Endocannabinoids and Synaptic Function in the CNS

YUKI HASHIMOTODANI, TAKAKO OHNO-SHOSAKU, and MASANOBU KANO

Marijuana affects neural functions through the binding of its active component (Δ^9-THC) to cannabinoid receptors in the CNS. Recent studies have elucidated that endogenous ligands for cannabinoid receptors, endocannabinoids, serve as retrograde messengers at central synapses. Endocannabinoids are produced on demand in activity-dependent manners and released from postsynaptic neurons. The released endocannabinoids travel backward across the synapse, activate presynaptic CB1 cannabinoid receptors, and modulate presynaptic functions. Retrograde endocannabinoid signaling is crucial for certain forms of short-term and long-term synaptic plasticity at excitatory or inhibitory synapses in many brain regions, and thereby contributes to various aspects of brain function including learning and memory. Molecular identities of the CB1 receptor and enzymes involved in production and degradation of endocannabinoids have been elucidated. Anatomical studies have demonstrated unique distributions of these molecules around synapses, which provide morphological bases for the roles of endocannabinoids as retrograde messengers. CB1-knockout mice exhibit various behavioral abnormalities and multiple defects in synaptic plasticity, supporting the notion that endocannabinoid signaling is involved in various aspects of neural function. In this review article, the authors describe molecular mechanisms of the endocannabinoid-mediated synaptic modulation and its possible physiological significance. NEUROSCIENTIST 13(2):127–137, 2007. DOI: 10.1177/1073858406296716

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Cannabinoid Receptors

The plant *Cannabis sativa* and its derivatives such as marijuana and hashish have been used for thousands of years. They affect neural functions and cause various psychomotor symptoms, including euphoria, appetite stimulation, sedation, analgesia, altered perception, and impairments of motor control, cognition, and memory. The major active component Δ^9-tetrahydrocannabinol (Δ^9-THC), which was identified in 1964 (Gaoni and Mechoulam 1964), and its related compounds have been called cannabinoids. The first breakthrough for cannabinoid research is the discovery of the receptors that bind Δ^9-THC (cannabinoid receptors) in animal tissues. So far, two types of cannabinoid receptors, CB1 (Matsuda and others 1990) and CB2 (Munro and others 1993), have been identified. They are both seven-transmembrane-domain receptors coupled to $G_{i/o}$ protein. Whereas CB1 is dominantly expressed in the CNS, CB2 is expressed mainly in the immune system. Therefore, it is assumed that the CB1 receptor is responsible for most, if not all, of the psychoactivity of cannabis. CB1 is distributed in virtually all regions of the CNS including the neocortex, hippocampus, cerebellum, and basal ganglia (Howlett and others 2002). At the cellular level, however, CB1 is heterogeneously distributed. CB1 is abundantly expressed in subsets of neurons and localized on their presynaptic terminals or axons (Freund and others 2003). Activation of the presynaptic CB1 leads to suppression of release of neurotransmitters including glutamate and GABA (Schlicker and Kathmann 2001). A recent study has shown that CB2 is also expressed in the brainstem and has suggested that the CB2 might be functionally coupled to inhibition of emesis in concert with CB1 (Van Sickle and others 2005). Moreover, presence of additional cannabinoid receptors in the brain has been suggested from pharmacological and genetic studies. It was claimed that so-called CB3 is present at hippocampal excitatory synapses (Hajos and others 2001; Hajos and Freund 2002). This notion was based on the observation that a cannabinoid agonist suppressed hippocampal excitatory transmission even in CB1-knockout mice (Hajos and others 2001) and also on the immunohistochemical data showing the absence of CB1 on hippocampal excitatory presynaptic terminals (Katona and others 1999; Hajos and others 2000). However, recent studies have challenged the CB3 hypothesis. Two groups independently showed that the

From the Department of Neurophysiology, Graduate School of Medicine, Osaka University, Suita, Japan (YH); Department of Impairment Study, Graduate School of Medical Science, Kanazawa University, Kanazawa, Japan (TO-S); and Department of Cellular Neuroscience, Graduate School of Medicine, Osaka University, Suita, Japan (MK).

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Address correspondence to: Masanobu Kano, Department of Cellular Neuroscience, Graduate School of Medicine, Osaka University, Suita 565-0871, Japan (e-mail: mkano@cns.med.osaka-u.ac.jp).

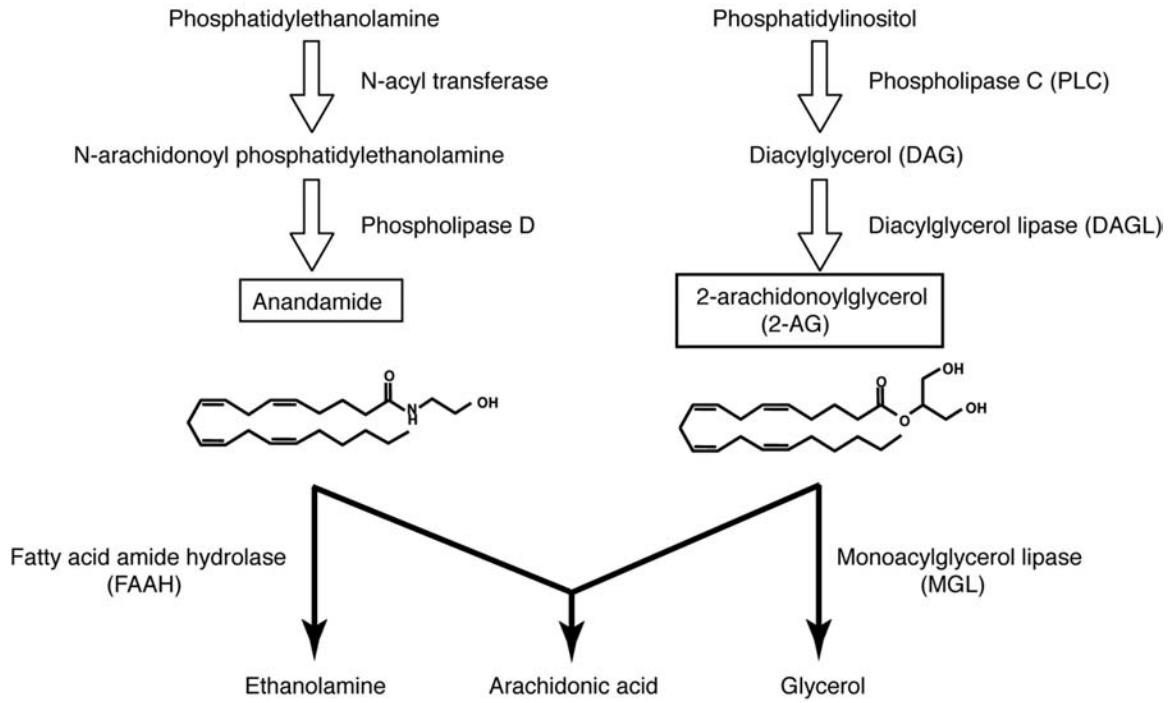

Fig. 1. Main pathways of biosynthesis and degradation of two endocannabinoids, anandamide and 2-arachidonyolglycerol.

