Calcium signaling and synaptic modulation: Regulation of endocannabinoid-mediated synaptic modulation by calcium

Takako Ohno-Shosaku, Yuki Hashimotodani, Takashi Maejima, Masanobu Kano

Abstract

Postsynaptic Ca\(^{2+}\) signal influences synaptic transmission through multiple mechanisms. Some of them involve retrograde messengers that are released from postsynaptic neurons in a Ca\(^{2+}\)-dependent manner and modulate transmitter release through activation of presynaptic receptors. Recent studies have revealed essential roles of endocannabinoids in retrograde modulation of synaptic transmission. Endocannabinoid release is induced by either postsynaptic Ca\(^{2+}\) elevation alone or activation of postsynaptic G\(_{q/11}\)-coupled receptors with or without Ca\(^{2+}\) elevation. The former pathway is independent of phospholipase C\(_{252}\) (PLC\(_{252}\)) and requires a large Ca\(^{2+}\) elevation to a micromolar range. The latter pathway requires PLC\(_{252}\) and is facilitated by a moderate Ca\(^{2+}\) elevation to a submicromolar range. This facilitation is caused by Ca\(^{2+}\)-dependency of receptor-driven PLC\(_{252}\) activation. The released endocannabinoids then activate presynaptic cannabinoid receptor type 1 (CB1), and suppress transmitter release from presynaptic terminals. Both CB1 receptors and G\(_{q/11}\)-coupled receptors are widely distributed in the brain. Thus, the endocannabinoid-mediated retrograde modulation may be an important and widespread mechanism in the brain, by which postsynaptic events including G\(_{q/11}\)-coupled receptor activation and Ca\(^{2+}\) elevation can retrogradely influence presynaptic function.

Keywords: Calcium signaling; G\(_{q/11}\)-coupled receptor; Endocannabinoid; Phospholipase C\(_{252}\)

1. Introduction

In the nervous system, postsynaptic Ca\(^{2+}\) ions play important roles in the regulation of synaptic transmission [1]. Elevation of postsynaptic Ca\(^{2+}\) concentration triggers several forms of short- and long-term synaptic modulations through multiple mechanisms. The Ca\(^{2+}\) elevation can modulate functions of postsynaptic receptors through phosphorylation/dephosphorylation processes [2–4]. The Ca\(^{2+}\) elevation can also induce a change in the number of surface receptors on postsynaptic membrane through insertion/endocytosis processes [5,6]. In addition to these postsynaptic changes, presynaptic function can also be controlled by postsynaptic Ca\(^{2+}\) signal through retrograde messengers [7]. Endocannabinoids are the most widely accepted substances as retrograde messengers in the brain. The production/release of endocannabinoids is known to depend on Ca\(^{2+}\)-signaling in postsynaptic neurons [7–12]. In this review, we focus on the roles of postsynaptic Ca\(^{2+}\) signaling in endocannabinoid-mediated retrograde modulation of presynaptic function.

2. Cannabinoid receptors and endocannabinoids

Cannabinoid receptor type 1 (CB1) is one of the most abundant G protein-coupled seven-transmembrane-domain receptors in the brain. This receptor is located densely on axons and axon terminals of certain types of neu-
CB1 receptors, and suppress transmitter release [7–12]. In below), and assumed to diffuse out across the cell membrane are synthesized in response to certain stimuli (described whereas 2-AG is mainly produced by phospholipase C (PLC) and diacylglycerol (DAG) lipase [22–24]. These molecules are synthesized in response to certain stimuli (described below), and assumed to diffuse out across the cell membrane immediately after their production. The endocannabinoids released from postsynaptic neurons then activate presynaptic CB1 receptors, and suppress transmitter release [7–12]. In the following sections, we describe the roles of postsynaptic Ca2+ signaling in generating endocannabinoids that mediate retrograde synaptic modulation.

