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Endocannabinoid-Mediated Control of Synaptic Transmission

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Departments of Neurophysiology and Pharmacology, Graduate School of Medicine, The University of Tokyo, Tokyo; Department of Impairment Study, Graduate School of Medical Science, Kanazawa University, Kanazawa; and Department of Anatomy, Hokkaido University School of Medicine, Sapporo, Japan

Kano M, Ohno-Shosaku T, Hashimotodani Y, Uchigashima M, Watanabe M. Endocannabinoid-Mediated Control of Synaptic Transmission. *Physiol Rev* 89: 309–380, 2009; doi:10.1152/physrev.00019.2008.—The discovery of cannabinoid receptors and subsequent identification of their endogenous ligands (endocannabinoids) in early 1990s have greatly accelerated research on cannabinoid actions in the brain. Then, the discovery in 2001 that endocannabinoids mediate retrograde synaptic signaling has opened up a new era for cannabinoid research and also established a new concept how diffusible messengers modulate synaptic efficacy and neural activity. The last 7 years have witnessed remarkable advances in our understanding of the endocannabinoid system. It is now well accepted that endocannabinoids are released from postsynaptic neurons, activate presynaptic cannabinoid CB_1 receptors, and cause transient and long-lasting reduction of neurotransmitter release. In this review, we aim to integrate our current understanding of functions of the endocannabinoid system, especially focusing on the control of synaptic transmission in the brain. We summarize recent electrophysiological studies carried out on synapses of various brain regions and discuss how synaptic transmission is regulated by endocannabinoid signaling. Then we refer to recent anatomical studies on subcellular distribution of the molecules involved in endocannabinoid signaling and discuss how these signaling molecules are arranged around synapses. In addition, we make a brief overview of studies on cannabinoid receptors and their intracellular signaling, biochemical studies on endocannabinoid metabolism, and behavioral studies on the roles of the endocannabinoid system in various aspects of neural functions.

I. INTRODUCTION

Marijuana and other derivatives of the plant *Cannabis sativa* have been used for thousands of years for their therapeutic and mood-altering properties. Their psychotropic actions include euphoria, appetite stimulation, sedation, altered perception, and impairments of memory and motor control (3). Δ^9-Tetrahydrocannabinol (Δ^9-THC) was identified as the major psychoactive component of cannabis in 1964 (172). Since then, a number of biologically active analogs of Δ^9-THC have been synthesized. These compounds are collectively called cannabinoids because of their cannabimimetic actions and have been used for laboratory animals to produce various behavioral symptoms analogous to those in humans (234).

A marked advance has been made in the cannabinoid research by the discovery of the receptors that bind Δ^9-THC (cannabinoid receptors) in animal tissues. The first canabinoid receptor (CB_1) was cloned and characterized in 1991 (339), and the second receptor (CB_2) was identified in 1993 (369). They are both G protein-coupled seven-transmembrane domain receptors and differ in their tissue distributions. The CB_1 receptor is abundantly expressed in the central nervous system (CNS), whereas the CB_2 receptor is present mainly in the immune system. The development of selective antagonists, SR141716A (434) for CB_1 and SR144528 (435) for CB_2, and the generation of genetically engineered mice lacking CB_1 (292, 583) or CB_2 (58) have enabled us to determine relative contribution of each cannabinoid receptor to pharmacological effects of cannabinoids. It is now evident that the CB_1 receptor is responsible for most, if not all, of the psychotropic actions of Δ^9-THC and other cannabinoids. Another great advance in this field has been brought about by the discovery of endogenous ligands for cannabinoid receptors (endocannabinoids). *N*-arachidonylethanolamide was first identified as an endocannabinoid, and named anandamide (118). Subsequently, 2-arachidonylglycerol (2-AG) (350, 495) was identified as the second endocannabinoid. Biochemical studies have shown that these molecules are produced on demand in an activity-dependent manner, and released to the extracellular space.

The year 2001 was the turning point of the cannabinoid research. In this year, endocannabinoids were discovered to mediate retrograde signaling at central synapses (285, 314, 394, 564), which opened up a new era in cannabinoid research, and also established a new concept of how diffusible messengers like endocannabinoids modulate synaptic efficacy and neural activity. Before this discovery, neurophysiologists had been searching for candidate molecule(s) mediating retrograde synaptic signaling for nearly 10 years. In the early 1990s, Llano et al. in the cerebellum (304) and Pitler and Alger in the hippocampus (426) demonstrated that depolarization of postsynaptic neurons induces a transient suppression of inhibitory synaptic transmission. This phenomenon was termed depolarization-induced suppression of inhibition (DSI). Because DSI is triggered by elevation of postsynaptic Ca^{2+} concentration and is associated with reduction of transmitter release from presynaptic terminals (426, 545), possible involvement of retrograde signaling was strongly suggested. Since then, many attempts have been made to identify the nature of retrograde signaling. In 2001, our group (394) and Wilson and Nicoll (564) re-

ported at the same time that an endocannabinoid functions as a retrograde messenger in DSI, using cultured hippocampal neurons (394) and hippocampal slices (564). Concurrently, Kreitzer and Regehr (285) discovered that the counterpart of DSI for excitatory synaptic transmission, termed depolarization-induced suppression of excitation (DSE), is also mediated by endocannabinoids in cerebellar Purkinje cells (285). In the same year, our group and Alger's group discovered another form of endocannabinoid-mediated short-term depression (eCB-STD) in the cerebellum (314) and hippocampus (537), respectively. Activation of group I metabotropic glutamate receptors (mGluRs) of postsynaptic neurons induced a transient suppression of synaptic transmission at excitatory synapses on cerebellar Purkinje cells (314) and inhibitory synapses on CA1 pyramidal cells (537). This mGluR-driven suppression was also demonstrated to utilize an endocannabinoid as a retrograde messenger. This form of eCB-STD is now considered to be physiologically more important than DSI and DSE (205, 209, 315). In 2002, retrograde endocannabinoid signaling was shown to be responsible for long-term depression (LTD) (175). The striatal LTD, which is induced by high-frequency stimulation of corticostriatal afferents, was prevented by pharmacological or genetic depletion of CB_1, indicating the involvement of endocannabinoids. Soon after this report, a similar endocannabinoid-mediated LTD (eCB-LTD) was found in the nucleus accumbens (437). So far, various forms of eCB-STD (Table 1–4) and eCB-LTD (Table 5) have been reported in many different brain regions.

In parallel with these electrophysiological studies, many behavioral studies have been carried out to clarify the roles of the endocannabinoid system in the CNS, by using CB_1 antagonists and CB_1-knockout mice. These studies have revealed that the endocannabinoid system is involved in various aspects of neural functions. For example, blocking the endocannabinoid system suppresses the extinction of aversive memory (330), relearning of the water maze test (540), cerebellum-dependent eyeblink conditioning (277), drug addiction (323), feeding behavior (407), a certain form of stress-induced analgesia (232), and the recovery of neurobehavioral function after brain injury (411). Involvement of the endocannabinoid system in various functions of the CNS under physiological and pathological conditions suggests that the molecules involved in endocannabinoid signaling may be promising targets for clinical management of disturbed neural functions or pathological conditions.

This review focuses on the major results of electrophysiological and anatomical studies conducted during the past several years to elucidate functional significance of the endocannabinoid system in the CNS. Electrophysiological studies showing how the synaptic transmission is regulated by endocannabinoid signaling will be discussed in sections V–VII. Anatomical studies showing subcellular distribution of the molecules involved in endocannabinoid signaling will be described in section VIII. In the rest of this review, we will make a brief overview of studies on cannabinoid receptors (sect. II) and their intracellular signaling (sect. III), biochemical studies on endocannabinoid metabolism (sect. IV), and behavioral studies on the roles of the endocannabinoid system in various aspects of neural functions (sect. IX). Excellent general reviews are available for the history of cannabinoid research (236), the cannabinoid receptors (235), the endocannabinoid system (111, 167, 439), and the endocannabinoid-mediated synaptic modulation (86, 206, 312, 422). Review articles for more specialized topics will be cited in each chapter.

II. CANNABINOID RECEPTORS

CB_1 and CB_2 are the two major cannabinoid receptors, but the distribution is strikingly different. The abundance of CB_1 and scarcity of the CB_2 in the CNS imply that the CB_1 receptor is primarily responsible for the psychoactivity of exogenous cannabinoids and physiological actions of endocannabinoids in the CNS (146). The studies using CB_1-knockout mice and CB_1-specific antagonists have confirmed this notion (146, 292). Additional cannabinoid receptors have been suggested to exist in the brain by pharmacological and genetic studies (23). In this section, we briefly summarize the main features of cannabinoid receptors, by referring to only essential studies on CB_1, CB_2, and some other related receptors. For more details, see the following review (235).

A. CB_1 Receptor

1. Structure

A 473-amino acid G protein-coupled receptor encoded by a rat brain cDNA clone was identified as a cannabinoid receptor in 1990 (339), and named CB_1. Later, a human homolog of 472 amino acids (174) and a mouse homolog of 473 amino acids (75) have been reported. These three CB_1 receptors have 97–99% amino acid sequence identity.

In humans, the gene encoding the CB_1 receptor is located on chromosome 6. Two types of NH_2-terminal splice variants, short-length receptors, have been reported (450, 472). These variants show altered ligand binding properties compared with the full-length receptor and are expressed at very low levels in a variety of tissues (450). A number of genetic polymorphisms have been described in the CB_1 receptor, and their correlation with various conditions has been examined (386). Although the results are rather controversial, some of the polymorphisms have been reported to link to obesity-related phe-

TABLE 1. *eCB-STD in the hippocampus*

Postsynaptic Neuron	Input	Type of STD	Dependence	Independence	DSI/DSE Enhancement	Reference Nos.
CA1	I	DSI	Ca^{2+}		BAY K 8644, AChR	426
			$G_{i/o}$ protein (pre)	G protein (post)		425
					mAChR	331
			CB_1	mGluR, vesicular release		564
			CB_1	PKA, PP		563
			CB_1		I-mGluR	537
			CB_1		mAChR	272
			Ca^{2+}	PLC, DGL		84
			Ca^{2+} store			243
				PLC, DGL	I-mGluR, mAChR	138
			CB_1	DGL		503
			CB_1, NO		mAChR	319
				PKA, RIM1α		85
		I-mGluR	CB_1			537
				Ca^{2+}		272
				PLC, DGL		138
			CB_1	Ca^{2+}		382
		mAChR	CB_1, G protein (post)	Ca^{2+}		272
			DGL	PLC		138
			CB_1	Ca^{2+}		382
		CCK	CB_1, G protein (post)			158
	E	DSE	CB_1			399
CA3	I	DSI	Ca^{2+}	II-mGluR		362
CCK-IN	I (CCK-IN)	DSI	CB_1			7
DGC	I	DSI	CB_1, Ca^{2+}, Ca^{2+} store			242
	E (MCF)	DSE	CB_1, Ca^{2+}	DGL	AChR, I-mGluR	88
MC	I	DSI	CB_1, Ca^{2+}		mAChR	227
Culture	I	DSI	Ca^{2+}			397
			CB_1, Ca^{2+}	mGluR, $GABA_B$		394
					mGluR5	398
			CB_1		M_1/M_3	395
				PLCβ1		209
			DGL			207
			DGL	PLCδ1, -δ3, -δ4		208
			VGCC			393
		NMDAR	CB_1, Ca^{2+}, DGL	VGCC	mAChR, I-mGluR	393
		I-mGluR	CB_1			398
			PLCβ1			209
			DGL			208
		M_1/M_3	CB_1			168
			PLCβ1, Ca^{2+}			209
			DGL			207
	E	DSE	CB_1			399
			CB_1, VGCC, DGL, Ca^{2+} store	NO		489
					mAChR, I-mGluR	490
		I-mGluR	CB_1			490
		mAChR	CB_1, PLC			490

CCK-IN, CCK-positive interneuron; DGC, dentate granule cell; MC, mossy cell; I, inhibitory; E, excitatory; MCF, mossy cell fiber; I-mGluR or II-mGluR, group I or group II metabotropic glutamate receptor; pre, presynaptic; post, postsynaptic; PP, protein phosphatase; VGCC, voltage-gated Ca^{2+} channel; BAY K 8644, Ca^{2+} channel activator.

notypes (173, 448), hebephrenic schizophrenia (78, 530), childhood attention deficit/hyperactivity disorder (429), and depression in Parkinson's disease (20).

Binding properties of cannabinoids to the CB_1 receptor have been elucidated. With the use of site-directed mutagenesis, binding sites of cannabinoids were shown to be embedded in the transmembrane helices of the receptor (481). NMR experiments support the hypothesis that a cannabinoid laterally diffuses within one membrane leaflet, and interacts with a hydrophobic groove formed by helices 3 and 6 of CB_1 (322, 512).

It is proposed that the CB_1 receptor likely exists as a homodimer in vivo (548). The extent of CB_1 dimerization was suggested to be regulated by agonists (311). The CB_1 receptor can also exist as a heteromer (311). One example is the heteromer between CB_1 and D_2 (268). It was demonstrated that receptor stimulation promotes the formation of CB_1/D_2 complex and alters the CB_1 signaling. Another example is the heteromer between CB_1 and orexin 1 receptor (OX1R). The CB_1 activation potentiated the OX1R signaling (218), suggesting the interaction of these two receptors. Interaction of their surface distribu-

tion was also reported. Coexpressed CB_1 and OX1R were shown to form a heteromeric complex (145). It is still unclear, however, whether these two receptors are interacting in vivo.

2. Distribution

This subsection summarizes general distribution of cannabinoid receptors in the brain and spinal cord, which corresponds, if not exactly, to distribution of the CB_1 receptor. The detailed distribution in several neural regions will be described in section VIII.

A) BINDING SITES OF RADIOLABELED SYNTHETIC CANNABINOID IN THE CNS. Distribution of cannabinoid receptors in the brain was first demonstrated by ligand binding using the radiolabeled synthetic cannabinoid [^{3}H]CP55,940 (214, 215, 318). Ligand binding sites are distributed widely in the brain at various levels depending on the regions and also the neuron types within a given region. High levels of [^{3}H]CP55,940 binding are observed in innermost layers of the olfactory bulb, hippocampus (particularly high in the dentate molecular layer and the CA3 region), lateral part of the striatum, target nuclei of the striatum (i.e., globus pallidus, entopeduncular nucleus, substantia nigra pars reticulata), and cerebellar molecular layer. Moderate levels are noted in other forebrain regions and a few nuclei in the brain stem and spinal cord. They include the cerebral cortex (higher in the frontal, parietal, and cingulated areas than other cortical areas), septum, amygdala (nucleus of lateral olfactory tract), hypothalamus (ventromedial hypothalamus), lateral subnucleus of interpeduncular nucleus, parabrachial nucleus, nucleus of solitary tract (caudal and commissural portions), and spinal dorsal horn. The thalamus, other nuclei in the brain stem, and spinal ventral horn are low in ligand binding. These overall binding properties are preserved across mammals (215).

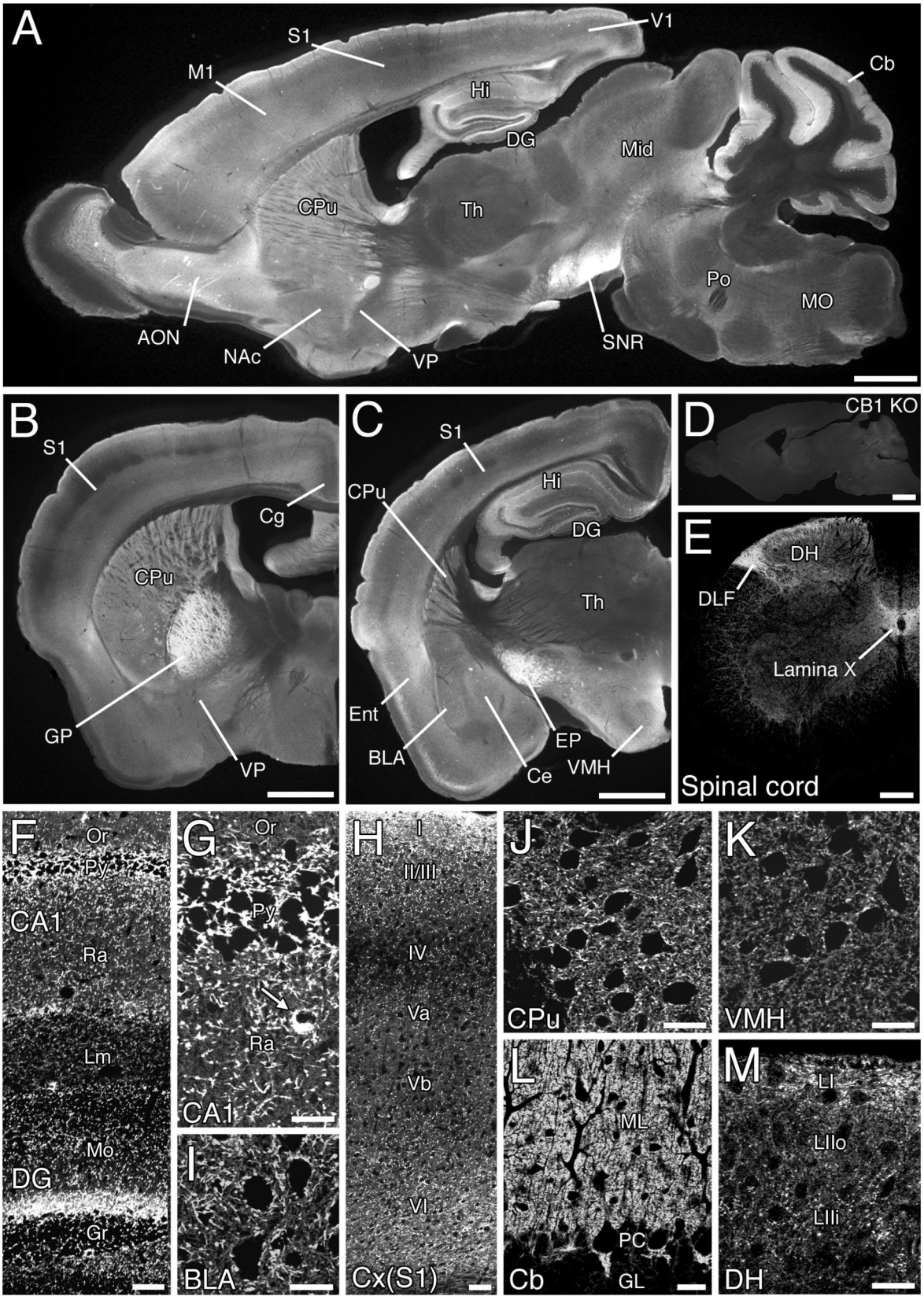
These high levels of ligand binding sites in the telencephalic and cerebellar regions are compatible with the effects of cannabinoids on motor and cognitive functions. In contrast, generally low levels of ligand binding in the lower brain stem areas that control cardiovascular and respiratory functions may explain why high doses of cannabinoids are not lethal (214, 318). Likewise, moderate binding level in the spinal dorsal horn is likely to be involved in analgesic action of intrathecally administered cannabinoids. Since the caudal solitary nucleus sends viscerosensory information via the parabrachial nucleus to the hypothalamus and amygdala, and the ventromedial hypothalamic nucleus is the satiety center for controlling appetite and feeding behavior, moderate levels in these nuclei seem to explain antianorexic and antiemetic actions of cannabinoids. Cannabimimetic drugs are now used in treatments for nausea and vomiting associated with cancer chemotherapy and for appetite suppression and cachexia in acquired immunodeficiency syndrome (AIDS) patients.

B) GENERAL FEATURES OF CB_1 mRNA EXPRESSION AND CB_1 PROTEIN DISTRIBUTION IN THE CNS. Soon after the first report of ligand binding study by Herkenham et al. (215), Matsuda et al. (339) cloned a cDNA of the first cannabinoid receptor CB_1. The cloning of CB_1 cDNA led to investigation of regional and cellular distribution of CB_1 mRNA by in situ hybridization and to cellular and subcellular localization of CB_1 by immunohistochemistry.

Since then, a number of histochemical studies have uncovered characteristic features of CB_1 expression in the nervous system (Fig. 1). First, although CB_1 is expressed widely and richly in the nervous system, two distinct patterns of CB_1 mRNA expression, i.e., uniform and nonuniform labelings, are noted depending on brain regions (318, 338). Uniform labeling resulting from mRNA expression in major neuronal populations is found in the striatum, thalamus, hypothalamus, cerebellum, and lower brain stem. For example, CB_1 mRNA is expressed in medium spiny neurons and parvalbumin-positive interneurons within the striatum, and in cerebellar granule cells, basket cells, and stellate cells within the cerebellar cortex. In contrast, nonuniform expression reflecting the presence of a few cell types expressing high CB_1 mRNA is found in the cerebral cortex, hippocampus, and amygdala. In these regions, strong expression is seen in cholecystokinin (CCK)-positive interneurons, whereas no expression in parvalbumin-positive ineterneurons and generally low expression in principal (or excitatory) neurons are noted (229, 261–263, 267, 329, 346, 520).

Second, CB_1 is preferentially targeted to presynaptic elements (Figs. 1 and 2). As a result, regional distributions of CB_1 mRNA and immunoreactivity sometimes dissociate. This is particularly conspicuous when CB_1 is predominantly expressed in projection neurons. For example, medium spiny neurons are the output neurons in the striatum, and very intense CB_1 immunoreactivity is detected in the target regions rather than within the striatum (Fig. 1, *A–C*). CB_1 immunoreactivity is strong along the striatonigral and striatopallidal pathways as well as in substantia nigra pars reticulata and the globus pallidus (Fig. 1*A*) (342), in both of which CB_1 mRNA is not expressed. In contrast to intense presynaptic immunolabeling, perikarya of CB_1 expressing cells are very low or negative in most regions with uniform labeling of CB_1 mRNA (252, 342, 528). Clear perikaryal labeling is seen in CCK-positive basket cells of the cerebral cortex, hippocampus, and amygdala (44, 262, 263).

Third, within presynaptic elements, CB_1 is often condensed in perisynaptic portions of axons. This is often apparent at the light microscopic level as close but dissociated distributions of CB_1 and vesicular transporters, such as vesicular GABA/glycine transporter (VGAT or VIAAT) and vesicular glutamate transporters (VGluTs)



(267, 528). At the electron microscopic level, CB_1 density in the perisynaptic portion is higher than that in synaptic and extrasynaptic portion of axons in the hippocampus and cerebellum (267, 391). Furthermore, when CB_1 density is compared between the synaptic and opposite sides of axolemma, the density in the synaptic side is twice as high as that in the opposite side in cerebellar parallel fibers (575). CB_1 thus accumulates on the synaptic side of perisynaptic axolemma, which appears ideal for binding endocannabinoids that are produced at the perisynaptic and extrasynaptic surface of dendritic shafts and spines of postsynaptic neurons (264, 575).

Fourth, inhibitory synapses generally have higher levels of CB_1 than excitatory synapses among CB_1-expressing synapses within given neural regions. Moreover, the enrichment of CB_1 receptors at inhibitory synapses varies greatly depending on brain regions. For example, the density of CB_1 immunogold labeling on inhibitory synaptic elements is higher than excitatory synapses by 30 times for hippocampal CA1 pyramidal cells (Fig. 2), six times for cerebellar Purkinje cells, and three to four times for striatal medium spiny neurons (267, 528). The difference in distribution, density, and regulation of CB_1 expression between excitatory and inhibitory synapses will provide molecular and anatomical bases for biphasic psychomotor and perceptual actions of marijuana that appear in time- and dose-dependent manners.

B. CB_2 Receptor

1. Structure

A human cDNA clone encoding another type of cannabinoid receptor was identified in 1993 and named CB_2 (369). It is a G protein-coupled receptor consisting of 360 amino acids. The human CB_2 receptor shares only 44% amino acid sequence identity with the human CB_1. Later, the mouse (471) and rat (55, 186) CB_2 genes were cloned. The mouse CB_2 is 13 amino acids shorter at the COOH terminal and has 82% amino acid sequence identity with the human CB_2. The rat CB_2 gene may be polymorphic and encodes a protein of 360 (186) or 410 amino acids (55).

2. Distribution

CB_2 was identified as a peripheral receptor expressed in macrophages (369). Subsequently, CB_2 expression in the brain has been established by using reverse transcription-polymerase chain reaction (RT-PCR), in situ hybridization, and immunohistochemistry. Although levels are much lower in the brain than in immune system organs (184), CB_2 is found in microglial cells, not in astrocytes (13, 387), and is upregulated in response to chronic pain (27, 325, 387, 578). In postmortem brains from patients with Alzheimer's disease, however, CB_2 is detected in neuritic plaque-associated astrocytes as well as microglia (31). A recent study showed that CB_2 in the brain stem was functionally coupled to inhibition of emesis in concert with CB_1 (534). However, Derbenev et al. (115) reported CB_2 mRNA was not detected in the brain stem by RT-PCR and immunoblot. There are several reports showing neuronal CB_2 expression in various regions of the brain (184, 404, 479), where CB_2 is distributed in neuronal somata and dendrites but not in terminals (184, 404).

C. "CB_3" Receptor

Presence of so-called "CB_3" at excitatory synapses was proposed (192, 195) based on the electrophysiological data showing the persisting effects of cannabinoid agonists on hippocampal excitatory transmission in CB_1-knockout mice (195). Previous immunohistochemical results showing the absence of CB_1 receptors on hippocampal excitatory presynaptic terminals (194, 263) were apparently in line with the "CB_3" hypothesis. This hypothesis was first challenged by the study using hippocampal cultures that showed unequivocally the absence of the effects of cannabinoid agonists on excitatory transmission in the neurons prepared

FIG. 1. Distribution of CB_1 receptors in the central nervous system of adult mice. *A–D*: overall distribution in parasagittal (*A* and *D*) and coronal (*B* and *C*) brain sections of wild-type (*A–C*) and CB_1-knockout (*D*) mice immunolabeled with a high-titer polyclonal antibody against the COOH terminus of mouse CB_1 receptor [443–473 amino acid residues, GenBank accession no. NM007726; Fukudome et al. (167)]. CB_1 immunoreactivity is highest along striatal output pathways, including the substantia nigra pars reticulata (SNR), globus pallidus (GP), and entopeduncular nucleus (EP). High levels are also observed in the hippocampus (Hi), dentate gyrus (DG), and cerebral cortex, such as the primary somatosensory cortex (S1), primary motor cortex (M1), primary visual cortex (V1), cingulate cortex (Cg), and entorhinal cortex (Ent). High levels are also noted in the basolateral amygdaloid nucleus (BLA), anterior olfactory nucleus (AON), caudate putamen (CPu), ventromedial hypothalamus (VMH), and cerebellar cortex (Cb). Virtual lack of immunostaining in CB_1-knockout (KO) mice indicates the specificity of the CB_1 immunolabeling. *E*: CB_1 immunolabeling in the spinal cord. Note that striking CB_1 immunoreactivity is seen in the superficial dorsal horn (DH), dorsolateral funiculus (DLF), and lamina X. *F–M*: high-power views in the hippocampal CA1 (*F* and *G*), dentate gyrus (*F*), primary somatosensory cortex (*H*), basolateral amygdaloid nucleus (*I*), caudate putamen (*J*), ventromedial hypothalamus (*K*), cerebellar cortex (*L*), and spinal dorsal horn (*M*). CB_1 immunoreactivity shows a punctate or meshwork pattern in all of these regions. CB_1-labeled perikarya are occasionally found in particular interneurons in cortical areas (arrow, *G*). In addition, CB_1 immunoreactivity also shows laminar patterns in the hippocampus (*F* and *G*), dentate gyrus (*F*), cerebral cortex (Cx; *H*), cerebellar cortex (*L*), and spinal dorsal horn (*M*), reflecting different amounts of CB_1 among afferents. In the primary somatosensory cortex, the layer IV is characterized by lower density of CB_1 immunopositive afferents (*H*). NAc, nucleus accumbens; VP, ventral pallidum; Ce, central amygdaloid nucleus; Th, thalamus; Mid, midbrain; Po, pons; MO, medulla oblongata; Or, stratum oriens; Py, pyramidal cell layer; Ra, stratum radiatum; Lm, lacunosum moleculare layer; Mo, dentate molecular layer; Gr, dentate granular layer; ML, cerebellar molecular layer; PC, Purkinje cell layer; GL cerebellar granular layer; LI, lamina I; LIIo, outer lamina II; LIIi, inner lamina II. Scale bars: 1 mm (*A–C*, *E*); 200 μm (*D*); 50 μm (*F* and *H*); 20 μm (*G*, *I*, *J–M*).

