying mechanism is distinct from DSI/DSE. Since then, many

studies have reported similar endocannabinoid-mediated retrograde modulations driven by  $G_{q/11}$ -coupled receptors, which include group I mGluRs [27-31],  $M_1/M_3$  muscarinic receptors [32,33], and the orexin receptor [34], in various brain regions. A current model for the receptor-driven endocannabinoid release is illustrated in Figure 1 (blue and black arrows) [7,8]. Activation of  $G_{q/11}$ -coupled receptors stimulates  $PLC\beta$  and the enzymatic product diacylglycerol (DG) is then converted to 2-AG by DGL. Anatomical studies demonstrated that  $DGL\alpha$ , one of the two isozymes of DGL, is localized on postsynaptic sites facing to CB1R-expressing terminals in the cerebellum and hippocampus, suggesting that these endocannabinoid system-related molecules are finely organized at synapses [35,36].

### **Combination of postsynaptic depolarization and receptor activation**

As described above, endocannabinoid release is induced by either depolarization or  $G_{q/11}$ -coupled receptor activation through distinct processes. However, when these two stimuli are combined, the endocannabinoid release is markedly facilitated [27,28,32,37]. Recent studies have revealed that  $PLC\beta$  is the key molecule to detect the coincidence of the two stimuli [16,38]. The study using hippocampal neurons clearly demonstrated that the muscarinic receptor-driven endocannabinoid release required  $PLC\beta 1$  and exhibited a sharp  $Ca^{2+}$  dependency within a physiological range. The muscarinic receptor-driven PLC activation, which was monitored by DG-sensitive TRPC6 channels, was also  $PLC\beta 1$ -dependent and exhibited a similar  $Ca^{2+}$  dependency. These results strongly suggest that  $PLC\beta 1$  detects the coincidence through its  $Ca^{2+}$  dependency and induces 2-AG synthesis in the hippocampus [38]. The study using cerebellar slices came to a similar conclusion [16]. The study showed that the mGluR1-driven endocannabinoid release was dependent on intracellular  $Ca^{2+}$  level, and that the enhanced endocannabinoid release by the combination of  $Ca^{2+}$  elevation and mGluR1 activation was abolished in  $PLC\beta 4$ -knockout mice. This  $PLC\beta 4$ -dependent enhancement of endocannabinoid release was induced by a submicromolar range of  $Ca^{2+}$  elevation, which was much lower than the level required for DSE [15,16]. The endocannabinoid release induced by simultaneous stimulation of small  $Ca^{2+}$  elevation and weak receptor activation has been termed  $Ca^{2+}$ -assisted receptor-driven endocannabinoid release (Ca-RER) [16]. A current model for Ca-RER is included in Figure 1 (green, blue and black arrows). When the activation of  $G_{q/11}$ -coupled receptors coincides with  $Ca^{2+}$  elevation,  $PLC\beta$  is effectively activated and

yields DG, which is then converted to 2-AG by DGL. It should be noted that the receptor-PLC $\beta$  signaling cascade may behave in a complicated manner due to the Ca $^{2+}$ -dependency of PLC $\beta$ . Under physiological conditions, it is known that the receptor-G $_{q/11}$ -PLC $\beta$  signaling induces a Ca $^{2+}$  elevation through inositol 1,4,5-trisphosphate receptors (IP $_3$ R) or receptor-operated Ca $^{2+}$ -permeable channels (TRPC channels or some other Ca $^{2+}$  entry channels). Therefore, the PLC $\beta$  signaling might be activated in a regenerative manner through the positive feedback loop between PLC $\beta$  and Ca $^{2+}$  (Fig. 1, purple arrows). It is also likely that the receptor-PLC $\beta$  signaling may be influenced by preceding conditions that modulate the basal Ca $^{2+}$  level or the filling state of Ca $^{2+}$  stores.

