Control of synaptic transmission in the CNS through endocannabinoid-mediated retrograde signaling

CONTROL OF SYNAPTIC TRANSMISSION IN THE CNS THROUGH ENDOCANNABINOID-MEDIATED RETROGRADE SIGNALING

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1. INTRODUCTION

Psychological and physiological effects of marijuana are caused by binding of its active component (Δ^9-tetrahydrocannabinol) to cannabinoid receptors. The cannabinoid receptors belong to a family of G protein-coupled seven-transmembrane-domain receptors, and consist of type 1 (CB1) and type 2 (CB2) receptors with different distributions (Matsuda *et al.*, 1990; Munro *et al.*, 1993; Felder and Glass, 1998). The CB1 receptor is expressed in the CNS, whereas the CB2 receptor is found in the immune system of the periphery (Klein *et al.*, 1998). Activation of the CB1 receptor induces various effects on neural functions (Di Marzo *et al.*, 1998; Felder and Glass, 1998), including suppression of neurotransmitter release (Gifford and Ashby, 1996; Ishac *et al.*, 1996; Shen *et al.*, 1996; Katona *et al.*, 1999; Hoffman and Lupica, 2000). Several molecules are identified as candidate endogenous ligands for cannabinoid receptors (endocannabinoids). Arachidonylethanolamide (anandamide) and 2-arachidonoylglycerol (2-AG), two major endocannabinoids, are reported to be synthesized from membrane phospholipids in an activity- and a Ca^{2+}-dependent manners (Cadas *et al.*, 1996; Stella *et al.*, 1997; Di Marzo *et al.*, 1998; Bisogno *et al.*, 1999; Piomelli *et al.*, 2000). It is thought that they can diffuse out across the cell membrane. The released endocanabinoids are removed from the extracellular space through uptake and enzymatic degradation (Mechoulam *et al.*, 1998). All these findings suggest that endocannabinoids can work as a diffusible and short-lived mediator that is released from activated neurons, binds to cannabinoid receptors on neighboring neurons and modulate their functions.

Recent electrophysiological studies have revealed that endocannabinoids play an important role in retrograde modulation of synaptic transmission in the CNS (Kreitzer

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and Regehr, 2001b; Maejima *et al.*, 2001a; Ohno-Shosaku *et al.*, 2001; Wilson and Nicoll, 2001). Endocannabinoids are released from postsynaptic neurons in response to either depolarization or activation of $G_{q/11}$-coupled receptors such as group I metabotropic glutamate receptors (mGluRs) and M_1/M_3 muscarinic acetylcholine receptors. The released endocannabinoids then activate presynaptic cannabinoid receptors and suppress transmitter release (Maejima *et al.*, 2001b; Alger, 2002; Kano *et al.*, 2002; Kreitzer and Regehr, 2002; Wilson and Nicoll, 2002; Freund *et al.*, 2003; Kano *et al.*, 2003; Piomelli, 2003). Thus, the endocannabinoid signaling is an important mechanism by which postsynaptic neuronal activity can retrogradely influence presynaptic functions. In this review, we introduce recent electrophysiological studies on endocannabinoid-mediated retrograde modulation and discuss its possible physiological roles in the CNS.