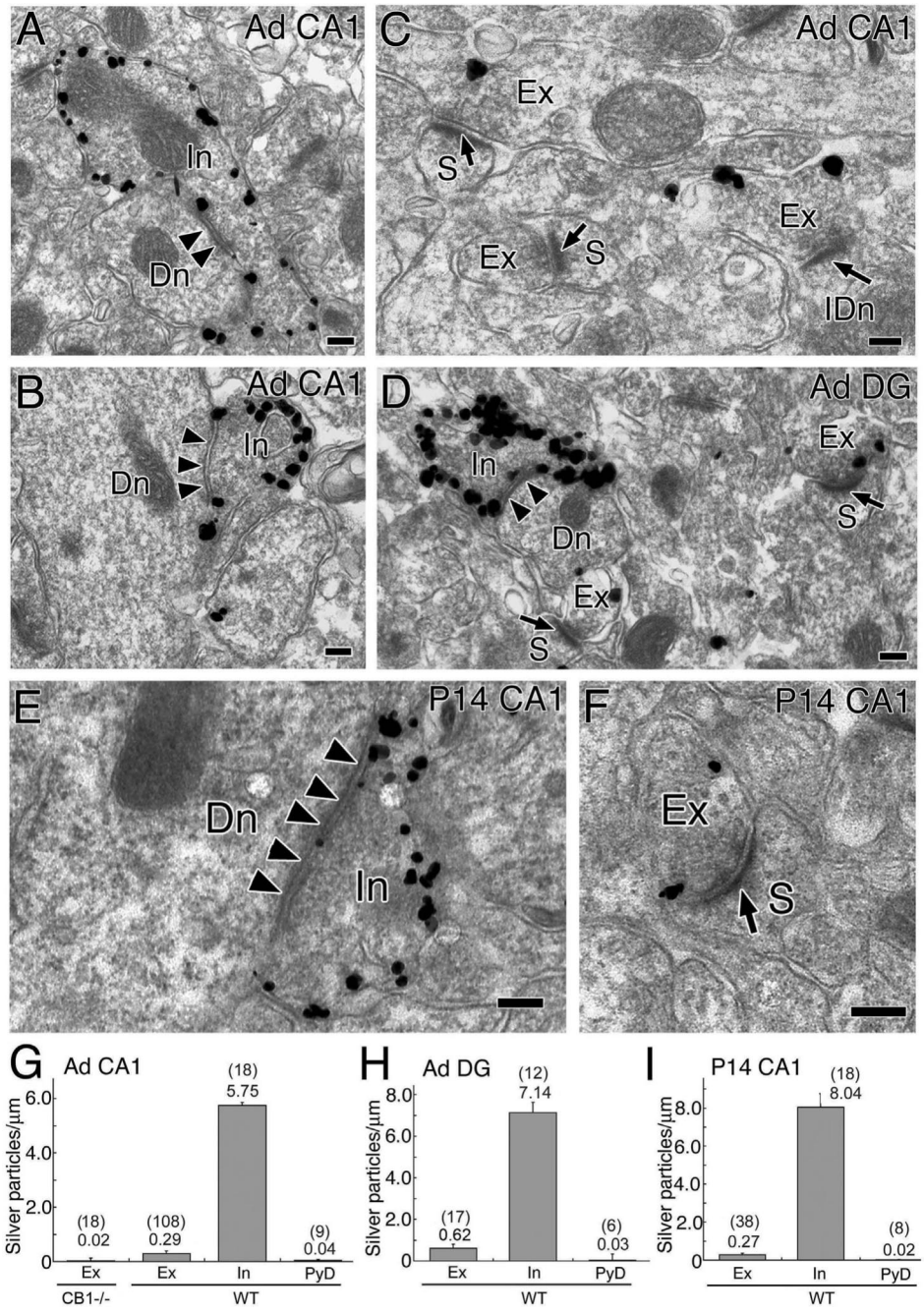


FIG. 2. Immunoelectron microscopy showing presynaptic localization of CB_1 receptors in the hippocampus. Ultrathin sections were prepared from adult (*A–D*, *G*, *H*) or P14 (*E*, *F*, *I*) mice. *A–F*: preembedding silver-enhanced immunogold for CB_1 in the stratum radiatum of the CA1 region (*A–C*, *E*, *F*) and in the innermost molecular layer of the dentate gyrus (*D*). Arrowheads and arrows indicate symmetrical and asymmetrical synapses, respectively. Dn, dendrite; Ex, excitatory terminal; IDn, interneuronal dendrite; In, inhibitory terminal; S, dendritic spine. Scale bar: 100 nm. *G–I*: summary bar graphs showing the number of silver particles per 1 μm of plasma membrane in excitatory terminals (Ex), inhibitory terminals (In), pyramidal cell dendrites (PyD), and granule cell dendrites (GCD) in the CA1 (*G* and *I*) and dentate gyrus (*H*). In wild-type mice (WT), the densities in excitatory terminals are significantly higher ($P < 0.05$) than the background level of PyD or GCD (*G–I*). Furthermore, the density in excitatory terminals in adult wild-type mice is significantly higher ($P < 0.01$) than the noise level, which was estimated from immunogold particle density in excitatory terminals of CB_1-knockout mice (*G*). The numbers in and out of parentheses on the top of each column (*G–I*) indicate the sample size and the mean density of silver particles, respectively. Error bars indicate SE. [From Kawamura et al. (267).]

from CB_1-knockout mice (399). Consistent with the results on hippocampal cultures, recent electrophysiological studies on slice preparations also showed the lack of cannabinoid effects on hippocampal excitatory transmission in CB_1-knockout mice (267, 504). A possible explanation for this discrepancy is that the different results might be due to the difference in concentration of the cannabinoid agonist WIN55,212-2. A high dose of WIN55,212-2 might suppress the excitatory transmission in CB_1-knockout mice through a direct effect on Ca^{2+} channels (380). Recent immunohistochemical studies with newly produced antibodies against CB_1 revealed the presence of CB_1 on hippocampal excitatory terminals (264, 267, 575). Furthermore, the study with conditional CB_1-knockout mice demonstrated that the excitatory transmission is modulated by presynaptic CB_1 receptors in the cortex and amygdala (130). The single-cell RT-PCR experiments confirmed the expression of CB_1 in cortical pyramidal neurons (219). All these studies support that the CB_1 receptor is the major, if not exclusive, cannabinoid receptor at excitatory synapses in these brain regions and indicate that there is no evidence for the presence of "CB_3" receptor.

D. TRPV1 Receptor

A functional vanilloid receptor consisting of 828 amino acids (originally named VR1) was first cloned in 1997 (72). VR1 is a Ca^{2+}-permeable, nonselective cation channel that belongs to the transient receptor potential (TRP) family, and thus called also TRPV1. It is expressed in primary sensory neurons with somata in dorsal root and trigeminal ganglia (189). These neurons have small to medium-sized cell bodies and are thought to convey nociceptive information. The study with TRPV1-knockout mice showed that it is essential for certain modalities of pain sensation and for tissue injury-induced thermal hyperalgesia (71).

Interestingly, the TRPV1 receptor is also distributed in the brain, where its activation by noxious heat or acids seems unlikely, which suggests the existence of endogenous ligands for TRPV1 receptors. So far, several endogenous substances have been found to activate TRPV1 receptors. They are called endovanilloids and include anandamide, *N*-arachidonoyldopamine (see sect. IV*A*), and several lipoxygenase products of arachidonic acid (484). The TRPV1 receptor is not activated by 2-AG and several synthetic cannabinoids and thus not characterized as a cannabinoid receptor (584). However, the fact that anandamide can exert actions through TRPV1 as well as CB_1/CB_2 cannabinoid receptors implies a possible cross-talk between the endocannabinoid and endovanilloid systems under some physiological or pathological conditions (310). For more detailed discussion of the mechanisms and roles of the endovanilloid signaling, see a recent review (484).

E. GPR55 Receptor

GPR55, an orphan G protein-coupled receptor, is proposed as a novel cannabinoid receptor and has recently attracted particular interest among cannabinoid researchers (18, 54, 418). GPR55 is targeted by a number of cannabinoids, but its pharmacological property is somewhat different from those of CB_1 and CB_2 receptors. Primarily using guanosine 5′-O-(3-thiotriphosphate) (GTPγS) binding in HEK293 cells stably expressing GPR55, Ryberg et al. (449) found that GPR55 can be activated by Δ^9-THC, CP55,940, and endocannabinoids including anandamide, 2-AG, noladin ether, and virodhamine (see sect. IV*A*), but not by WIN55,212-2, the most widely used agonist for CB_1 and CB_2 receptors. As another unique feature of GPR55, the authors reported that a widely used cannabinoid antagonist, AM251, behaves not as an antagonist but as an agonist. Moreover, GPR55 was shown to be activated by palmitoylethanolamide and oleoylethanolamide, which are not the ligands for CB_1 and CB_2 receptors (449). Using HEK293 cells transiently expressing GPR55, Lauckner et al. (289) found that GPR55-dependent Ca^{2+} response was evoked by Δ^9-THC and anandamide, but not by WIN55,212-2, CP55,940, 2-AG, and virodhamine. The inability of the latter three compounds to increase Ca^{2+} level might be due to a functional selectivity of different GPR55 agonists.

GPR55 mRNA is found in a number of organs including the adrenal glands, gastrointestinal tract, spleen, and brain. In the brain, GPR55 mRNA is widely distributed, but the levels are significantly lower than those for CB_1 (449). Although GPR55 mRNA is detected, it is not evident that functional GPR55 proteins are actually expressed in the brain. [^{3}H]CP55,940, a synthetic cannabinoid, has been used to examine the distribution of cannabinoid receptors. Because CP55,940 is also a potent ligand for GPR55, it is expected that the distribution of GPR55 proteins can be detected by applying [^{3}H]CP55,940 to CB_1/CB_2-knockout mice. This is not the case, however, because a previous study shows a lack of specific binding of [^{3}H]CP55,940 to the brain of CB_1-knockout mice (583). Similarly, the spleen membranes derived from CB_2-knockout mice have no detectable binding of [^{3}H]CP55,940 (58). It is possible that the expression level of GPR55 is too low to be detected, compared with those of CB_1 and CB_2 receptors (449).

III. CB_1 RECEPTOR SIGNALING

Binding of cannabinoid agonists to cannabinoid receptors causes various effects through multiple signaling pathways. Mechanisms of cellular signaling driven by CB_1 or CB_2 receptors have been intensively investigated and discussed in a number of excellent reviews (114, 126, 233, 237, 344, 367). Here we just make a brief overview of CB_1 receptor signaling.

A. Intracellular Signaling Pathways

Agonist stimulation of CB_1 receptors activates multiple signal transduction pathways primarily via the $G_{i/o}$ family of G proteins, which is supported by the studies examining [^{35}S]GTPγS binding and pertussis toxin (PTX) sensitivity of cannabinoid effects (419). The CB_1 activation inhibits adenylyl cyclase or cAMP production in many preparations, which include neuronal cells with native CB_1 receptors and cell lines expressing recombinant CB_1. The CB_1-mediated inhibition of adenylyl cyclase is sensitive to PTX, confirming the involvement of $G_{i/o}$ proteins. Under certain conditions, however, the coupling of CB_1 to G_s and the consequent increase in cAMP level have been reported. The types of adenylyl cyclase isoforms expressed in the tested cells are suggested to influence the outcome of CB_1 activation (114, 235). Moreover, the CB_1 activation evokes a transient Ca^{2+} elevation in a

phospholipase C (PLC)-dependent manner through either $G_{i/o}$ (494) or G_q proteins (288).

Activation of CB_1 receptors modulates various types of ion channels and enzymes in a cAMP-dependent or -independent manner. In neurons or CB_1-transfected cells, application of a cannabinoid agonist activates A-type (198) and inwardly rectifying K^+ channels (313) and inhibits N- and P/Q-type Ca^{2+} channels (524) and D- and M-type K^+ channels (365, 466). The enzymes that are influenced by CB_1 activation include focal adhesion kinase (116), mitogen-activated protein kinase (460), phosphatidylinositol 3-kinase (45), and some enzymes involved in energy metabolism (190).

B. Suppression of Transmitter Release

There are a number of studies demonstrating that the CB_1 activation inhibits neurotransmitter release, by using electrophysiological and biochemical techniques (464). The neurotransmitters reported to be controlled by the CB_1 receptor include glutamate (297), GABA (502), glycine (247), acetylcholine (176), norepinephrine (241), dopamine (61), serotonin (374), and CCK (26).

The suppression of glutamate release by cannabinoid agonists was first reported in cultured hippocampal neurons (468). The cannabinoid agonist WIN55,212-2 was shown to suppress excitatory postsynaptic currents (EPSCs) with an increase in the coefficient of variation, indicating reduction of transmitter release. The suppression of hippocampal EPSCs by WIN55,212-2 was later shown to be sensitive to the CB_1-specific antagonist SR141716A, confirming the involvement of CB_1 receptors. A similar CB_1-dependent suppression of glutamate release has been reported in various brain regions including the cerebellum, striatum, and cortex (464).

The inhibitory effects of cannabinoids on GABA release were first reported in neurons in the striatum (502) and substantia nigra pars reticulata (76). In these neurons, WIN55,212-2 suppressed GABAergic inhibitory postsynaptic currents (IPSCs), but not the postsynaptic response to exogenously applied GABA or the $GABA_A$-receptor agonist muscimol, indicating a presynaptic site of action. The antagonistic effects of SR141716A on the suppression of IPSCs confirmed the involvement of CB_1 receptors (77, 502). A similar CB_1-dependent suppression of GABA release has been reported in various brain regions including the hippocampus, cerebellum, and nucleus accumbens (NAc) (464).

As to the mechanisms, the involvement of voltage-gated Ca^{2+} channels has been proposed for the suppression of GABA release in the hippocampus (224) and glutamate release at the corticostriatal synapses (238), cerebellar parallel fiber-Purkinje cell synapses (57), and calyx of Held synapses (286). The possible involvement of K^+ channels has also been suggested for the suppression of glutamate release at the cerebellar PF-PC synapses (106, 107) and in the NAc (436). Additional involvement of the sites downstream of Ca^{2+} influx has been demonstrated for the presynaptic suppression of inhibitory (505) and excitatory transmission (570) in the cerebellum. Thus the presynaptic mechanisms underlying the suppression of transmitter release might be different at different synapses.

C. Morphological Changes

There are several studies showing that the CB_1 activation induces morphological changes of neurons. The CB_1 activation has been shown to induce inhibition of new synapse formation in cultured hippocampal neurons (270), neurite retraction in neuroblastoma N1E-115 cells (579), chemorepulsion of growth cones in cortical GABAergic neurons (32), and neurite outgrowth in Neuro-2A cells (251). The inhibition of synapse formation and neurite retraction involves cAMP-dependent signaling pathways. The repulsion of growth cones is mediated by activation of RhoA. The neurite outgrowth is proposed to involve Rap1, Ral, Src, Rac, JNK, and Stat3 (211).

IV. BIOCHEMISTRY OF ENDOCANNABINOIDS

A. Endocannabinoids

The first endocannabinoid *N*-arachidonoylethanolamide (Fig. 3) was isolated from pig brain (118) and was named "anandamide" based on the Sanskrit word *ananda* that means "bliss." Anandamide behaves as a partial agonist at both CB_1 and CB_2 receptors (493), and also as an endogenous ligand for TRPV1 (see sect. II*D*). Therefore, it can activate both the endocannabinoid and endovanilloid systems. Another major endocannabinoid, 2-AG (Fig. 3), was originally isolated from canine gut (350) and rat brain (495). 2-AG is a rather common molecule and is present in the brain at concentrations on the order of nanomoles per gram tissue, which is much higher than that of anandamide (492). 2-AG acts as a full agonist in various assay systems and is strictly recognized by CB_1 and CB_2 receptors, suggesting that 2-AG is a true natural ligand for the cannabinoid receptors (492). There is good evidence to show that these endocannabinoids are synthesized and released from neurons in an activity-dependent manner and play physiological roles as intercellular signaling molecules, as described below.

Other putative endocannabinoids include dihomo-γ-linolenoyl ethanolamide (200), docosatetraenoyl ethanolamide (200), 2-arachidonyl glycerol ether (noladin ether) (199), *O*-arachidonoylethanolamine (virodhamine) (430),

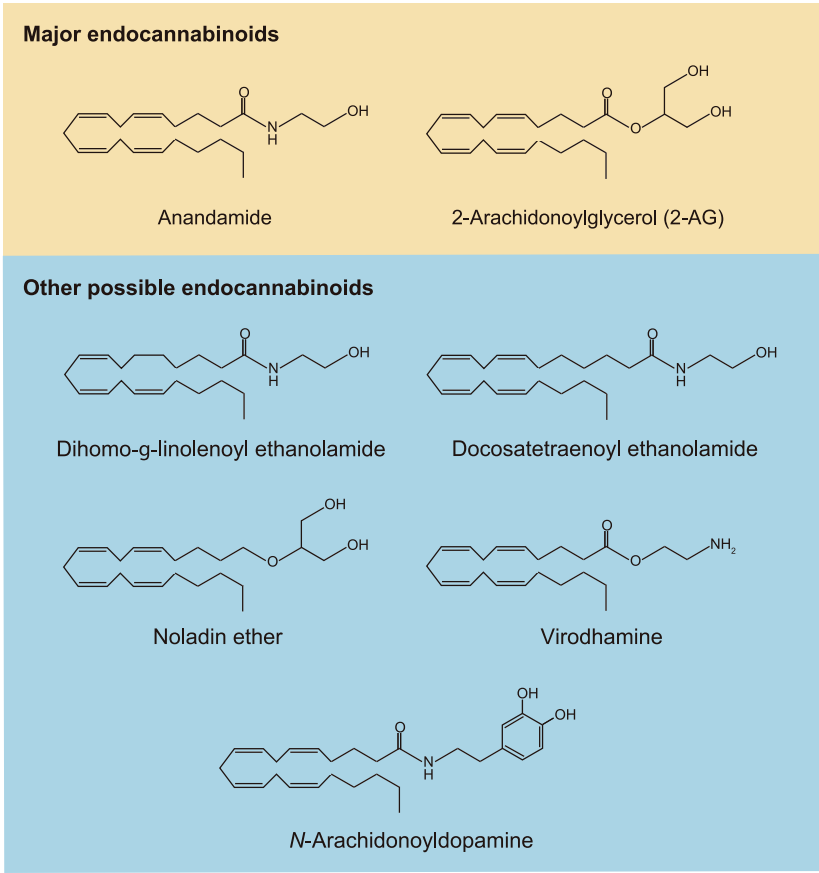


FIG. 3. Molecular structures of endocannabinoids.

and *N*-arachidonoyldopamine (239) (Fig. 3). Dihomo-γ-linolenoyl ethanolamide and docosatetraenoyl ethanolamide, which are members of the *N*-acylethanolamide family like anandamide, are present in the brain and bind to CB_1 receptors (152, 200). These *N*-acylethanolamides have lower affinities for CB_2 (153). Noladin ether was originally synthesized to prepare a metabolically stable analog of 2-AG, and its agonistic action on CB_1 receptors was confirmed (496). Later, it was isolated from porcine brain, and assumed as an endocannabinoid (199), although another study reported that noladin ether was not detected in the brain (400). Noladin ether binds to CB_1 receptors, but shows much lower affinity for CB_2 receptors (199). Virodhamine was isolated from rat brain and identified as a CB_2 agonist (430). It acts as a full agonist for CB_2 receptors, but acts as an antagonist or a partial agonist for CB_1 receptors. *N*-arachidonoyldopamine was shown to be present in rat and bovine nervous tissues (239). Like anandamide, it binds to both the cannabinoid and TRPV1 receptors (40). Although these endogenous lipids can bind to cannabinoid receptors, it is still not clear whether these molecules actually function as intercellular signals.

In the following sections, we introduce biochemical studies that have revealed the metabolic pathways for formation and degradation of the two major endocannabinoids anandamide and 2-AG. For the other endocannabinoids, see a specific review (47). Because endocannabinoid metabolism has been extensively discussed by several other reviews (21, 38, 402, 492, 536), we will refer only to representative studies.

B. Biosynthesis of Anandamide

Activity-dependent production of anandamide in intact neurons was first reported in 1994 (119). When rat striatal or cortical neurons were exposed to the Ca^{2+} ionophore ionomycin or depolarized by a high K^+ solution, anandamide was produced and released to the extracellular space. This anandamide production was blocked by chelating extracellular Ca^{2+} with EGTA. The Ca^{2+}-dependent production of anandamide was also induced in cultured cortical neurons by applying both glutamate and the acetylcholine receptor agonist carbachol (486). This production was not blocked by chelating extracellular Ca^{2+} with EGTA, but blocked by the treatment with BAPTA-AM, a membrane-permeable Ca^{2+} chelator. Importantly, anandamide production can be induced by electrical stimulation in the nervous tissues. In rat hypothalamic slices, high-frequency stimulation (HFS; 100 Hz, 1 s, twice) induced an increase in anandamide level (122),

which was measured by mass spectrometric analysis. This increase in anandamide level was abolished by blocking both AMPA- and NMDA-type glutamate receptors.

As to the biochemical pathways for Ca^{2+}-dependent production of anandamide, earlier studies suggested the "transacylation-phosphodiesterase pathway" composed of two enzymatic reactions (60). The first step is the transfer of an arachidonate group from the *sn*-1 position of phospholipids to the primary amino group of phosphatidylethanolamine (PE), yielding *N*-arachidonoyl PE. This reaction is catalyzed by *N*-acyltransferase (NAT). The second step is the hydrolysis of *N*-arachidonoyl PE to anandamide and phosphatidic acid, and catalyzed by *N*-acylphosphatidylethanolamine-hydrolyzing phospholipase D (NAPE-PLD). The NAT activity is potently stimulated by Ca^{2+}, and generally thought to be the rate-limiting step in anandamide production. The NAT activity is high in the brain and widely distributed in various brain regions. Its cDNA has not yet been cloned. Recently, another type of NAT, which is rather Ca^{2+}-independent and referred as Ca^{2+}-independent NAT (iNAT), was cloned (249). Its mRNA level is the highest in testes among various organs, suggesting that this enzyme may be responsible for the formation of anandamide in testes. NAPE-PLD was molecularly cloned from mouse, rat, and human, and the amino acid sequences were determined (401). The activity of purified recombinant NAPE-PLD is enhanced by Mg^{2+} as well as Ca^{2+}. Taking into account the presence of Mg^{2+} at millimolar levels, this enzyme should be constitutively active in cells. The NAPE-PLD activity is high in the brain, but its regional distribution is not necessarily consistent with that of CB_1 receptors. Recently, NAPE-PLD-knockout mice were generated (295), which are viable and show no obvious abnormality in their behavior in cage. The studies using NAPE-PLD-knockout mice suggest that anandamide can be produced through NAPE-PLD-independent pathways (402).

C. Biosynthesis of 2-AG

Generation of 2-AG as an endocannabinoid was first described in 1997. Elevation of 2-AG level was reported in ionomycin-treated N18TG2 neuroblastoma cells (42) and in hippocampal slices in response to electrical stimulation (487). Later, many biochemical studies showed stimulus-induced generation of 2-AG in various cell types including neurons. The elevation of 2-AG level was observed in NMDA-stimulated cortical neurons (486), hypothalamic slices after HFS (122), ATP-stimulated microglia (566) or astrocytes (552), and cerebellar (315), corticostriatal, or hippocampal slices (254) after exposure to the group I metabotropic glutamate receptor (mGluR) agonist DHPG.

Biochemical studies have revealed several pathways for 2-AG generation (Fig. 4). The main pathway is the combination of PLC and diacylglycerol lipase (DGL). As the first step, PLC hydrolyzes arachidonic acid-containing membrane phospholipid such as phosphatidylinositol and produces arachidonic acid-containing diacylglycerol. Then, 2-AG is produced from the diacylglycerol by the action of DGL. Involvement of these enzymes has been demonstrated by using metabolic inhibitors in the ionomycin-treated cultured neurons (487), Ca^{2+}-exposed brain homogenates (280), and DHPG-stimulated brain slice cultures (254). Two closely related genes encoding

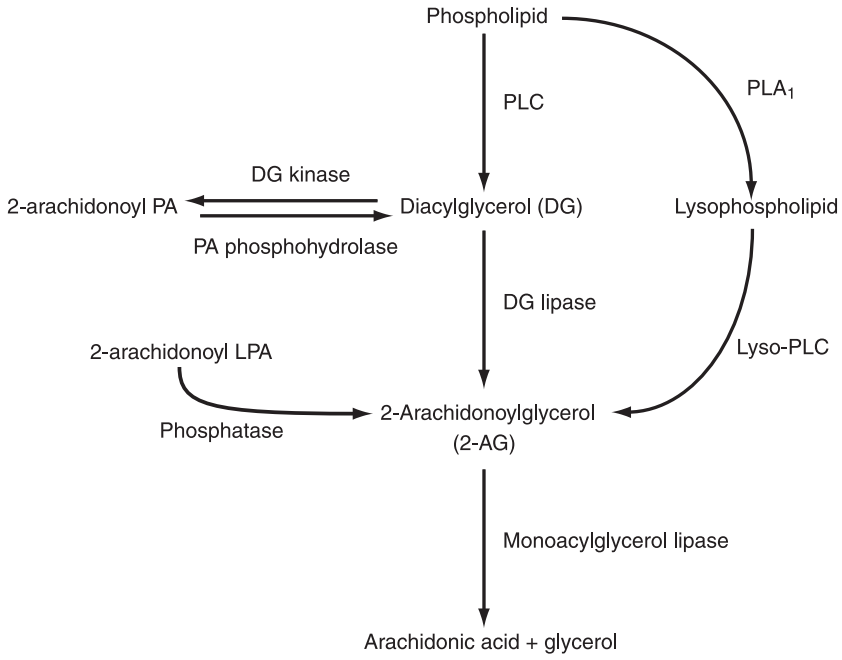


FIG. 4. Postulated pathways of biosynthesis and degradation of 2-arachidonylglycerol. PLC, phospholipase C; PLA_1, phospholipase A_1; PA, phosphatidic acid; LPA, lysophosphatidic acid.

DGL activity were cloned and named DGLα and DGLβ (37). These enzymes were confirmed to be blocked by DGL inhibitors including RHC-80267 and tetrahydrolipstatin (THL). The 2-AG level was increased by overexpression of DGLα and was decreased by a DGL inhibitor, THL, or by RNA interference in ionomycin-stimulated cells (37) and DHPG-stimulated neuroblastoma cells (253). The results indicate the major contribution of DGL to 2-AG synthesis. Other pathways for 2-AG generation so far proposed include the sequential reactions by phospholipase A_1 (PLA_1) and lysoPI-specific PLC (495, 522, 529), the conversion from 2-arachidonoyl lysophosphatidic acid to 2-AG by phosphatase (373), and the formation from 2-arachidonoyl phosphatidic acid through 1-acyl-2-arachidonoylglycerol (41, 68) (Fig. 4). The biosynthetic pathways for 2-AG might be different in different tissues and cells. They might also be dependent on conditions of stimulation.

D. Degradation of Endocannabinoids

Endocannabinoids can be degraded through two different pathways, hydrolysis and oxidation (536). The enzymes that catalyze the first pathway include fatty acid amide hydrolase (FAAH) for anandamide and monoacylglycerol lipase (MGL) for 2-AG. The second pathway involves the well-known cyclooxygenase (COX) and lipoxygenase (LOX), which induce oxidation of the arachidonic moiety of the endocannabinoids.

The enzymatic activity responsible for anandamide degradation was first reported in neuroblastoma and glioma cells as "anandamide amidase," later identified as "anandamide amidohydrolase" in the brain, and finally renamed as FAAH, when purified and cloned from rat liver (96). Rat, mouse, and human FAAH proteins are all 579 amino acids in length. FAAH is detected in many organs including brain. FAAH is able to recognize a variety of fatty acid amides, but its preferred substrate is anandamide. FAAH also catalyzes the hydrolysis of the ester bond of 2-AG in vitro. The esterase activity of FAAH is, however, less important in vivo. FAAH-knockout mice were generated by Cravatt et al. (95). The knockout mice exhibit increased responsiveness to exogenous administration of anandamide. Recently, another membrane-associated FAAH was identified and named "FAAH-2" (558). FAAH-2 is present in several species including human and primates, but absent in murids. High levels of human FAAH-2 expression are seen in the kidney, liver, lung, and prostate, but not in the brain.

MGL was identified in 1976 (513) and first cloned from a mouse adipocyte cDNA library (257). MGL is now recognized as the main enzyme catalyzing the hydrolysis of 2-AG in vivo (128, 129, 536). Mouse, rat, and human MGL proteins are all 303 amino acids in length (128, 257, 258). MGL mRNA is present in various organs including brain (128). Several studies suggest the existence of additional 2-AG hydrolyzing enzymes in the brain (43, 366, 453). With the use of a functional proteomic strategy to assemble a complete and quantitative profile of mouse brain 2-AG hydrolyzing enzymes, it was clearly shown that MGL accounts for ~85% of 2-AG hydrolysis and that the remaining 15% is mostly catalyzed by two uncharacterized enzymes, ABHD6 and ABHD12 (43). This study also showed distinct subcellular distributions of MGL, ABHD6, and ABHD12, suggesting that they may have preferred access to distinct pools of 2-AG in vivo.

COX enzymes in mammalian tissues include three forms: COX-1, COX-2, and COX-3. Among them, COX-1 and COX-2 preferably recognize arachidonic acid (AA) as a substrate. When incubated with anandamide, purified COX-2, but not COX-1, acts on anandamide and produces prostaglandin-ethanolamides, although the affinity for anandamide is lower than that for AA (536). In contrast, 2-AG is a substrate as effective as AA for COX-2 (536). COX-2-knockout mice were generated in 1995 (127, 361). These mice show various phenotypes including severe nephropathy, but effects of endocannabinoids are not known. LOXs are widely expressed in mammals and plants. Anandamide and 2-AG are the substrates of several kinds of LOXs. The genetically modified mice lacking 5-LOX or leukocyte-type 12/15-LOX have been generated (82, 185, 497). However, effects of endocannabinoids have not been assessed in these knockout mice.

E. Endocannabinoid Transport

Endocannabinoids are removed from the extracellular space by a two-step process: the transport into cells and the subsequent enzymatic degradation (164, 222, 347). Anandamide uptake has been observed in a number of preparations including primary neuronal cell cultures (29, 119, 221). Anandamide uptake is saturable and temperature dependent. Several structural analogs of anandamide, such as AM404, have been reported to inhibit the anandamide uptake (29, 423), and they are called anandamide transport inhibitors. However, their molecular identities have not been clarified yet.

There are at least three models proposed for anandamide uptake by cells. The first model is that anandamide is transported by a carrier protein, which binds and translocates anandamide from one side of the membrane to the other (151, 302). The second model is that anandamide passes through the membrane not by carrier but by simple diffusion, which is facilitated by the concentration gradient made by intracellular enzymatic degradation (178). The third model is that anandamide undergoes endocytosis through a caveolae-related uptake process (348). In contrast to the intensive

studies on the mechanisms of anandamide uptake, there is relatively little information concerning 2-AG uptake. There are several studies suggesting that 2-AG and anandamide are transported by the same system (28, 39, 423).

F. Lipid Raft

The compartmentalization of endocannabinoids into lipid rafts has been reported. Lipid rafts are specialized membrane domains enriched in cholesterol and sphingolipids. Various physiological roles have been attributed to lipid rafts (8). They compartmentalize neurotransmitter signaling elements and either enhance or inhibit the signaling. They are also involved in endocytosis and trafficking of signaling molecules. The role of lipid rafts in endocannabinoid signaling has been investigated (19). In a dorsal root ganglion cell line, DGLα, 2-AG, and its precursor arachidonoyl-containing diacylglycerol, but not other diacylglycerols lacking arachidonoyl moiety, were found to be localized to lipid rafts (433). Because DGLα has no selectivity for substrate acyl chain length or saturation (37), it is suggested that the selective trafficking of arachidonoyl-containing DAG to lipid rafts may be crucial for the selective production of 2-AG by DGLα (433). In the same study, anandamide exhibited no selective localization to lipid rafts, at least, under basal conditions (433).

V. ENDOCANNABINOID-MEDIATED SHORT-TERM DEPRESSION

Since the first reports in 2001 (285, 314, 394, 564), many studies have clarified that endocannabinoids mediate retrograde signaling at various synapses in the CNS and contribute to several forms of short-term and long-term synaptic plasticity. Endocannabinoids are produced and released from postsynaptic neurons either phasically in an activity-dependent manner or tonically under basal conditions. The released endocannabinoids activate presynaptic CB_1 receptors and suppress transmitter release either transiently (eCB-STD) or persistently (eCB-LTD). In this and the following chapters, we describe how the synaptic transmission is modulated by retrograde endocannabinoid signaling in each brain region, and what enzymatic pathways are involved in the generation and degradation of endocannabinoids.