### **Functional significance of Ca $^{2+}$ -assisted receptor-driven endocannabinoid release in synaptic plasticity**

Recent studies have suggested that Ca-RER plays crucial roles in induction of endocannabinoid-mediated short-term or long-term plasticity. In the cerebellum, a brief burst of PF stimulation (for example, 50-100 Hz, 10 pulses), but not granular layer stimulation [39,40], induced endocannabinoid release from PCs and suppressed the transmitter release from PF terminals [16,41]. This type of synaptic suppression was dependent on both mGluR1 activation and postsynaptic Ca $^{2+}$  elevation and abolished in PLC $\beta$ 4-knockout mice [16], indicating the contribution of Ca-RER. A similar type of suppression induced by presynaptic firing alone is observed in local interneurons of the cerebellum [42] and dopamine neuron of the ventral tegmental area [43]. An associative type of short-term plasticity that involves Ca-RER was also found in the cerebellum. Brenowitz and Regehr showed that coactivation of PF and CF markedly facilitated endocannabinoid-mediated suppression of PF synapses [44]. When PF stimulation was combined with CF stimulation (100 Hz, 5 pulses), only two to five pulses to PFs were enough to induce endocannabinoid release. This associative short-term plasticity was shown to be dependent on both mGluR1 activation and postsynaptic Ca $^{2+}$  elevation. Ca-RER can also play a role in integrating the inputs of different neurotransmitters. Cholinergic inputs enhanced the depolarization-induced endocannabinoid release and augmented the suppression of GABAergic inputs in the hippocampus [32,38] and striatum [45]. Because the postsynaptic depolarization is generally evoked by glutamatergic inputs, PLC $\beta$  could play a role in integrating glutamatergic and cholinergic

inputs and modulating GABAergic inputs through the endocannabinoid signal in these neurons.

There are several studies suggesting the involvement of Ca-RER in long-term plasticity, which includes cerebellar long-term depression (LTD) [24] and cortical spike-timing-dependent plasticity (STDP) [46,47]. Cerebellar LTD, which is induced by repeating conjunctive PF and CF stimulation, has been shown to require postsynaptic  $\text{Ca}^{2+}$  elevation and mGluR1 activation [48]. A recent study has demonstrated that cerebellar LTD requires 2-AG release and CB1R activation [24], suggesting that Ca-RER triggered by postsynaptic  $\text{Ca}^{2+}$  elevation and mGluR1 activation is involved in cerebellar LTD. STDP is induced by pre- and postsynaptic firings with a certain pre-to-post timing. At excitatory synapses in the sensory cortex, repeated presynaptic firing preceding postsynaptic firing by 0-20 ms generally induces long-term potentiation (tLTP), whereas pairing with the inverse timing induces long-term depression (tLTD). In a classical model of STDP, postsynaptic NMDA receptors (NMDARs) function as the sole coincidence detector for induction of both tLTP and tLTD [49,50]. Alternatively, two coincidence detector model for STDP is proposed [51], which is supported by recent studies. Bender et al. investigated STDP at excitatory layer 4 synapses on layer 2/3 (L2/3) pyramidal cells in somatosensory cortex [46]. They found that postsynaptic NMDARs were required for tLTP but not for tLTD, whereas mGluR5, voltage-gated  $\text{Ca}^{2+}$  channels,  $\text{IP}_3\text{R}$  and CB1Rs were required for tLTD. Nevian and Sakmann reported that tLTD at basal dendrites of L2/3 pyramidal cells in the somatosensory cortex involved mGluR-PLC pathway and endocannabinoid signaling, but did not require postsynaptic NMDARs [47]. The authors proposed that mGluRs function as a postsynaptic switch, which determines the direction of STDP. Interestingly, these tLTDs is independent of postsynaptic NMDARs, but require non-postsynaptic (probably presynaptic) NMDAR activation, as reported at tLTD in visual cortex [52]. A presumed mechanism of endocannabinoid release for inducing tLTD is as follows. Postsynaptic firing causes a rise of postsynaptic  $\text{Ca}^{2+}$ , which boosts the mGluR-PLC $\beta$  cascade driven by the subsequent presynaptic firing and effectively triggers the retrograde endocannabinoid signal. In this model, PLC $\beta$  plays as the second coincidence detector.