2. ENDOCANNABINOID-MEDIATED RETROGRADE MODULATION OF SYNAPTIC TRANSMISSION

2.1. Depolarization-Induced Suppression of Inhibition (DSI)

More than ten years ago, it was found that depolarization of a postsynaptic neuron induces transient suppression of inhibitory postsynaptic currents (IPSCs) recorded from the depolarized neuron in the cerebellum (Llano *et al.*, 1991) and the hippocampus (Pitler and Alger, 1992; Ohno-Shosaku *et al.*, 1998). This phenomenon was termed depolarization-induced suppression of inhibition (DSI). DSI is induced by a depolarizing voltage pulse with a long duration (several seconds). However, a train of action potentials can also induce DSI (Pitler and Alger, 1992; Morishita and Alger, 2001; Ohno-Shosaku *et al.*, 2001), suggesting that DSI may occur physiologically *in vivo*. DSI lasts for tens of seconds and the magunitude of DSI depends on the duration of depolarization (Ohno-Shosaku *et al.*, 1998; Lenz and Alger, 1999). Injection of a fast Ca^{2+} cheleter, BAPTA, into the postsynaptic neuron inhibits DSI (Pitler and Alger, 1992; Vincent and Marty, 1993; Ohno-Shosaku *et al.*, 2001), indicating that elevation of postsynaptic Ca^{2+} concentration is required for the induction of DSI. DSI accompanies a clear increase in the paired-pulse ratio (Ohno-Shosaku *et al.*, 1998; Wilson and Nicoll, 2001), a widely used indicator of presynaptic modulation (Zucker and Regehr, 2002), but not a comparable decrease in the postsynaptic sensitivity to the inhibitory transmitter GABA (Pitler and Alger, 1992; Ohno-Shosaku *et al.*, 2001). These data indicate that the depolarization-induced elevation of intracellular Ca^{2+} concentration in the postsynaptic neuron induces suppression of GABA release from presynaptic terminals. Thus, it is evident that DSI requires a retrograde signal from depolarized postsynaptic neurons to presynaptic terminals.

Recent electrophysiological studies have revealed that endocannabinoids mediate the retrograde signal of DSI, by using hippocampal cultures (Ohno-Shosaku *et al.*, 2001), hippocampal slices (Wilson and Nicoll, 2001) and cerebellar slices (Kreitzer and Regehr, 2001a; Diana *et al.*, 2002; Yoshida *et al.*, 2002). Our study on hippocampal cultures clearly demonstrates that hippocampal inhibitory synapses are heterogeneous in cannabinoid sensitivity (Ohno-Shosaku *et al.*, 2001). The cannabinoid agonist WIN55,212-2 is effective only at a subset of inhibitory synapses, and suppresses GABA release through activation of the CB1 receptor (Ohno-Shosaku *et al.*, 2001). DSI can be induced only at cannabinoid-sensitive synapses, and blocked by CB1 antagonists (AM

281 and SR141716A) (Fig. 1). These results clearly indicate that DSI is mediated by endocannabinoids (Ohno-Shosaku *et al.*, 2001). Another study on hippocampal slices (Wilson and Nicoll, 2001) shows essentially the same results as those in cultures. Besides, DSI is not affected by postsynaptically-applied botulinum toxin (Wilson and Nicoll, 2001), suggesting that vesicular release is not involved in DSI. In cerebellar slices, it is demonstrated that DSI of Purkinje cells is completely occluded by WIN55,212-2-induced suppression of inhibitory transmission, blocked by CB1 antagonists and deficient in CB1-knockout mice (Kreitzer and Regehr, 2001a; Diana *et al.*, 2002; Yoshida *et al.*, 2002). Involvement of endocannabinoids in DSI has also been reported in other regions of the CNS including the basal ganglia (Wallmichrath and Szabo, 2002) and the neocortex (Trettel and Levine, 2003; Trettel *et al.*, 2004). These findings strongly suggest that endocannabinoids mediate DSI widely in the CNS.
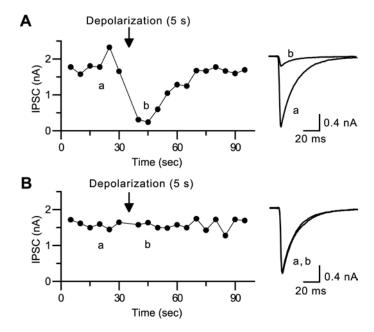

Figure 1. Retrograde modulation induced by depolarization at hippocampal inhibitory synapses. In the experiments shown in Figs. 1-4, inhibitory postsynaptic currents (IPSCs) were recorded from cultured hippocampal neurons prepared from rats, as described previously (Ohno-Shosaku *et al.*, 2001). A. An example of DSI. When a postsynaptic neuron was depolarized from –80 mV to 0 mV for 5 sec, the amplitude of IPSCs was transiently decreased. B: After the treatment with the cannabinoid antagonist AM281 (0.3 μM), the depolarization failed to induce DSI. IPSC traces acquired at the indicated time points are shown on the right.