A. Endocannabinoid as a Retrograde Messenger

Depolarization-induced suppression of GABAergic inhibitory inputs was originally discovered in the cerebellum by Llano et al. (304). They recorded spontaneous IPSCs from Purkinje cells in cerebellar slices and found that the IPSCs were transiently suppressed following depolarizing voltage pulses. A similar observation was reported in the hippocampus by Pitler and Alger (426). They found that spontaneous inhibitory postsynaptic potentials (IPSPs) or IPSCs recorded from CA1 pyramidal cells in the hippocampal slices were transiently suppressed following a train of postsynaptic action potentials, and termed this phenomenon "depolarization-induced suppression of inhibition," or DSI (6, 425). Later, DSI was found to be induced in culture preparation of dissociated hippocampal neurons by Ohno-Shosaku et al. (397).

The first step of DSI induction was suggested to be Ca^{2+} entry, because cerebellar DSI was inhibited by removing extracellular Ca^{2+} or adding Cd^{2+} to the bath solution (304). Furthermore, DSI was shown to be enhanced by the L-type Ca^{2+} channel activator BAY K 8644 and prevented by intracellular application of high concentrations of Ca^{2+} chelators such as BAPTA and EGTA (426, 545). From these results, it was proposed that depolarization-induced Ca^{2+} entry through voltage-gated Ca^{2+} channels causes a significant elevation of Ca^{2+} concentration in the postsynaptic neuron, and then elicits DSI. To determine the site of DSI expression, postsynaptic sensitivity to exogenously applied GABA or the amplitude and frequency of miniature synaptic events were measured. In cerebellar Purkinje cells, DSI was associated with a decrease in the frequency of miniature IPSCs (mIPSCs) recorded in the presence of tetrodotoxin, and with an increase, not a decrease, in the amplitude of GABA-induced currents, indicating that DSI is expressed presynaptically (304). In CA1 neurons of the hippocampus, postsynaptic depolarization decreased neither the amplitude of mIPSCs (425) nor the response of postsynaptic neuron to applied GABA (426). These results unequivocally indicate that DSI is expressed as a suppression of GABA release from presynaptic terminals. The finding that Ca^{2+} elevation in the postsynaptic neuron induces a presynaptic change strongly suggests the involvement of retrograde synaptic signaling (6, 304).

As a candidate retrograde messenger, glutamate was first proposed (179, 363). Because DSI was attenuated by mGluR antagonists, i.e., by L-AP3 in the cerebellum (179) and MCPG in the hippocampus (363), it was suggested that glutamate or a glutamate-like substance might be released from the postsynaptic neuron and suppress GABA release through activation of presynaptic mGluRs. In 2001, this "glutamate hypothesis" gave way to the model that endocannabinoids mediate retrograde signaling of DSI (394, 564), which is now widely accepted. Our group in hippocampal cultures (394) and Wilson and Nicoll in hippocampal slices (564) demonstrated at the same time that DSI is blocked completely by the CB_1 antagonists SR141716A, AM251,

or AM281 (Fig. 5), but not by the mGluR antagonist MCPG. We found a heterogeneity in the cannabinoid sensitivity of inhibitory presynaptic terminals and clearly showed that DSI can be induced only at cannabinoid-sensitive synapses (394). Wilson and Nicoll (2001) found that DSI was occluded by the cannabinoid agonist WIN55,212-2. Importantly, they found that inhibition of membrane fusion by postsynaptic application of botulinum toxin E light chain did not affect DSI (564), indicating that the release of retrograde messengers does not require vesicular fusion, which is necessary for the release of classical neurotransmitters. Concurrent with the two papers on hippocampal DSI, Kreitzer and Regehr (285) discovered a DSI-like phenomenon at excitatory synapses in cerebellar Purkinje cells. They observed that excitatory transmission to Purkinje cells was transiently suppressed by postsynaptic depolarization and termed this phenomenon "depolarization-induced suppression of excitation," or DSE. DSE was accompanied by an increase in the paired-pulse ratio. Moreover, Kreitzer and Regehr (285) presented direct evidence for presynaptic locus of DSE that the presynaptic Ca^{2+} transients in response to stimulation of excitatory climbing fibers were suppressed during DSE. Similarly to DSI, DSE was prevented by postsynaptic BAPTA injection, occluded by the CB_1 agonist WIN55,212-2, and blocked by the CB_1 antagonist AM251, but not by the antagonists for mGluRs, $GABA_B$, and adenosine A_1 receptors. All these data unequivocally indicate that DSI/DSE is mediated by endocannabinoids, not by glutamate.

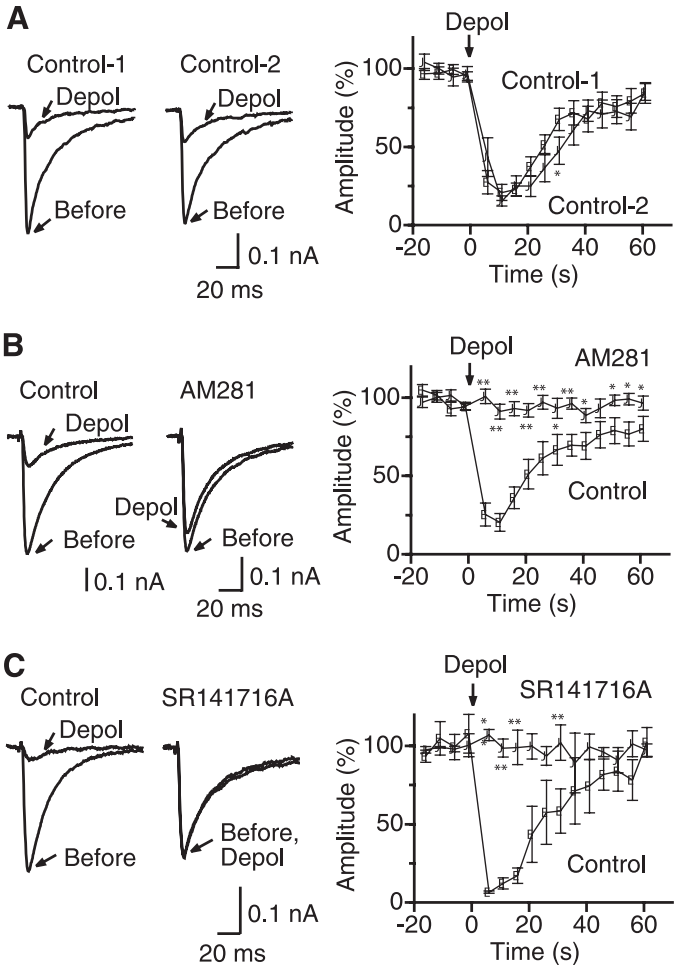


FIG. 5. Blockade of depolarization-induced suppression of inhibition (DSI) by CB_1 antagonists in rat cultured hippocampal neurons. *A*: examples of inhibitory postsynaptic currents (IPSCs) (*left*) and the summary (*right*) of the results showing that DSI can be elicited repeatedly without any rundown of its magnitude. Traces acquired before and 6 s after the first (*control 1*) or the second (*control 2*) depolarization (to 0 mV for 5 s) are shown. Averaged time courses of the changes in IPSC amplitudes induced by the first (open circles) and the second (closed circles) depolarization ($n = 10$). *B* and *C*: examples of IPSCs (*left*) and the averaged time courses of DSI (*right*) before and after the treatment with 0.3 μM AM281 ($n = 11$) and 0.3 μM SR141716A ($n = 3$). The asterisks represent statistically significant differences from the control (*$P < 0.05$; **$P < 0.01$; paired *t*-test). [From Ohno-Shosaku et al. (394), with permission from Elsevier.]

In the same year, Maejima et al. (314) in our laboratory found a totally distinct form of eCB-STD at excitatory synapses on cerebellar Purkinje cells. Maejima et al. (314) reported that application of a selective group I mGluR agonist, DHPG, caused a reversible suppression of EPSCs (Fig. 6). Eight members of the mGluR family (mGluR1-mGluR8) are classified into three groups (groups I–III), and the group I mGluRs (mGluR1 and mGluR5) are coupled to the $G_{q/11}$ type of heterotrimeric G proteins (465). Several lines of evidence indicate that the DHPG-induced suppression is triggered by postsynaptic activation of mGluR1, and eventually expressed as a suppression of glutamate release. First, inactivation of postsynaptic G proteins by intracellular application of GTPγS or GDPβS, a nonhydrolyzable analog of GTP, to Purkinje cells prevented DHPG-induced suppression of EPSCs. Second, the effects of DHPG were abolished in mGluR1-knockout mice and restored in Purkinje cell-specific mGluR1-rescue mice. Third, DHPG decreased the frequency, but not the amplitude, of quantal EPSCs recorded in the presence of Sr^{2+} to induce asynchronous release. Fourth, the suppression of EPSCs was associated with an increase in the paired-pulse ratio and the coefficient of variation, both of which are widely used indices reflecting presynaptic change in transmitter release. Furthermore, it was shown that the DHPG-induced suppression was occluded by the CB_1 agonist WIN55,212-2 and blocked by the CB_1 antagonists SR141716A and AM281 (Fig. 6), indicating that an endocannabinoid is involved in this phenomenon as a retrograde messenger. In striking contrast to DSI/DSE, the DHPG-induced suppression was not prevented by injecting BAPTA to the postsynaptic neuron to prevent Ca^{2+} elevation. From these results, Maejima et al. (314) concluded that the activation of mGluR1 located in postsynaptic Purkinje cells induces a suppression of glutamate release by releasing an endocannabinoid as a retrograde messenger and activating presynaptic CB_1 receptors. Shortly after this publication, a similar phenomenon at the hippocampal inhibitory synapses was reported by

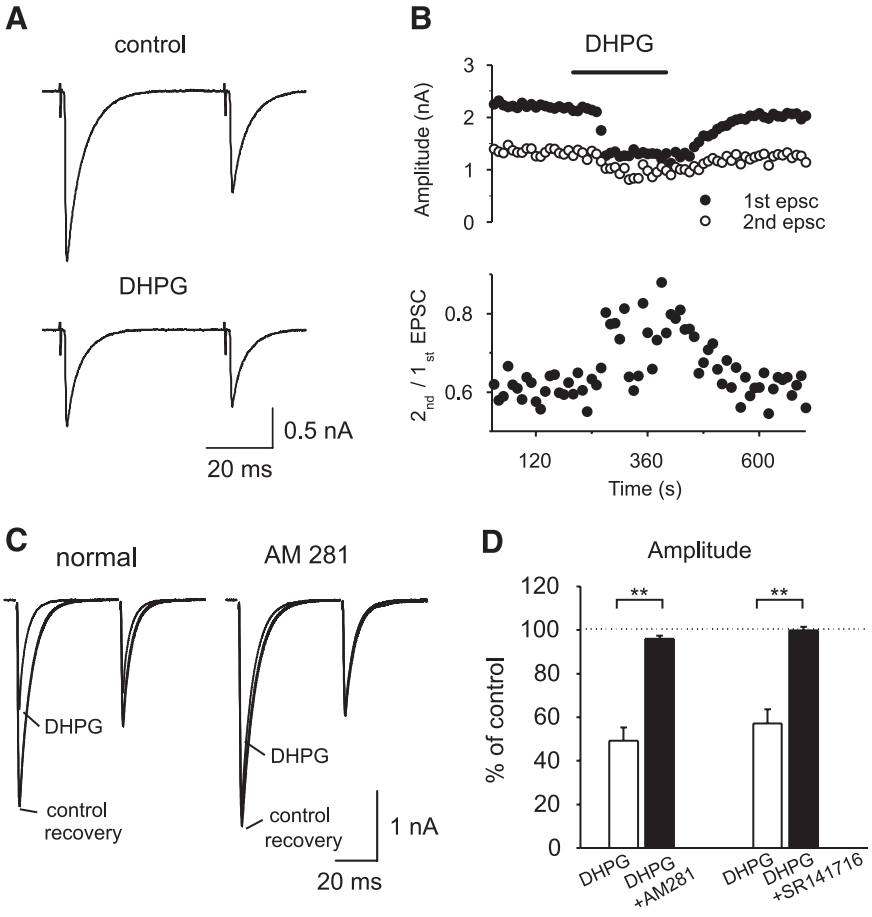


FIG. 6. Blockade of mGluR-driven retrograde suppression by CB_1 antagonists in mouse cerebellar Purkinje cells. *A*: examples of climbing fiber (CF)-mediated EPSCs (CF-EPSCs) (average of 6–12 consecutive responses) in response to paired stimuli (50-ms interval) obtained before and during bath application of 50 μM DHPG. *B*: time course of DHPG-induced suppression of CF-EPSCs, which was accompanied by an increase in the paired-pulse ratio. *C*: the DHPG-induced suppression of CF-EPSCs was abolished by the treatment with 1 μM AM281. *D*: summary of the effects of 50 μM DHPG on the first CF-EPSC amplitudes before and after the treatment with AM281 (1 μM, $n = 9$) or SR141716A (1 μM, $n = 5$). [Modified from Maejima et al. (314), with permission from Elsevier.]

Varma et al. (537). In this study, the CB_1 dependence of DHPG-induced synaptic suppression was confirmed by using CB_1-knockout mice. This study also demonstrated for the first time that DSI is significantly enhanced by low concentrations of DHPG.

Since these pioneering works in 2001, various forms of eCB-STD including DSI, DSE, and mGluR-driven suppression have been reported in various regions of the brain (Tables 1–4). In the next section, we introduce electrophysiological studies reporting eCB-STD in each brain region, by using brain slices or cultured neurons prepared from rats or mice.

B. eCB-STD in Various Brain Regions

1. Hippocampus

The hippocampal formation is required for declarative memory in humans (143) and spatial memory in laboratory animals (500). Dentate granule cells, CA3 pyramidal cells, and CA1 pyramidal cells are glutamatergic and form main excitatory networks. In addition, multiple types of GABAergic inhibitory neurons are distributed in the hippocampus, each of which is characterized by morphology, electrophysiology, and immunocytochemistry for cell marker proteins including neuropeptides and calcium-binding proteins (166). In this brain area, various forms of eCB-STD have been reported (Table 1).

A) DSI. By measuring spontaneous or evoked IPSCs/IPSPs, neurophysiologists have shown that DSI can be induced in various hippocampal neurons including CA1 pyramidal cells (426, 564), CA3 pyramidal cells (362), dentate granule cells (242), hilar mossy cells (227), CCK-positive interneurons (7), and cultured hippocampal neurons (394, 397).

Hippocampal DSI was first found in CA1 pyramidal neurons by Pitler and Alger in 1992 (426). Since then, the properties and mechanisms of DSI in these cells have been studied intensively. In these neurons, DSI can be induced by applying a depolarizing voltage pulse (e.g., from −60 to 0 mV, 1–5 s) in the presence or absence of carbachol, which was originally used to increase spontaneous synaptic events, but later found to enhance DSI (272, 395). As described in section V*A*, early studies demonstrated that DSI is induced by postsynaptic Ca^{2+} increase, and expressed as a suppression of GABA release from presynaptic terminals. In 2001, Wilson and Nicoll (564) presented clear evidence that DSI in CA1 pyramidal

neurons is mediated by endocannabinoids, which are released from postsynaptic neurons, activate presynaptic CB_1 receptors, and suppress GABA release.

The DSI in CA3 pyramidal neurons was reported to be Ca^{2+} dependent (362), but its CB_1 dependency has not been determined. The DSI in granule cells, mossy cells, and cultured hippocampal neurons was confirmed to be Ca^{2+} dependent and CB_1 dependent (227, 242, 394). As for the ability of inhibitory neurons to induce DSI, different results have been reported. Some studies with hippocampal slices showed that DSI was absent in the interneurons located in the stratum radiatum and stratum oriens (226, 415). Because IPSCs recorded from these interneurons were shown to be supppressed by a cannabinoid agonist (226), it was suggested that interneurons might be unable to produce sufficient amount of endocannabinoids. Another study with slice preparations, however, demonstrated that DSI could be induced at inhibitory synapses between CCK-positive Schaffer collateral associated interneurons in the stratum radiatum (7), indicating that interneurons can release endocannabinoids. The study using cultured hippocampal neurons demonstrated that DSI can be induced in inhibitory neurons as effectively as in excitatory neurons (397).

Both anatomical and electrophysiological data indicate that only a subpopulation of inhibitory presynaptic terminals is sensitive to cannabinoids. In cultured hippocampal neurons, we made paired whole cell recordings and recorded unitary IPSCs arising from single inhibitory neuron (394). Application of the cannabinoid agonist WIN55,212-2 suppressed IPSCs in about half of the neuron pairs, but had no effect in the rest of the neuron pairs. Importantly, DSI was readily induced in the cannabinoid-sensitive pairs, but not in the cannabinoid-insensitive pairs. A similar heterogeneity in cannabinoid sensitivity and susceptibility to DSI among IPSCs was observed in hippocampal slices (332, 563). These electrophysiological data are consistent with the anatomical data that show the existence of CB_1-positive and CB_1-negative inhibitory terminals in the hippocampus (263).

B) DSE. We first reported hippocampal DSE in CA1 pyramidal cells and cultured neurons in 2002 (399). We showed that evoked EPSCs recorded from CA1 pyramidal cells or pairs of cultured neurons are transiently suppressed by postsynaptic depolarization. This DSE was blocked by CB_1 receptor antagonists and totally absent in neurons prepared from CB_1-knockout mice. Compared with DSI, DSE was smaller in magnitude and required a longer duration of postsynaptic depolarization. These differences between DSI and DSE are attributed to the difference in cannabinoid sensitivity between inhibitory and excitatory presynaptic terminals, which is consistent with the anatomical data showing a lower level of CB_1 expression at excitatory terminals compared with inhibitory ones (267) (see sect. VIII). By measuring autaptic EPSCs, a similar DSE was reported in microisland culture of hippocampal neurons (489). In dentate granule cells of hippocampal slices, an input-specific expression of DSE was found (88). The study showed that depolarization of granule cells induced DSE at glutamatergic inputs from mossy cells but not from lateral perforant paths. This input specificity might be due to the difference in cannabinoid sensitivity of these two types of inputs.

C) NMDAR-DRIVEN eCB-STD. In DSI and DSE, endocannabinoid release is triggered by elevation of intracellular Ca^{2+} concentration in postsynaptic neuron that is caused by Ca^{2+} influx through voltage-gated Ca^{2+} channels. Then, a question arises as to whether Ca^{2+} influx through other Ca^{2+}-permeable channels can also trigger endocannabinoid release. We recorded cannabinoid-sensitive IPSCs in cultured hippocampal neurons and examined possible contribution of highly Ca^{2+}-permeable NMDA-type glutamate receptors to eCB-STD (393). Under the conditions that minimize Ca^{2+} influx through voltage-gated Ca^{2+} channels, application of NMDA induced a transient suppression of IPSCs. This NMDA-induced suppression was prevented by an NMDA receptor antagonist and a CB_1 antagonist and reduced by postsynaptic loading with BAPTA. Treatment with a cocktail of Ca^{2+} channel blockers for P/Q type (AgTX), R type (SNX-482), and L-type (nifedipine), but not for N type, which is required for synaptic transmission at these synapses, largely suppressed DSI, but not the NMDA-induced suppression of IPSCs. These results indicate that Ca^{2+} influx through NMDA receptors induces endocannabinoid release and suppresses IPSCs. In this study, however, both synaptic and extrasynaptic NMDA receptors were activated by exogenously applied NMDA. It remains to be determined whether local activation of NMDA receptors by synaptically released glutamate is enough to induce eCB-STD.

D) mGluR-DRIVEN eCB-STD. In the hippocampus, mGluR-driven eCB-STD has been found at inhibitory synapses on CA1 neurons and at both inhibitory and excitatory synapses of cultured hippocampal neurons. In hippocampal slices, Varma et al. (537) reported that activation of group I mGluRs by DHPG decreased the amplitude of IPSCs in CA1 neurons and that this suppression was abolished in CB_1-knockout mice, indicating the involvement of endocannabinoids. In cultured hippocampal neurons, we observed a similar suppression of IPSCs by DHPG application (398). This DHPG-induced suppression at inhibitory synapses was blocked by a CB_1 antagonist and the mGluR5-specific antagonist MPEP, indicating major contribution of mGluR5 to endocannabinoid release in the hippocampus. In micro-island culture of hippocampal neurons, a similar phenomenon was reported at autaptic excitatory synapses (490). DHPG application induced suppression of autaptic EPSCs, which was absent in CB_1-knockout mice. In these studies on mGluR-driven eCB-

STD, DHPG was applied only for a short time. In this condition, IPSCs/EPSCs were recovered after washout of DHPG. When the DHPG application was prolonged (10–20 min), however, long-lasting suppression was induced in some preparations, indicating the induction of eCB-LTD (84, 282). This long-term effect will be discussed in section VI.

E) mAChR-DRIVEN eCB-STD. In the studies on hippocampal DSI, the cholinergic agonist carbachol was often included in the bath solution to increase the frequency of spontaneous IPSCs. Meanwhile, Kim et al. (272) found that carbachol itself has a suppressing effect on evoked IPSCs recorded from CA1 pyramidal cells. The carbachol-induced suppression of IPSCs was blocked by a CB_1 antagonist, and absent in CB_1-knockout mice, although some extent of suppression remained with a high concentration of carbachol (25 μM). The type of cholinergic receptors responsible for this effect was pharmacologically examined, and the involvement of muscarinic acetylcholine receptors (mAChRs) rather than nicotinic receptors was confirmed. We found a similar mAChR-driven eCB-STD at inhibitory synapses in cultured hippocampal neurons (168). In this study, the subtype of mAChRs involved in mAChR-driven eCB-STD was determined by using knockout mice. Among five subtypes of mAChRs, M_1, M_3 and M_5 receptors are coupled positively to PLC through $G_{q/11}$ protein, whereas M_2 and M_4 receptors are coupled negatively to adenylyl cyclase through $G_{i/o}$ protein (73). Using the genetically engineered mice that are deficient in one of the five subtypes of mAChRs, we (168) revealed that M_1 and M_3 receptors are responsible for mAChR-driven eCB-STD. In contrast, M_2 receptor was found to mediate direct presynaptic suppression caused by muscarinic agonists (168). The mAChR-driven eCB-STD was found also at autaptic excitatory synapses in cultured hippocampal neurons (490).

F) CCK RECEPTOR-DRIVEN eCB-STD. In hippocampal slices, Foldy et al. (158) examined effects of CCK on IPSCs in CA1 pyramidal cells (158). Hippocampal basket cells include two types, CCK-positive and parvalbumin-positive basket cells (166). Application of CCK (CCK-8S) increased the frequency of spontaneous IPSCs (158). This effect was attributed to the depolarizing action of CCK on parvalbumin-positive basket cells. By recording unitary IPSCs in paired recordings with synaptically connected interneurons and CA1 pyramidal cells, Foldy et al. (158) found that CCK induced suppression of IPSCs derived from CCK-positive basket cells but not those from parvalbumin-positive basket cells. The suppressing effect of CCK on IPSCs was abolished by the CB_1 antagonist AM251 and postsynaptic application of guanosine 5′-*O*-(2-thiodiphosphate) (GDPβs). From these results, Foldy et al. (158) concluded that activation of CCK receptors, which are dominantly coupled to $G_{q/11}$ protein (134), induces suppression of GABA release by releasing endocannabinoids and activating CB_1 receptors on CCK-positive basket cell terminals. This study revealed that CCK acts on the two types of inhibitory inputs in the opposite ways, namely, activation of one type and inhibition of the other. In agreement with this notion, it was reported that CCK selectively suppresses carbachol-induced spontaneous IPSPs, which reflect the inputs from CB_1- and CCK-positive basket cells (259). The CB_1 dependency of this effect remains to be determined.

2. Cerebellum

The cerebellum is involved in coordination, control, and learning of movements (245). The basic neuronal circuit of the cerebellar cortex has been studied in detail (245), which includes Purkinje cells, granule cells, and three types of interneurons, i.e., basket cells, stellate cells, and Golgi cells. Purkinje cells receive two distinct excitatory inputs from parallel fibers (PFs) and climbing fibers (CFs). PFs are the axons of granule cells and form synapses on the spines of Purkinje cell's dendrites. Synaptic inputs from individual PFs are weak, but the number of PFs innervating a single Purkinje cell is as many as 100,000–200,000. CFs originate from the inferior olive in the contralateral medulla and form contacts directly on Purkinje cells. In contrast to PFs, only one CF innervates a single Purkinje cell in the adult cerebellum, but each CF makes strong synaptic contacts on Purkinje cell's proximal dendrites. Purkinje cells provide the sole output pathway of the cerebellar cortex to their target neurons in the vestibular and cerebellar nuclei. As described in section V*A*, pioneering studies on eCB-STD have been conducted in the cerebellar cortex (Table 2).

A) DSI. In 1991, Llano et al. (304) made the first report of DSI that spontaneous IPSCs recorded from Purkinje cells in cerebellar slices were transiently suppressed following a depolarizing voltage pulse (e.g., +30 mV, 0.2 s). Early studies by this group have revealed that DSI is induced by postsynaptic Ca^{2+} elevation and expressed as a suppression of GABA release, which suggests the involvement of a retrograde messenger. Later, a crucial role of an endocannabinoid as a retrograde messenger in cerebellar DSI was proven independently by three research groups (125, 284, 576). By recording spontaneous or evoked IPSCs from Purkinje cells in cerebellar slices, DSI was shown to be blocked by CB_1 antagonists (125, 284, 576) but not by the antagonists of mGluRs or $GABA_B$ receptors (284), and completely abolished in CB_1-knockout mice (576). As for other cell types in the cerebellar cortex, DSI was found to be absent in Golgi cells (24). Whether DSI can be induced in other cell types in the cerebellar cortex remains to be investigated.

B) DSE. DSE was originally found in cerebellar Purkinje cells by Kreitzer and Regehr in 2001 (285). In Purkinje cells of cerebellar slices, they found that PF-EPSCs

TABLE 2. *eCB-STD in the cerebellum*

Postsynaptic Neuron	Input	Type of STD	Dependence	Independence	DSI/DSE Enhancement	Reference Nos.
PC	I	DSI	Ca^{2+}			304
			CB_1	mGluR, $GABA_B$		284
			CB_1			125
			CB_1	mGluR		576
			CB_1, DGL, CaMKII	PLC		503
					mGluR1	135
		I-mGluR	CB_1, G protein (post), PLC, DGL	Ca^{2+}		170
		PF-stim	CB_1, mGluR			170
		CF-stim				136
	E (CF)	DSE	CB_1, Ca^{2+}	mGluR, $GABA_B$, A_1		285
			CB_1			314
			PLCβ4 (for DSE enhancement)	PLCβ4 (for DSE)	mGluR1	315
			DGL			208
		I-mGluR	CB_1, mGluR1, G protein (post)	Vesicular release, Ca^{2+}		314
			PLCβ4, Ca^{2+}			315
			CB_1			367
		PF-stim	CB_1, mGluR1			314
	E (PF)	DSE	CB_1, Ca^{2+}	mGluR, $GABA_B$, A_1		285
				DGL		455
			CB_1, DGL			503
		mGluR1	iGluR, Ca^{2+}			296
			CB_1			267
		PF-stim	CB_1, mGluR1			56
			CB_1, Ca^{2+}, mGluR1, DGL	Ca^{2+} store		315
			DGL			455
		PF&CF-stim	CB_1, Ca^{2+}, mGluR1	Ca^{2+} store		51
BC	E (PF)	DSE	CB_1			25
SC	E (PF)	DSE	CB_1			25
		PF-stim	CB_1, mGluR1, NMDAR, DGL, Ca^{2+}			25
GC	E (PF)	no DSE				24

PC, Purkinje cell; BC, basket cell; SC, stellate cell; GC, Golgi cell; I, inhibitory; E, excitatory; CF, climbing fiber; PF, parallel fiber; I-mGluR, group I metabotropic glutamate receptor; CaMKII, Ca^{2+}/calmodulin-dependent protein kinase II; post, postsynaptic; pre, presynaptic; iGluR, ionotropic glutamate receptor; A_1, A_1 adenosine receptor.

and CF-EPSCs were both transiently suppressed by postsynaptic depolarization. This suppression was prevented by postsynaptic BAPTA injection, occluded by the cannabinoid agonist WIN55,212-2, and blocked by the CB_1 antagonist AM251. From these results, the authors concluded that depolarization-induced Ca^{2+} elevation releases endocannabinoids and causes a transient suppression of glutamate release by activating presynaptic CB_1 receptors.

PFs form excitatory synapses not only on Purkinje cells but also on basket cells, stellate cells, and Golgi cells (245). Whether DSE could be induced at the PF synapses on these interneurons was investigated in cerebellar slices (24, 25). In both basket and stellate cells, DSE could readily be induced in a CB_1-dependent manner (25). The magnitude of DSE was, however, smaller in these interneurons than in Purkinje cells. Because there was no difference in cannabinoid sensitivity of PF-EPSCs between these interneurons and Purkinje cells, the difference in DSE magnitude was attributed to the difference in the capability of postsynaptic neurons to release endocannabinoids. In contrast, DSE was absent at PF-Golgi cell synapses, although these synapses were also shown to be cannabinoid sensitive (24). Therefore, it seems likely that Golgi cells cannot produce or release endocannabinoids sufficiently. Consistent with this notion, Golgi cells failed to exhibit DSI.

c) mGluR-DRIVEN eCB-STD. As detailed in section VA, mGluR-driven eCB-STD was first discovered at CF-Purkinje cell synapses by Maejima et al. in our laboratory in 2001 (314). We demonstrated clearly that activation of mGluR1 located on postsynaptic Purkinje cells induces the endocannabinoid release, and thereby suppresses the transmitter release through activation of presynaptic CB_1 receptors. This suppression did not require elevation of intracellular Ca^{2+} concentration in postsynaptic Purkinje cells (314). A similar mGluR-driven eCB-STD was found at inhibitory synapses on Purkinje cells (170). In this study, paired recordings were made from interneurons (basket or stellate cells) and Purkinje cells. Unitary IPSCs re-

corded from Purkinje cells were suppressed by application of DHPG. This suppression of IPSCs was dependent on both mGluR1 and CB_1 receptors, and independent of Ca^{2+} elevation in postsynaptic Purkinje cells, indicating that the mGluR-driven eCB-STD of IPSCs shares the same mechanisms with that of EPSCs (314).