## **Mechanisms of terminating endocannabinoid signal**

The magnitude and time-course of endocannabinoid signaling is determined by the balance between the generation and degradation of endocannabinoids. The major endocannabinoid 2-AG is hydrolyzed by monoacylglycerol lipase (MGL) [53], which is localized at axon terminals [54]. It was originally reported that MGL is responsible for termination of hippocampal DSI by using a newly introduced MGL inhibitor, URB754 [55]. However, later studies failed to confirm the effects of this compound on MGL activity and DSI [21,56,57]. This discrepancy was recently solved by the correction showing that the previously reported effects of URB754 are not caused by URB754 itself, but caused by some contaminated substances [58]. Instead of URB754, the contribution of MGL activity to terminating the endocannabinoid signal was confirmed by showing the prolongation of DSI/DSE by generally used MGL inhibitors [21,59]. Interestingly, it was found that these MGL inhibitors suppressed the basal synaptic transmission in a CB1-dependent manner [21], suggesting that 2-AG is constitutively released and degraded by presynaptic MGL even at basal conditions. The blockade of another endocannabinoid degradation enzyme, cyclooxygenase-2 (COX-2), also prolonged DSI [18]. In contrast to MGL, COX-2 was suggested to degrade 2-AG within postsynaptic neurons before it is released [18]. The termination mechanism of endocannabinoid signal is shown in Figure 1 (brown arrows).

## **Conclusions and future directions**

After the first discovery of retrograde endocannabinoid signaling, a growing body of evidence has been presented that the endocannabinoid signal is widely used for induction of short-term and long-term synaptic plasticity throughout the brain. Although endocannabinoid release can be induced by multiple mechanisms, Ca-RER, which depends on both of PLC $\beta$  and Ca<sup>2+</sup>, seems most physiologically important. In this review, we introduced several studies suggesting that Ca-RER plays a crucial role in endocannabinoid-mediated synaptic plasticity, which is induced by presynaptic repetitive firing, associated activities of two distinct synaptic inputs, or combined pre- and postsynaptic firings with a certain pre-to-post timing. In either case, PLC $\beta$  appears to detect the coincidence of Ca<sup>2+</sup> elevation and G<sub>q/11</sub>-coupled receptor activation in the postsynaptic neuron and effectively generate the endocannabinoid signal, which then activates presynaptic CB1Rs. It is not clear how presynaptic CB1R activation causes a



long-lasting change in transmitter release. In other forms of endocannabinoid-mediated LTD, a similar question still remains open. The downstream signaling cascades from CB1R activation to long-lasting modification of transmitter release should be determined in future studies.

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16• (Maejima et al., 2005)

This study shows that mGluR1-driven endocannabinoid release requires PLC $\beta$ 4 and is enhanced by intracellular Ca<sup>2+</sup> elevation in cerebellular PCs. The study also demonstrates that this Ca<sup>2+</sup>-assisted mGluR1-PLC $\beta$ 4 cascade is essential for endocannabinoid release triggered by a short train of PF stimulation. Furthermore, the authors show convincingly by biochemical analysis that combination of weak mGluR1 activation and mild depolarization in PCs effectively induces 2-AG production in a PLC $\beta$ 4-dependent manner.

35• (Yoshida et al., 2006)

This study demonstrates subcellular localization of DGL $\alpha$  in the cerebellum and hippocampus. Anatomical data indicate that in the cerebellum, DGL $\alpha$  is predominantly expressed in PCs and is concentrated at the base of spine neck and somatodendritic surface but not in the spine head. By contrast, in the hippocampal pyramidal cells, DGL $\alpha$  is distributed in either spine head or neck or both. By examining anatomical relationship of DGL $\alpha$  distribution with other endocannabinoid signaling molecules including mGluR1, PLC $\beta$ 4 and CB1R, the authors show that these elements are finely organized at excitatory synapses.

36• (Katona et al., 2006)

This paper also shows the localization of DGL $\alpha$  in the hippocampus. *In situ* hybridization analysis shows that principal neurons express high levels of DGL $\alpha$  mRNA throughout the hippocampal formation. Immunostaining data indicate that DGL $\alpha$  is concentrated in the spine heads of principal neurons. This study also shows that CB1Rs are located on the glutamatergic presynaptic terminals opposite to the DGL $\alpha$ -expressing postsynaptic site.