cannabinoid agonist WIN55,212-2 failed to suppress excitatory transmission in the hippocampus of CB1-knockout mice (Kawamura and others 2006; Takahashi and Castillo 2006). Moreover, immunohistochemical studies with newly produced antibodies against CB1 have now clearly demonstrated the presence of CB1 on hippocampal excitatory terminals (Katona and others 2006; Kawamura and others 2006; Yoshida and others 2006). Thus, CB1 appears to be the major cannabinoid receptor at excitatory synapses in the CNS.

Endocannabinoids

The second breakthrough in cannabinoid research is the discovery of endogenous ligands for cannabinoid receptors (endocannabinoids). The first identified endocannabinoid is *N*-arachidonoylethanolamide, which was named anandamide after the Sanskrit word *ananda*, which means "bliss" (Devane and others 1992). Anandamide is biosynthesized from membrane lipids by *N*-acyltransferase and phospholipase D (Fig. 1) (Freund and others 2003). The second identified endocannabinoid is 2-arachidonoylglycerol (2-AG) (Mechoulam and others 1995; Sugiura and others 1995). 2-AG is present much more abundantly than anandamide in the brain. 2-AG is produced from membrane lipids mainly through two enzymatic steps by phospholipase C (PLC) and diacylglycerol lipase (DAGL) (Fig. 1) (Freund and others 2003). Although there are some other endogenous substances that can interact with cannabinoid receptors, such as 2-arachidonoylglycerol ether (noladin ether) and *O*-arachidonoylethanolamine (virodhamine), it is unclear whether they can actually work as neuromodulators. Endocannabinoids are metabolized by intracellular enzymes. Anandamide and 2-AG are primarily hydrolyzed in the brain by fatty acid amide hydrolase (FAAH) and monoacylglycerol lipase (MGL), respectively (Fig. 1). FAAH is expressed in the soma and dendrites of neurons (Egertova and others 2003), whereas MGL is distributed in presynaptic terminals (Gulyas and others 2004).

Endocannabinoid-Mediated Short-Term Plasticity

The third breakthrough in cannabinoid research is the discovery that endocannabinoids function as retrograde messengers at synapses in the CNS (Kreitzer and Regehr 2001; Ohno-Shosaku and others 2001; Wilson and Nicoll 2001). In 2001, the three groups independently revealed that endocannabinoids are released from postsynaptic neurons, act retrogradely on presynaptic CB1 receptors, and transiently suppress transmitter release. Since then, dozens of papers have been published that have confirmed the role of endocannabinoids as retrograde messengers in various regions of the CNS. It is now established that endocannabinoid release can be induced by four stimulation protocols, namely, postsynaptic depolarization, activation of postsynaptic G_q-coupled receptors, combined G_q-coupled receptor activation with depolarization, and repetitive synaptic activation. In the following sections, we describe mechanisms of endocannabinoid release by each of the four stimulation protocols.

Depolarization-Induced Endocannabinoid Release

In the cerebellum (Llano and others 1991) and the hippocampus (Pitler and Alger 1992), it was originally found that depolarization of principle neurons induced transient suppression of inhibitory synaptic inputs to the depolarized neurons. This phenomenon was termed depolarization-induced suppression of inhibition (DSI) (Fig. 2). Because DSI was induced by postsynaptic Ca^{2+} elevation and was attributable to suppression of transmitter release (Pitler and Alger 1992; Vincent and Marty 1993), involvement of some retrograde signal from postsynaptic neurons to presynaptic terminals was suggested. Since then, many studies have been done to identify the retrograde signal. In 2001, two research groups finally discovered that endocannabinoids mediate the retrograde signal for hippocampal DSI, by showing that DSI can be mimicked by a synthetic cannabinoid agonist (WIN55,212-2) and suppressed by CB1 antagonists (AM281 and SR141716A) (Fig. 2) in cultured hippocampal neurons (Ohno-Shosaku and others 2001) and hippocampal slices (Wilson and Nicoll 2001). Concurrently, Kreitzer and Regehr reported a counterpart of DSI at excitatory synapses, termed depolarization-induced suppression of excitation (DSE) (Kreitzer and Regehr 2001). Depolarization of cerebellar Purkinje cells (PCs) induced transient suppression of excitatory synaptic transmission onto PCs from climbing fibers (CFs) or parallel fibers (PFs). DSE was blocked by postsynaptic injection of BAPTA to prevent Ca^{2+} elevation and was attributable to suppression of transmitter release, indicating the involvement of some retrograde signal. Furthermore, they found that DSE was prevented by a CB1 antagonist and concluded that cerebellar DSE is also mediated by endocannabinoids. Since the first discovery in 2001, many studies have reported the endocannabinoid-mediated DSI/DSE in various brain regions, which include the cerebellum, neocortex, substantia nigra, brainstem, and hypothalamus for DSI, and the hippocampus, ventral tegmental area (VTA), hypothalamus, and striatum for DSE (Chevaleyre and others 2006). Importantly, Brenowitz and others have reported recently that depolarization-induced endocannabinoid release is likely to occur at physiologically relevant Ca^{2+} elevation after burst firing of cerebellar PCs (Brenowitz and others 2006).