D) SYNAPTICALLY DRIVEN eCB-STD. An important question is whether eCB-STD could be induced under physiological conditions, namely, by synaptic activity, rather than by depolarizing postsynaptic neurons or by pharmacological activation of $G_{q/11}$-coupled receptors. Induction of eCB-STD has been attempted by using physiologically relevant synaptic stimulation protocols. The cerebellum is the most intensively studied region for synaptically driven eCB-STD. We made the first demonstration of synaptically driven eCB-STD in 2001 (314). We found that repetitive stimulation of PFs (25 or 50 stimuli at 100 Hz) induces transient suppression of CF-mediated EPSCs. This suppression was blocked by the CB_1 antagonist SR141716 and the mGluR1 antagonist CPCCOEt, indicating that PF synaptic activity heterosynaptically induced eCB-STD of CF-Purkinje cell synapses (314). Following this report (314), synaptically driven eCB-STD has been examined by using several stimulation protocols at excitatory as well as inhibitory synapses in the cerebellum.

Brown et al. (56) in Regehr's laboratory demonstrated that PF stimulation induces endocannabinoid release, which homosynaptically affects the activated PFs. In this study, presynaptic inhibition of excitatory transmission at PF-Purkinje cell synapses was monitored by measuring Ca^{2+} transients in PF terminals. A brief train of PF stimulation reduced presynaptic Ca^{2+} transients in a CB_1-dependent manner. This endocannabinoid-mediated presynaptic inhibition was dependent on both the number and the frequency of PF stimulation. A half-maximal effect was obtained with three to five stimuli at 50 Hz. The effect of PF stimulation was reduced by the mGluR1 antagonist CPCCOEt, and completely blocked by coapplication of CPCCOEt and the AMPA receptor blocker NBQX, suggesting that the activation of both mGluR1 and AMPA receptors is necessary for synaptically driven endocannabinoid release. This study also reported a synapse specificity of endocannabinoid signaling. The endocannabinoid-mediated presynaptic inhibition was observed at the stimulated inputs, but not at the nonstimulated inputs 20 μm apart from the stimulated point. In accordance with this observation, it was observed that postsynaptic Ca^{2+} signals were spatially restricted to a small region of the dendritic arbor, when PFs were stimulated in a train of 10 or 50 stimuli at 50 Hz. These results indicate that PF activity induces eCB-STD in an input-specific manner. Regehr's group (24, 25) further examined eCB-STD at PF-interneuron synapses and reported that synaptically driven eCB-STD can be induced by PF stimulation (10 stimuli at 50 Hz) at PF-stellate cell synapses, but not at PF-Golgi cell synapses. Interestingly, synaptically driven eCB-STD at PF-stellate cell synapses was shown to involve mGluR1 and NMDA receptors (25).

When more intense PF stimulation protocol is used, endocannabinoids can spread from PF synapses to CF or inhibitory synapses on the same Purkinje cell. As mentioned previously, we found that stimulation of PFs in a train of 25 or 50 stimuli at 100 Hz causes a suppression of CF-EPSCs in a CB_1-dependent manner (314). Galante and Diana (170) observed that stimulation of PFs in a train of 10 stimuli at 100 Hz induced a transient suppression of spontaneous IPSCs in a CB_1-dependent manner. In contrast, spread of endocannabinoids from CF synapses to other synapses is under debate. Galante and Diana (170) found that CF activity (5 stimuli at 20 Hz) failed to affect spontaneous IPSCs. Duguid and Smart (136) reported that stimulation of CFs (10 stimuli at 2 Hz) induced a transient suppression of spontaneous IPSCs in P11–P14 animals. The same or stronger stimulation protocol (10–60 stimuli at 2 Hz) was, however, shown to be without effects on spontaneous IPSCs in P15-P17 animals (50).

Importantly, Brenowitz and Regehr (51) found the associative nature of combined PF and CF stimulation for eCB-STD. The number of PF stimulation required for induction of eCB-STD at PF-Purkinje cells is reduced when PF stimulation is combined with CF stimulation. The timing of PF and CF stimulations is critical for the associative enhancement of eCB-STD, and a temporal window is within several hundred milliseconds. This associative nature of eCB-STD is similar to that of cerebellar LTD, which will be discussed in section VI*A5*.

3. Basal ganglia

Basal ganglia are known to play important roles in motor control and reinforcement learning (562) and consist of four nuclei, the striatum, globus pallidus, substantia nigra (pars reticulata and pars compacta), and subthalamic nucleus. Medium spiny neurons are principle neurons of the striatum and send their GABAergic outputs to the substantia nigra and the globus pallidus (562). Anatomical studies show abundant distribution of CB_1 receptors in the basal ganglia, suggesting a physiological importance of the endocannabinoid system. In this region, several forms of eCB-STD have been reported (Table 3).

A) DSI. Medium spiny neurons receive GABAergic inputs from interneurons including parvalbumin-positive fast-spiking interneurons (266). Narushima et al. (377) in our laboratory found that DSI is readily induced at the fast-spiking interneuron-derived inhibitory synapses on medium spiny neurons in the dorsal striatum. In this study, unitary IPSCs were monitored by paired recordings with a presynaptic fast-spiking interneuron and a postsynaptic medium spiny neuron in brain slices. The amplitude

TABLE 3. *eCB-STD in the basal ganglia, nucleus accumbens, cerebral cortex and amygdala*

Region	Postsynaptic Neuron	Input	Type of STD	Dependence	Independence	DSI/DSE Enhancement	Reference Nos.
Striatum	MSN	I	DSI	CB_1			377
				CB_1		I-mGluR	165
						mAChR (M_1)	376
				DGL		I-mGluR	528
			I-mGluR	CB_1			165
				DGL			528
				CB_1, mGluR5			74
				CB_1, mGluR5, GSH, DGL			310
			mAChR (M_1)	CB_1, G protein (post)			376
				DGL			528
		E	DSE	CB_1		I-mGluR	375
				DGL		I-mGluR	528
			I-mGluR	CB_1, VGCC (L)			282
				CB_1			375
				DGL			528
			Input ($+D_2$)	CB_1, Ca^{2+}, mGluR1, Ca^{2+} store, PLC			574
GP		I	DSI	CB_1			147
SNr		I	DSI	CB_1			551
				CB_1		AChR	571
				CB_1, DGL			503
SNc		I	DSI	CB_1			571
NAc	MSN	E	I-mGluR	CB_1			437
Cortex	L2/3 PyC	I	DSI	CB_1, Ca^{2+}			517
						AChR	161
	L5 PyC	I	DSI				44
			no DSI				160
		E	DSE	CB_1, Ca^{2+}	mGluR		160
	Culture	I	DSI				488
BLA	Principle cell	I	DSI	CB_1			581
	Isolated cell	I	DSI	CB_1, Ca^{2+}			581

GP, globus pallidus; SNr, substantia nigra pars reticulata; SNc, substantia nigra pars compacta; NAc, nucleus accumbens; BLA, basolateral amygdala; MSN, medium spiny neuron; PyC, pyramidal cell; I, inhibitory; E, excitatory; I-mGluR, group I metabotropic glutamate receptor; GSH, glutathione; post, postsynaptic; VGCC, voltage-gated Ca^{2+} channel; L, L-type.

of IPSCs was transiently suppressed by depolarization (0 mV, 1–5 s) of medium spiny neurons. This DSI was prevented by a CB_1 antagonist. By recording evoked IPSCs from medium spiny neurons, Freiman et al. (165) reported that depolarization (0 mV, 100 ms, 9 pulses at 1 Hz) failed to induce DSI under normal conditions, but induced prominent DSI in the presence of the group I mGluR agonist DHPG. Importantly, Narushima et al. (376) reported that ambient acetylcholine derived from tonically active cholinergic interneurons constitutively upregulates DSI in medium spiny neurons. This muscarinic enhancement of DSI is mediated by M_1 muscarinic receptors in medium spiny neurons (376). Therefore, activity of cholinergic interneurons in the striatum may control striatal output by modulating DSI in medium spiny neurons.

Globus pallidus neurons receive GABAergic inputs from striatal medium spiny neurons via the striatopallidal pathway (562). Engler et al. (147) showed that this pathway is cannabinoid sensitive and exhibits DSI in a CB_1-dependent manner (147). The authors recorded IPSCs from globus pallidus neurons in slice preparations in response to stimulation in the caudata-putamen. Application of a cannabinoid agonist decreased the amplitude of IPSCs. Depolarization (30 mV, 5 s) induced a transient suppression of IPSCs, and the suppression was blocked by a CB_1 antagonist. These data are consistent with the anatomical data showing that medium spiny neurons express CB_1 receptor mRNA and that the receptor density is high in the globus pallidus (140, 229, 318, 519).

Substantia nigra pars reticulata (SNr) and pars compacta (SNc) receive GABAergic input from striatonigral fibers. Wallmichrath and Szabo (551) demonstrated that the striatonigral GABAergic input exhibits DSI in SNr neurons. In sagittal slices containing the striatum and SNr, stimulation was applied to the striatum, and the evoked IPSCs were recorded from SNr neurons. Postsynaptic depolarization (+30 mV, 5 s) induced a suppression of IPSCs, which was mimicked by a cannabinoid agonist and blocked by a CB_1 antagonist. Another study compared the size of DSI induced by depolarization (0 mV, 10 s) between SNr and SNc neurons (571). DSI was larger in SNr neurons than in SNc neurons. This difference in DSI size was not attributable to the difference in cannabinoid sensitivity of presynaptic fibers, because the cannabinoid agonist WIN55,212-2 suppressed IPSCs similarly in SNr and SNc neurons. It should be noted, however, that IPSCs were evoked by stimulation within the substantia nigra, and therefore might be derived from several

sources of GABAergic inputs including the striatum, globus pallidus, and interneurons within the substantia nigra.

B) DSE. In contrast to DSI, DSE cannot easily be induced in this region. Depolarization (0 mV, 5 s) of medium spiny neurons failed to induce DSE at corticostriatal synapses in the dorsolateral striatum under normal conditions (375). DSE was induced only in the presence of a high dose of the group I mGluR agonist DHPG (50 μM) (375), or in the presence of both 5 μM DHPG and 0.5 μM oxo-M, a muscarinic agonist (528).

C) mGluR-DRIVEN eCB-STD. At the corticostriatal synapses of medium spiny neurons in the dorsal striatum, mGluR-driven endocannabinoid release has been reported by two research groups (282, 375, 528). Application of the group I mGluR agonist DHPG (50 μM) induced a suppression of evoked EPSCs, which was blocked by a CB_1 receptor antagonist (375). Another study reported a similar suppression of EPSCs by DHPG (282). The authors found that the suppressing effect of DHPG was more prominent when the postsynaptic neuron was slightly depolarized (from -70 to -50 mV). This facilitating effect was blocked by the L-type Ca^{2+} channel blocker nitrendipine, suggesting that the enhancement of the DHPG-induced suppression is caused by the elevated Ca^{2+} level in medium spiny neurons (see sect. V*C3*). Interestingly, this study showed that after the treatment with 10 μM DHPG for 10–20 min, EPSCs did not fully recover from suppression, suggesting that eCB-LTD was induced (see sect. VI*A1*). At the cortico-NAc synapses of the medium spiny neurons, similar mGluR-driven eCB-STD and eCB-LTD were reported (437). Bath-applied DHPG (100 μM, 10 min) caused a suppression of EPSCs in a CB_1-dependent manner. EPSCs were only partially recovered after washout of DHPG.

At inhibitory synapses on the medium spiny neurons in the dorsal striatum, mGluR-driven endocannabinoid release is also found (74, 165, 310). Application of DHPG (50 μM) was shown to induce a suppression of IPSCs in a CB_1-dependent manner (74, 165, 310). The amplitude of evoked IPSCs and the frequency of spontaneous or miniture IPSCs were recovered to the original level after washout of DHPG (74, 310), indicating that DHPG application induces eCB-STD rather than eCB-LTD in these synapses.

D) mAChR-DRIVEN eCB-STD. In the dorsal striatum, Narushima et al. (376) in our laboratory reported mAChR-driven eCB-STD at inhibitory synapses on medium spiny neurons. Application of the muscarinic agonist oxo-M caused a suppression of evoked IPSCs. This suppression was blocked by the muscarinic antagonist atropine, a CB_1 receptor antagonist, and postsynaptic application of GDPβS, confirming the involvement of retrograde endocannabinoid signaling. The effect of oxo-M was blocked by the M_1-preferring antagonist pirenzepine and abolished in M_1-knockout mice. From these results, Narushima et al. (376) concluded that M_1 muscarinic receptors are responsible for mAChR-driven eCB-STD in these neurons.

E) SYNAPTICALLY DRIVEN eCB-STD. In the dorsal striatum, Yin and Lovinger (574) reported synaptically driven eCB-STD at corticostriatal synapses. When synaptic stimulation (2 or 3 pulses at 20 Hz) of corticostriatal pathways and application of the D_2 agonist quinpirole were combined, glutamatergic transmission was suppressed at the corticostriatal synapses. This effect was blocked by bath application of the mGluR1 antagonist CPCCOEt, and by postsynaptic injection of the Ca^{2+} chelator BAPTA, the intracellular Ca^{2+} pump inhibitor thapsigargin, or the PLC inhibitor U73122, suggesting the involvement of group I mGluR and store-dependent Ca^{2+} elevation. Furthermore, this suppression of synaptic transmission was completely blocked by the CB_1 antagonist AM251 and was absent in CB_1-knockout mice, confirming that this phenomenon is a form of eCB-STD. Although the site of action of the D_2 agonist was not determined, the activation of D_2 receptors might be involved in facilitation of endocannabinoid production in cooperation with group I mGluRs.

F) NEUROTENSIN-INDUCED eCB-STD. Yin et al. (573) reported that application of neurotensin induced a suppression of EPSCs recorded from medium spiny neurons in the dorsolateral striatum. This suppression was dependent on CB_1 receptors, group I mGluRs, and D_2 receptors. The dependence on group I mGluRs and D_2 receptors indicated the possibility that the activation of neurotensin receptors might induce the endocannabinoid release not directly, but indirectly through modulating glutamatergic or dopaminergic system. Although it is not clear where and how neurotensin acts, this finding is important because neurotensin is proposed as an endogenous antipsychotic.

4. Cerebral cortex

Disruption of cognition and impaiment of working memory are examples of the major symptoms induced by marijuana inhalation. Despite the importance of the cerebral cortex as a target for cannabinoids, information about eCB-STD in the cerebral cortex is relatively limited. So far only DSI and DSE have been reported (Table 3).

A) DSI. DSI has been reported in several types of cortical neurons, including layer 2/3 pyramidal cells (161, 516, 517) and layer 2 and 5 pyramidal cells (44) of the sensory cortex and cultured cortical neurons (488).

Involvement of retrograde endocannabinoid signaling in short-term plasticity in the cerebral cortex was first reported in layer 2/3 pyramidal cells of the sensory cortex by Trettel and Levine in 2003 (517). Evoked IPSCs were found to be suppressed transiently by applying depolarizing voltage pulses (0 mV, 150 ms, 10 pulses at 0.3 Hz) to the postsynaptic pyramidal cells. This suppression was

associated with an increase in the paired-pulse ratio and blocked by either postsynaptic BAPTA injection or bath application of the CB_1 antagonist AM251, confirming the involvement of endocannabinoids. The same group further investigated the synaptic specificity of DSI expression (516). In this study, DSI of spontaneous IPSCs was induced by postsynaptic depolarization (0 mV, 1 s) in the presence of 5 μM carbachol. By using local application technique, DSI was selectively induced at perisomatic inhibitory inputs to pyramidal cells. In a subsequent study by the same group, DSI was induced under a more physiological condition, namely, by brief action potential trains in the postsynaptic neuron (e.g., 20 Hz, 1 s) under the current-clamp mode (161). Spike probability of pyramidal cells was increased during DSI, and this change was blocked by AM251. These data indicate that the endocannabinoid system regulates the output of pyramidal cells through suppression of inhibitory inputs.

Bordor et al. (44) compared endocannabinoid signaling between layers 2 and 5B pyramidal cells in the somatosensory cortex. While most layer 2 pyramidal cells exhibited DSI, only a small portion of layer 5B pyramidal cells did so. This study also demonstrated the difference in density of CB_1-positive terminals among cortical layers, suggesting that inhibitory inputs to pyramidal cells might be regulated differently at different layers in the cerebral cortex. Fortin and Levine reported similar results (160). In this study, IPSCs were evoked by stimulation in either layer 5 or layer 2/3, and recorded from layer 5 pyramidal cells in the somatosensory cortex. In most of the recorded cells, depolarization failed to induce DSI. The lack of DSI was attributed not to the lack of endocannabinoid release from postsynaptic neurons but to cannabinoid insensitivity of the presynaptic terminals. The authors suggested that the majority of inhibitory inputs to layer 5 pyramidal cells are insensitive to cannabinoids. Storozhuk et al. (488) reported DSI of cultured neocortical neurons. Depolarization (to -10 mV for 5 s) of postsynaptic neurons induced a transient suppression of unitary IPSCs, which was accompanied by an increase in the paired-pulse ratio. However, whether this transient suppression was CB_1 dependent or not was not examined.

Zilberter's group reported an endocannabinoid-independent DSI-like phenomenon in the cerebral cortex (204, 582). At the synapses between fast-spiking, nonaccommodation interneurons and layer 2/3 pyramidal cells, postsynaptic trains of 10 action potentials at 50 Hz suppressed IPSPs. This suppression was shown to be induced by dendritic Ca^{2+} elevation like DSI, but to be resistant to CB_1 antagonists, and was suggested to be mediated by glutamate. The CB_1 independence of this suppression is consistent with the anatomical data showing the absence of CB_1 receptors on the axon terminals of fast-spiking, nonaccommodation interneurons (204).

B) DSE. Cortical DSE was reported in layer 5 pyramidal cells of the somatosensory cortex by Fortin and Levine (160). Postsynaptic depolarization (to 0 mV for 10 ms, 60 pulses at 20 Hz) transiently suppressed EPSCs that were evoked by stimulation in layer 5. This suppression was blocked by CB_1 antagonists and by postsynaptic BAPTA injection, but not by the mGluR antagonist MCPG, confirming the involvement of endocannabinoids. Although the EPSCs evoked by layer 5 stimulation exhibited DSE, the EPSCs evoked by layer 2/3 stimulation failed to exhibit DSE. In accordance with these results, bath application of the cannabinoid agonist WIN55,212-2 suppressed the EPSCs evoked from layer 5, but not the EPSCs evoked from layer 2/3. The study also showed that depolarization of layer 5 pyramidal cells results in a reduction of spike probability on the mixed synaptic potentials evoked by layer 5 stimulation in the absence of neurotransmitter receptor antagonists. This result exhibits a striking contrast to the data from layer 2–3 pyramidal cells showing that the net effect of postsynaptic depolarization is an increase in excitability (161). Thus it is conceivable that eCB-STD can regulate the excitability of postsynaptic neurons in two opposite directions, depending on the relative predominance of DSI and DSE.

5. *Amygdala*

Although the amygdala is crucial for the acquisition, storage, and expression of fear memory (293), there is only one report as to the endocannabinoid-mediated synaptic modulation in this brain structure (Table 3). Zhu and Lovinger (581) reported DSI, using acutely isolated neurons from basolateral amygdala. In these neurons, spontaneous IPSCs were observed, indicating that they contained functional synaptic boutons. Postsynaptic depolarization (0 mV, 4 s) induced a suppression of spontaneous IPSCs in a CB_1-dependent manner. Unlike the DSI observed in other brain regions, this suppression tended to be long-lasting. In the presence of the mGluR5 antagonist MPEP, however, the same depolarizing protocol induced a transient suppression, which can be recognized as DSI. Therefore, it is likely that the apparent long-lasting DSI may be caused by the augmentation of DSI by tonic mGluR5 activation (see sect. v*C3*). Zhu and Lovinger also used acute slices of basolateral amygdala and confirmed the expression of DSI.

6. *Hypothalamus*

The hypothalamus is a brain region that controls autonomic and endocrine functions. The studies on hypothalamic eCB-STD have been concerned with the lateral hypothalamus (LH), which is essential for the control of food intake (157), magnocellular neurons of the supraoptic nucleus (SON), and paraventricular nucleus (PVN), which release the neuropeptides oxytocin and vasopres-

sin, and parvocellular neurons of PVN, which release corticotropin-releasing hormone (Table 4).

A) DSI. Hypothalamic DSI was reported in perifornical LH neurons by Jo et al. (250). Postsynaptic depolarization (to 0 mV for 5 s) induced a suppression of IPSCs in a CB_1-dependent manner. Interestingly, DSI was blocked by bath application of leptin, a peptide involved in the control of appetite and body weight. This effect of leptin was explained by its inhibitory effect on voltage-gated Ca^{2+} channels through JAK2- and MAPK-dependent pathways. This explanation was further supported by the experiments with leptin-deficient mice (*ob/ob* mice). Perifornical LH neurons of *ob/ob* mice exhibited more robust DSI and larger Ca^{2+} currents than those of wild-type mice. Since CB_1 antagonists suppress appetite (121, 407), the authors hypothesized that the endocannabinoid system regulates excitability of perifornical LH neurons and thereby controls food intake (250).

B) DSE. Hypothalamic DSE was reported in magnocellular neurons of SON by Pittman's group (223, 279). In these neurons, injection of depolarizing current pulses (1 s, 2–4 pulses) or a voltage pulse (to 0 mV for 1 s) induced a transient suppression of EPSCs (223, 279). In an early study, this suppression was suggested to be mediated by neuropeptides, because the suppression was mimicked by the neuropeptide oxytocin and blocked by oxytocin receptor antagonists (279). Later, the same group suggested that the suppression is mediated by endocannabinoids, because DSE was mimicked by the cannabinoid agonist WIN55,212-2 and blocked by the CB_1 antagonist AM251 (223). Therefore, the depolarization-induced suppression in SON is similar to DSE in other brain regions. The authors presented the hypothesis that endocannabinoid release, which is required for DSE, is facilitated when dendritically released oxytocin activates postsynaptic oxytocin receptors. The ability of SON magnocellular neurons to induce DSE was confirmed by another group (122). By measuring the amount of endocannabinoids, the authors demonstrated that endocannabinoids were produced in an activity-dependent fashion and might be capable of shaping spiking activity of magnocellular neurons through suppression of excitatory inputs.

C) OXYTOCIN-DRIVEN eCB-STD. As described above, application of oxytocin was found to induce suppression of EPSCs in SON magnocellular neurons (279). At first, this suppression was considered to be caused via presynaptic oxytocin receptors. Later, this suppression was demonstrated to be mediated by endocannabinoids that are released in response to activation of postsynaptic oxytocin receptors (223). A similar oxytocin-driven eCB-STD was found for inhibitory transmission (403). In this study, oxytocin- and vasopressin-producing magnocellular neurons were immunohistochemically identified and characterized electrophysiologically. Application of oxytocin suppressed IPSCs in oxytocin-producing cells, but not in vasopressin-producing cells. Interestingly, IPSCs in oxytocin cells were tonically suppressed through activation of oxytocin and CB_1 receptors. From these results, Oliet et al. (403) suggested that oxytocin is tonically released from oxytocin-producing cells and activates oxytocin re-

TABLE 4. *eCB-STD in the hypothalamus and brain stem*

Region	Postsynaptic Neuron	Input	Type of STD	Dependence	Independence	DSE Enhancement	Reference Nos.
LH	LH neuron	I	DSI	CB_1, Ca^{2+}			250
SON	Magnocellular neuron	I	Oxytocin	CB_1, Ca^{2+}			403
		E	DSE	Oxytocin receptor			279
				CB_1, oxytocin receptor			223
				CB_1			122
			Oxytocin				279
				CB_1			223
			Glucocorticoid	CB_1, G protein (post)			124
PVN	Magnocellular neuron	E	Glucocorticoid	CB_1, G protein (post)			124
	Parvocellular neuron	E	Glucocorticoid	CB_1, G protein (post)			123
HGN	Motoneuron	I (Gly)	DSI	CB_1, Ca^{2+}			368
			NMDAR	CB_1, Ca^{2+}			368
VTA	DA neuron	E	DSE	CB_1, Ca^{2+}		D_2	352
			Input-stim	CB_1, mGluR1, PLC, DGL, Ca^{2+}, Ca^{2+} store	mGluR5, D_2, NMDAR		351
DCN	Fusiform cell	E (PF)	DSE	CB_1			527
	Cartwheel cell	E (PF)	DSE	CB_1			527
MNTB	Principle cell	E (Calyx)	I-mGluR	CB_1, Ca^{2+}			286
PAG		I	I-mGluR				132
				CB_1			131
DRN	5-HT neuron	E	Orexin	CB_1, G protein (post), PLC, DGL	Ca^{2+}		191

LH, lateral hypothalamus; SON, supraoptic nucleus; PVN, paraventricular nucleus; HGN, hypoglossal nucleus; VTA, ventral tegmental area; DCN, dorsal cochlear nucleus; MNTB, medial nucleus of the trapezoid body; PAG, periaqueductal gray; DRN, dorsal raphe nucleus; DA, dopamine; I, inhibitory; E, excitatory; Gly, glycinergic; NMDAR, NMDA receptor; I-mGluR, group I metabotropic glutamate receptor.

ceptors, which in turn induces endocannabinoid release and suppresses GABA release through presynaptic CB_1 receptors. Because the excitatory transmission to these neurons is also cannabinoid sensitive, it is not clear how the net excitability of postsynaptic neurons is regulated by tonically released endocannabinoids. It has been suggested that oxytocin autoregulates the excitability of magnocellular neurons (307). Endocannabinoid signaling might be involved in this autoregulation by oxytocin.

D) GLUCOCORTICOID-DRIVEN eCB-STD. Steroid hormones have diverse actions. Di and co-workers (123, 124) found that glucocorticoids can induce retrograde endocannabinoid signaling in PVN and SON. In parvocellular neurons of PVN, bath application of the glucocorticoid dexamethasone caused a decrease in the frequency, but not the amplitude of miniature EPSCs (mEPSCs) (123). This effect was very rapidly induced, in contrast to traditional actions of steroid hormones through binding to intracellular receptors and regulating transcription (148). Several lines of evidence suggested that the effect of glucocorticoids is mediated by postsynaptic activation of membrane receptors. First, membrane-impermeant dexamethasone-BSA was similarly effective. Second, intracellular application of dexamethasone was without effects. Third, the effect of dexamethasone was not blocked by the incubation of intracellular corticosteroid receptor antagonists, but blocked by intracellular application of GDPβS. The glucocorticoid-induced suppression of mEPSCs was blocked by the CB_1 receptor antagonist AM251 and was mimicked and occluded by the cannabinoid agonist WIN55,212-2. From these results, the authors concluded that activation of membrane glucocorticoid receptors induces endocannabinoid release, and thereby suppresses glutamate release through activation of presynaptic CB_1 receptors.

The same research group further examined effects of glucocorticoids on excitatory and inhibitory synaptic transmission to magnocellular neurons of PVN and SON (124). The study demonstrated that glucocorticoids decreased the frequency of mEPSCs in a CB_1-dependent manner, as described for PVN parvocellular neurons. In contrast, glucocorticoids increased the frequency of mIPSCs. This effect was blocked by the CB_1 antagonist AM251, but was not mimicked by cannabinoid agonists, which suppressed mIPSCs, and not blocked by the vanilloid receptor antagonist capsazepine. From these data, the authors suggested that presynaptic AM251-sensitve, noncannabinoid/vanilloid receptors might be responsible for the glucocorticoid-induced facilitation of inhibitory transmission.

7. Brain stem

Multiple forms of eCB-STD have been reported in several structures located in the brain stem including the hypoglossal nuclei, ventral tegmental area (VTA), cochlear nucleus, medial nucleus of the trapezoid body (MNTB), periaqueductal grey (PAG), and dorsal raphe nucleus (Table 4).

A) DSI. In hypoglossal motoneurons, Mukhtarov et al. (368) reported DSI at glycinergic inhibitory synapses. This paper is the first report on glycinergic DSI. In brain stem slices, postsynaptic depolarization (to 0 mV for 1 s, 20–40 pulses) induced a transient suppression of evoked glycinergic IPSCs, but failed to suppress IPSCs when the postsynaptic neuron was loaded with BAPTA. This DSI of evoked IPSCs was associated with an increase in the paired-pulse ratio. Depolarization also induced a decrease in the frequency, but not the amplitude, of mIPSCs, confirming that the suppression is of presynaptic origin. The suppression of IPSCs was blocked by the CB_1 antagonist SR141716A and occluded by the cannabinoid agonist WIN55,212-2. All these properties of glycinergic DSI are essentially the same as those of GABAergic DSI.

B) DSE. DSE was reported in the VTA and the dorsal cochlear nucleus. In VTA dopamine neurons, Melis et al. (352) found that depolarization (+40 mV, 10 s) elicited a transient suppression of evoked EPSCs. This DSE was blocked by a CB_1 antagonist and postsynaptic BAPTA injection and occluded by the cannabinoid agonist WIN55,212-2. DSE was enhanced by the D_2 agonist quinpirole and suppressed by the D_2 antagonist eticlopride. This finding is somewhat surprising, because D_2 receptors are coupled to $G_{i/o}$ proteins, while the receptors that have been reported to enhance DSI/DSE are coupled to $G_{q/11}$ proteins. The authors speculated that the activation of D_2 receptors might stimulate PLC, like other $G_{q/11}$-coupled receptors, and thereby enhance 2-AG production (see sect. v*C3*). This possibility is supported by the studies suggesting that D_2 receptors play an important role in endocannabinoid production in the striatum (283, 574).

In the dorsal cochlear nucleus, glutamatergic parallel fibers, which resemble those in the cerebellar cortex, innervate fusiform principle neurons and cartwheel interneurons (392). Parallel fiber inputs were shown to exhibit DSE in these neurons in a CB_1-dependent manner (527). Postsynaptic depolarization (to 0 mV for 1 s) induced a large suppression of parallel fiber-mediated EPSCs in cartwheel cells, and a small suppression in fusiform cells (527). Anatomical data suggest that the difference in DSE size is attributable to the difference in distribution of presynaptic CB_1 receptors between these two types of synapses.