3. Endocannabinoid release triggered by depolarization-induced Ca2+ elevation

In various brain regions, it has been demonstrated that postsynaptic depolarization-induced Ca2+ elevation causes endocannabinoid release, and suppresses transmitter release through activation of presynaptic CB1 receptors. [25–30]. This phenomenon was originally found at inhibitory synapses in the cerebellum [31] and then in the hippocampus [32], and was named depolarization-induced suppression of inhibition (DSI). Later, a similar phenomenon induced by postsynaptic depolarization was found at excitatory synapses on cerebellar Purkinje cells [27], and was termed depolarization-induced suppression of excitation (DSE). Both DSI and DSE involve endocannabinoids as retrograde messengers [25–30], and require a large Ca2+ elevation to a micromolar range [33,34]. Several studies estimated the Ca2+ level required for DSI/DSE induction. The estimated Ca2+ level required for DSI/DSE induction is ~4 μM for hippocampal DSI [34] and ~15 μM for cerebellar DSI/DSE to attain the half-maximal effect [33]. Since DSI/DSE requires strong Ca2+ elevation, its physiological significance might be limited. In fact, a study on hippocampal slices has demonstrated that normal firing patterns of hippocampal neurons that occur in vivo do not elicit DSI [35]. The molecular identity of endocannabinoids that mediate DSI/DSE is controversial. In a study on hippocampal DSI, both the PLC inhibitor U73122 and the DAG lipase inhibitor RHC-80267 failed to suppress DSI, suggesting that the retrograde messenger is not 2-AG [36]. Another study on hippocampal DSI showed that inhibition of cyclooxygenase-2 (COX-2), but not fatty acid amidase hydrolase (FAAH), prolonged DSI [37]. FAAH mainly degrades anandamide, whereas COX-2 can degrade both 2-AG and anandamide. Thus, the negative effect of FAAH inhibitor on DSI suggests that 2-AG rather than anandamide is released during DSI. In either case, it is still unclear which enzyme is activated by Ca2+.

4. Endocannabinoid release triggered by activation of Gq/11-coupled receptors

In addition to strong Ca2+ elevation, postsynaptic activation of Gq/11-coupled receptors also causes endocannabinoid-mediated retrograde suppression without Ca2+ elevation [38–40]. This type of modulation was originally found in cerebellar Purkinje cells [38]. In this study, application of DHPG, an agonist of Gq/11-metabotropic glutamate receptors (mGluRs), induced suppression of excitatory transmission from climbing fibers to Purkinje cells in cerebellar slices. By examining the mechanisms of this DHPG-induced suppression, it has been demonstrated that activation of group I mGluRs on Purkinje cells induces release of endocannabinoids, which retrogradely act on presynaptic CB1 receptors and suppress glutamate release from climbing fiber terminals. Importantly, this mGluR-induced endocannabinoid release does not require Ca2+ elevation in Purkinje cells [38]. The endocannabinoid-mediated retrograde modulation triggered by activation of group I mGluRs was also found at hippocampal inhibitory synapses [39,40]. Similar to group I mGluRs, activation of postsynaptic M1/M3 muscarinic receptors also causes endocannabinoid release and retrogradely suppresses transmitter release by activating presynaptic CB1 receptors [41]. Since both group I mGluRs and M1/M3 receptors are coupled to PLC through Gq/11, proteins, it is most likely that the molecule that mediates these forms of suppression is 2-AG rather than anandamide.

5. Endocannabinoid release triggered by activation of Gq/11-coupled receptors and Ca2+ elevation

As described above, endocannabinoid release is triggered by either strong Ca2+ elevation or activation of Gq/11-coupled receptors such as group I mGluRs and M1/M3 muscarinic receptors. Although these two stimuli can drive endocannabinoid signaling independently, they can also work in a cooperative manner to produce endocannabinoids. It has been reported that the endocannabinoid release is markedly facilitated when Ca2+ elevation is combined with the activation of group I mGluRs or M1/M3 receptors [39,42,43]. In our studies on cultured hippocampal neurons, the amount of released endocannabinoids is estimated to be several times higher than the simple sum of the amounts induced by each stimuli alone [39,42]. It should be noted that weak receptor activation by a low dose of agonist and small Ca2+ elevation by a short depolarization, both of which do not induce endocannabinoid release when applied separately, can drive endocannabinoid signaling when applied simultaneously [39,42].
Fig. 1. Receptor-driven endocannabinoid release is dependent on intracellular Ca\textsuperscript{2+} concentration. (A) Cannabinoid-sensitive inhibitory postsynaptic currents (IPSCs) were recorded from rat cultured hippocampal neurons dialyzed with various pCa solutions. Application of the muscarinic agonist oxotremorine-M (oxo-M) suppressed IPSCs in a Ca\textsuperscript{2+}-dependent manner. (B) Averaged data for oxo-M-induced suppression of IPSCs at three different pCa levels. (Modified from[50].).