A current model for the depolarization-induced endocannabinoid release is illustrated in Figure 3. Strong depolarization of a postsynaptic neuron activates voltage-gated Ca^{2+} channels, induces Ca^{2+} entry, and causes an elevation of intracellular Ca^{2+} concentration, which in turn triggers the biosynthesis of endocannabinoids. The newly formed endocannabinoids are then released from the depolarized postsynaptic neuron, activate presynaptic CB1 receptors, and suppress the transmitter release from inhibitory (Fig. 3, *left*) or excitatory (Fig. 3, *right*) presynaptic terminals. Molecular identity of the DSI/DSE-mediating endocannabinoid has not been proven conclusively. However, available evidence suggests that 2-AG is the more likely candidate than anandamide, as discussed later. The enzymes responsible for Ca^{2+}-induced endocannabinoid biosynthesis are still unknown.

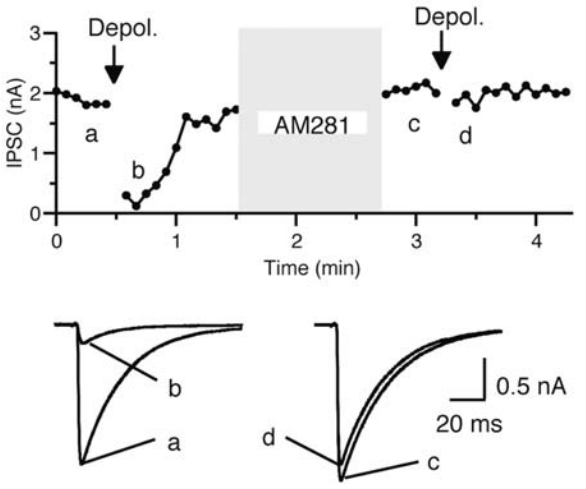

Fig. 2. Representative data showing depolarization-induced suppression of inhibition (DSI). In a pair of cultured hippocampal neurons with inhibitory synaptic connections, the presynaptic neuron was stimulated and inhibitory postsynaptic currents (IPSCs) were recorded from the postsynaptic neuron. Amplitudes of IPSCs were plotted as a function of time. Depolarizing pulse (to 0 mV, 5 sec) to the postsynaptic neuron (arrow) induced a transient suppression of IPSCs (DSI). After treatment of the CB1 antagonist AM281, DSI was abolished.

Receptor-Driven Endocannabinoid Release

Another form of endocannabinoid-mediated short-term plasticity, which is driven by receptor activation, was first found in the cerebellum (Maejima and others 2001). Application of DHPG, a selective agonist of group I metabotropic glutamate receptor (mGluR), induced transient suppression of the excitatory transmission from CFs to PCs. Group I mGluRs are known to couple to G_q protein and consist of mGluR1 and mGluR5. The DHPG-induced suppression was blocked by an mGluR1-prefering antagonist, was eliminated in mGluR1-knockout mice, and was restored in the mGluR1-rescue mice that express mGluR1 only on PCs in the cerebellum. Moreover, the DHPG-induced suppression was abolished by blocking G protein cascades in postsynaptic PCs by GTPγS or GDPβS. These results clearly indicate that the DHPG-induced suppression is caused by activation of mGluR1 in postsynaptic PCs. On the other hand, the site of expression was determined to be presynaptic. The DHPG-induced suppression accompanied clear changes in several presynaptic parameters, such as paired-pulse ratio, coefficient of variation, and frequency of quantal EPSCs. The finding that the DHPG-induced suppression is induced postsynaptically and expressed presynaptically implied the involvement of some retrograde signal. In further experiments, it was found that the suppression was occluded by the cannabinoid agonist WIN55,212-2 and eliminated by the CB1 antagonists. From these results, Maejima and others concluded that activation of mGluR1-induced release of endocannabinoids and suppressed transmitter release

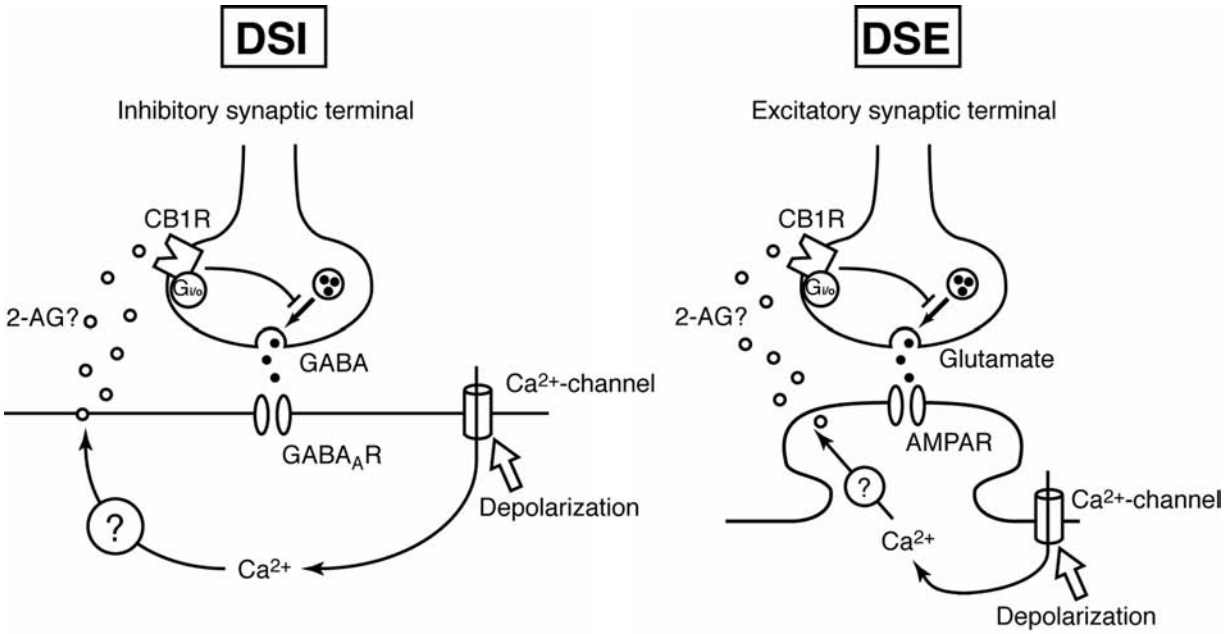

Fig. 3. Current models for the mechanisms of depolarization-induced suppression of inhibition (DSI) and depolarization-induced suppression of excitation (DSE). Postsynaptic depolarization induces Ca^{2+} influx through voltage-gated Ca^{2+} channels. Elevation of intracellular Ca^{2+} concentration triggers biosynthesis of endocannabinoids. Endocannabinoids are then released from postsynaptic neurons, activate presynaptic CB1 receptors, and suppress GABA (DSI, *left*) or glutamate (DSE, *right*) release.

through activation of presynaptic CB1 receptors. Importantly, this mGluR1-driven endocannabinoid release did not require postsynaptic Ca^{2+} elevation. Therefore, mechanisms of the mGluR1-driven endocannabinoid release were assumed to be distinct from those of DSI/DSE. Later, similar endocannabinoid release driven by group I mGluRs was also found at other synapses in several brain regions, which include inhibitory synapses in the hippocampus (Varma and others 2001; Ohno-Shosaku and others 2002) and cerebellum (Galante and Diana 2004), and excitatory synapses in the medial nucleus of the trapezoid body (Kushmerick and others 2004) and dorsal striatum (Narushima and others 2006). In these synapses, DHPG application induced suppression of transmitter release, and the suppression was completely eliminated by the CB1 antagonists.