C) NMDAR-DRIVEN eCB-STD. In the study reporting glycinergic DSI in hypoglossal motoneurons, effects of NMDA application on glycinergic IPSCs were also examined (368). Bath application of NMDA suppressed glycinergic IPSCs, and this suppression was blocked by postsynaptic BAPTA injection and by bath application of the CB_1 antagonist SR141716A. In this study, however, the possibil-

ity could not be excluded that the activation of NMDA receptors evoked local depolarization, which in turn induced DSI through activation of voltage-gated Ca^{2+} channels. Therefore, it is unclear whether this phenomenon is a genuine NMDAR-driven eCB-STD, as demonstrated in hippocampal neurons (393). Interestingly, NMDA application induced a potentiation of IPSCs in SR141716A-treated neurons. This potentiation was shown to be Ca^{2+} dependent and attributed to the change in postsynaptic response to glycine. The authors suggested that postsynaptic Ca^{2+} elevation through activation of NMDA receptors or voltage-gated Ca^{2+} channels produces bidirectional effects on glycinergic inputs. The predominant effect is presynaptic inhibition through retrograde endocannabinoid signaling, and the other is potentiation of postsynaptic glycine receptors. Physiological significance of this bidirectional regulation remains to be elucidated.

D) mGluR-DRIVEN eCB-STD. In the MNTB, Kushmerick et al. (286) found group I mGluR-driven eCB-STD at calyx of Held synapses. This study demonstrated that the group I mGluR agonist DHPG triggers endocannabinoid release from MNTB principle cells and induces a transient suppression of EPSCs. Taking advantage of giant synapses, whole cell patch-clamp recordings were made from the presynaptic terminals and Ca^{2+} currents were simultaneously recorded. In 65% of the cells tested, application of DHPG reduced presynaptic Ca^{2+} currents. This inhibition of presynaptic Ca^{2+} currents was mimicked by the cannabinoid agonist WIN55,212-2. These results provide direct evidence that presynaptic Ca^{2+} channels are actually inhibited during eCB-STD.

The midbrain PAG is a major site of the analgesic actions of opioids and cannabinoids. In PAG neurons, Drew and Vaughan (132) observed that IPSCs were suppressed by application of mGluR agonists including DHPG through a presynaptic mechanism. In a later study by the same group, the DHPG-induced suppression of IPSCs was demonstrated to be CB_1 dependent (131). A similar CB_1-dependent suppression of IPSCs was induced by glutamate transport blocker TBOA, suggesting that glutamate spillover could modulate GABAergic transmission through group I mGluRs and endocannabinoid signaling.

E) OREXIN-INDUCED eCB-STD. Orexin is a neuropeptide involved in the regulation of arousal and feeding behavior (79, 456). In serotonergic neurons of the dorsal raphe nucleus, Haj-Dahmane and Shen (191) reported orexin-induced eCB-STD at excitatory synapses. In these neurons, application of orexin-B induced a suppression of evoked EPSCs with an increase in the paired-pulse ratio. Orexin-B also induced a change in the frequency, but not the amplitude, of mEPSCs. The orexin-induced suppression was blocked by postsynaptic application of GDPβS, occluded by the cannabinoid agonist WIN55,212-2, and prevented by the CB_1 antagonist AM251. These results suggest that endocannabinoids are released in response to activation of postsynaptic orexin receptors, and activate presynaptic CB_1 receptors to suppress the glutamate release. Then, a question arises whether orexin-induced endocannabinoid signaling is related to wakefulness. It is interesting to figure out how orexin regulates net excitability of serotonergic neurons in the dorsal raphe nucleus and contributes to wakefulness.

F) SYNAPTICALLY DRIVEN eCB-STD. In the VTA dopamine neurons, Melis et al. (351) found that a brief train of stimuli (10 pulses at 5 Hz) to afferent fibers induced a transient suppression of EPSCs in a CB_1-dependent manner. This synaptically driven eCB-STD was blocked by the mGluR1 antagonist CPCCOEt, the DGL inhibitor THL, and the PLC inhibitor U73122, and by postsynaptic BAPTA injection. These data suggest that the presynaptically released glutamate causes postsynaptic mGluR1 activation and Ca^{2+} elevation, which in turn induces 2-AG production via PLCβ and DGL, and finally suppresses the glutamate release via presynaptic CB_1 receptors.

C. Mechanisms of ecb-std

Mechanisms of eCB-STD have been investigated in various brain regions. Unlike classical neurotransmitters such as amino acids, amines, or neuropeptides, endocannabinoids are not stored in vesicles but produced on demand and released immediately. How does depolarization, activation of $G_{q/11}$-coupled receptors, or synaptic stimulation trigger endocannabinoid production in neurons? Most studies are consistent with the hypothesis that endocannabinoids are produced through two different pathways. One is PLCβ-independent and driven by a large increase in intracellular Ca^{2+} concentration alone (CaER), and the other is PLCβ-dependent and driven by activation of $G_{q/11}$-coupled receptors at basel (basal RER) or elevated Ca^{2+} levels (Ca^{2+}-assisted RER).

1. CaER

Many studies consistently show that postsynaptic Ca^{2+} elevation is indispensable for DSI (294, 394, 397, 426, 545, 564), DSE (285), and NMDAR-driven eCB-STD (393). Uncaging Ca^{2+} in the postsynaptic neuron mimicked DSI in the hippocampus (564), indicating that postsynaptic Ca^{2+} elevation is sufficient to induce eCB-STD. Therefore, it is reasonable to assume that, in these forms of eCB-STD, endocannabinoid release is induced by Ca^{2+} elevation itself, which we proposed to term CaER (210, 315). The term *CaER*, however, should be used with caution, because DSI/DSE may sometimes include component other than CaER. Phenomenologically, DSI/DSE is often enhanced by simultaneous activation of $G_{q/11}$-coupled receptors such as group I mGluRs and M_1/M_3 muscarinic

receptors. The enhanced component of DSI/DSE is not attributable to CaER but to Ca^{2+}-assisted RER (see sect. v*C3*). Therefore, the studies dealing with the enhanced form of DSI/DSE are not included in this subsection.

The main source of postsynaptic Ca^{2+} elevation is Ca^{2+} entry through voltage-gated Ca^{2+} channels for DSI/DSE (393, 426) or Ca^{2+} entry through NMDA receptors for NMDAR-driven eCB-STD (393). In some cases, Ca^{2+} release from intracellular Ca^{2+} stores may also contribute to CaER (243). In the cerebellum, direct recordings from dendrites of Purkinje cells clearly provided the evidence that endocannabinoid release is linked with local dendritic Ca^{2+} spikes (431). Several studies have estimated the Ca^{2+} concentration required for CaER. In hippocampal CA1 neurons, Ca^{2+} elevation to 4 μM was required for inducing half-maximum DSI (553). In cerebellar Purkinje cells, half-maximum DSE/DSI was induced by Ca^{2+} transients with peak levels around 16 μM (52), 10 μM (315), or submicromolar range (180). In globus pallidus neurons, postsynaptic depolarization (from -60 to $+30$ mV, 5 s) induced Ca^{2+} transients with peak values of 14.5 μM in the soma and 9.9 μM in dendrites, which induced prominent DSI (147). From these studies, it is conceivable that Ca^{2+} elevation to micromolar level is required for CaER. However, the Ca^{2+} concentration required for CaER may be dependent on the duration of Ca^{2+} elevation and, therefore, can be lowered when Ca^{2+} elevation is prolonged (50).

Although it is evident that Ca^{2+} elevation is the first step of CaER, it remains unclear how Ca^{2+} elevation stimulates endocannabinoid synthesis. To elucidate this mechanism, attempts have been made to determine the molecular identity of endocannabinoid that is released during CaER. As described in section iv*D*, biochemical studies show that COX-2 degrades both 2-AG and anandamide and that FAAH and MGL preferently degrade anandamide and 2-AG, respectively. Hippocampal DSI was shown to be prolonged by inhibition of COX-2 (271) and MGL (207, 321), but not by inhibition of FAAH (271, 321). Similarly, hippocampal DSE was prolonged by MGL inhibition (207), but not affected by pharmacological or genetical blockade of FAAH (489). Cerebellar DSI was also prolonged by MGL inhibition (503). These data are all consistent with the notion that 2-AG rather than anandamide mediates DSI/DSE.

If 2-AG mediates the retrograde signal for CaER-dependent forms of eCB-STD, Ca^{2+} elevation should somehow facilitate 2-AG synthesis. Biochemical studies suggest that the main pathway for 2-AG production is the combination of PLC and DGL, the latter of which converts diacylglycerol to 2-AG (see sect. iv*C*). There are many studies reporting the effects of DGL inhibitors on CaER, but the results are controversial. As DGL inhibitors, THL (also called orlistat) and RHC-80267 are widely used. We reported that THL effectively blocked hippocampal DSI (207, 208), hippocampal NMDAR-driven eCB-STD (393), cerebellar DSE (208), and striatal DSI/DSE (528). Straiker and Mackie (489) reported that RHC-80267 attenuated hippocampal DSE. Szabo et al. (503) reported that THL blocked cerebellar DSI/DSE and substantia nigra DSI, but failed to block hippocampal DSI. Edwards et al. (138) reported that neither THL nor RHC-80267 blocked hippocampal DSI. Similarly, Safo and Regehr (455) reported the resistance of cerebellar DSE to these DGL inhibitors. The resistance of hippocampal DSI/DSE to these inhibitors was also reported by Castillo's group (84, 88). Reasons for these apparently conflicting data are not clear. Different results might be partly due to the difference in the method of drug application. Intracellular application of inhibitors is widely used to examine the involvement of 2-AG-producing enzymes in CaER. However, we noticed that THL was less effective with intracellular loading from patch pipettes than with bath application. In addition, bath-applied THL was more effective when the bath solution contained bovine serum albumin (unpublished observations). Therefore, we used this "bath application with albumin" method to treat slice preparations with THL and obtained positive effects on DSI/DSE (208, 528). However, we cannot entirely exclude the possibility that THL blocked DSI/DSE by acting on the enzymes other than DGL. Whether DGL is required for CaER should be directly determined by using DGL-knockout mice in future studies.

There is no evidence indicating the involvement of PLC in CaER. The treatment with the PLC inhibitor U73122 failed to prevent DSI in the hippocampus and cerebellum (84, 138, 503). Hippocampal DSI and cerebellar DSE were intact in PLCβ1- and PLCβ4-knockout mice, respectively (209, 315). Hippocampal DSI was also intact in PLCδ1-, PLCδ3-, or PLCδ4-knockout mice (208). How Ca^{2+} elevation stimulates 2-AG synthesis is still a mystery.

A current model of CaER underlying DSI/DSE and NMDAR-driven eCB-STD is illustrated in Figure 8*A*. When a large Ca^{2+} elevation (micromolar range) is induced by "strong" activation of voltage-gated Ca^{2+} channels or NMDA receptors, 2-AG is produced through an undetermined pathway, which is PLCβ independent, and presumably DGL dependent. 2-AG is then released from postsynaptic neurons and suppresses transmitter release by activating presynaptic CB_1 receptors.

2. *Basal receptor-driven endocannabinoid release (basal RER)*

The mGluR-driven eCB-STD was originally reported in the cerebellum (314). This study clearly demonstrated that this form of eCB-STD is resistant to postsynaptic loading of BAPTA (314), making a striking contrast to DSI/DSE. The resistance of mGluR- or mAChR-driven

eCB-STD to postsynaptic BAPTA was confirmed by later studies (170, 272, 382). Therefore, activation of these G protein-coupled receptors can induce endocannabinoid release even at basal Ca^{2+} levels presumably through some intracellular pathway different from CaER, which we proposed to term RER (205, 210, 315). The G protein-coupled receptors that were reported to induce eCB-STD include group I mGluRs (mGluR1/5), M_1/M_3 muscarinic receptors, glucocorticoid receptors, oxytocin receptors, and orexin receptors. All these receptors are known to stimulate PLCβ through $G_{q/11}$ proteins. Therefore, it is likely that the activation of $G_{q/11}$-coupled receptors induces 2-AG production through sequential enzymatic reactions by PLCβ and DGL.

The involvement of PLC in RER has been investigated by using pharmacological tools and genetically modified mice. The PLC inhibitor U73122 reduced mGluR-driven eCB-STD at inhibitory synapses on cerebellar Purkinje cells (170), orexin-induced eCB-STD at excitatory synapses on serotonergic neurons in the dorsal raphe nucleus (191), and mAChR-induced eCB-STD at excitatory autapses in cultured hippocampal neurons (490). In contrast, U73122 failed to block both mGluR-driven and mAChR-driven eCB-STDs at hippocampal inhibitory synapses (138). Although pharmacological data are rather conflicting, the studies using genetically modified mice have clearly demonstrated that RER is dependent on PLCβ (209, 315). PLCβ consists of four isozymes (PLCβ1-4), and each isozyme is uniquely distributed in the brain (256, 556). In situ hybridization studies show that the main isozyme of PLCβ is PLCβ1 in the hippocampus, and PLCβ4 in the rostral portion of the cerebellum (256, 556). Hippocampal neurons prepared from PLCβ1-knockout mice exhibited neither mGluR-driven eCB-STD nor mAChR-driven eCB-STD (209). Similarly, mGluR-driven eCB-STD was absent in cerebellar Purkinje cells of PLCβ4 knockout mice (315). These results strongly suggest that RER is PLCβ dependent, although it is necessary to confirm whether other forms of RER are also abolished in PLCβ-knockout mice.

Contribution of DGL to RER has been determined by using pharmacological tools. The DGL inhibitor RHC-80267 reduced mGluR-driven eCB-STD in the cerebellum (170) and orexin-driven eCB-STD in the dorsal raphe nucleus (191). Another DGL inhibitor, THL, blocked both mGluR-driven and mAChR-driven eCB-STDs at inhibitory synapses in cultured hippocampal neurons (207, 208) and in the dorsal striatum (528). Edwards et al. (138) reported complicated results showing that DGL inhibitors RHC-80267 and THL blocked mAChR-driven eCB-STD, but not mGluR-driven eCB-STD, suggesting the difference in downstream signaling between mGluRs and mAChRs. Except for some conflicting data, pharmacological data reported so far are generally consistent with the hypothesis that RER is DGL dependent.

A current model of basal RER underlying several forms of receptor-driven eCB-STD at basal Ca^{2+} levels is presented in Figure 8*B*. The word *basal* is added to this type of RER to distinguish it from "Ca^{2+}-assisted" RER (210) described in the next section. Activation of postsynaptic $G_{q/11}$-coupled receptors stimulates PLCβ and produces diacylglycerol, which is then converted to 2-AG by DGL. 2-AG released from postsynaptic neurons activates presynaptic CB_1 receptors and suppresses the transmitter release. It should be noted that "strong" receptor activation with a relatively high concentration of agonist is required for basal RER to occur compared with "weak" receptor activation required for Ca^{2+}-assisted RER (see next section).

3. Ca^{2+}-assisted RER

Many studies demonstrate apparent enhancement of DSI, DSE, or NMDAR-driven eCB-RER by activation of receptors that can induce eCB-STD. DSI is enhanced by cholinergic or muscarinic agonists in the hippocampus (227, 272, 331, 395), striatum (376), substantia nigra (571), and layer 2/3 pyramidal cells of the cerebral cortex (161). DSI is also enhanced by the group I mGluR agonist DHPG in the hippocampus (398, 537) and striatum (165, 528). DSE is enhanced by cholinergic or muscarinic agonists in the hippocampus (88, 490) and by DHPG in the hippocampus (88, 490), cerebellum (315), and striatum (375). NMDAR-driven eCB-STD is enhanced by activation of mAChRs or group I mGluRs in cultured hippocampal neurons (393). Enhancement of DSE by D_2 receptor activation was also reported in the VTA dopamine neurons (352). Although D_2 receptors are generally coupled to the $G_{i/o}$-type of G proteins, this DSE enhancement was speculated to be mediated by stimulation of PLCβ like the DSE enhancement by other $G_{q/11}$-coupled receptors (352).

Mechanisms underlying these phenomena have been investigated in several preparations and are now well understood. By roughly estimating the endocannabinoid concentration around synapses from the magnitude of synaptic suppression, our group (395, 398) and Melis et al. (352) demonstrated that the amount of endocannabinoids released by depolarization (Ca^{2+} elevation) combined with the receptor activation is several times larger than the algebraic sum of the estimated value for depolarization alone and that for receptor activation alone. These results indicate a synergistic effect of Ca^{2+} elevation and receptor activation on endocannabinoid production. At this stage, there were at least two possibilities for the mechanism of the synergistic effect, i.e., enhancement of CaER by activation of $G_{q/11}$-coupled receptors and enhancement of RER by Ca^{2+} elevation. Our subsequent studies have unequivocally demonstrated that the latter is the case.

In cultured hippocampal neurons, mAChR-driven eCB-STD exhibits Ca^{2+} dependence, which is attributable to the Ca^{2+}-dependent nature of PLCβ activity (209). We examined the Ca^{2+} dependence of mAChR-driven eCB-STD by loading postsynaptic neurons with solutions containing various concentrations of free Ca^{2+} (1–1,000 nM). The magnitude of mAChR-driven eCB-STD exhibited a strong positive correlation with the free Ca^{2+} concentration of intracellular solutions (Fig. 7, *A* and *B*). Next, we examined the Ca^{2+} dependence of PLCβ1 activity by using TRPC6 channels as a biosensor for the PLC product diacylglycerol (113, 515). The TRPC6 channel is a nonselective cation channel and is activated by diacylglycerol. In hippocampal neurons expressing exogenous TRPC6 channels, application of the muscarinic agonist oxo-M induced a prominent inward current, which was confirmed to be the cation currents through TRPC6 channels. The agonist-induced TRPC6 current was absent in PLCβ1-knockout mice, indicating that this inward current reflects PLCβ1 activity. Using this bioassay system, we demonstrated that the Ca^{2+} dependence of agonist-induced PLCβ1 activation is similar to that of mAChR-driven eCB-STD. Furthermore, the agonist-induced PLCβ1 activation was confirmed to be enhanced by a transient Ca^{2+} elevation induced by depolarization. Essentially the same results were obtained when the group I mGluR agonist DHPG was used to activate PLCβ1. Importantly, the enhancement of DSI by oxo-M or DHPG was totally absent in PLCβ1-knockout mice (Fig. 7, *E* and *F*). From these results, we concluded that the facilitated endocannabinoid release by combined Ca^{2+} elevation and $G_{q/11}$-coupled receptor activation results from the Ca^{2+} dependence of receptor-driven PLCβ activation. Although this effect is often described phenomenologically as "an enhancement of DSI by receptor agonist," the enhanced component is clearly distinct from the basal DSI that depends on CaER. It is RER that is enhanced during combined Ca^{2+} elevation and $G_{q/11}$-coupled receptor activation.

In parallel with the study on hippocampal eCB-STD, we performed similar experiments on cerebellar slices and confirmed the Ca^{2+} dependence of mGluR-driven eCB-STD (315). By recording PF- or CF-EPSCs from Purkinje cells, we examined the effects of depolarization-induced Ca^{2+} elevation and activation of group I mGluRs by DHPG. A small Ca^{2+} elevation in the submicromolar

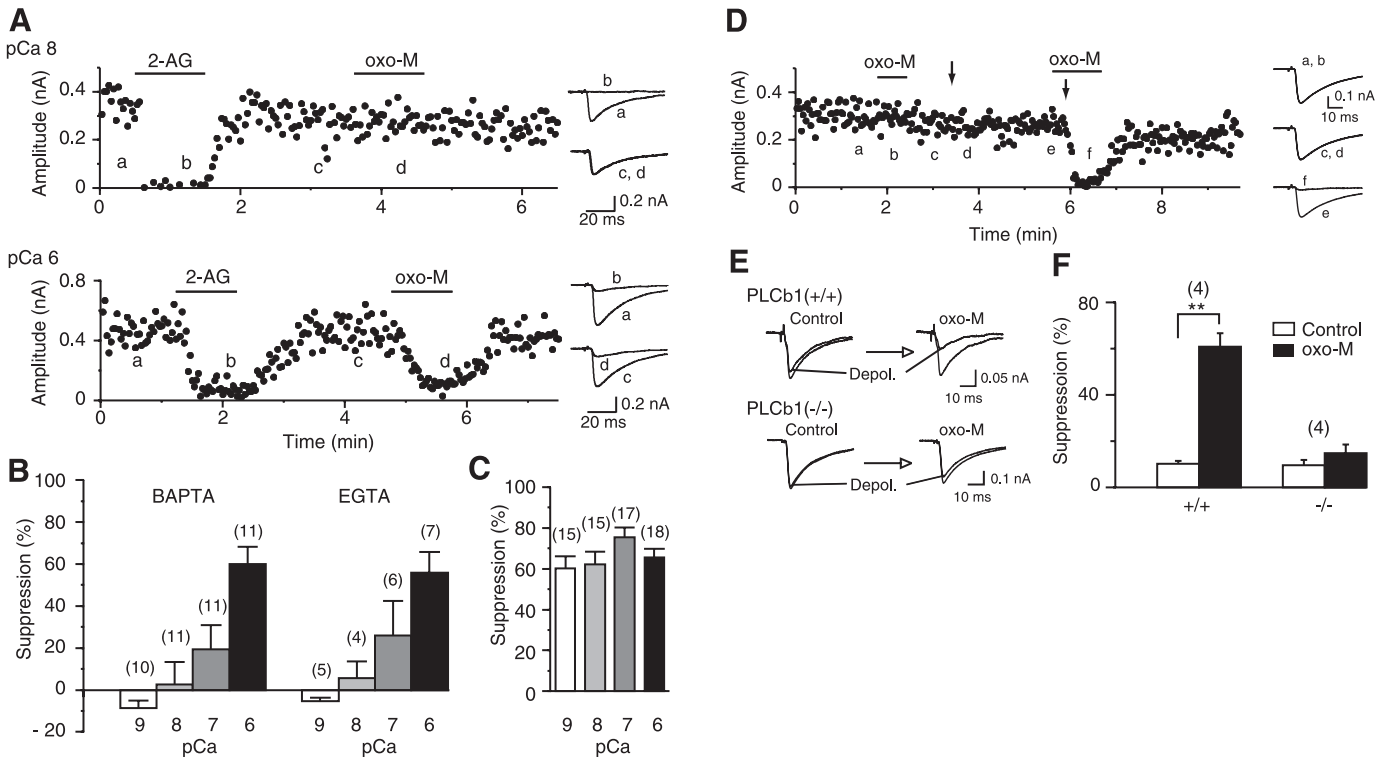


FIG. 7. Ca^{2+} dependence of mAChR-driven eCB-STD at inhibitory synapses in cultured hippocampal neurons. *A*: examples representing the effects of 2-AG (30 nM) and the muscarinic agonist oxo-M (0.3 μM) in rat neurons at two different pCa levels buffered with 30 mM BAPTA. IPSC traces acquired at the indicated time points are shown on the *right*. *B*: averaged data for oxo-M-induced suppression of IPSCs at four different pCa levels buffered with 10–30 mM BAPTA or 10 mM EGTA. *C*: averaged data for 2-AG-induced suppression at four different pCa levels. *D*: examples representing the effects of oxo-M (0.3 μM) application and postsynaptic depolarization (arrows) on IPSCs in rat neurons dialyzed with a pCa 8 solution containing 10 mM BAPTA. Note that a marked suppression of IPSCs was induced when these two stimuli were combined. *E* and *F*: sample traces (*E*) and averaged data (*F*) for depolarization-induced suppression of IPSCs in the absence or presence of 0.3 μM oxo-M. Neurons prepared from wild-type or PLCβ1-knockout mice were dialyzed with a solution containing 5 mM EGTA. [Modified from Hashimotodani et al. (209), with permission from Elsevier.]

range failed to induce any suppression of EPSCs. Similarly, application of a low dose of DHPG did not affect EPSCs. However, when these two subthreshold stimuli were combined, prominent eCB-STD was induced, which is phenomenologically "an enhancement of DSE." This apparent DSE enhancement was abolished in PLCβ4-knockout mice. The size of mGluR-driven eCB-STD was dependent on the postsynaptic Ca^{2+} level. These results indicate that RER is Ca^{2+} dependent in the cerebellum as well as in the hippocampus and that the apparent DSE enhancement is caused by the enhancement of RER by Ca^{2+} elevation.

A current model of the enhancement of RER by Ca^{2+} elevation, which we proposed to term Ca^{2+}-assisted RER, is illustrated in Figure 8*C*. When "weak" activation of postsynaptic $G_{q/11}$-coupled receptors is combined with a small Ca^{2+} elevation (submicromolar range), PLCβ is effectively activated. In this scheme, PLCβ is the key molecule that functions as a coincidence detector to integrate $G_{q/11}$-coupled receptor activation and Ca^{2+} elevation (210). Thus enhancement of DSI/DSE or NMDAR-driven eCB-STD by $G_{q/11}$-coupled receptor activation can be explained by Ca^{2+}-assisted RER. However, we cannot exclude the possibility that CaER mechanism itself is enhanced by activation of $G_{q/11}$-coupled receptors. To test this possibility, the enzyme(s) involved in CaER should be identified. In this regard, a missing link remains to be discovered.

It is now established that basal RER and Ca^{2+}-assisted RER share the same molecular mechanism (209, 315). Because Ca^{2+} dependence of PLCβ is continuous, there is virtually no clear distinction between basal RER and Ca^{2+}-assisted RER. However, basal RER and Ca^{2+}-assisted RER are induced in different experimental conditions, and they are clearly distinguishable. In most cases, basal RER is studied with a high concentration of Ca^{2+} chelator in the recording pipette. To induce RER without elevation of intracellular Ca^{2+} concentration, strong receptor activation with a relatively high concentration of agonist is required. In contrast, when a small Ca^{2+} elevation is combined, Ca^{2+}-assisted RER is readily induced by weak receptor activation with an agonist concentration that is 5–10 times lower than that required for

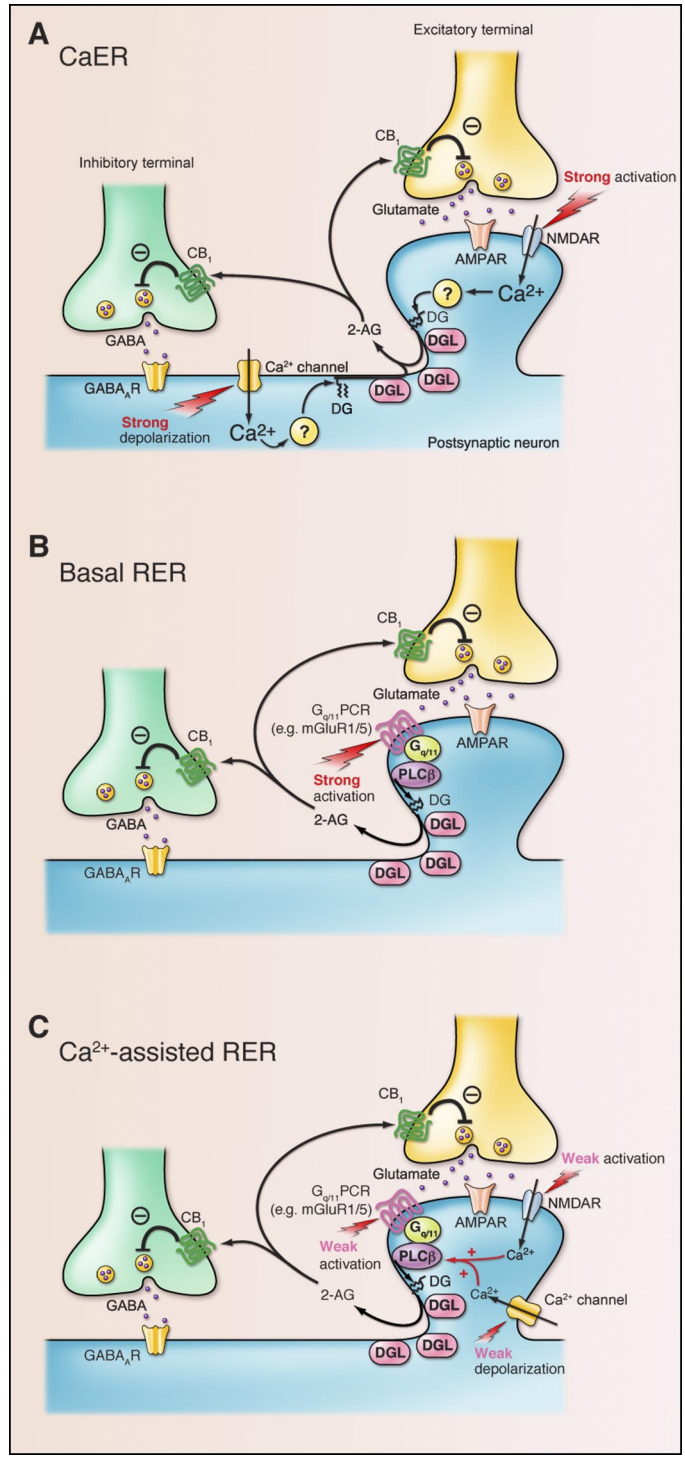


FIG. 8. Schematic diagrams illustrating the three modes of retrograde endocannabinoid signaling. *A*: Ca^{2+}-driven endocannabinoid release (CaER). Strong postsynaptic depolarization causes influx of Ca^{2+} through voltage-gated Ca^{2+} channels. The resultant large increase in intracellular Ca^{2+} concentration to the micromolar range induces production of diacylglycerol (DG) through unknown pathways (?). DG is converted to 2-arachidonoylglycerol (2-AG) by diacylglycerol lipase (DGL). The produced 2-AG is then released from the postsynaptic neuron. When a large Ca^{2+} elevation is caused by activation of NMDA receptors (NMDR), 2-AG is produced and released through the same pathways. *B*: basal receptor-driven endocannabinoid release (RER). At basal Ca^{2+} levels, strong activation of $G_{q/11}$-coupled receptors (e.g., mGluR1/5) stimulates PLCβ, which hydrolyzes phosphatidylinositol 4,5-bisphosphate into DG and IP_3. 2-AG is then produced from DG by DGL, and released. *C*: Ca^{2+}-assisted RER. When weak activation of $G_{q/11}$-coupled receptors coincides with small Ca^{2+} elevation (submicromolar range) through weak activation of either voltage-gated Ca^{2+} channels or NMDA receptors, PLCβ activation is enhanced. In this condition, 2-AG production can be induced and released even by weak activation of $G_{q/11}$-coupled receptors, which is subthreshold for basal RER. Note the difference in Ca^{2+} levels required for CaER and Ca^{2+}-assisted RER, which is expressed as the difference in letter sizes. In any of the three modes, the released 2-AG binds to presynaptic CB_1 receptors (CB_1R) and suppresses neurotransmitter release.

basal RER. Therefore, the requirement for Ca^{2+}-assisted RER can be fulfilled much more easily than that for basal RER under physiological conditions. In light of physiological relevance of endocannabinoid signaling, we propose distinguishing basal RER and Ca^{2+}-assisted RER.