2.2. Depolarization-Induced Suppression of Excitation (DSE)

Endocannabinoids released from depolarized postsynaptic neurons can also suppress transmitter release from excitatory presynaptic terminals. Depolarization of cerebellar Purkinje cells induces transient suppression of excitatory postsynaptic currents (EPSCs) elicited by stimulation of climbing fibers or parallel fibers (Kreitzer and Regehr,

2001b; Maejima *et al.*, 2001a). This depolarization-induced suppression of excitation (DSE) lasts for tens of seconds, is blocked by postsynaptic injection of BAPTA, and accompanies a clear change in the paired-pulse ratio (Kreitzer and Regehr, 2001b). DSE is blocked by CB1 antagonists (Kreitzer and Regehr, 2001b), and deficient in CB1-knockout mice (our unpublished data). All these properties are quite similar to those of DSI. These results indicate that endocannabinoids are released from depolarized Purkinje cells, and suppress the release of the excitatory transmitter glutamate through activation of the presynaptic CB1 receptor.

DSE can also be induced in other brain regions including the hippocampus (Ohno-Shosaku *et al.*, 2002b) and ventral tegmental area (Melis *et al.*, 2004). In the hippocampus, however, DES is less prominent than DSI. The duration of depolarization required for DSE induction is longer than that for DSI, and the magnitude of DSE is much smaller than that of DSI when the neuron is fully depolarized. It is demonstrated that EPSCs are less sensitive to WIN55,212-2 than the cannabinoid-sensitive IPSCs. Thus, presynaptic cannabinoid sensitivity appears to be a major factor that determines the extent of endocannabinoid-mediated suppression.

While the cerebellar DSE is clearly CB1-dependent, identity of the cannabinoid receptor involved in the hippocampal DSE is controversial. It is reported that the third yet unidentified subtype of cannabinoid receptor (CB3) exists at excitatory synapses in adult hippocampal slices (Hajos *et al.*, 2001; Kofalvi *et al.*, 2003). This is based on the experimental results that the suppression of excitatory synaptic transmission (Hajos *et al.*, 2001) or glutamate release (Kofalvi *et al.*, 2003) by WIN55,212-2 is present in CB1-knockout mice, but the effect is sensitive to the cannabinoid antagonist SR141716A. However, our results on cultured hippocampal neurons indicate that both DSE and the suppression of EPSCs by WIN55,212-2 are totally deficient in CB1-knockout mice (Ohno-Shosaku *et al.*, 2002b). The reason for this discrepancy is unclear. It is possible that the putative CB3 receptor is not expressed in immature neurons, but appears later in adult animals. The molecular identity and the expression pattern of the putative CB3 receptor, if exists, remain to be elucidated.

2.3. Retrograde Modulation Induced by mGluR Activation

In addition to depolarization, postsynatpic activation of certain types of receptors can induce endocannabinoid-mediated retrograde suppression. This type of modulation was originally found in cerebellar Purkinje cells (Maejima *et al.*, 2001a). In mouse cerebellar slices, application of a group I mGluR agonist, (RS)-3,5-dihydroxyphenylglycine (DHPG), induces suppression of climbing fiber-mediated EPSCs (CF-EPSCs) recorded from Purkinje cells. Several lines of evidence indicate that this suppression is caused by activation of mGluR subtype 1 (mGluR1), a member of group I mGluRs, at postsynaptic Purkinje cells. Firstly, the DHPG-induced suppression is blocked by postsynaptic injection of GDP-β-S or GTP-γ-S. Secondly, the suppression is absent in the mGluR1-knockout mouse. Finally, this defect is restored in the mGluR1-rescue mouse in which rat mGluR1α is introduced into the mGluR1-knockout mouse by using a Purkinje cell specific promotor. Although the induction is postsynaptic, the expression of this suppression is clearly presynaptic. The DHPG-induced suppression is associated with a clear increase in the paired-pulse ratio, indicating that the suppression is caused by the reduction of glutamate release from presynaptic terminals. Therefore, some retrograde signal must exist for this synaptic modulation. It is demonstrated that this