In addition to group I mGluRs, other types of G_q-coupled receptors also induce endocannabinoid release. It was reported that activation of muscarinic acetylcholine receptors induced transient suppression of inhibitory synaptic transmission through releasing endocannabinoids in the hippocampus (Kim and others 2002). The later study proved that M_1 and M_3 receptors, both of which are coupled to G_q protein, are responsible for this effect, using knockout mice for each subtype of muscarinic receptors (Fukudome and others 2004). In the dorsal raphe nucleus, orexin receptor, another $G_{q/11}$-coupled receptor, was found to drive endocannabinoid release (Haj-Dahmane and Shen 2005).

The endocannabinoid release driven by these G_q-coupled receptors is dependent on phospholipase Cβ (PLCβ). There are four types of PLCβ isozymes (PLCβ1-4), and each isozyme exhibits a unique distribution in the brain (Watanabe and others 1998). The major isozyme is PLCβ1 in the hippocampus, and PLCβ4 in the cerebellum. In the PLCβ1-knockout mice, activation of muscarinic receptors or group I mGluRs failed to induce the cannabinoid-dependent suppression of hippocampal inhibitory transmission (Hashimotodani and others 2005). In the PLCβ4-knockout mice, activation of group I mGluRs failed to induce the cannabinoid-dependent suppression of excitatory transmission onto cerebellar PCs (Maejima and others 2005). Importantly, DSI and DSE were intact in these two mouse strains (Hashimotodani and others 2005; Maejima and others 2005), indicating that PLCβ is not involved in the depolarization-induced endocannabinoid release.

Models for the receptor-driven endocannabinoid release at cerebellar excitatory synapses and hippocampal inhibitory synapses are illustrated in Figure 4. In the cerebellum, activation of mGluR1 in PCs stimulates PLCβ4 and yields diacylglycerol, which is then converted to 2-AG by DAGL. Then, 2-AG is released from the postsynaptic neuron, activates presynaptic CB1 receptors, and suppresses the glutamate release (Fig. 4*A*). In the hippocampus, activation of G_q-coupled receptors, such as mGluR1/5 and M_1/M_3 muscarinic receptors, stimulates PLCβ1 and induces the production and release of 2-AG

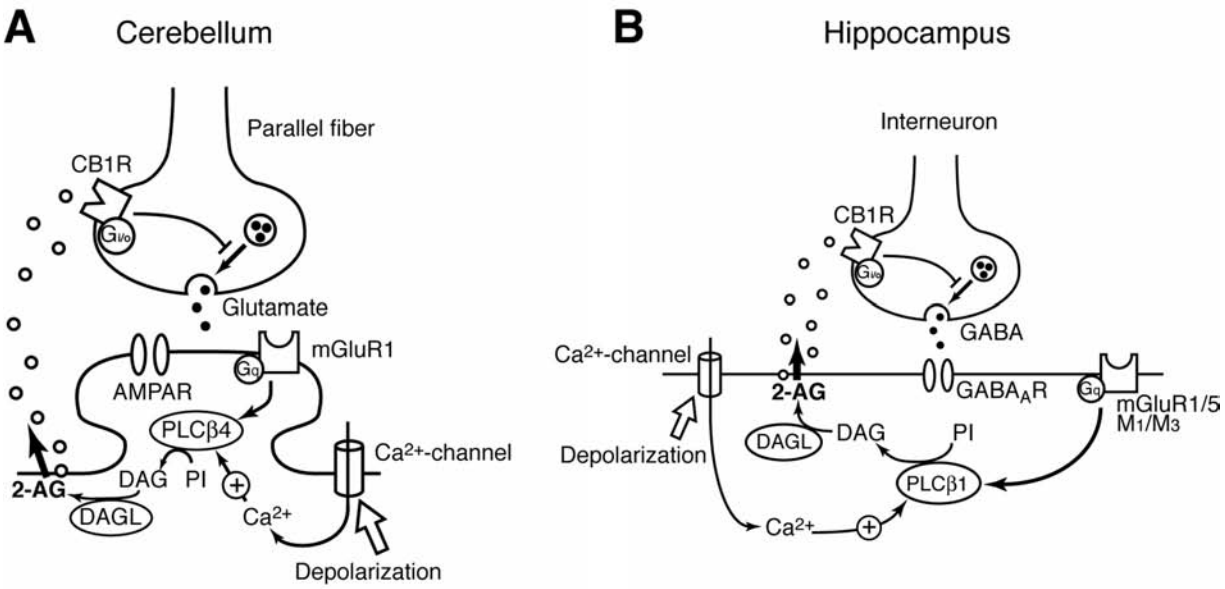

Fig. 4. Current models for the mechanisms of receptor-driven endocannabinoid release and Ca^{2+}-assisted receptor-driven endocannabinoid release. *A*, In the cerebellum, activation of mGluR1 in Purkinje cells stimulates PLCβ4 and yields diacylglycerol (DAG). DAG is converted to 2-arachidonoylglycerol (2-AG) by diacylglycerol lipase (DAGL). 2-AG is then released from postsynaptic neurons, activates presynaptic CB1 receptors on parallel fibers, and suppresses glutamate release. *B*, In the hippocampus, activation of mGluR1/5 or M_1/M_3 muscarinic receptors stimulates PLCβ1-DAGL cascade and induces the production of 2-AG. As a retrograde messenger, 2-AG activates presynaptic CB1 receptors and suppresses GABA release. These signaling pathways in *A* and *B* are facilitated by depolarization-induced Ca^{2+} elevation because of the Ca^{2+} dependency of PLCβ1/4 (Ca^{2+}-assisted receptor-driven endocannabinoid release). PLC = phospholipase C.

through DAGL activity. 2-AG then activates presynaptic CB1 receptors and suppresses the GABA release (Fig. 4*B*).