4. Synaptically driven eCB-STD

Synaptic activity could drive CaER, basal RER, and Ca^{2+}-assisted RER, depending on the stimulation protocol and recording conditions. These three modes can be distinguished by blocking postsynaptic Ca^{2+} elevation and $G_{q/11}$-coupled receptor activation. A good example is the synaptically driven eCB-STD at PF-Purkinje cell synapses in the cerebellum (315). The eCB-STD induced by repetitive PF stimulation of 10 pulses at 100 Hz was blocked either by the postsynaptic loading with 30 mM BAPTA or by the bath application of mGluR1 antagonist CPCCOEt, indicating predominant contribution of Ca^{2+}-assisted RER. In contrast, the eCB-STD induced by intense PF stimulation of 100 pulses at 100 Hz persisted in the presence of CPCCOEt. Therefore, it is most likely that the eCB-STD under this condition is dependent on CaER due to Ca^{2+} influx through voltage-gated Ca^{2+} channels that are activated by AMPA receptor-mediated local depolarization.

The mGluR1-dependent synaptically driven eCB-STD at PF-Purkinje cell synapses was blocked by RHC-80267 (455) and THL (315, 455). In contrast, synaptically driven eCB-STD at PF-stellate cell synapses required activation of mGluR1 and NMDA receptors and was blocked by RHC-80267 and THL (25). In the VTA dopaminergic neurons, synaptically driven eCB-STD was mGluR1-dependent and prevented by U73122 and THL (351). These studies indicate that synaptically driven eCB-STD under physiological conditions involves PLC-DGL pathway and resultant production of 2-AG as a retrograde messenger.

5. Termination of eCB-STD

There are several reports for the effects of endocannabinoid transporter inhibitors on the magnitude and time course of DSI/DSE. The transporter inhibitor AM404 potentiated and prolonged DSI in the cerebral cortex (517), whereas AM404 failed to change the time course of DSI in the hippocampus (564). Another transporter inhibitor, UCM-707, did not potentiate, but rather attenuated, DSE in cultured hippocampal neurons (489). These conflicting data may be partially attributable to the difference in experimental conditions, such as temperature (193), or possible side effects of these inhibitors, such as an agonistic action on TRPV1 receptors.

Several research groups have examined the effects of inhibiting endocannabinoid degradation enzymes on DSI/DSE. Makara et al. (321) reported that DSI in hippocampal slices was prolonged by inhibition of the 2-AG hydrolyzing enzyme MGL by URB602 (232) and URB754 that were claimed to be selective MGL inhibitors. Later, the inhibitory effects of URB602 and URB754 on MGL were questioned (452, 535). Consistent with these reports, we observed that URB754 failed to prolong DSI in cultured hippocampal neurons (207). Soon after these reports, Makara et al. (320) corrected their previous notion and reported that the inhibitory action of "URB754" on MGL in their report in 2005 was exerted not by genuine URB754 but by some contaminated substances. King et al. (273) reexamined the effects of URB602 and found that it inhibits purified recombinant rat MGL only weakly (IC_{50} = 223 μM).

Effects of MGL inhibition on DSI were examined by using other MGL inhibitors, MAFP and ATFMK (207, 503). We demonstrated that MAFP prolonged not only DSI/DSE, but also the suppression of IPSCs/EPSCs by short (10 s) focal application of 2-AG. These results, together with the morphological data showing presynaptic localization of MGL (188), indicate that presynaptic MGL activity is crucial in termination of eCB-STD (207). Because MAFP and ATFMK are broad serine hydrolase inhibitors, the possibility cannot be excluded that some unidentified 2-AG hydrolyzing enzymes other than MGL are sensitive to these inhibitors and contribute to termination of eCB-STD. Interestingly, novel 2-AG hydrolyzing enzymes have recently been identified in microglial cells (366) and in the mouse brain (43) (see sect. IV*D*). It remains to be elucidated how these novel enzymes contribute to termination of endocannabinoid signaling.

Inhibition of COX-2 also prolonged hippocampal DSI (271). Because anatomical data show that COX-2 is expressed at postsynaptic sites (265), the authors suggested that 2-AG might be degraded by COX-2 within the postsynaptic neurons before it is released (271). This hypothesis is consistent with the observation that inhibition of COX-2 failed to affect the IPSC suppression induced by exogenously applied 2-AG (207). In contrast, there is no evidence indicating the contribution of FAAH to termination of eCB-STD. The FAAH inhibitor URB597 (260) has no effects on DSI/DSE (88, 271, 321, 489, 503).

A current model for termination of 2-AG signaling is illustrated in Figure 9. After biosynthesis, 2-AG is partially degraded by postsynaptic COX-2, and partly released to the extracellular space. The released 2-AG enters into the membrane lipid bilayer of presynaptic terminals and activates CB_1 receptors through lateral diffusion (see sect. II*A1*). The 2-AG located in membrane lipids is then removed and degraded by presynaptic MGL. According to this model, the magnitude and time course of eCB-STD is determined by the balance between the postsynaptic production and the postsynaptic/presynaptic degradation of 2-AG.

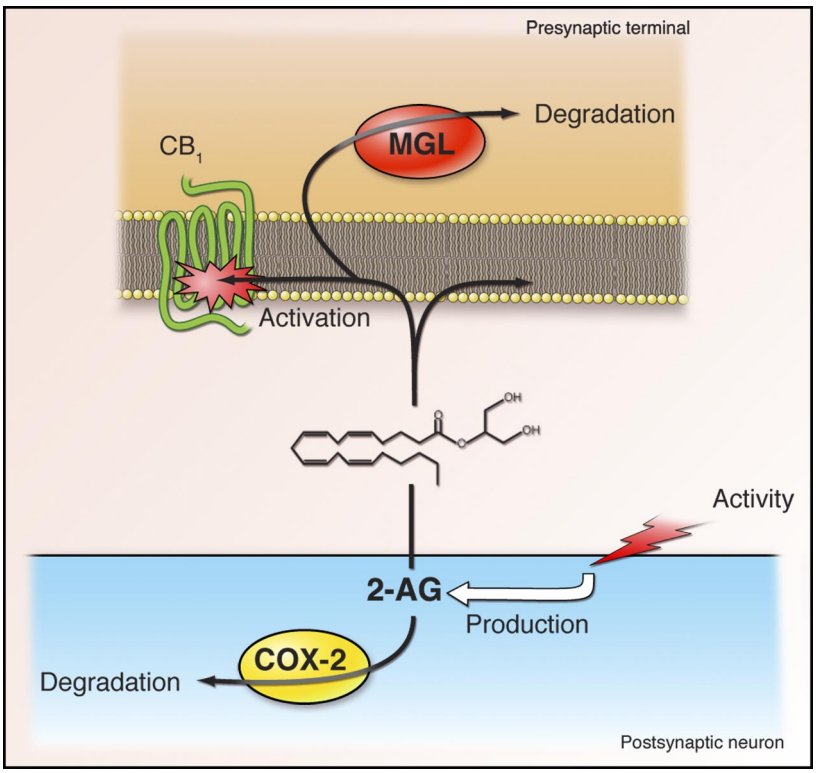


FIG. 9. Schematic diagram illustrating how 2-AG signaling is terminated. 2-AG is partially degraded by COX-2 at the postsynaptic site after it is produced. 2-AG is released from the postsynaptic neuron and enters into the membrane lipid bilayer of presynaptic terminals. At presynaptic terminals, 2-AG activates CB_1 receptors through fast lateral diffusion across the membrane lipid bilayer. Then, 2-AG is degraded mostly by MGL in the cytoplasm of presynaptic terminals and axons.

6. Spread of eCB-STD

Since the discovery of the role of endocannabinoids as a diffusible messenger, considerable attention has been paid to the question as to how far endocannabinoids can diffuse from the site of production. It is evident that endocannabinoids can act on nonstimulated synapses or nonstimulated neurons under certain conditions (170, 281, 564).

In the hippocampus, Wilson and Nicoll (564) reported spread of endocannabinoids in a short distance by using DSI protocol. IPSCs were simultaneously recorded from two neighboring pyramidal cells in hippocampal slices, and DSI was induced in one neuron of the pair. When the two neurons were separated by 20 μm or less, IPSCs recorded from the nondepolarized neuron was suppressed. In this study, the distance between the two neurons was defined as the distance between the tips of the recording electrodes. The data indicate that the endocannabinoids released from a pyramidal cell can influence the synaptic input to nearby neurons in the hippocampus. Significant spread of DSI to nondepolarized neurons was also reported in cultured hippocampal neurons (396).

In the cerebellum, spread of DSE, mGluR-driven eCB-STD, and synaptically driven eCB-STD has been investigated. In the 2001 paper by Maejima et al. (314), we demonstrated that mGluR-driven eCB-STD does not spread to neighboring Purkinje cells. Kreitzer et al. (281) reported that spread of DSE from one Purkinje cell to another can be observed at room temperature (24°C), but not at physiological temperature (34°C). By examining synaptically driven eCB-STD, the same group demonstrated that endocannabinoid signaling generated at the stimulated synapses does not spread to the unstimulated synapses 20 μm apart from the site of stimulation on the same Purkinje cell (56). In an early study by Vincent and Marty (545), spread of DSI from one Purkinje cell to another at a distance of 70 μm was observed. This spread of DSI, however, does not necessarily indicate a long-distance spread of endocannabinoids to presynaptic terminals, because the effect was not observed in the presence of tetrodotoxin. Later, Kreitzer et al. (281) demonstrated that the spread of DSI is caused by diffusion of endocannabinoids from the depolarized Purkinje cell to the soma of nearby interneurons, but not to the inhibitory terminals on nondepolarized Purkinje cells. On the soma of interneurons, endocannabinoids are thought to activate K^+ channels (presumably G protein-activated inward rectifier potassium channels), and thereby suppress the outputs to many Purkinje cells, which can be recognized as a spread of DSI in the target Purkinje cells.

Spread of endocannabinoid signaling might be differently controlled at different brain regions and synapses. Available evidence is mostly obtained from the studies using the hippocampus and cerebellum. Further studies with other brain regions are necessary to understand how

far endocannabinoids can spread during eCB-STD as well as eCB-LTD.

7. Other possible mechanisms of retrograde synaptic modulation

There are several studies reporting involvement of glutamate in retrograde synaptic modulation. Crepel's group (296) reported that glutamate might mediate STD at PF terminals on Purkinje cells in the cerebellum. They claimed that activation of mGluR1 in Purkinje cells induced glutamate release from dendrites and activated presynaptic ionotropic glutamate receptors, which in turn increased spontaneous transmitter release and consequently decreased evoked synaptic transmission (296). Recently, they showed that endocannabinoids contributed predominantly to DSE and mGluR1-driven STD at PF terminals in juvenile rodents, while glutamate also contributed to these forms of retrograde synaptic suppression in mature animals (98, 99). However, in our preliminary data, both DSE and mGluR1-driven STD in Purkinje cells of mature mice were completely blocked by the CB_1 antagonist AM281 (unpublished data). Therefore, it is still an open question whether glutamate mediates retrograde suppression at PF-Purkinje cell synapses. On the other hand, Duguid and Smart (136) showed facilitating effects of glutamate, which acted retrogradely on inhibitory synaptic terminals of cerebellar Purkinje cells. They showed that glutamate was released from dendrites following depolarization of Purkinje cells, and activated presynaptic NMDA receptors, resulting in enhancement of GABA release. In a later study, the same group reported additional action of glutamate released from Purkinje cell dendrites (135). They showed that depolarization of Purkinje cells released glutamate from dendrites, which activated mGluR1 on the same dendrites in an autocrine fashion. Then activated mGluR1 caused enhancement of endocannabinoid production and consequently enhanced DSI. In the cerebral cortex, Zilberter's group (204) reported that glutamate was released from depolarized postsynaptic neurons and activated presynaptic mGluRs to suppress the GABA release, as described in section v*B4*.

Makara et al. (319) suggested that nitric oxide (NO) may be involved in hippocampal DSI as a retrograde messenger. They found that DSI was prevented not only by inhibition of CB_1 receptors but also by blocking NO signaling pathway at various points, including inhibition of NO synthase, inhibition of NO-sensitive guanylyl cyclase, and application of NO scavenger. However, this NO dependence of DSI was observed only in the presence of carbachol. Since carbachol is a broad-spectrum cholinergic agonist, it may have multiple modulatory actions including postsynaptic depolarization through nicotinic receptor activation and apparent enhancement of DSI through mAChR activation. Therefore, it is conceivable that NO is not a key messenger in DSI, but a modulator of retrograde endocannabinoid signaling. Recently, Maccarrone et al. (310) proposed a new model of eCB-STD in the striatum. They reported that activation of postsynaptic mGluR5 increased the glutathione levels in medium spiny neurons, which might activate DGL and facilitate 2-AG production. It is not clear whether this mechanism is unique to the striatum or it is applicable to other brain regions. Significance of NO and glutathione in retrograde endocannabinoid signaling remains to be determined.

VI. ENDOCANNABINOID-MEDIATED LONG-TERM DEPRESSION

Endocannabinoid-mediated long-term depression (eCB-LTD) induced by stimulation of synaptic inputs has been reported in several brain regions (Table 5). In the following sections, we review the studies on eCB-LTD and discuss the three fundamental issues: *1*) What pattern of input activity induces eCB-LTD in each brain region? *2*) How does afferent stimulation cause endocannabinoid release? *3*) How does CB_1 receptor activation cause long-lasting effects on synaptic transmission?

A. eCB-LTD in Various Brain Regions

1. Dorsal striatum

a) HFS-INDUCED LTD. Involvement of endocannabinoids in long-term synaptic plasticity was first reported at excitatory synapses in the dorsal striatum in 2002 (175). HFS of corticostriatal glutamatergic inputs to medium spiny neurons is known to induce LTD (63). This LTD requires postsynaptic Ca^{2+} elevation (64) and is accompanied by a decrease in the probability of glutamate release (89, 90). These previous studies suggest that LTD is induced postsynaptically and expressed presynaptically, indicating the involvement of retrograde synaptic signaling. Gerdeman et al. (175) attempted to identify the retrograde messenger and found that endocannabinoids mediate striatal LTD (175). LTD was induced by HFS (100 Hz for 1 s, repeated 4 times at 10-s interval) of corticostriatal pathways paired with postsynaptic depolarization. This LTD of corticostriatal synapse was blocked by the CB_1 antagonist SR141716A and was abolished in CB_1 knockout mice, indicating the involvement of endocannabinoid signaling. The same group also reported that CB_1 receptor activation is required for induction but not maintenance of LTD (442). The CB_1 dependence of striatal LTD was later confirmed by Kreitzer and Malenka with the same induction protocol (282).

How does the HFS induce endocannabinoid release? Striatal LTD was reported to be dependent on group I mGluRs (498) and L-type Ca^{2+} channels (64). Kreitzer and

TABLE 5. *eCB-LTD in the brain*

Region	Postsynaptic Neuron	Input	Induction	Dependence	Independence	Reference Nos.
Dorsal striatum	MSN	E	HFS+Depol	CB_1, Ca^{2+}		175
				CB_1, I-mGluR, VGCC (L), D_2	NMDAR	282, 283
				CB_1, D_2, Cav1.3, VGCC (L)		555
			MFS	CB_1, D_2	mGluR, VGCC (L), NMDAR, Ca^{2+}	443
				mGluRs (pre)	CB_1, NMDAR, mGluR1/5 (post)	282
NAc	MSN	E	MFS	CB_1, Ca^{2+}, mGluR5, Ca^{2+} store	D_1, D_2, NMDAR, mGluR2/3	437
				PKA (pre), VGCC (P/Q)	VGCC (N, L)	337
Sensory cortex	L5 PyC (V)	E	PS (postpre)	CB_1, NR2B, Ca^{2+}	NMDAR (post)	477
	L2-3 PyC (S)	E (L4)	PS (postpre)	CB_1, VGCC, mGluR5, IP_3R, DGL, NMDAR	NMDAR (post)	30
				CB_1, VGCC, mGluR, NMDAR, PLC	NMDAR (post), IP_3R	383
PrF cortex	L5/6 PyC	E	MFS	CB_1, mGluR5, PLC, DGL, Ca^{2+}	NMDAR, D_1, D_2	287
DCN	Cartwheel cell	E (PF)	PS (prepost)	CB_1, NMDAR (post), Ca^{2+}	mGluR1/5	527
Cerebellum	Purkinje cell	E (PF)	PF+CF stim	CB_1, DGL, Ca^{2+}, NO		455
BLA	Principle cell	I	LFS	CB_1, PKA, mGluR1	DGL, PLC, Ca^{2+}, mGluR5	330, 16
Hippocampus	CA1 PyC	I	HFS (SR)	CB_1, I-mGluR, PLC, DGL	NMDAR, Ca^{2+}	84
			MFS, TBS (SR)	CB_1		83
			TBS (SR)	cAMP/PKA (pre), RIM1α	PKA (post)	85
		E	Hetero-HFS	CB_1, mGluR1/5, K^+ channel (pre)	NMDAR, Ca^{2+}	572

NAc, nucleus accumbens; PrF, prefrontal; DCN, dorsal cochlear nucleus; BLA, basolateral amygdala; MSN, medium spiny neuron; PyC, pyramidal cell; V, visual cortex; S, somatosensory cortex; E, excitatory; PF, parallel fiber; I, inhibitory; HFS, high-frequency stimulation; Depol, postsynaptic depolarization; MFS, medium-frequency stimulation; PS, pairing stimulation; LFS, low-frequency stimulation; SR, stratum radiatum; TBS, θ-burst stimulation; Hetero, heterosynaptic; I-mGluR, group I metabotropic glutamate receptor; VGCC, voltage-gated Ca^{2+} channel; L, L-type; P/Q, P/Q-type; N, N-type; pre, presynaptic; post, postsynaptic; NMDAR, NMDA receptor.

Malenka (282) found that the HFS-induced LTD was blocked by the mGluR antagonist LY341495, and mimicked by bath application of DHPG (100 μM, 10 min), and that this DHPG-induced LTD was blocked by the CB_1 antagonist AM251. Bath application of DHPG resulted in LTD when the postsynaptic membrane potential was held at −50 mV, but not at −70 mV, and the LTD induction by DHPG at −50 mV was prevented by the L-type Ca^{2+} channel blocker nitrendipine. These results indicate that the activation of group I mGluRs by HFS of corticostriatal inputs and Ca^{2+} entry into postsynaptic neurons through L-type Ca^{2+} channels are both necessary for induction of eCB-LTD. Therefore, it is likely that the conjoint activation of group I mGluRs and Ca^{2+} elevation by the LTD induction protocol (HFS + depolarization) induces endocannabinoid release through Ca^{2+}-assisted RER.

The aforementioned studies consistently indicate that CB_1 receptor activity is necessary for the induction of corticostriatal LTD. The next question is whether CB_1 activation is sufficient for LTD induction. Ronesi et al. (442) examined the effects of bath application of the CB_1 agonist WIN55,212-2 for 20 min on corticostriatal EPSCs that were evoked by test pulses delivered at 0.05 Hz. The EPSC amplitude was decreased by WIN55,212-2 and completely reversed by removing WIN55,212-2 and simultaneously applying the CB_1 receptor antagonist SR141716A. The authors concluded that the CB_1 receptor activation is necessary, but not sufficient, to induce LTD. Kreitzer and Malenka (282) performed similar experiments, but obtained different results. They observed that after the 20-min treatment with WIN55,212-2, the EPSC amplitude was not reversed after washout of WIN55,212-2 and application of the CB_1 antagonist AM251. The authors interpreted these data as the evidence that the CB_1 activation itself is sufficient to induce LTD. This notion was modified in a subsequent paper of the same group (475). In this study, activity dependence of HFS- and WIN55,212-2-induced LTD was examined. By performing two-pathway experiments, the authors showed that 20-min application of WIN55,212-2 induced LTD at the input that received test pulses at 0.05 Hz, but not at the other input that did not receive any stimulation during the application of WIN55,212-2. HFS-LTD exhibited a similar dependence on presynaptic activity. From these results, the authors concluded that striatal eCB-LTD requires low-frequency presynaptic activity coincident with activation of CB_1 receptors. This study also suggested that this dual requirement might be suitable for input-specific induction of LTD.

Striatal LTD is known to be suppressed by D_2 antagonists (65, 282), suggesting its dependence on D_2 dopamine receptor. How D_2 receptors contribute to LTD is, however, controversial. Wang et al. (555) suggested that D_2 receptors on cholinergic interneurons dominantly contribute to the control of LTD induction. They used transgenic mouse lines in which D_1 receptor- or D_2 receptor-expressing medium spiny neurons were labeled with EGFP, and showed that LTD could be induced in both D_1 receptor- and D_2 receptor-expressing neurons. LTD induction in both neurons was blocked by the D_2 antagonist sulpiride, suggesting that activation of D_2 receptors lo-

cated on postsynaptic medium spiny neurons is not necessary for LTD induction. The authors further examined the effects of Ca^{2+} channel blockade and changes in basal activity of muscarinic M_1 receptors, and proposed the following model of corticostriatal LTD. Activation of D_2 receptors located on cholinergic interneurons suppresses interneuron firing, and reduces acetylcholine release, resulting in a decrease in M_1 receptor tone. The reduced M_1 tone increases the activity of an L-type Ca^{2+} channel, Cav1.3, and thereby enhances Ca^{2+} entry, which ultimately enhances endocannabinoid release and promotes LTD induction.

Meanwhile, Kreitzer and Malenka (283) presented a different model, by performing experiments similar to those by Wang et al. (555). They also used the transgenic mouse lines to discriminate between direct-pathway medium spiny neurons (corresponding to D_1 receptor-expressing neurons) and indirect-pathway medium spiny neurons (corresponding to D_2 receptor-expressing neurons). They showed that striatal LTD was expressed only in the indirect-pathway neurons. Moreover, DHPG-induced LTD was shown to be elicited only in indirect-pathway neurons, despite similar cannabinoid sensitivity of excitatory transmission to direct-pathway and indirect-pathway neurons. This study also showed that the DHPG-induced LTD in indirect-pathway neurons was enhanced by activation of D_2 receptors, which is consistent with the reports that D_2 receptor activation enhanced endocannabinoid release (177, 352). From these results, Kreitzer and Malenka (283) proposed that activation of D_2 receptors located on indirect-pathway medium spiny neurons facilitates mGluR-driven endocannabinoid release, and consequently promotes LTD. The authors also suggested that this synapse-specific LTD might be important for motor coordination.

Reasons for these conflicting results from the two groups are not clear. Kreitzer and Malenka (283) showed that glutamatergic presynaptic terminals on direct-pathway neurons (D_1 receptor-expressing neurons) have CB_1 receptors and can exhibit LTD when cannabinoid agonist application and presynaptic activity are combined (283). Therefore, endocannabinoids released from indirect-pathway neurons (D_2 receptor-expressing neurons) might have spread to glutamatergic presynaptic terminals on direct-pathway neurons and induced HFS-LTD in the study by Wang et al. (555).

B) LTD INDUCED BY MEDIUM-FREQUENCY STIMULATION. A new form of LTD, which was induced by medium-frequency stimulation (MFS), was reported at corticostriatal excitatory synapses on medium spiny neurons in the dorsal striatum. Ronesi and Lovinger (443) found that MFS (10 Hz, 5 min) induced LTD, which was accompanied by an increase in the paired-pulse ratio, and dependent on CB_1 and D_2 receptors, but not on mGluRs and L-type Ca^{2+} channels (443). From these results, the authors concluded that this type of LTD is also mediated by endocannabinoids. Kreitzer and Malenka (282) performed similar experiments and confirmed that a similar induction protocol (10 Hz, 10 min) induced a reliable LTD. Surprisingly, however, this MFS-induced LTD was not blocked by the CB_1 antagonist AM251, indicating that the LTD is not mediated by endocannabinoids. Reasons for this discrepancy between these two studies are unclear.

2. NAc

The eCB-LTD in the NAc, a major component of the ventral striatum, was first reported by Robbe et al. in 2002 (437). MFS (13 Hz, 10 min) of prelimbic cortical afferents to NAc induced a reliable LTD of EPSCs. This LTD was blocked by the CB_1 antagonist SR141716A, occluded by the cannabinoid agonist WIN55,212-2, and abolished in CB_1-knockout mice. LTD was also blocked by postsynaptic BAPTA injection, bath application of the mGluR5 antagonist MPEP, and depletion of Ca^{2+} store by thapsigargin or ryanodine, but not by D_1 and D_2 receptor antagonists. Bath application of DHPG (100 μM, 10 min) induced LTD in wild-type mice but not in CB_1-knockout mice. From these results, the authors concluded that postsynaptic activation of mGluR5 and store-derived Ca^{2+} elevation are required for the induction of LTD. Presynaptic mechanisms of eCB-LTD was further studied by the same group (337). The eCB-LTD was blocked by the protein kinase A (PKA) inhibitor KT5720, and occluded by selective blockade of P/Q-type Ca^{2+} channels with ω-agatoxin-IVA, but not by blockade of N-type or L-type Ca^{2+} channels. These data suggest that the expression of eCB-LTD requires the inhibition of cAMP/PKA cascade and of P/Q-type Ca^{2+} channels.

Interestingly, eCB-LTD of the NAc was found to be impaired by chronic (225) or a single in vivo exposure (336) to Δ^9-THC. In the study by Hoffman et al. (225), rats were given a single, daily intraperitoneal injection of Δ^9-THC (2 mg/ml, 10 mg/kg) or vehicle for 7 consecutive days. When the brain slices prepared from Δ^9-THC-treated and vehicle-treated rats were examined, induction of eCB-LTD by MFS (10 Hz, 5 min) was impaired in Δ^9-THC-treated slices. The Δ^9-THC treatment also decreased the cannabinoid sensitivity of excitatory and inhibitory synaptic transmissions in the NAc. These data indicate that chronic cannabinoid exposure blocks synaptic plasticity in the NAc through functional tolerance of CB_1 receptors. Similar results were reported by Mato et al. (336). In this study, mice were injected once with Δ^9-THC (3 mg/kg) or vehicle 15–20 h before the experiment. The eCB-LTD, which was induced by MFS (13 Hz, 10 min), was abolished in Δ^9-THC-injected mice. This suppression of LTD was shown to be reversible within 3 days. In this study, hippocampal eCB-LTD (sect. VI*A7*) was also shown to be impaired in Δ^9-THC-injected mice. These data indicate

that administration of cannabis derivatives influences neural functions, not only by acutely activating CB_1 receptors but also by persistently blocking endocannabinoid-mediated synaptic plasticity.

3. Cerebral cortex

Spike-timing-dependent plasticity is a form of long-term synaptic plasticity induced by pairing of presynaptic and postsynaptic action potentials in a millisecond time window. The direction of change, either long-term potentiation (LTP) or LTD, is determined by the precise timing of the presynaptic and postsynaptic spikes (35, 154, 327). In the cerebral cortex, pairing protocols with presynaptic spiking preceding postsynaptic firing (pre-to-post protocols) generally induce LTP (spike-timing-dependent LTP; tLTP), while those with the inverse order of spiking (post-to-pre protocols) induce LTD (spike-timing-dependent LTD, tLTD). Therefore, spike-timing-dependent plasticity follows the Hebbian rule (212). For the induction of tLTP, postsynaptic NMDA receptors are reported to play a critical role as a timing detector (104, 473). Mechanisms of tLTD induction, however, remained unclear.

At excitatory synapses between layer 5 pyramidal neurons of the visual cortex, Sjostrom et al. (478) successfully induced tLTD by a post-to-pre pairing protocol (e.g., post-pre interval of 10 ms, 0.1–20 Hz, 50–75 spike pairings in total). Using this induction protocol, they found that tLTD is CB_1 dependent, and proposed that tLTD induction requires the coincident activation of CB_1 and NMDA receptors at presynaptic terminals (477). They found that tLTD is expressed presynaptically and blocked by the CB_1 receptor antagonist AM251 and the NR2B subunit-specific NMDA receptor antagonist ifenprodil. Presynaptic firing in the presence of a cannabinoid agonist (anandamide or a related molecule) without postsynaptic firing induced a similar LTD. This LTD was resistant to postsynaptic BAPTA injection and blocked by the NMDA receptor antagonist APV. Based on these results, they proposed a model that postsynaptic spiking induces endocannabinoid release and activates presynaptic CB_1 receptors, while presynaptic spiking releases glutamate and activates presynaptic NMDA receptors, and the coincident activation of both receptors at presynaptic terminals induces tLTD (477). In line with this model, inhibition of degradation or uptake of endocannabinoids broadened the time window of tLTD. The same research group further reported that a similar eCB-LTD can be induced by pairing stimulation of presynaptic spiking and postsynaptic subthreshold depolarization instead of postsynaptic spiking (476). This form of LTD is also dependent on CB_1 and NMDA receptors and appears to share the same mechanisms with tLTD.

At excitatory synapses on layer 2/3 pyramidal neurons in the somatosensory cortex, a similar CB_1-dependent tLTD was found independently by two research groups (30, 383). Bender et al. compared the properties of tLTP and tLTD and clearly showed that postsynaptic activation of NMDA receptors is necessary for tLTP, but not for tLTD. Pharmacological data in this study indicate that tLTD induction requires voltage-gated Ca^{2+} channels, mGluR5, IP_3 receptors, and CB_1 receptors. Similar results were reported by Nevian and Sakmann (383) that tLTD, which was induced by a post-to-pre pairing protocol, was dependent on CB_1 receptors, nonpostsynaptic (presumably presynaptic) NMDA receptors, voltage-gated Ca^{2+} channels, and mGluR-PLC pathway. The authors presented a model that the activation of voltage-gated Ca^{2+} channels by postsynaptic firing and the subsequent mGluR activation by presynaptically released glutamate trigger the production and release of endocannabinoids in a PLC-dependent manner, and induce LTD through activation of presynaptic CB_1 receptors.

As described in section VI*A2*, Robbe et al. (437) found that the glutamatergic inputs from the prelimbic area of the prefrontal cortex to the NAc exhibit eCB-LTD. They further examined whether a similar eCB-LTD could be induced within the prefrontal cortex and found that MFS (10 Hz, 10 min) of layer 2/3 afferents to layer 5/6 pyramidal neurons induced a robust LTD of excitatory inputs (287). This LTD was accompanied by a change in the coefficient of variation (a presynaptic indicator) and blocked by the CB_1 antagonist AM251. The LTD was blocked by the mGluR5 antagonist MPEP, the PLC inhibitor U73122, the DGL inhibitor THL, and BAPTA applied to postsynaptic neurons. In contrast, the LTD was not affected by the NMDA receptor antagonist MK801, the D_1 antagonist SCH23390, and the D_2 antagonist sulpiride. Subthreshold stimulation (10 Hz, 5 min) induced LTD in the presence of the MGL inhibitor URB602, but not in the presence of the FAAH inhibitor URB597. From these results and anatomical data showing localization of mGluR5, DGLα, and CB_1 receptor around these synapses, the authors concluded that MFS induces LTD by releasing 2-AG through mechanisms depending on postsynaptic mGluR5-PLC cascade, Ca^{2+} elevation and DGL.