Thus, it is expected that some mechanisms must exist to detect the coincidence of the two stimuli for endocannabinoid release. It is known that G\textsubscript{q/11}-coupled receptors including group I mGluRs and M\textsubscript{1}/M\textsubscript{3} receptors [44,45] activate PLC\textsubscript{β}, one of the five types (I, II, III, IV, and V) of PLC. It is also known that all of the five types of PLC require Ca\textsuperscript{2+} for their catalytic function, although Ca\textsuperscript{2+} concentration required for maximum activation is different in different PLC isozymes [44,46–49]. Thus, if PLC\textsubscript{β} activation is required for endocannabinoid production and sensitive to Ca\textsuperscript{2+} elevation in a physiological range, PLC\textsubscript{β} can work as a coincidence detector for triggering endocannabinoid generation. We have recently tested this possibility [50]. By measuring cannabinoid-sensitive synaptic currents, we have found that the receptor-driven endocannabinoid release is dependent on physiological levels of intracellular Ca\textsuperscript{2+} concentration (Fig. 1) and is markedly enhanced by depolarization-induced Ca\textsuperscript{2+} elevation. By using exogenous TRPC6 channel as a biosensor for the PLC product diacylglycerol, we have demonstrated that the receptor-driven PLC activation exhibits a similar Ca\textsuperscript{2+}-dependence to that of endocannabinoid release (Fig. 2). Neither endocannabinoid release nor PLC activation was induced by receptor activation in PLC\textsubscript{β1}-knockout mice. From these results, we conclude that PLC\textsubscript{β1} serves as a coincidence detector through its Ca\textsuperscript{2+}-dependency for triggering endocannabinoid release in hippocampal neurons. In contrast, the endocannabinoid-release induced by strong Ca\textsuperscript{2+} elevation alone was intact in the PLC\textsubscript{β1}-knockout mouse, indicating that strong Ca\textsuperscript{2+} elevation can drive PLC\textsubscript{β1}-independent pathway for endocannabinoid release [50].

Fig. 2. Receptor-driven PLC\textsubscript{β} activation is dependent on intracellular Ca\textsuperscript{2+} concentration. (A) Rat cultured hippocampal neurons expressing TRPC6 channel were dialyzed with various pCa solutions. Application of oxo-M induced an inward TRPC6 current, which reflects PLC\textsubscript{β} activity, in a Ca\textsuperscript{2+}-dependent manner. (B) Averaged data for oxo-M-induced suppression of TRPC6 current at four different pCa levels. (Modified from[50].).

6. Current models of endocannabinoid-mediated retrograde modulation

Fig. 3 shows current models for the mechanisms of endocannabinoid-mediated retrograde modulation. Postsynaptic depolarization triggers Ca\textsuperscript{2+} influx by activating voltage-gated Ca\textsuperscript{2+} channels, and causes transient elevation of intracellular Ca\textsuperscript{2+} concentration. When Ca\textsuperscript{2+} concentration is elevated to a micromolar range, endocannabinoids can be produced through PLC\textsubscript{β1}-independent pathway and are released from postsynaptic neurons (Fig. 3A). Activation of G\textsubscript{q/11}-coupled receptors such as group I mGluRs and M\textsubscript{1}/M\textsubscript{3} muscarinic receptors stimulates PLC\textsubscript{β}, and causes 2-AG production through DAG lipase activity (Fig. 3B). This PLC\textsubscript{β1}-dependent endocannabinoid release is markedly facilitated by Ca\textsuperscript{2+} elevation because of the Ca\textsuperscript{2+}-dependency of PLC\textsubscript{β1} (Fig. 3B). When receptors are strongly activated by a high dose of agonist, the endocannabinoid release can be induced even at a resting level of Ca\textsuperscript{2+} concentration. When receptors are activated weakly by a low dose of agonist, the endocannabinoid release is not induced at a resting Ca\textsuperscript{2+} level, but can be induced effectively when combined with Ca\textsuperscript{2+} elevation. The Ca\textsuperscript{2+} level required for this effect is a submicromolar range. This Ca\textsuperscript{2+}-assisted drive of PLC\textsubscript{β1}-dependent pathway seems of physiological significance. Through this pathway, mild receptor activation combined with small Ca\textsuperscript{2+} elevation, both of which appear to occur under physiological conditions, can trigger endocannabinoid release effectively. The released endocannabinoids diffuse retrogradely and bind to CB1 receptor on excitatory or inhibitory presynaptic terminals. The binding of endocannabinoids to the CB1 receptor activates G\textsubscript{i/o} proteins and suppresses the release of glutamate or GABA, presumably by inhibiting voltage-gated Ca\textsuperscript{2+} outflow.
Fig. 3. Current models of endocannabinoid-mediated retrograde suppression of central synapses. (A) Strong postsynaptic depolarization induces endocannabinoid production through PLC-$\gamma$-independent pathway. This pathway requires elevation of intracellular Ca$^{2+}$ concentration to a micromolar range. (B) Postsynaptic activation of G$_{q/11}$-coupled receptors induces the production of endocannabinoid, presumably 2-AG, through PLC-$\gamma$-dependent pathway. This pathway is facilitated by mild Ca$^{2+}$ elevation to a submicromolar range.