Ca^{2+}-Assisted Receptor-Driven Endocannabinoid Release

As described above, the endocannabinoid production is initiated by two distinct stimuli, Ca^{2+} elevation and G_q coupled receptor activation, which activate PLCβ-independent (Fig. 3) and PLCβ-dependent (Fig. 4) pathways, respectively. It has been reported that the combination of the two stimuli markedly enhanced endocannabinoid release (Kim and others 2002; Ohno-Shosaku and others 2002; Ohno-Shosaku and others 2003). When Ca^{2+} elevation and receptor activation by group I mGluR or muscarinic agonist were combined, the amount of endocannabinoids released was estimated to be several times higher than the simple sum of the amounts produced by individual stimuli applied separately (Ohno-Shosaku and others 2002; Ohno-Shosaku and others 2003). Recently, it has been demonstrated clearly that this enhancement is attributable to Ca^{2+} dependency of PLCβ (Hashimotodani and others 2005; Maejima and others 2005) (Fig. 4). In hippocampal cultures, endocannabinoid release driven by muscarinic receptors depended on intracellular Ca^{2+} concentration. The receptor-driven PLCβ activation could be successfully monitored in hippocampal neurons by measuring inward currents through overexpressed DAG-sensitive TRPC6 channels. With this technique, it was demonstrated that the receptor-driven PLCβ activation exhibited a Ca^{2+} dependency similar to that of receptor-driven endocannabinoid release. The enhancement of endocannabinoid release by the combination of Ca^{2+} elevation and muscarinic activation was eliminated in PLCβ1-knockout mice (Hashimotodani and others 2005). In cerebellar slices, the mGluR1-driven endocannabinoid release was Ca^{2+}-dependent, and the enhancement of endocannabinoid release by the combination of Ca^{2+} elevation and mGluR1 activation was eliminated in PLCβ4-knockout mice (Maejima and others 2005). Thus, this mode of endocannabinoid release was termed "Ca^{2+}-assisted receptor-driven endocannabinoid release" (Maejima and others 2005).

This mode of endocannabinoid release seems physiologically important, because mild Ca^{2+} elevation and mild receptor activation, both of which are subthreshold for inducing endocannabinoid release when applied alone, can effectively induce endocannabinoid release when applied conjointly. In this Ca^{2+}-assisted receptor-driven endocannabinoid release, PLCβ detects the coincidence of Ca^{2+} elevation reflecting postsynaptic activity and the receptor activation reflecting presynaptic activity. In this regard, PLCβ and the endocannabinoid signal can work as a coincidence detector and a coincidence signal, respectively, for activity-dependent synaptic modulation.

Synaptically Triggered Endocannabinoid Release

Endocannabinoid release can be induced experimentally by the aforementioned three stimulation protocols, strong depolarization (large Ca^{2+} elevation), strong activation of G_q-coupled receptors, and combined mild depolarization (small Ca^{2+} elevation) and mild receptor activation. The first protocol drives the PLCβ-independent pathway, whereas the second and the third protocols drive the same PLCβ-dependent pathway. Under physiological conditions, the endocannabinoid signaling should be triggered by neural activities. Then, what pattern of neural activity can induce endocannabinoid release? Which pathway is involved in such physiologically relevant endocannabinoid release? There are several studies that examined endocannabinoid release induced by synaptic activity. In the cerebellum, brief bursts of PF stimulation (for example, 50–100 Hz, 10 pulses) induced endocannabinoid release from PCs and suppressed the transmitter release from PF terminals (Brown and others 2003; Maejima and others 2005). This type of synaptic suppression was dependent on both mGluR1 activation and postsynaptic Ca^{2+} elevation (Maejima and others 2005). 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 (Brenowitz and Regehr 2005). This associative short-term plasticity induced by coactivation of PF and CF synapses was also dependent on both mGluR1 activation and postsynaptic Ca^{2+} elevation. In VTA dopamine neurons, brief-burst stimulation (5 Hz, 10 pulses) of excitatory inputs from the prefrontal cortex induced endocannabinoid-mediated suppression of the excitatory transmission, which was sensitive to both mGluR1 antagonist and the blockade of postsynaptic Ca^{2+} elevation (Melis and others 2004). All these results indicate the importance of Ca^{2+}-assisted receptor-driven pathway for synaptically triggered endocannabinoid release. In addition, the other two pathways, namely, the depolarization-induced pathway being driven by Ca^{2+} elevation alone (Brenowitz and Regehr 2005) and the receptor-driven pathway being triggered by G_q-coupled receptor activation alone (Maejima and others 2001), can also be involved in the synaptically triggered endocannabinoid release. Besides the temporal pattern, the spatial pattern of synaptic activation is also important for endocannabinoid signaling in the cerebellum. Although spatially dispersed synapse activation failed to induce retrograde endocannabinoid signaling, activation of nearby synapses induced retrograde inhibition of PF-PC synapses, because of activation of mGluRs by glutamate spillover from nearby active synapses (Marcaggi and Attwell 2005).

Presynaptic Mechanisms Underlying CB1-Dependent Suppression of Transmitter Release