4. Dorsal cochlear nucleus

The dorsal cochlear nucleus is thought to integrate auditory and somatosensory inputs and play an important role in the sound localization and the orientation of the head toward sounds of interest (343, 499). The dorsal cochlear nucleus structurally resembles the cerebellum (392). Parallel fibers of granule cells make synapses with both fusiform principal cells and cartwheel interneurons in the molecular layer. Fusiform cells are glutamatergic and receive other excitatory inputs at basal dendrites from auditory nerve fibers. Cartwheel cells are glycinergic and inhibit fusiform cells through feed-forward inhibition.

Tzounopoulos et al. (526) found cell type-specific and opposing forms of spike-timing-dependent plasticity at parallel fiber synapses onto fusiform cells and cartwheel interneurons. The parallel fiber-fusiform cell synapse exhibited a Hebbian form of plasticity so that LTP was induced by a pre-to-post pairing protocol. In contrast, the plasticity of the parallel fiber to cartwheel cell synapse was anti-Hebbian so that LTD was induced by the same pairing protocol. The same group further examined the mechanisms of these forms of plasticity and found that this anti-Hebbian form of tLTD in cartwheel cells is mediated by retrograde endocannabinoid signaling (527). In cartwheel cells, tLTD is induced by a protocol in which a postsynaptic spike is triggered 5 ms after the onset of a parallel fiber-evoked excitatory postsynaptic potentials (EPSP) (5 pairs at 10 Hz, repeated 10 times at 5-s interval). This tLTD was associated with a change in the coefficient of variation and blocked by NMDA receptor antagonists (bath-applied APV or intracellularly applied MK-801) and postsynaptic BAPTA, but not by mGluR antagonists. Application of the CB_1 antagonist AM251 not only prevented tLTD, but also unmasked tLTP. These results suggest that the pre-to-post pairing protocol causes postsynaptic Ca^{2+} elevation by activation of NMDA receptors and induces both LTD and LTP, the former being dominant and mediated by endocannabinoids. This form of eCB-LTD is unique in the sense that postsynaptic NMDA receptors are required for endocannabinoid release.

5. *Cerebellum*

Cerebellar LTD, which is induced by conjunctive stimulation of PF and CF inputs to Purkinje cells, is one of the most well-investigated forms of long-term synaptic plasticity (244). Many lines of evidence indicate that LTD induction is dependent on several signal transduction cascades including mGluR1-$G_{q/11}$-PLCβ4-protein kinase C (PKC) cascade and postsynaptic Ca^{2+} elevation, and that LTD expression is attributable to endocytosis of postsynaptic AMPA receptors (244). Safo and Regehr (455) showed that this postsynaptically expressed LTD at PF-Purkinje cell synapses required endocannabinoid signaling. This finding is surprising, because all other forms of eCB-LTD in various brain regions are shown to be presynaptically expressed. In this study (455), LTD was induced by a protocol in which a burst of 10 PF stimuli at 100 Hz followed by two CF stimuli at 20 Hz was repeated 30 times with an interval of 10 s. This LTD was blocked by the CB_1 antagonist AM251, the DGL inhibitors RHC-80267 and THL, and postsynaptic BAPTA application and was deficient in CB_1-knockout mice. Activation of CB_1 receptors was necessary, but not sufficient, for the induction of cerebellar LTD. From these results, the authors proposed a model that the combined PF and CF stimulation causes both mGluR1 activation and postsynaptic Ca^{2+} elevation, which elicits LTD through the mechanisms including the retrograde endocannabinoid signaling. Moreover, this cerebellar LTD was confirmed to be expressed postsynaptically. Then, how does the activation of presynaptic CB_1 receptors induce endocytosis of postsynaptic AMPA receptors? The authors suggested that CB_1 activation might promote the release of NO, which mediates an anterograde signal from PFs to Purkinje cells, and somehow contributes to the postsynaptic modulation of AMPA receptors. The observation that application of the NO synthase inhibitor L-NAME blocked LTD in the presence of WIN55,212-2 is consistent with this possibility.

Regehr's group further examined the timing dependence of cerebellar eCB-LTD (454) and compared the timing dependence with that of synaptically driven eCB-STD (51) (see sect. v*B2*). The study on eCB-STD clearly demonstrated that the endocannabinoid release was sharply dependent on the timing of PF and CF activation (51). The eCB-LTD exhibited a similar timing dependence, and LTP was most effectively induced when CF activity followed PF activity by ~80 ms (454). These data strongly suggest that the property of endocannabinoid release determines the timing dependence of cerebellar LTD.

6. *Amygdala*

Aforementioned forms of eCB-LTD are all expressed at excitatory synapses. The endocannabinoid-mediated LTD expressed at inhibitory synapses (LTDi) was first reported in the amygdala (330) and later in the hippocampus (84). At inhibitory synapses on principle neurons of the basolateral amygdala, low-frequency stimulation (LFS; 100 pulses at 1 Hz) of afferents induced a suppression of IPSCs (330). This suppression lasted for more than 20 min and was termed LTDi. The LTDi was associated with an increase in the paired-pulse ratio, blocked by the CB_1 receptor antagonist SR141716A, and completely abolished in CB_1-knockout mice. The same group further examined the mechanisms of LTDi and found that the induction of LTDi required postsynaptic mGluR1 activation but not postsynaptic Ca^{2+} elevation (16). From the pharmacological data, the authors suggested that LTDi involves activation of adenylyl cyclase-PKA pathway and release of anandamide. Long-lasting suppression of inhibitory synaptic inputs is expected to increase the excitability of neurons. Consistent with this expectation, HFS-induced LTP at excitatory synapses was enhanced when LFS was applied to induce LTDi 10 min before the HFS in the amygdala (16).

7. *Hippocampus*

Hippocampal eCB-LTDi was reported in CA1 pyramidal neurons by Chevaleyre and Castillo (84). Under the blockade of AMPA- and NMDA-type glutamate receptors but not mGluRs, HFS (2 trains of 100 pulses at 100 Hz,

separated by 20 s) in the stratum radiatum induced LTDi (Fig. 10, *A* and *B*). LTDi was associated with an increase in the paired-pulse ratio (Fig. 10*C*), indicating that it is of presynaptic origin. LTDi was blocked by the CB_1 antagonist AM251 (Fig. 10*D*), mGluR antagonists, the PLC inhibitor U73122, the DGL inhibitor RHC-80267, and postsynaptic GDPβS injection, but not by postsynaptic loading of BAPTA. By varying the timing of AM251 application, the authors showed that several minutes of CB_1 receptor activation following HFS was required for the induction of LTDi (Fig. *10E)*. Application of the group I mGluR agonist DHPG mimicked and occluded LTDi. HFS in the stratum pyramidale, in which excitatory fibers are sparsely distributed, failed to induce LTD. From these results, the authors proposed that HFS releases glutamate and activates group I mGluRs, which in turn produces 2-AG through PLC-DGL pathway and eventually induces LTDi by prolonged activation of presynaptic CB_1 receptors for several minutes.

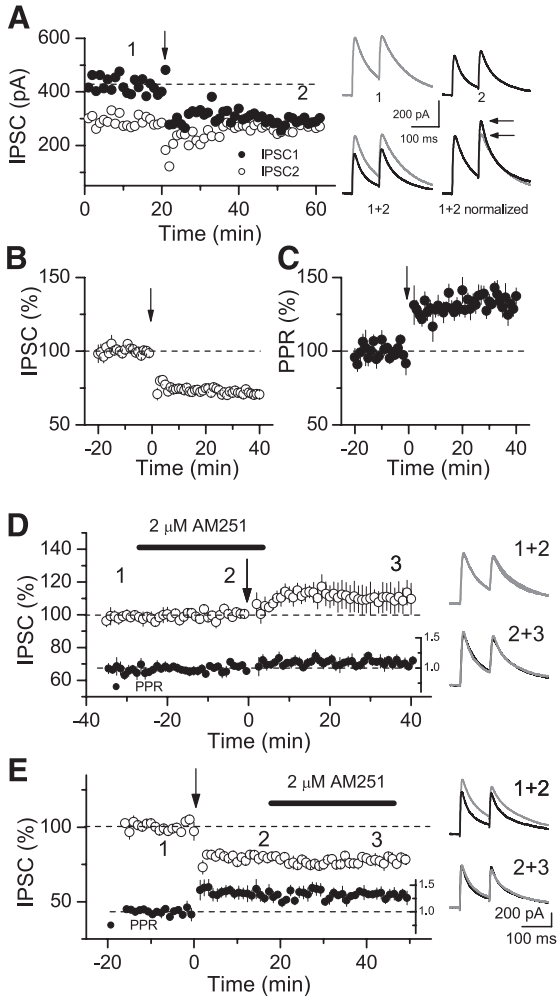


FIG. 10. CB_1 receptors are necessary for long-term depression of inhibitory inputs to CA1 pyramidal neurons. *A*: representative experiment in which IPSCs were recorded from a CA1 pyramidal neuron. Synaptic currents were evoked by paired-pulse stimulation (100 ms apart), and the amplitude of both IPSCs is plotted against time. High-frequency stimulation (HFS) was given at the time indicated by the arrow. Averaged sample traces taken during the experiment (indicated by numbers) are depicted on the *right*. Traces are superimposed and also normalized (bottom row) to point out the change in the paired-pulse ratio (PPR) (horizontal arrows). *B* and *C*: summary graph of the first IPSC amplitude (*B*) and PPR (*C*) from 21 experiments performed as in *A*. *D*: bath application of the CB_1 antagonist AM251 for 30 min (horizontal bar) had no effect on inhibitory basal synaptic transmission or PPR but completely blocked the LTD ($n = 7$). Sample traces from a representative experiment are shown on the *right*. *E*: when bath applied 20 min after LTD induction, AM251 had no effect on IPSC amplitude or PPR ($n = 5$). [Modified from Chevaleyre and Castillo (84), with permission from Elsevier.]

Physiological significance of this eCB-LTDi was further investigated by Chevaleyre and Castillo (83). In this study, the spread of LTDi along the dendritic compartment of CA1 pyramidal cells was measured by using focally applied theta-burst stimulation (TBS). LTDi was shown to be highly localized in a very small dendritic areas (<10 μm from the stimulation point). Then, the authors sought stimulation parameters capable of inducing LTDi without affecting excitatory synapses by using bulk stimulation. They found that LTDi could be induced by MFS (2 trains of 100 pulses at 10 Hz, 20 s apart), which induced neither LTP nor LTD at excitatory synapses. Using this MFS protocol, they examined whether LTDi expression affected subsequent LTP induction at excitatory synapses. After priming with the MFS, the subsequent induction of LTP at excitatory synapses was enhanced. This priming effect of the MFS was blocked by the CB_1 antagonist AM251 and mGluR antagonists and was abolished in CB_1-knockout mice. These data indicate that eCB-LTDi causes local disinhibition and selectively primes nearby excitatory synapses, and thereby facilitates subsequent induction of LTP at the primed excitatory synapses.

Castillo's group (85) investigated the presynaptic mechanisms of eCB-LTDi. TBS-induced LTDi was blocked by incubation of adenylyl cyclase activator forskolin and PKA inhibitors but not by postsynaptic application of a membrane-impermeant PKA inhibitor, and was impaired in RIM1α-knockout mice. These treatments that blocked LTDi were without effects on DSI. These data suggest that presynaptic cAMP/PKA and RIM1α are involved in the signaling from CB_1 receptors to the release machinery, which causes LTDi. Moreover, this study showed that LFS-induced LTDi in the amygdala was also impaired in RIM1α-knockout mice, suggesting that presynaptic mechanisms underlying eCB-LTDi might be the same in the hippocampus and amygdala.

Yasuda et al. (572) found that heterosynaptic LTD at excitatory synapses was mediated by endocannabinoids in CA1 pyramidal cells of the developing hippocampus. In this study, two independent Schaffer collateral pathways were alternately stimulated, and field EPSPs or EPSCs were recorded. HFS (20 pulses at 100 Hz, repeated 5 times at 20-s intervals) applied to one pathway elicited LTP in the stimulated pathway, and LTD in the other unstimu-

lated pathway. This heterosynaptic LTD was prominent at postnatal days 2–10 (P2–P10), attenuated during development, and disappeared at P42. The LTD was associated with decrease in fiber volley amplitude, and suppressed by the CB_1 antagonist AM251, mGluR antagonists (LY367385 plus MPEP), and K^+ channel blockers including Ba^{2+}, 4-AP, and α-DTX. These results suggest that endocannabinoids are released upon mGluR activation and induce heterosynaptic LTD through activation of presynaptic K^+ channels (572).

B. Mechanisms of Endocannabinoid Release in eCB-LTD

Mechanisms of endocannabinoid release during the induction of eCB-LTD might be different depending on experimental conditions and brain regions. Pharmacological data strongly suggest that Ca^{2+}-assisted RER dominantly contributes to eCB-LTD in most brain regions, including the dorsal striatum (282), NAc (437), cerebellum (244, 455), prefrontal cortex (287), and sensory cortex (30, 383). On the other hand, basal RER appears to contribute to LTDi in the hippocampus (84), whereas CaER seems to play a major role in anti-Hebbian form of tLTD in the dorsal cochlear nucleus (527).

Contribution of Ca^{2+}-assisted RER is supported by the following findings. The HFS-induced LTD in the dorsal striatum requires both activation of group I mGluRs and Ca^{2+} entry through L-type Ca^{2+} channels (282). The MFS-induced LTD in the NAc requires both mGluR5 and store-derived Ca^{2+} elevation (437). The cerebellar LTD induced by conjunctive stimulation of PFs and CFs requires mGluR1, PLCβ4, DGL, and Ca^{2+} elevation (244, 455), all of which are involved in Ca^{2+}-assisted RER in Purkinje cells (315). The MFS-induced LTD in the prefrontal cortex requires mGluR5, PLC, DGL, and Ca^{2+} increase (287). The Hebbian form of tLTD in the layer 2/3 pyramidal cells of the sensory cortex requires voltage-gated Ca^{2+} channels, mGluR5, and IP_3 receptors (30, 383). In these forms of eCB-LTD, Ca^{2+}-assisted RER is likely to be driven by simultaneous activation of postsynaptic group I mGluRs and Ca^{2+} elevation in the postsynaptic neurons. The former is achieved by glutamate released from excitatory presynaptic terminals during repetitive stimulation, and the latter is induced either by Ca^{2+} influx through voltage-gated Ca^{2+} channels or by Ca^{2+} release from internal stores.

In the hippocampus, eCB-LTDi was shown to require group I mGluRs, PLC, and DGL, but not Ca^{2+} elevation in postsynaptic neurons (84). Therefore, basal RER may be sufficient to release endocannabinoids in this form of eCB-LTD. Under physiological conditions, however, it is likely that stimulation of excitatory inputs causes not only group I mGluR activation but also local postsynaptic Ca^{2+} elevation, which may drive Ca^{2+}-assisted RER. It remains to be determined whether LTDi in the hippocampus is always caused by basal RER or depends on Ca^{2+}-assisted RER under certain conditions, especially when LTDi is induced by a weak induction protocol.

In contrast to eCB-LTD in other brain regions, the anti-Hebbian form of tLTD in cartwheel cells of the dorsal cochlear nucleus appears to use CaER. This LTD requires postsynaptic NMDA receptors and Ca^{2+} elevation, but not mGluRs (527). In this form of LTD, CaER is likely to be driven by Ca^{2+} influx through NMDA receptors, which are activated by presynaptically released glutamate and the coincident postsynaptic depolarization. NMDA receptors and the machinery required for CaER are both widely distributed in the brain. Nevertheless, this form of eCB-LTD has been reported only in this cell type. It is possible that this form of LTD might require a special molecular organization so that NMDA receptors and the elements required for CaER are all closely packed in the membrane domain that is faced to the glutamate release site.

C. Presynaptic Mechanisms of eCB-LTD

Except cerebellar LTD, eCB-LTD is expressed as a long-term reduction of transmitter release from presynaptic terminals. Presynaptic CB_1 receptors need to be activated for only several minutes to trigger LTD (84) and are not involved in the maintenance of LTD (437). It is not consistent among studies on eCB-LTD whether the activation of CB_1 receptors for several minutes is sufficient to trigger LTD. At excitatory synapses in the NAc (437) and inhibitory synapses in the hippocampus (85), short application of cannabinoid agonists alone can induce LTD, suggesting that the CB_1 activation is sufficient. At excitatory synapses in the dorsal striatum, however, LTD cannot be induced by CB_1 activation alone (442), but LTD induction requires CB_1 activation and coincident low-frequency presynaptic activity (475). At excitatory synapses between layer 5 pyramidal cells in the visual cortex, LTD induction is postulated to require coincident activation of CB_1 and NMDA receptors at presynaptic terminals (477).

The next question is how short activation of CB_1 receptor induces long-term change in transmitter release. There are only a few studies addressing this issue. Mato et al. (337) suggested that the expression of MFS-induced LTD in the NAc requires inhibition of cAMP/PKA cascade and P/Q-type voltage-gated Ca^{2+} channels at presynaptic terminals. Chevaleyre et al. (85) demonstrated that the expression of LTDi in the hippocampus and amygdala requires presynaptic cAMP/PKA signaling and RIM1α, which might be involved in the signaling from CB_1 receptor to release machinery. Yasuda et al. (572) suggested that heterosynaptic LTD in the developing hippocampus

involves activation of presynaptic K^+ channels. Further investigation is needed to understand how these and other signaling molecules contribute to the long-term change in transmitter release during eCB-LTD.

VII. OTHER PROPERTIES OF ENDOCANNABINOID SIGNALING

A. Modulation of Endocannabinoid-Independent Synaptic Plasticity

Endocannabinoid signaling can either suppress or enhance the LTP that is in itself cannabinoid-independent. A presynaptic form of LTP at cerebellar PF-Purkinje cell synapses is induced by PF stimulation with a short duration and high frequency (e.g., 8 Hz, 15 s) (457). Induction of presynaptic PF-LTP depends on the activation of Ca^{2+}-sensitive adenylyl cyclase and the subsequent activation of PKA. In rat cerebellar slices, this PF-LTP was reported to be suppressed by coactivation of CFs (532). This suppression was blocked by the CB_1 antagonist AM251 and mimicked by the cannabinoid agonist WIN55,212-2. The data suggest that the CF activity suppresses the presynaptic PF-LTP by releasing endocannabinoids and interfering presynaptic adenylyl cyclase-PKA cascade through CB_1 activation.

Enhancement of excitatory LTP during inhibitory eCB-STD or eCB-LTD was reported in hippocampal CA1 neurons. Induction of LTP at hippocampal excitatory synapses is known to be dependent on NMDA receptors, and independent of endocannabinoid signaling. In the CA1 region of rat hippocampal slices, a subthreshold stimulation to induce excitatory LTP becomes effective to trigger LTP, when applied during DSI (67). The disinhibition caused by DSI is thought to enhance the subthreshould EPSP to the level that they can trigger NMDA receptor-dependent LTP. More selective and long-lasting enhancement of excitatory LTP by inhibitory eCB-LTD was reported in rat hippocampal slices (83). As described in the previous section, endocannabinoid-independent, excitatory LTP in CA1 pyramidal cells is enhanced by precedently induced inhibitory eCB-LTD. Therefore, local activation of excitatory inputs not only triggers excitatory LTP, but also primes nearby excitatory synapses for subsequent induction of LTP by inducing inhibitory eCB-LTD.

These studies demonstrate multiple actions of endocannabinoid signaling on synaptic plasticity. First, endocannabinoid signaling constitutes a key component of the signaling cascade for the induction of eCB-STD or eCB-LTD in various brain regions. Second, endocannabinoid signaling can modify other endocannabinoid-independent forms of synaptic plasticity by directly acting on their presynaptic processes. Third, endocannabinoid signaling can indirectly affect inducibility of endocannabinoid-independent forms of synaptic plasticity by influencing excitability of postsynaptic neurons as a consequence of eCB-STD or eCB-LTD.

B. Regulation of Excitability

Endocannabinoids have been shown to modulate excitability of neurons in several brain regions. In rat cerebellar slices, depolarization of Purkinje cells transiently reduces the firing rate of nearby interneurons in a CB_1-dependent manner (281). The most likely explanation for this phenomenon is that endocannabinoids released from a depolarized neuron bind to CB_1 receptors on nearby interneurons, cause hyperpolarization through activation of K^+ channels, and suppress the firing of interneurons. This suppression leads to disinhibition of surrounding Purkinje cells. Because a cerebellar interneuron projects over several hundred micrometers, the spatial extent of this disinhibition is well beyond the limits of endocannabinoid diffusion.

In rat somatosensory cortical slices, low-threshold-spiking (LTS) interneurons, but not fast-spiking (FS) interneurons, exhibited a long-lasting self-inhibition through endocannabinoid signaling (17). LTS interneurons contain CCK, whereas FS cells do not. In LTS cells, action potential trains at 10–50 Hz were followed by a long-lasting hyperpolarization, which was accompanied by a long-lasting increase in membrane conductance. Induction of this hyperpolarization was dependent on Ca^{2+} elevation, blocked by the CB_1 antagonist AM251, and mimicked by transient application of the endocannabinoid 2-AG. The data suggest that the long-lasting hyperpolarization after self-firing is mediated by the autocrine release of endocannabinoids.

These two studies clearly show that endocannabinoid signaling can affect not only presynaptic function but also postsynaptic properties through activation of CB_1 receptors presumably on somatodendritic regions. The time course of change in excitability is, however, quite different between these two cases, being transient in cerebellar Purkinje cells and long-lasting in cortical neurons. These two types of modulation can be considered as the somatodendritic counterparts of eCB-STD and eCB-LTD, respectively. It is not clear whether these types of modulation are unique to specific neurons, or more general phenomenon throughout the brain. Detailed morphological studies on subcellular distribution of CB_1 receptors might be helpful to address this issue.

C. Basal Activity of Endocannabinoid Signaling

It has been debated whether CB_1 receptors exhibit constitutive activity in the absence of its agonists. Studies using recombinant expression systems have reported the

constitutive activity for many types of G protein-coupled receptors including exogenously expressed CB_1 receptors. However, evidence that native CB_1 receptors exhibit constitutive activity is relatively poor (233). The constitutive activity has been suggested mainly by the finding that the CB_1 antagonist SR141716 inhibits basal G protein signaling in native brain preparations. This effect of SR141716 as an inverse agonist, however, requires higher concentrations (micromolar levels) than the effect as a competitive antagonist (nanomolar levels) (474). Importantly, SR141716 at micromolar range was found to similarly inhibit the basal G protein signaling in brain membranes from CB_1-knockout mice (49). A later study demonstrated that CB_1 receptors are not constitutively active and that the inhibitory effect of SR141716 at micromolar range can be interpreted as the action on constitutively active adenosine receptor (463).

Even if native CB_1 receptors are not constitutively active, CB_1 receptors may have basal activity because of tonically released endocannabinoids. Such a basal activity of CB_1 receptors has been shown in several reports (213, 382, 403, 480, 523). In CB_1-transfected cell line and native hippocampal neurons, agonist-induced G protein activation and the number of CB_1 receptors on cell membranes, which reflects the agonist-induced CB_1 internalization, were measured in the presence and absence of the DGL inhibitor THL (523). The treatment with THL decreased the basal activity of CB_1 receptors and increased the number of CB_1 receptors. From these results, the authors concluded that cell-derived endocannabinoids, presumably 2-AG, are responsible for the basal activity of CB_1 receptors in both neurons and nonneural cells. By using paired whole cell recordings from presynaptic CCK-positive basket cells and postsynaptic CA1 pyramidal cells, Neu et al. (382) examined the effects of the CB_1 antagonist AM251 on unitary IPSCs. Application of AM251 facilitated the inhibitory transmission, but had no effects when BAPTA was injected into the postsynaptic pyramidal cells, suggesting that endocannabinoids might be tonically released from pyramidal cells in a Ca^{2+}-dependent manner (382). The CB_1 blockade by SR141716 or AM281 also augmented field EPSPs in the CA1 region, even under the blockade of $GABA_A$ and $GABA_B$ receptors (480). Conversely, the inhibition of COX-2, but not COX-1, decreased EPSPs partly in a CB_1-dependent manner (480). These results suggest that COX-2 might regulate the excitatory transmission through changing the endocannabinoid level in the hippocampus. In mouse brain slices containing the hypothalamus, Hentges et al. (213) recorded IPSCs and EPSCs from proopiomelanocortin (POMC) neurons and non-POMC neurons. The CB_1 antagonist AM251 selectively augmented IPSCs of POMC neurons. In contrast, other synaptic currents of POMC neurons and both IPSCs and EPSCs of non-POMC neurons were not affected, although these synaptic currents were also sensitive to cannabinoids. The augmentation of the POMC-IPSCs by AM251 was abolished by injecting BAPTA to the postsynaptic POMC neuron under recording. These data indicate that POMC neurons tonically release endocannabinoids in a Ca^{2+}-dependent manner under basal conditions. In rat hypothalamic slices, Oliet et al. (403) recorded IPSCs from magnocellular neurosecretory cells in the supraoptic and paraventricular nuclei. Based on the data with agonists and antagonists for CB_1 receptors and oxytocin receptors, they suggested that IPSCs of oxytocin-producing neurosecretory cells are tonically suppressed by endocannabinoids, which are released through activation of oxytocin receptors by tonically released oxytocin. All these studies indicate that the activities of postsynaptic neurons primarily determine the basal tone of presynaptic CB_1 activity. Alternatively, the activities of presynaptic neurons might also influence the activity of presynaptic CB_1 receptors. In rat hippocampal neurons, outputs of a unique class of inhibitory neurons were found to be kept silenced by persistently active CB_1 receptors and switched on by high-frequency presynaptic stimulations (305). The mechanisms responsible for this cell-type specific tonic CB_1 activity, however, have not been determined. Because partial inhibition of presynaptic MGL could activate presynaptic CB_1 receptors through accumulation of continuously released 2-AG (207), control of presynaptic MGL activity may be an important factor that determines the basal tone of CB_1 activity.

D. Plasticity of Endocannabinoid Signaling

The endocannabinoid system can be up- or downregulated by several manipulations. DSI was shown to be persistently enhanced in the rat hippocampus following a single episode of experimental seizures during early postnatal development (81). This potentiation of endocannabinoid signaling is attributable to an increase in the number of CB_1 receptors on CCK-positive inhibitory terminals. A further study by the same group demonstrated that potentiation of DSI can be induced in vitro by tetanic stimulation of Schaffer collateral synapses in hippocampal slice preparations (80). Importantly, the study also revealed that the induction of the DSI potentiation requires CB_1 activation. Application of CB_1 antagonists during febrile seizures in vivo as well as during tetanic stimulation in vitro blocked the DSI potentiation and prevented the long-lasting effects on limbic excitability, which might be clinically important. Upregulation of endocannabinoid signaling was also induced by repetitive low-frequency stimulation in hippocampal CA1 inhibitory synapses (580).

Downregulation of endocannabinoid signaling was reported in the NAc and hippocampus. Chronic exposure

to Δ^9-THC or WIN55,212-2 decreased the cannabinoid sensitivity of both excitatory and inhibitory synapses and blocked eCB-LTD at excitatory synapses in the NAc (225). Moreover, even a single in vivo exposure to Δ^9-THC decreased the cannabinoid sensitivity and abolished eCB-LTD in both the NAc and hippocampus (336). These findings might be helpful to understand how cannabis derivatives alter cognitive functions and motivational behaviors.

E. Actions of Endocannabinoid-Derived Oxygenated Products by COX-2

Anandamide and 2-AG are degraded mainly by FAAH and MGL, and COX-2 contributes little to removal of endocannabinoids (see sect. IV*D*). However, its action might have important roles under certain conditions, because it produces additional biologically active compounds from endocannabinoids (162). This alternative metabolic pathway may become more important when the primary hydrolytic enzymes FAAH and MGL are blocked.

Prostamides (prostaglandin-ethanolamides) are COX-2-derived oxidative products of anandamide. PGE_2-ethanolamide (PGE_2-EA) is the first prostamide to be described (577). Now it is known that cells can produce a range of prostamides, which include PGD_2-, PGE_2-, and $PGF_{2\alpha}$-EAs, when incubated with relatively high concentrations of anandamide (162). However, little information is available concerning the levels of prostamides in intact animals. The levels of PGD_2-EA + PGE_2-EA, which are occasionally hard to separate, and $PGF_{2\alpha}$-EA in liver, kidney, lung, and small intestine were measured in wild-type and FAAH-knockout mice with or without administration of anandamide (50 mg/kg) (557). In FAAH-knockout mice, detectable levels of these prostamides were produced only when anandamide was intravenously injected. Anandamide-treated control mice produced detectable but lower levels of PGD_2-EA + PGE_2-EA, but not $PGF_{2\alpha}$-EA, in kidney and lung. Pharmacological properties of prostamides are now beginning to be elucidated. They have several biological actions including contraction of iris sphincter and modulation of synaptic transmission, presumably through prostamide-specific receptors that have not been cloned (162, 568). In mouse cultured hippocampal neurons, effects of prostamides on mIPSCs were examined (462). PGD_2-EA, but not PGE_2-EA or $PGF_{2\alpha}$-EA, increased the frequency of mIPSCs. In contrast, anandamide and PGD_2 markedly decreased the frequency of mIPSCs, suggesting that the prostamide-induced effect is mediated by undefined receptors other than cannabinoid and prostanoid receptors.

Oxidation of 2-AG by COX-2 produces prostaglandin glycerol esters (PG-Gs) (162). Incubation of cultured cells with 2-AG resulted in the production of PGE_2-G and $PGF_{2\alpha}$-G. Synthesis of PG-Gs from endogenous 2-AG was also demonstrated in activated macrophages. In macrophage-like cell line, PGE_2-G, but not PGD_2-G or $PGF_{2\alpha}$-G, triggered Ca^{2+} mobilization, IP_3 synthesis, and activation of PKC (384). In mouse hippocampal neurons, all three PG-Gs (PGE_2-G, PGD_2-G, and $PGF_{2\alpha}$-G) increased the frequency of mIPSCs (462). The study also reported that inhibition of COX-2 reduced mIPSCs and augmented DSI, whereas the enhancement of COX-2 augmented mIPSCs and abolished DSI. These data suggest that PG-Gs are endogenously produced by COX-2 and that any changes in COX-2 activity can influence endocannabinoid signaling. A later study by the same group reported that PGE_2-G increased the frequency of mEPSCs as well as mIPSCs, and also induced neuronal cell death, which was attenuated by blockade of NMDA receptors (461). Since 2-AG is present at higher levels in the brain and a more effective substrate for COX-2 than anandamide, PG-Gs may play important roles in pathophysiological functions in the brain.