retrograde signal is also mediated by endocannabinoids. The DHPG-induced suppression of CF-EPSCs is mimicked and occluded by the cannabinoid agonist WIN55,212-2, and blocked by CB1 antagonists. Thus, activation of mGluR1 on postsynaptic Purkinje cells stimulates production and release of endocannabinoids, which retrogradely act on the presynaptic CB1 receptor and suppress glutamate release from climbing fiber terminals. Importantly, this mGluR-induced endocannabinoid release does not require elevation of intracellular Ca^{2+} concentration in postsynaptic Purkinje cells, forming a striking contrast to the depolarization-induced release of endocannabinoids. Thus, the endocannabinoid-mediated retrograde modulation can be initiated by two distinct stimuli, namely the depolarization-induced Ca^{2+} elevation and the mGluR1 activation. Furthermore, it should be noted that the activation of mGluR1 by synaptically released glutamate after repetitive stimulation of parallel fibers, the other excitatory inputs to Purkinje cells, causes transient cannabinoid-dependent suppression of CF-EPSCs (Maejima *et al.*, 2001a). This result suggests that the mGluR1-mediated generation of the endocannabinoid signal is functional *in vivo* and may contribute to the control of presynaptic functions.
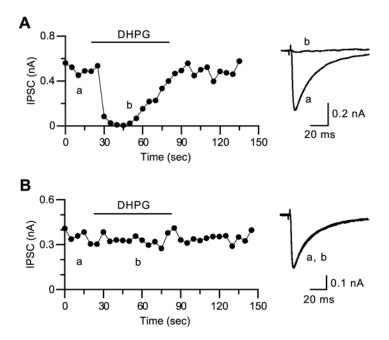

Figure 2. Retrograde modulation induced by activation of group I mGluRs at hippocampal inhibitory synapses. A. Application of a group I mGluR agonist, DHPG (50 µM), induced a suppression of IPSCs. B. This DHPG-induced suppression was blocked by the AM281 treatment.

The endocannabinoid-mediated retrograde modulation triggered by activation of group I mGluRs was also found at hippocampal inhibitory synapses (Varma *et al.*, 2001; Ohno-Shosaku *et al.*, 2002a). A study on cultured hippocampal neurons demonstrates that DHPG suppresses cannabinoid-sensitive IPSCs (Fig. 2A), but not cannabinoid-insensitive IPSCs (Ohno-Shosaku *et al.*, 2002a). This effect is largely blocked by an antagonist

specific to mGluR subtype 5 (mGluR5), the other member of group I mGluRs. The DHPG-induced suppression is associated with an increase in the paired-pulse ratio, and blocked by CB1 antagonists (Fig. 2B) (Ohno-Shosaku *et al.*, 2002a), indicating that the activation of group I mGluRs (mostly mGluR5) produces endocannabinoids and suppresses GABA release from cannabinoid-sensitive presynaptic terminals through activation of the CB1 receptor.