Activation of presynaptic CB1 receptors suppresses transmitter release reversibly. Three mechanisms have been suggested for the CB1-mediated suppression of transmitter release, which include inhibition of voltage-gated Ca^{2+} channels, activation of K^+ channels, and inhibition of release machinery. First, the possibility of Ca^{2+} channel inhibition has been most intensively studied and well supported by many lines of evidence. Whole-cell Ca^{2+} current measurements showed that cannabinoid agonists inhibited L-type, N-type, and Q-type Ca^{2+} channels (Howlett and others 2002). The Ca^{2+}-imaging studies on cerebellar CF and PF synapses demonstrated a clear decrease in action potential–induced presynaptic Ca^{2+} entry during DSE (Kreitzer and Regehr 2001; Brown and others 2003). The studies using Ca^{2+}-channel blockers and K^+-channel blockers concluded that the cannabinoid-mediated suppression was caused by inhibition of presynaptic Ca^{2+} channels rather than activation of K^+ channels in the hippocampus and cerebellum (Hoffman and Lupica 2000; Brown and others 2004). Furthermore, direct measurement of Ca^{2+} currents from calyx of Held terminals in the medial nucleus of the trapezoid body demonstrated a clear inhibition of presynaptic Ca^{2+} currents by the cannabinoid agonist WIN55,212-2 and also by the endocannabinoids that were released by DHPG application (Kushmerick and others 2004). Second, the possibility of K^+-channel activation has also been suggested (Daniel and Crepel 2001; Diana and Marty 2003; Daniel and others 2004). It is thought that activation of K^+ channels changes the action potential wave form and indirectly suppresses the Ca^{2+} influx into presynaptic terminals. Third, inhibition of the release machinery has also been suggested to contribute to CB1-mediated suppression of transmitter release (Takahashi and Linden 2000; Yamasaki and others 2006). In cerebellar PCs, CB1 activation had no effect on basal miniature postsynaptic events but selectively suppressed miniature postsynaptic events enhanced by Ca^{2+} elevation in presynaptic terminals (Yamasaki and others 2006). Thus, CB1 activation appears to regulate processes of spontaneous transmitter release by acting on the downstream of Ca^{2+} entry into presynaptic terminals.

Long-Term Depression Mediated by Endocannabinoids

Endocannabinoids are reported to mediate not only short-term but also long-term synaptic plasticity in various brain regions. The first report for endocannabinoid-mediated long-term depression (eCB-LTD) appeared in 2002 (Gerdeman and others 2002). In dorsal striatal neurons, high-frequency stimulation of corticostriatal afferents induced LTD of excitatory synaptic transmission (LTDe). This LTDe was prevented by CB1 antagonists and was absent in CB1-knockout mice, indicating the involvement of endocannabinoid signaling. Soon after this publication, similar eCB-LTDe was reported in the nucleus accumbens (NAc) (Robbe and others 2002). Later, eCB-LTD at inhibitory synapses (eCB-LTDi) was found in the basolateral amygdala (Marsicano and others 2002) and hippocampus (Chevaleyre and Castillo 2003). Another form of eCB-LTDe was discovered in the visual cortex (Sjostrom and others 2003). In layer 5 pyramidal neurons, LTDe was induced by pairing stimulation of pre- and postsynaptic neurons with postsynaptic spiking preceding

the presynaptic activity. This spike timing-dependent LTDe required coactivation of presynaptic CB1 and NMDA receptors (Sjostrom and others 2003). The aforementioned forms of eCB-LTD are all expressed presynaptically as persistent decrease in transmitter release. By contrast, cerebellar LTD, which is well known to be expressed postsynaptically, was reported to require endocannabinoid signaling (Safo and Regehr 2005). In the following sections, we describe these studies in more detail and discuss the functional significance of the endocannabinoid signaling in neural functions.

Dorsal Striatum

In medium spiny neurons of the dorsal striatum, high-frequency stimulation (for example, 100 Hz, 1 s) of corticostriatal glutamatergic afferents was known to induce LTD of the excitatory input. This striatal LTDe required postsynaptic Ca^{2+} elevation and was suggested to be expressed as long-lasting suppression of glutamate release, implying the involvement of retrograde signal. It was also reported that CB1 activation suppresses glutamate release in the striatum (Gerdeman and Lovinger 2001). Based on these findings, Lovinger and his co-workers expected that endocannabinoids might work as retrograde messengers in LTDe induction. They found that LTDe was blocked by CB1 antagonist and was absent in CB1-knockout mice, and concluded that striatal LTDe was mediated by endocannabinoids (Gerdeman and others 2002). The same group reported subsequently that postsynaptic loading with anandamide transporter inhibitors prevented striatal LTDe, suggesting the requirement of transporter-mediated release of anandamide for LTDe induction (Ronesi and others 2004). On the other hand, Kreitzer and Malenka reported that dopamine modulated striatal endocannabinoid release and LTDe induction in a manner dependent on the "state" of medium-spiny neuron's membrane potential (i.e., "up" or "down" state) (Kreitzer and Malenka 2005). The dorsal striatum is an important brain area for motor control and habit learning. Because the striatum receives excitatory inputs from the cortex and thalamus, synaptic plasticity of excitatory synapses in this area is thought to be crucial for such striatum-related functions. The endocannabinoid signaling might play a role in the striatal functions through the contribution to LTDe.

Nucleus Accumbens

Similar eCB-LTDe was observed in NAc (Robbe and others 2002). NAc-LTDe was induced by repetitive stimulation of prelimbic cortical glutamatergic afferents in a naturally occurring frequency range (10 min at 13 Hz). Like striatal LTDe, NAc-LTDe was blocked by CB1 antagonists and postsynaptic Ca^{2+} chelation and was abolished in CB1-knockout mice. NAc-LTDe required both activation of mGluR5 and Ca^{2+} release from intracellular stores. Interestingly, in vivo exposure to Δ^9-THC blocked NAc-LTDe, which was explained by a functional tolerance of CB1 (Hoffman and others 2003; Mato and others 2004). Because NAc is crucial for behaviors related to motivation and reward, these results suggest that NAc-LTDe might be related to drug addiction.

Amygdala

eCB-LTD at inhibitory synapses was first found in the basolateral amygdala (Marsicano and others 2002). Low-frequency stimulation (1 Hz, 100 pulses) of afferents induced LTD of GABAergic inhibitory transmission (LTDi). This LTDi was shown to be expressed presynaptically and required CB1 activation. A further study reported that induction of LTDi was dependent on mGluR1 activation but not on postsynaptic Ca^{2+} influx (Azad and others 2004). Pharmacological experiments with inhibitors of PLC and DAGL suggested that anandamide rather than 2-AG was involved in LTDi. Consistent with this idea, LTDi was enhanced in mice lacking the anandamide degradation enzyme FAAH (Azad and others 2004). Although it is not clear how mGluR1 activation causes anandamide production, the authors suggested that adenylyl-cyclase and protein kinase A might be involved. The amygdala is crucial for acquisition and storage of fear memory (LeDoux 2000) and presumably also for its extinction. Marcicano and others found that the extinction of fear memory in an auditory fear-conditioning paradigm was strongly impaired in CB1-knockout mice and was suppressed by systemic application of the CB1 antagonist SR141716A before extinction trials, indicating the involvement of the endocannabinoid system in extinction of fear memory. eCB-LTDi in the amygdala may underlie this process.