38•• (Hashimotodani et al., 2005)

This study demonstrates that endocannabinoid release driven by group I mGluR or muscarinic receptors in hippocampal neurons requires PLC $\beta$ 1 and exhibits a sharp Ca<sup>2+</sup> dependence within a physiological range. The study shows that combination of muscarinic receptor activation and depolarization markedly facilitates endocannabinoid release in a PLC $\beta$ 1-dependent manner and the timing



of two stimuli is critical for this enhancement. Using DG-sensitive TRPC6 channels as a real-time monitor of PLC activity, the authors confirm that muscarinic receptor-driven PLC $\beta$ 1 activation exhibits the same Ca<sup>2+</sup>-dependency, and conclude that PLC $\beta$ 1 serves as a coincidence detector of Ca<sup>2+</sup> elevation and G<sub>q/11</sub>-coupled receptor activation for induction of endocannabinoid release.

44•• (Brenowitz et al., 2005)

In this paper, the authors demonstrate that endocannabinoid release evoked by PF stimulation is greatly enhanced by coactivation of CF synapses in cerebellar PCs. The authors suggest that this short-term form of associative synaptic plasticity involves two mechanisms. First, PF-induced mGluR1 activation reduces the Ca<sup>2+</sup> requirement of endocannabinoid release. Second, PF and CF coactivation induces supralinear dendritic calcium transients.

46•• (Bender et al., 2006)

This study supports the two coincidence detector model for STDP. The study shows that in the somatosensory cortex spike-timing-dependent LTP requires postsynaptic NMDARs, whereas LTD requires mGluR5, Ca<sup>2+</sup> influx through voltage-gated Ca<sup>2+</sup> channels, IP<sub>3</sub>Rs, retrograde endocannabinoid signaling, and non-postsynaptic, probably presynaptic NMDARs.

47•• (Nevian & Sakmann, 2006)

This study also supports the two coincidence detector model for STDP. The authors demonstrate that spike-timing-dependent LTD requires postsynaptic Ca<sup>2+</sup> elevation, activation of mGluRs and PLC, and endocannabinoid signaling, but not postsynaptic NMDARs. Importantly, this study shows that the amplitude of spine Ca<sup>2+</sup> increase is not correlated to the direction of plasticity (LTP or LTD). The authors suggest that mGluR-coupled signaling cascade is important for determination of the direction of plastic change.

## Figure legend

### Figure 1

Schematic models of retrograde endocannabinoid signaling. Strong postsynaptic depolarization causes large intracellular  $\text{Ca}^{2+}$  elevation, which induces DG production through unknown pathways (red arrows). Activation of  $\text{G}_q$ -coupled receptor stimulates  $\text{PLC}\beta$  and yields DG (blue arrows). When  $\text{G}_q$ -coupled receptor activation coincides with mild  $\text{Ca}^{2+}$  elevation induced by weak postsynaptic depolarization (green arrows), DG is produced more effectively than without  $\text{Ca}^{2+}$  elevation because of the  $\text{Ca}^{2+}$  dependency of  $\text{PLC}\beta$ . Another  $\text{PLC}\beta$  product,  $\text{IP}_3$ , might facilitate the receptor- $\text{PLC}\beta$  signaling by releasing  $\text{Ca}^{2+}$  from endoplasmic reticulum (ER) through  $\text{IP}_3\text{Rs}$  (purple arrows). DG is then converted to 2-AG through DGL activity (black arrow). 2-AG is released, binds to presynaptic  $\text{CB1Rs}$ , probably through fast lateral diffusion across the membrane lipid bilayer, and causes suppression of neurotransmitter release. Degradation of 2-AG is mediated by postsynaptic  $\text{COX-2}$  and presynaptic  $\text{MGL}$  activity (brown arrows). In this scheme, synapse is generalized and not specified as either excitatory or inhibitory. PI, phosphatidylinositol; VGCC, voltage-gated  $\text{Ca}^{2+}$  channel.