2.4. Retrograde Modulation Induced by Muscarinic Activation

Activation of muscarinic receptors produces various effects on neuronal functions in the CNS. Application of cholinergic or muscarinic agonists depresses synaptic transmission in various regions of the brain, primarily by suppressing transmitter release. Recently, it was demonstrated that muscarinic suppression of hippocampal inhibitory transmission is partly cannabinoid-dependent in slices (Kim *et al.*, 2002) and cultured neurons (Fukudome *et al.*, 2004). In the latter study, it is shown that two distinct mechanisms mediate the muscarinic suppression. At a subset of synapses, activation of the presynaptic M_2 receptor suppresses GABA release directly. In contrast, at a different subset of synapses, activation of the postsynatpic M_1/M_3 receptors causes endocannabinoid release and subsequently suppresses GABA release by activating the presynaptic CB1 receptor (Fig. 3).
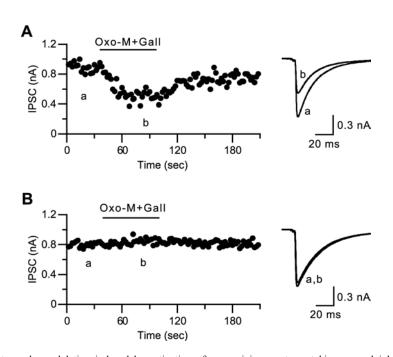

Figure 3. Retrograde modulation induced by activation of muscarinic receptors at hippocampal inhibitory synapses. A. Application of a muscarinic agonist, oxotremoline-M (3 μM), induced suppression of IPSCs under the blockade of the presynaptic M2 muscarinic receptor by gallamine (100 μM). B. This suppression was blocked by the AM281 treatment.

Since both group I mGluRs and M_1/M_3 receptors are coupled to $G_{q/11}$ proteins and capable to trigger endocannabinoid production, it is possible that other $G_{q/11}$-coupled receptors can also be involved in the retrograde modulation by triggering endocannabinoid signaling in the CNS. In this context, it is interesting that the activation of G-protein-coupled corticosteroid receptor triggers the endocannabinoid release and suppresses the glutamate release retrogradely in the hypothalamus (Di *et al.*, 2003), although the molecular identity of this receptor is not clear.

2.5. Interaction between the Depolarization and the Activation of $G_{q/11}$-coupled Receptors

As described above, endocannabinoid release can be triggered by the two distinct stimuli, namely the depolarization-induced Ca^{2+} elevation and the activation of $G_{q/11}$-coupled receptors. These two pathways may interact each other, because simultaneous application of these two stimuli produces a synergistic effect (Varma *et al.*, 2001; Kim *et al.*, 2002; Ohno-Shosaku *et al.*, 2002a; Ohno-Shosaku *et al.*, 2003).

The activation of group I mGluRs by DHPG enhances DSI in hippocampal slices (Varma *et al.*, 2001; Ohno-Shosaku *et al.*, 2002a) and cultures (Fig. 4A) (Ohno-Shosaku *et al.*, 2002a). This enhancement is much more prominant (about 7-times higer) than what is expected from the simple summation of the depolarization-induced and the group I mGluR-induced endocannabinoid release (Ohno-Shosaku *et al.*, 2002a). DHPG causes no change in depolarization-induced Ca^{2+} transients, indicating that the enhanced DSI by DHPG is not due to the augmentation of Ca^{2+} influx. These results suggest that the two pathways work in a cooperative manner to release endocannabinoids, at least partly, through a common intracellular cascade.
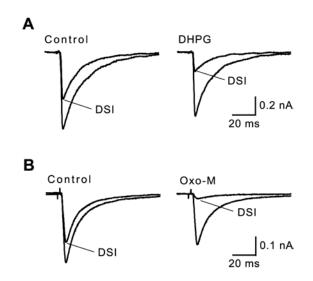

Figure 4. Synergistic effect of depolarization and receptor activation on retrograde suppression. A. DSI induced by a brief depolarization (1 sec) was enhanced by 5 µM DHPG. B. DSI induced by a brief depolarization (1 sec) was enhanced by 0.3 µM oxotremoline-M.