Hippocampus

eCB-LTDi was also observed in the hippocampus (Chevaleyre and Castillo 2003). Two trains of high-f requency stimulation (100 Hz, 100 pulses) or theta burst stimulation in the stratum radiatum of the CA1 region caused LTD at GABAergic inhibitory synapses. This hippocampal LTDi was presynaptically expressed, blocked by CB1 antagonist (Chevaleyre and Castillo 2003) and eliminated in CB1 knockout mice (Chevaleyre and Castillo 2004), indicating the involvement of endocannabinoid signal. LTDi was prevented by pharmacological blockade of mGluR1/5, PLC, and DAGL, but not by postsynaptic loading of the Ca^{2+} chelator BAPTA, suggesting that the generation of endocannabinoid signal through the mGluR1/5-PLCβ-DAGL pathway is required for LTDi induction. By examining the effects of CB1 antagonist at different time periods before, during, and after LTDi, the induction of LTDi was shown to require continuous activation of CB1 for 5 to 10 minutes. Possible mechanisms of LTDi induction are proposed as follows (Fig. 5*A*). High-frequency stimulation of Schaffer collaterals releases glutamate, which activates postsynaptic mGluR1/5, leading to the production of 2-AG via the PLCβ-DAGL pathway. 2-AG is then released, activates presynaptic CB1 on nearby GABAergic interneurons, and causes long-term suppression of GABA release, if CB1 activation continues for several minutes (Chevaleyre and Castillo 2003). These authors extended their study on eCB-LTDi and demonstrated that

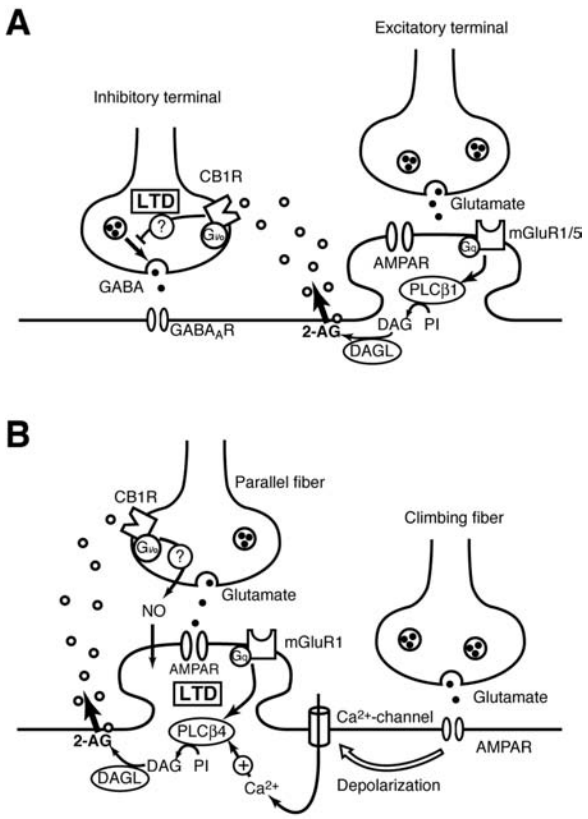

Fig. 5. Current models for the mechanisms of endocannabinoid release involved in endocannabinoid-mediated long-term depression (eCB-LTD). *A*, Hippocampal LTD at inhibitory synapses (LTDi). High-frequency stimulation of Schaffer collaterals induces glutamate release and activates mGluR1/5 at excitatory synapses. 2-arachidonoylglycerol (2-AG) is produced through the mGluR1/5-PLCβ1-DAGL pathway and released from postsynaptic neurons. 2-AG travels to the neighboring GABAergic terminals and causes sustained activation of CB1 receptors leading to LTD expression by unknown mechanisms. *B*, Cerebellar LTD of excitatory synaptic transmission (LTDe). Conjunctive stimulation of parallel fibers and climbing fibers triggers production of 2-AG through the mGluR1-PLCβ4-DAGL pathway. This pathway may be enhanced by Ca^{2+} elevation that is evoked by climbing fiber stimulation. The released 2-AG activates presynaptic CB1 receptors on parallel fiber terminals. Because cerebellar LTDe is expressed postsynaptically, it is necessary to send a presynaptic message to postsynaptic neurons. It is proposed that nitric oxide (NO) may mediate this anterograde message. DAG = diacylglycerol; DAGL = diacylglycerol lipase; PLC = phospholipase C.

the expression of LTDi facilitated subsequent induction of long-term potentiation (LTP) at excitatory synapses (Chevaleyre and Castillo 2004). Therefore, the endocannabinoid signal could contribute to the memory formation through the induction of local metaplasticity. Possible involvement of the endocannabinoid system in extinction processes has been suggested in a behavioral study (Varvel and Lichtman 2002). This study evaluated performance of CB1-knockout mice in the Morris water maze test, a standard task for examination of hippocampus-dependent spatial memory in rodents. CB1-knockout mice exhibited a deficit in learning a new platform location during the reversal text. One possible explanation for this deficit is that the endocannabinoid signal plays a key role in facilitating extinction processes. In contrast, CB1-knockout mice exhibited normal acquisition and extinction of trace eyeblink conditioning that is known to be dependent on the hippocampus (Kishimoto and Kano 2006). It remains to be investigated whether and how eCB-LTDi contributes to extinction processes of hippocampus-dependent learning.

Neocortex

It has been reported that neocortical synapses exhibit spike-timing-dependent plasticity, which is induced by pre- and postsynaptic firings with a certain pre-to-post timing (Dan and Poo 2004). When presynaptic firing repeatedly precedes postsynaptic firing by 0 to 20 ms, LTP is usually induced. By contrast, when presynaptic firing follows postsynaptic firing with a delay up to 100 ms, LTD is induced. In visual cortical layer 5 pyramidal neuron, Nelson and his co-workers demonstrated that this timing-dependent LTD (tLTD) at glutamatergic synapses (tLTDe) was expressed presynaptically and was dependent on activation of CB1 (Sjostrom and others 2003). Interestingly, they demonstrated that tLTDe required coincident activation of presynaptic CB1 and NMDA receptors. Modeling studies have suggested that the spike timing-dependent plasticity can provide a fundamental mechanism, by which neural networks can perform computational functions, such as causality detection and sequence learning (Dan and Poo 2004). The endocannabinoid signaling might contribute to these neural functions through mediating tLTDe in the neocortex.