7. Involvement of endocannabinoids in induction of long-term synaptic plasticity

Several recent studies have reported that the endocannabinoid signal is required for long-term synaptic plasticity. In the striatum, high-frequency stimulation of the input from the cortex leads to long-term depression (LTD) of excitatory transmission [55]. This striatal LTD was blocked by the CB1 antagonist SR141716A, and was absent in CB1-deficient mice [56]. Examination of the effects of SR141716A application at various time points suggests that CB1 activation is necessary for LTD induction but not for LTD maintenance [57]. A similar CB1-dependent LTD of excitatory transmission has been reported in the nucleus accumbens [58]. CB1-dependent LTD of inhibitory transmission, which is induced by low-or high-frequency stimulation, has been reported in the basolateral amygdala [59] and the hippocampus [36]. These studies demonstrated that LTD of inhibitory transmission (I-LTD) was blocked by CB1 antagonists and was absent in CB1-deficient mice [36,59]. The hippocampal I-LTD was blocked by group I mGluR antagonists, the PLC inhibitor U73122, and the DAG lipase inhibitor RHC-80267, suggesting that 2-AG is produced during LTD induction through group I mGluR-G$_{q/11}$-PLC pathway and DAG lipase [36]. These findings suggest that endocannabinoids are involved in certain forms of long-term synaptic plasticity as well as short-term synaptic modulation. Presynaptic mechanisms of these forms of synaptic plasticity remain to be elucidated.

8. Physiological significance of receptor-G$_{q/11}$-PLC$\gamma$ pathway in synaptic modulation

The receptor-G$_{q/11}$-PLC$\gamma$ signaling pathway is required for endocannabinoid generation, especially under physiological conditions. This pathway is also involved in multiple intracellular signaling pathways other than endocannabinoid generation. The PLC$\gamma$ product IP$_3$ drives Ca$^{2+}$ mobilization from internal stores through IP$_3$ receptors. The other PLC$\gamma$ product DAG activates protein kinase C (PKC) and plays important roles in short- and long-term synaptic modulations [60,61]. All these signaling pathways are under the control of the extent of G$_{q/11}$-coupled receptor activation and intracellular Ca$^{2+}$ level. Various types of G$_{q/11}$-coupled receptors are distributed in the brain, and are activated by the corresponding neurotransmitters or modulators. It is therefore likely that the receptor-G$_{q/11}$-PLC$\gamma$ pathway contributes to various forms of activity-dependent synaptic modulation through Ca$^{2+}$, PKC and endocannabinoids.

References

I. Llano, N. Leresche, A. Marty, Calcium entry increases the sensitivity of cerebellar Purkinje cells to applied GABA and decreases inhibitory synaptic currents, Neuron 6 (1991) 565–574.

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[53] H. Daniel, F. Crepel, Control of Ca\(^{2+}\) influx by cannabinoid and metabotropic glutamate receptors in rat cerebellar cortex requires K\(^+\) channels, J. Physiol. 537 (2001) 793-800.