Activation of muscarinic receptors also enhances DSI in hippocampal slices (Kim *et al.*, 2002) and cultures (Ohno-Shosaku *et al.*, 2003). The cholinergic agonist carbachol (CCh) markedly enhances DSI at 0.01-0.3 μM without changing the presynaptic cannabinoid sensitivity (Ohno-Shosaku *et al.*, 2003). The facilitating effect of CCh on DSI is mimicked by a muscarinic agonist (Fig. 4B), and blocked by a muscarinic antagonist. It is also blocked by intracellular injection of GDP-β-S to postsynaptic neurons. By using knockout mice lacking one or two subtypes of muscarinic receptors, it is shown that both M_1 and M_3 receptors are involved in the muscarinic enhancement of DSI (Ohno-Shosaku *et al.*, 2003). These results indicate that the activation of the postsynaptic M_1/M_3 receptors facilitates the depolarization-induced release of endocannabinoids in the hippocampus.

In addition to these $G_{q/11}$-coupled receptors, D_2 dopamine receptors might be also involved in the modulation of endocannabinoid signalling. In ventral tegmental area dopamine neurons, DSE is partially blocked by a D_2 antagonist, and enhanced by a D_2 agonist without changing the presynaptic cannabinoid sensitivity (Melis *et al.*, 2004). Although the D_2 receptor is $G_{i/o}$-coupled, the authors suggest that its enhancing effect on DSE might be caused by the stimulation of PLC, probably through βγ subunits.

2.6. Limited Diffusion of Endocannabinoids

The released endocannabinoids to the extracellular space are thought to be removed rapidly by uptake and enzymatic degradation (Di Marzo *et al.*, 1998; Piomelli *et al.*, 2000). The limited diffusion of endocannabinoids is reported in several electrophysiolial studies on hippocampal and cerebellar slices. In hippocampal slices, it is shown that endocannabinoids released by depolarization can affect only the synapses on neighboring neurons located within 20 μm from the depolarized neurons (Wilson and Nicoll, 2001). In cerebellar slices, it is demonstrated that endocannabinoids released from a Purkinje cell can not spread to the synaptic terminals onto neighboring Purkinje cells (Maejima *et al.*, 2001a) but can spread to closely located interneurons (Kreitzer *et al.*, 2002). These results indicate that the diffusion of endocannabinoids is rather limited in brain tissues. Thus, endocannabinoids can function as a spatially limited local signal in the CNS.

2.7. Current Model of Endocannabinoid-Mediated Retrograde Modulations

Figure 5 shows a current model for the mechanisms of endocannabinoid-mediated retrograde modulations. Postsynaptic depolarization triggers Ca^{2+} influx by activating voltage-gated Ca^{2+} channels, and causes transient elevation of intracellular Ca^{2+} concentration. This Ca^{2+} elevation and the activation of $G_{q/11}$–coupled receptors such as group I mGluRs and M_1/M_3 muscarinic receptors work in a cooperative manner to produce and release endocannabinoids. The released endocannabinoids diffuse retrogradely and bind to the CB1 receptor on excitatory or inhibitory presynaptic terminals. The binding of endocannabinoids to the CB1 receptor activates $G_{i/o}$ proteins and suppresses the release of glutamate or GABA, presumably by inhibiting voltage-gated Ca^{2+} channels (Hoffman and Lupica, 2000; Guo and Ikeda, 2004), activating K^+ channels (Daniel and Crepel, 2001; Guo and Ikeda, 2004) or inhibiting vesicular release machinery directly (Takahashi and Linden, 2000).
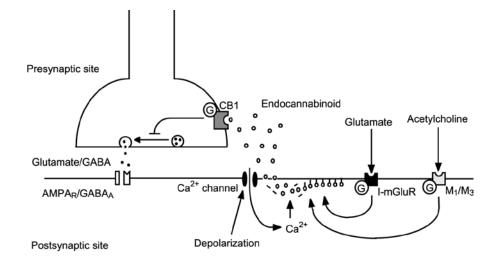
Figure 5. A current model of endocannabinoid-mediated retrograde modulations at central synapses.