Cerebellum

Although several forms of eCB-LTD described above are all expressed presynaptically, postsynaptically expressed eCB-LTD was reported in the cerebellum (Safo and Regehr 2005). It is well known that cerebellar LTD at PF to PC synapses is a cellular basis for certain forms of discrete motor learning (Ito 2001). This LTD is induced at PF-PC excitatory synapses by conjunctive stimulation of PFs and CFs. Endocannabinoid requirement for cerebellar LTDe was supported by the results that the induction of LTDe was blocked by a CB1 antagonist and abolished in CB1-knockout mice. Cerebellar LTDe was also blocked by postsynaptic loading of DAGL inhibitors, suggesting the involvement of 2-AG. Because cerebellar LTDe is known to be expressed postsynaptically (Ito 2001), presynaptic CB1 activation should eventually bring the message to postsynaptic neurons. Safo and Regehr proposed that nitric oxide (NO) might work as an anterograde messenger (Fig. 5*B*), based on the observation that inhibition of NO synthesis prevented LTD induction even in the presence of a CB1 agonist (Safo and Regehr 2005). The finding that cerebellar LTDe is cannabinoid-dependent suggests that cerebellum-dependent motor learning might

involve endocannabiniod-signaling. In support of this expectation, it was recently reported that classical eyeblink conditioning, a well-known form of cerebellum-dependent discrete motor learning, was severely impaired in CB1-knockout mice, and was blocked by intraperitoneal injection of the CB1 antagonist SR141716A (Kishimoto and Kano 2006). Interestingly, extinction of conditioned eyeblink response was not affected by SR141716A injection. These results indicate that the endocannabinoid signaling plays an important role in cerebellar LTDe and contributes to acquisition of cerebellum-dependent discrete motor learning.

Which Is the Retrograde Messenger, Anandamide or 2-AG?

There are a number of studies that aimed to determine which molecule, 2-AG or anandamide, is responsible for short-term and long-term forms of endocannabinoid-mediated synaptic plasticity. The molecular identity of the retrograde signal involved in DSI/DSE is not fully determined. In the hippocampus, two studies have suggested that 2-AG mediates DSI. Kim and Alger (2004) reported that DSI in CA1 pyramidal cells was not affected by FAAH inhibition but was prolonged by inhibition of COX-2, which can degrade both 2-AG and anandamide (Kim and Alger 2004). Because FAAH inhibition was shown to facilitate the effect of exogenously applied anandamide, the negative effect of FAAH inhibition on DSI suggests that anandamide does not play a dominant role in DSI. Makara and others (2005) also reported that inhibitors of MGL (URB754 and URB602) increased 2-AG levels and prolonged DSI in CA1 pyramidal cells, suggesting the involvement of 2-AG (Makara and others 2005). However, conflicting results have also been reported. In the hippocampus, it was shown that DSI was not affected by application of inhibitors of PLC and DAGL (Chevaleyre and Castillo 2003). Cerebellar DSE was also resistant to DAGL inhibitors (Safo and Regehr 2005). Because PLC and DAGL are key enzymes for 2-AG production, these results appear to be inconsistent with the 2-AG hypothesis. However, the data do not necessarily contradict the 2-AG hypothesis, because 2-AG can be synthesized through alternative pathways independent of PLC/DAGL (Sugiura and others 2006).

As for receptor-driven or synaptically triggered endocannabinoid release, 2-AG is likely to function as the retrograde messenger. The studies on hippocampal neurons of PLCβ1-knockout mice (Hashimotodani and others 2005) and cerebellar PCs of PLCβ4-knockout mice (Maejima and others 2005) demonstrated that the endocannabinoid release driven by group I mGluRs or muscarinic receptors was abolished in these PLCβ-knockout neurons. Synaptically triggered endocannabinoid release in cerebellar PCs (Maejima and others 2005; Safo and Regehr 2005) or in VTA dopaminergic neurons (Melis and others 2004), which were mainly driven by group I mGluR activation, was blocked by inhibition of DAGL. Moreover, biochemical measurements of 2-AG indicate that driving mGluR1-PLCβ4 cascade induces 2-AG production (Maejima and others 2005). In corticostriatal slice cultures, mGluR5 activation induced 2-AG formation through the PLC-DAGL cascade (Jung and others 2005).

As for the molecular identity of endocannabinoid for eCB-LTD, anandamide and 2-AG appear to function in different brain regions. Whereas anandamide is suggested to mediate LTDe in the striatum (Ronesi and others 2004) and LTDi in the amygdala (Azad and others 2004), 2-AG is assumed to contribute to hippocampal LTDi (Chevaleyre and Castillo 2003) and cerebellar LTDe (Safo and Regehr 2005). Because hippocampal LTDi and cerebellar LTDe require activation of group I mGluRs (Chevaleyre and Castillo 2003; Safo and Regehr 2005), it is reasonable to assume that 2-AG is produced downstream of group I mGluRs involving G_q-PLCβ cascade. It is less clear how LTD-inducing synaptic activity leads to production of anandamide in the striatum and amygdala. Further studies are required to determine the enzymes for the endocannabinoid production and the related signaling molecule involved in eCB-LTD.

Conclusion

In this review, we focused on roles of endocannabinoids in short-term and long-term forms of retrograde synaptic modulation. It is now widely accepted that endocannabinoids are released from postsynaptic neurons in an activity-dependent manner and induce transient or persistent suppression of transmitter release. These endocannabinoid-mediated forms of synaptic plasticity are observed in various brain regions including the neocortex, hippocampus, amygdale, hypothalamus, striatum, NAc, and cerebellum. Because CB1 is widely distributed in the CNS, it is expected that the endocannabinoid signaling may be involved in synaptic plasticity throughout the CNS and contribute to various aspects of brain functions. In addition to the roles as retrograde messengers at synapses, endocannabinoids have other important functions that are not included in this article because of limited space. For example, endocannabinoids are reported to control the excitability of neocortical GABAergic interneurons in an autocrine manner (Bacci and others 2004). Endocannabinoids also play an important role in neuroprotection (van der Stelt and Di Marzo 2005). As reviewed in this article, recent studies have greatly expanded our knowledge of the endocannabinoid system in the CNS. The development in endocannabinoid research is also very important from a clinical point of view, because the endocannabinoid system has been expected to provide potential targets for the treatment of neurological disorders.

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