2.8. Wide Distribution of the Presynaptic CB1 and Postsynaptic G_q/G_{11}-coupled Receptors in the CNS

Elevation of intracellular Ca^{2+} concentration that triggers endocannabinoid release can be induced by a variety of neural activity through voltage-gated Ca^{2+} channels, NMDA receptors and Ca^{2+} release channels on intracellular stores. Most neurons in the CNS express group I mGluRs on their postsynaptic sites (Abe *et al.*, 1992; Shigemoto *et al.*, 1992; Shigemoto *et al.*, 1997; Thomas *et al.*, 1997). M_1/M_3 muscarinic receptors are also widely distributed in the CNS. In addition, the CB1 receptor is expressed at presynaptic terminals in various regions of the brain (Herkenham *et al.*, 1990; Matsuda *et al.*, 1993; Egertova and Elphick, 2000). Therefore, the endocannabinoid-mediated retrograde modulation observed in the hippocampus and the cerebellum may be a general mechanism, through which postsynaptic Ca^{2+} elevation and/or activation of G_q/G_{11}-coupled receptors can influence presynaptic functions.

3. FUNCTIONAL SIGNIFICANCE OF THE ENDOCANNABINOID-MEDIATED RETROGRADE MODULATION

What could be a functional role of the endocannabinoid-mediated retrograde modulation in the CNS? If endocannabinoids released from an activated neuron suppress its excitatory and inhibitory inputs to the same extent, the excitatory-inhibitory balance of the activated neuron will remain unchanged. In the hippocampus, however, there is a substantial difference in cannabinoid sensitivity between excitatory and inhibitory inputs. When a small amount of endocannabinoid is released from the activated postsynatpic neuron, the cannabinoid-sensitive inhibitory input will be selectively suppressed because of its high cannabinoid sensitivity. Under this condition, the endocannabinoid signal will exert net excitatory action on the postsynaptic neuron. When a large amount of

endocannabinoid is released, both the excitatory and the cannabinoid-sensitive inhibitory inputs will be suppressed, but the cannabinoid-insensitive inhibitory input is intact. Under this condition, the endocannabinoid signal will exert net inhibitory action. Thus, endocannabinoids can control the excitatory-inhibitory balance depending on the postsynaptic activity in the hippocampus.

In addition to the short-term modulation, several recent studies suggest that the endocannabinoid system might also be involved in long-term synaptic plasticity, learning and memory. The induction of long-term potentiation (LTP) of the excitatory transmission in the hippocampus is facilitated during DSI (Carlson *et al.*, 2002). Long-term depression (LTD) of the excitatory transmission in the striatum (Gerdeman *et al.*, 2002; Robbe *et al.*, 2002; Ronesi *et al.*, 2004) and the neocortex (Sjöström *et al.*, 2003) is suppressed by cannabinoid antagonists, and the striatal LTP is absent in CB1-knockout mice. LTD of the inhibitory transmission in the basolateral amygdala (Marsicano *et al.*, 2002) and the hippocampus (Chevaleyre and Castillo, 2003) is also suppressed by cannabinoid antagonists. Furthermore, the extinction of certain forms of memory (Marsicano *et al.*, 2002; Varvel and Lichtman, 2002) is reported to be impaired in CB1-knockout mice. Precise mechanisms for the roles of the endocannabinoid system in long-term synaptic plasticity, learning and memory remain to be investigated.

4. UNRESOLVED QUESTIONS

Several important questions remain unanswered. (1) What molecule(s) (anandamide, 2-AG or some other endocannabinoids) contributes to the retrograde modulation induced by the depolarization and/or the receptor activation? (2) What biochemical pathways are involved and which enzyme(s) is rate-limiting in the generation of endocannabinoids? (3) How do the depolarization and the receptor activation interact in the production and release of endocannabinoid? (4) How are endocannabinoids released across the cell membrane to the extracellular space and uptaken into the cells? (5) How does the activation of the presynaptic CB1 receptor lead to the suppression of transmitter release? (6) How are endocannabinoids involved in long-term synaptic plasticity, learning and memory? (7) How does the endocannabinoid signaling contribute to higher brain functions? Future studies should answer these important questions from the molecular/cellular level to the system level by combining various methodologies.

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