F. Contribution of Astrocytes to Endocannabinoid Signaling

It is generally accepted that neurons communicate with each other through endocannabinoid signaling (86, 206, 422). It is highly likely that glial cells play important roles in communication among neurons. However, contribution of glial cells to endocannabinoid signaling has not been well understood. There are several studies reporting the presence of CB_1 receptors on cultured astrocytes or astrocytes in situ in several brain areas (378, 440). Various actions of Δ^9-THC or cannabinoids on astrocyte functions have been reported, which include gene expression, differentiation, cell survival, and glucose metabolism (183). Production and inactivation of endocannabinoids by astrocytes have also been shown in many studies (552). Therefore, astrocytes have the ability to communicate with neighboring neurons or other astrocytes through endocannabinoid signaling. It remains to be elucidated, however, how astrocytes utilize endocannabinoid signaling to achieve their physiological functions. Details of astrocytes' contributions to endocannabinoid signaling are not described in this review, but this issue and related studies have been extensively discussed in an excellent review by Stella (485).

VIII. SUBCELLULAR DISTRIBUTIONS OF ENDOCANNABINOID SIGNALING MOLECULES

Molecules involved in endocannabinoid signaling are arranged around synapses in highly integrated and stra-

tegic manners. In this section, we overview morphological studies by using in situ hybridization, immunofluorescence, and immunoelectron microscopy as to subcellular distributions of CB_1, $G_{q/11}$ protein-coupled receptors, Gq protein α-subunit, PLCβ, DGL, NAPE-PLD, MGL, FAAH, and COX-2.

A. CB_1 Receptor

1. Hippocampus

In the hippocampus, CB_1 mRNA is expressed at high levels in subsets of interneurons (216, 316, 318, 329, 338, 339, 561). These neurons coexpress glutamic acid decarboxylase 65k (GAD65) and CCK. At the protein level, CB_1 is abundantly localized in terminals and preterminal axons, which surround pyramidal neurons in a basketlike manner and are labeled for CCK, GABA, and vesicular GABA transporter (Fig. 1, *F* and *G*) (168, 194, 202, 262, 263, 267, 364), indicating that CB_1 is richly expressed in CCK-positive basket cells (262, 263, 329). In contrast, CB_1 mRNA and immunoreactivity cannot be detected in parvalbumin-positive basket cells (262, 263, 329). Low levels of CB_1 mRNA are found in other interneurons including CCK-positive or calbindin-positive interneurons (329). In calretinin-positive interneurons, CB_1 mRNA was below the detection threshold of in situ hybridization (329), but its immunoreactivity was observed in ~30% of the interneurons (520).

Although pyramidal neurons express CB_1 mRNA at low levels (318, 329, 338, 359, 561), CB_1 immunoreactivity had not long been detected in their perikarya and axons (101, 140, 142, 262, 263, 340, 519). In 2006, employment of high-titer CB_1 antibody successfully visualized specific CB_1 immunoreactivity in excitatory terminals forming asymmetrical synapses onto dendritic spines (264, 267). The density of immunogold labeling on excitatory terminals is 1/30 of that on inhibitory terminals. As for other cell types in the hippocampus, CB_1 immunoreactivity is detected in serotonergic fibers in the CA3 region (201), whereas cholinergic fibers lack CB_1 in the entire hippocampus (203).

2. Dentate gyrus

In the dentate gyrus, high levels of CB_1 mRNA are observed in a subset of GAD65-positive interneurons, which exist in the granular and subgranular layers and the majority (72.9%) coexpress CCK mRNA (329). Low levels of CB_1 mRNA are also observed in other CCK-positive interneurons (329). Consistent with this report, CB_1 immunoreactivity is detected in perikarya and axon terminals that are labeled for GABA and CCK (262, 306, 520). CB_1 mRNA is also detected in mossy cell, a type of excitatory neuron in the hilus of the dentate gyrus (329, 359). At the protein level, CB_1 is rich in mossy cell terminals forming asymmetrical synapses with granule cell spines in the inner one-third of the molecular layer (Fig. 1*F*) (142, 194, 267, 306, 520). In contrast, CB_1 mRNA is not expressed in granule cells, and their axons, mossy fibers are negative for CB_1 immunoreactivity. By using CB_1 knockout mice specific to excitatory or inhibitory neurons, Monory et al. (359) have demonstrated that CB_1 at dentate gyrus excitatory synapses plays an important role in prevention of kainate-induced epilepsy.

3. Cerebral cortex

In the cerebral cortex, CB_1 mRNA is expressed at low to high levels in particular types of GAD65-positive interneurons that coexpress CCK or calbindin (329). CB_1 immunoreactivity is detected in somata of large CCK-positive interneurons and calbindin-positive ones (44), but not in small CCK-positive interneurons. CB_1 immunoreactivity is strongly detected in inhibitory terminals and preterminal axons, which are distributed in a meshwork pattern and surround pyramidal neurons (44, 142, 202). CB_1 mRNA is also expressed at low but significant levels in pyramidal neurons, whereas no CB_1 immunoreactivity is detected in their somata (44, 140, 142). As is the case in the hippocampus, CB_1 immunoreactivity had not been detected in excitatory terminals (44, 142, 202), but a recent study using high-titer CB_1 antibody detected its expression in excitatory terminals forming asymmetrical synapses with mGluR5-expressing spines (287). Both CB_1 mRNA and immunoreactivity are rarely detected in interneurons positive for parvalbumin, calretinin, somatostatin, or vasoactive intestinal peptide (44, 329), whereas single-cell PCR analysis detects CB_1 mRNA in more than half of the latter two types of interneurons (219). CB_1 immunoreactivity is negative in cholinergic fibers (203).

CB_1 mRNA and immunoreactivity exhibit a laminar pattern of expression in the cerebral cortex, where neurons with high CB_1 mRNA are distributed in the layers II, III, V, and VI (44, 316, 318, 329, 338, 339, 561). Laminar pattern of CB_1 immunoreactivity varies depending on cortical areas and species. In the rodent, for example, CB_1 is dense in the layers II, III, Va, and VI in the somatosensory cortex (Fig. 1, *A–C* and *H*) (44, 117, 139, 140, 519), whereas it is intense in the layers II and VI in the entorhinal cortex (Fig. 1*C*) (519). In the association cortices, including the prefrontal cortex and cingulate cortex, the layer IV is the highest for CB_1 immunoreactivity in the monkey (142) but is the lowest in the rat (140).

4. Amygdala

In the amygdala, CB_1 mRNA and immunoreactivity are highly enriched in the basolateral nucleus (Fig. 1, *C* and *I*) (142, 261, 318, 329, 338, 339). In this nucleus, CB_1 mRNA is expressed at low to high levels in particular

types of interneurons that coexpress CCK or calbindin mRNA (329). CB_1 immunoreactivity is detected in somata of large CCK-positive interneurons, but not in small CCK-positive ones (263, 346). CB_1 immunoreactivity is detected on CCK-containing inhibitory terminals or preterminal axons forming symmetrical synapses onto amygdala neurons (261). Low levels of CB_1 mRNA are also detected in ~30% of calretinin-positive interneurons and in a few parvalbumin-positive ones (329, 346).

CB_1 mRNA is expressed at low levels in pyramidal neurons of the basolateral nucleus, but its immunoreactivity is not detected in their perikarya (140, 142, 261). CB_1 immunoreactivity becomes positive in pyramidal neuron perikarya after colchicine treatment (346), thus confirming low CB_1 expression at the protein level in pyramidal neurons. Although CB_1 immunoreactivity has not been detected in excitatory terminals in this nucleus (202, 261), it is possible that low levels of CB_1 can be detected in future studies, as is the case for the hippocampus or cerebral cortex (264, 267, 287). CB_1 immunoreactivity is found in serotonergic fibers projecting to the basolateral nucleus (201).

5. Basal ganglia

The striatum is the input nucleus of the basal ganglia and characterized by high and uniform expression of CB_1 mRNA (317, 318, 329, 338, 561). Similar to ligand binding study, CB_1 mRNA and immunoreactivity are distributed in gradient from the dorsolateral to ventromedial portions (Fig. 1, *A* and *B*) (101, 229, 317, 318, 329, 338, 342, 519). All of CB_1-expressing striatal neurons coexpress GAD65 mRNA (329). More than 90% striatal neurons are medium spiny neurons (MSNs), which are GABAergic projection neurons and classified into the direct pathway/D_1-MSN and the indirect pathway/D_2-MSN. CB_1 mRNA is expressed in both types of MSNs and also in parvalbumin-positive GABAergic interneurons (216, 229, 329). Notably, CB_1 immunoreactivity is strongly detected in their terminals surrounding neuronal somata and dendrites in a meshwork pattern, while their somata are labeled only faintly (Fig. 1*J*) (140, 342, 377, 520, 528). CB_1 immunoreactivity is extremely low or below the detection threshold in other types of interneurons containing somatostatin/NO synthase, calretinin, or acetylcholine (229, 329, 528).

Among afferents to the striatum, CB_1 immunoreactivity is detected at low levels in excitatory terminals arising from the cortical layer V (342, 528). In contrast, CB_1 immunoreactivity is below the detection threshold in excitatory terminals from the thalamus or in dopaminergic terminals from the SNc (528). Consistent with these immunohistochemical data, CB_1 mRNA is moderately expressed in layer V cortical neurons, but not in the parafascicular thalamic nucleus or SNc (252, 318, 338, 528, 561).

The highest level of CB_1 immunoreactivity in the brain is observed in the three major projection regions of MSNs, i.e., the SNr, globus pallidus, and entopeduncular nucleus (Fig. 1, *A–C*) (101, 140, 202, 252, 342, 420, 519). However, CB_1 mRNA is not detected in these regions (252, 317, 338, 561), thus reflecting striking levels of CB_1 protein in projecting axons of MSNs. As for other nuclei of the basal ganglia, CB_1 mRNA is expressed at high levels in the subthalamic nucleus (318, 338). In contrast to the dorsal striatum, CB_1 expression is generally low in the NAc (Fig. 1*A*) (140, 202, 216, 317, 318, 338, 436). In the VTA, CB_1 immunoreactivity is localized in terminals forming symmetrical and asymmetrical synapses (341).

6. Hypothalamus

CB_1 mRNA is uniformly expressed in the hypothalamus with higher levels in the ventromedial nucleus and preoptic area, i.e., centers of satiety and sexual behavior (216, 318, 329, 338). CB_1 mRNA is also expressed in hypothalamic hormone-producing neurons, such as neurons expressing corticotropin-releasing factor in the paraventricular nucleus (94). Moreover, CB_1 mRNA is expressed in hypothalamic neurons involving food intake: neurons expressing cocaine-amphetamine-regulated transcript, prepro-orexin, or melanin-concentrating hormone (94). In contrast, CB_1 mRNA is hardly detected in neurons expressing gonadotropin-releasing hormone (171). Notably, CB_1 mRNA is not detected in GAD65- or CCK-positive hypothalamic neurons (329).

At the protein level, CB_1 immunoreactivity shows a punctate or fibrous pattern in various hypothalamic regions, including the anterior nucleus, lateral hypothalamic area, paraventricular nucleus, and ventromedial nucleus (Fig. 1*K*) (70, 519, 567). Moreover, in support of the expression in hypothalamic hormone-producing neurons, CB_1-immunoreactive axons are distributed around the hypophysial portal veins in the median eminence (567). CB_1 immunoreactivity is also localized at inhibitory terminals that are positive for GAD67 or form symmetrical synapses in the paraventricular nucleus, arcuate nucleus, and supraoptic nucleus (70, 567). These CB_1-positive inhibitory terminals presumably belong to extrahypothalamic neurons, because of the absence of CB_1 expression in intrinsic GABAergic hypothalamic neurons (329). As to CB_1 expression at excitatory synapses, Castelli et al. (70) reported its lack in VGluT2-positive terminals, whereas Wittmann et al. (567) found its presence in terminals forming asymmetrical synapses.

7. Cerebellum

In the cerebellum, CB_1 mRNA is strongly expressed in the granular layer (316, 318, 338). In contrast, CB_1 immunoreactivity is very low in the granular layer and,

instead, densely accumulates in the molecular layer (Fig. 1*L*) (139, 140, 267, 420, 519). This reflects dense accumulation of CB_1 protein in PFs, i.e., granule cell axons forming excitatory synapses onto Purkinje cells (125, 139, 140, 267, 420, 519). CB_1 immunoreactivity highly accumulates in perisynaptic portions of PFs (267). In addition, CB_1 mRNA and immunoreactivity are highly expressed in basket and stellate cells, inhibitory interneurons projecting to Purkinje cells (125, 139, 140, 267, 316, 317, 338). Labeling density of CB_1 immunoreactivity is five times higher in inhibitory interneuron terminals than in PF terminals (267). Notably, CB_1 immunoreactivity is particularly high in the pinceau formation, the clustered axons and terminals of basket cells that surround the initial segment of Purkinje cells (267, 519). These expression patterns of CB_1 at PFs and inhibitory synaptic terminals are consistent with the results that eCB-STD is readily induced at these synapses. Although robust eCB-STD is also observed at CF to Purkinje cell synapses, CB_1 expression at CFs is very low or below the detection limit (267). CB_1 expression is lacking in Purkinje cells or deep cerebellar nucleus neurons (139, 316, 338).

8. Spinal cord

CB_1 mRNA is expressed in intrinsic dorsal horn neurons (4, 318). At the protein level, CB_1 is distributed at high levels in the lamina I and IIi, in which CB_1-positive fibers densely extend in the rostrocaudal direction (Fig. 1, *E* and *M*) (149, 269, 458, 519). CB_1 is colocalized in the lamina IIi with PKCγ, a marker for excitatory interneurons in the lamina IIi (149). CB_1 immunoreactivity is also reported to be expressed in inhibitory interneurons (459).

CB_1 mRNA and immunoreactivity are detected in some neurons in the dorsal root ganglion (DRG) (4, 9, 36, 53, 228, 230, 357, 458). Until now, however, there has been no report demonstrating overlap of CB_1 with markers for primary afferents, such as calcitonin gene-related peptide, isolectin B4, and VR1 (149, 269). CB_1 immunoreactivity in the dorsal horn showed no changes after dorsal root rhizotomy (149). Moreover, there was no difference in CB_1 immunoreactivity in the dorsal horn between wild-type mice and DRG neuron-specific CB_1-knockout mice (4). Therefore, it is still uncertain whether CB_1 protein is present in central branches of primary afferents, which is an important issue to understand the mechanism of cannabinoid-induced analgesia. CB_1 immunoreactivity is also expressed at high levels in the lamina X and dorsolateral funiculus (Fig. 1*E*) (149, 458, 519). CB_1 immunoreactivity in astrocytes was also reported in the dorsal horn (458).

B. $G_{q/11}$ Protein-Coupled Receptors

Major $G_{q/11}$ protein-coupled receptors expressed in the CNS are the group I metabotropic glutamate receptors mGluR1 and mGluR5; muscarinic acetylcholine receptors M_1, M_3, and M_5; serotonin receptors 5-HT_{2A}, 5-HT_{2B}, and 5-HT_{2C}; adrenoceptors α1A, α1B, and α1D; and histamine receptor H1. In addition, peptides such as leptin, ghrelin, and orexin/hypocretin modulate food intake, and their actions are influenced by CB_1 receptor-mediated signaling (100, 120, 145, 218, 250, 278). Among such peptide receptors, orexin receptor OX1R is a $G_{q/11}$ protein-coupled receptor (456). Although not all of these $G_{q/11}$ protein-coupled receptors have been shown to contribute to endocannabinoid signaling, they should have potential to produce endocannabinoids through basal RER or Ca^{2+}-assisted RER.

1. Group I metabotropic glutamate receptors

Among $G_{q/11}$ protein-coupled receptors, mGluR1, a member of the group I mGluR, has been best studied in terms of regional and cellular expression and of localization around synapse. mGluR1 is expressed in various brain regions (469) with high levels in the glomerular and external plexiform layers in the olfactory bulb, anterior olfactory nucleus, alveus of the hippocampus, hilus of the dentate gyrus, globus pallidus, ventral pallidum, thalamic nuclei except the reticular nucleus, superior colliculus, substantia nigra pars compacta, inferior olivary nucleus, and Purkinje cells and subpopulation of unipolar brush cells in the cerebellum (22, 333, 388, 506). In the cerebral cortex, hippocampus, and anterior olfactory nucleus, mGluR1 is expressed in somatostatin-positive interneurons, but not in principal neurons (22). Such an interneuronal expression is also conspicuous in the striatum, where principal neurons (medium spiny neurons) lack detectable levels of mGluR1 (528). However, medium spiny neurons in the monkey striatum were reported to express mGluR1 (413). Studies with immunoelectron microscopy have revealed that mGluR1 is exclusively present in somatodendritic or postsynaptic elements. mGluR1 is enriched in dendritic spines (Fig. 11) and highly accumulated at perisynaptic annulus surrounding excitatory synaptic junction in Purkinje cells (22, 181, 308, 309, 333, 389).

mGluR5, the other member of group I mGluR, displays regional and cellular expression almost reciprocal to mGluR1. mGluR5 is rich in the telencephalon including the cerebral cortex, hippocampus, dentate gyrus, olfactory tubercle, striatum, NAc, and lateral septum (1, 470, 510). In these regions, mGluR5 is mainly expressed in principal neurons with occasional labeling in certain hippocampal interneurons (308, 509, 528). mGluR5 is also expressed moderately in the superior and inferior colliculi, trigeminal spinal tract nucleus, and Golgi cells in the cerebellum (470). Similar to mGluR1, mGluR5 is also exclusively expressed at somatodendritic elements, enriched in dendritic spines, and accumulated at perisynaptic anulus (308). Compared with mGluR1 on Purkinje

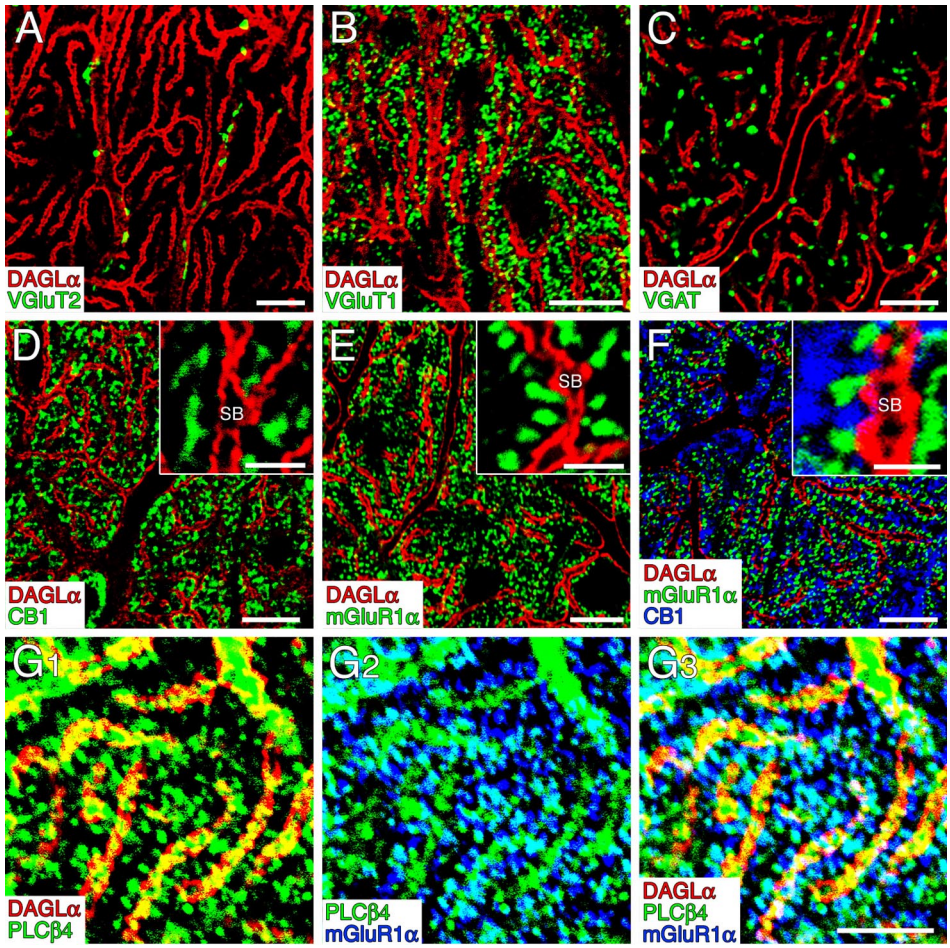


FIG. 11. Immunofluorescence showing diacylglycerol lipase-α (DGLα) distribution in mouse cerebellar Purkinje cells. In all images, DGLα is colored in red. *A–C*: lack of DGLα in VGluT2-labeled climbing fiber terminals (*A*), VGluT1-labeled parallel fiber terminals (*B*), and VGAT-labeled inhibitory interneuron terminals (*C*). *D–F*: DGLα on spiny branchlets (SB) is distributed close to, but separated from, CB_1 (green in *D*, blue in *F*) or mGluR1α (green in *E* and *F*). *G*: triple immunostaining for DGLα, PLCβ4 (green), and mGluR1α (blue). Note overlap of PLCβ4 with DGLα in dendritic shafts and with mGluR1α in dendritic spines. Scale bars: *A–F*, 10 μm; *G*, 5 μm; *insets* in *D–F*, 2 μm. [From Yoshida et al. (575).]

cells, which exhibits intensive spine accumulation and steep decline of receptor density with distance from the synaptic edge, the distribution of mGluR5 in hippocampal pyramidal cells and striatal medium spiny neurons is more widely distributed in somatodendritic elements and also within dendritic spines (309, 528). Labeling density of cell membrane-associated mGluR5 is in the order of spine > dendritic shaft > soma (528) (Fig. 12). While Purkinje cell synapses are completely surrounded by astroglial processes, the sealing of hippocampal synapses is loose (483, 542). Therefore, the different subcellular distributions of mGluR1 and mGluR5 appear to reflect the difference in extracellular glutamate levels around synapses of Purkinje cells and hippocampal neurons.

2. Muscarinic acetylcholine receptors

M_1 is concentrated in various telencephalic structures in a similar way to the distribution of mGluR5 (59, 299, 559). In the striatum, M_1 is detected in 78–85% of striatal neurons and expressed in striatonigral (D_1R/substance P-positive) and striatopallidal (D_2R/enkephalin-positive) types of medium spiny neurons (33, 217, 376). M_1 expression in striatal interneurons is below the detection threshold of immunohistochemistry (376). M_1 is also exclusive in pyramidal cells within the hippocampus and in granule cells within the dentate gyrus (298). On the basis of ultrastructural and lesion experiments, M_1 is shown to predominate in somatodendritic elements in both regions (217, 298, 376, 427, 446). In striatal medium spiny neurons, cell membrane-associated M_1 labeling is higher in dendritic shafts and somata than in spines (376) (Fig. 12).

M_3 is relatively abundant in the cerebral cortex, olfactory bulb, olfactory tubercle, and thalamic nuclei (anteroventral, ventrolateral, and midline thalamic nuclei, and habenula), whereas it is low in the dentate gyrus, striatum, and lateral septum (59, 559). Low levels are also noted in some brain stem nuclei. In the striatum and hippocampus, M_3 is mainly distributed in somatodendritic elements of principal neurons, and also found in excitatory terminals forming asymmetrical synapses (217, 427, 446).

M_5 is generally low in the brain compared with M_1 and M_3. Relatively high levels are noted in pyramidal cells in the hippocampal CA1, SNc, VTA, lateral habe-

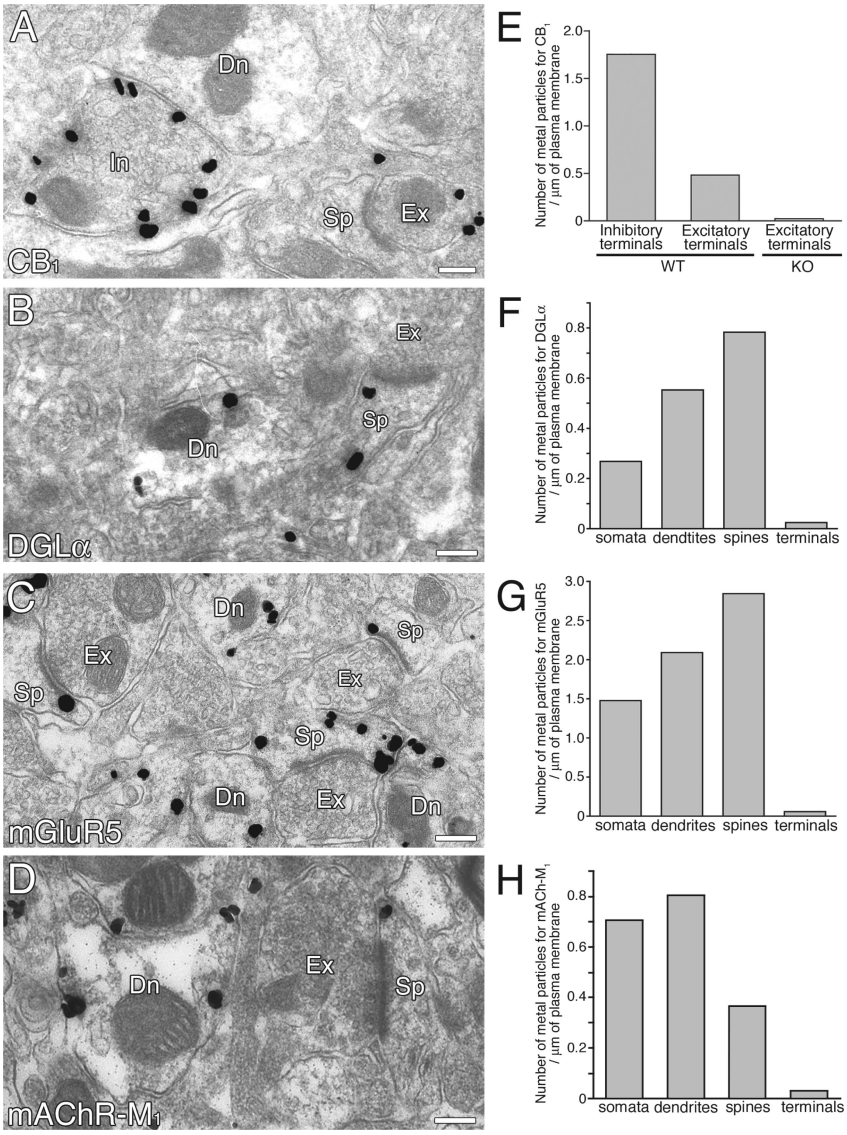


FIG. 12. Silver-enhanced immunogold and quantitative data for CB_1 (*A* and *E*), DGLα (*B* and *F*), mGluR5 (*C* and *G*), and mAChR-M_1 (*D* and *H*) in the mouse striatum. Bar graphs in *E–H* show the number of metal particles per 1 μm of the plasma membrane in each element. CB_1 is selectively distributed on presynaptic terminals, and particularly abundant in symmetrical (inhibitory) terminals (*E*). In contrast, DGLα, mGluR5, and M_1 are preferentially localized on the somatodendritic membranes. The density for DGLα and mGluR5 labeling is higher in spines than in somata and dendrites (*F* and *G*), whereas the relative density is opposite for M_1 labeling (*H*). Ex, excitatory terminal; In, inhibitory terminal; Dn, dendrite; Sp, spine. Scale bars: 200 nm. [Modified from Uchigashima et al. (528) and Narushima et al. (376).]

nula, ventromedial hypothalamic nucleus, and mammillary bodies (544). M_5 is also expressed in the endothelium and tunica media of cerebral arteries and is thought to mediate acetylcholine-induced vasodilation (11, 508).

In the hippocampus, M_2, a $G_{i/o}$-coupled mAChR, is expressed in cholinergic axons arising from the medial septum and diagonal band of Broca and also in GABAergic axons of parvalbumin-containing interneurons (196, 445, 446). M_2 and CB_1 are thus expressed in axons of distinct basket cells (168, 263). Consequently, the hippocampus undergoes two distinct forms of muscarinic suppression of GABA release, one through direct activation of M_2 on parvalbumin-containing basket cell axons and the other through M_1/M_3-mediated endocannabinoid release and subsequent activation of CB_1 on CCK-containing basket cell axons (168).

3. 5-HT$_2$ serotonin receptors

Although 5-HT_2 receptors were reported to facilitate 2-AG synthesis when expressed in the NIH3T3 cell line (414), information as to expression and localization in the brain is still limited. Its regional distribution was determined by binding of radiolabeled ligands (416, 417), in situ hybridization (353, 428), and light microscopic immunohistochemistry (2, 92, 137). 5-HT_{2B} was cloned from the stomach fundus (formerly named as 5-HT_{2F}), and its expression in the brain was below the detection threshold by in situ hybridization (428). 5-HT_{2A} and 5-HT_{2C} (formerly named as 5-HT_{1C}) display distinct regional expression in the brain. In general, high levels of 5-HT_{2A} binding and mRNA are rather confined to the frontal cortex, piriform cortex, hippocampal CA3, medial mammillary nucleus, pontine nuclei, and motor cranial nerve nuclei (416,

428). 5-HT_{2C} is present at very high levels in the choroid plexus, and also enriched in the retrosplenial cortex, piriform cortex, and entorhinal cortex, anterior olfactory nucleus, lateral septum, subthalamic nucleus, amygdala, subiculum, hippocampal CA3, lateral habenula, SNc, several brain stem nuclei, and spinal cord (353, 417, 428). 5-HT_3 is the only ligand-gated ion channel receptor for serotonin and exists as 5-HT_{3A} homomeric channels or 5-HT_{3A}/5-HT_{3B} heteromeric channels. (108, 133). CB_1 and 5-HT_{3A} were reported to be coexpressed with CB_1 in CCK-containing GABAergic neurons of the cerebral cortex, hippocampus, and amygdala (360). Since 5-HT_3 activation facilitates GABA release, serotonin and endocannabinoids could interact with each other at the molecular or circuit level for regulation of GABA release.

4. Adrenoceptors α1

Three adrenoceptors α1 mRNAs are expressed in various regions of the brain and spinal cord, showing overlapping and nonoverlapping patterns of spatial expression (110, 345, 421). α1A (formerly α1A/C) is highest in the olfactory bulb, magnocellular preoptic nucleus, several hypothalamic nuclei (paraventricular, supraoptic, and ventromedial nuclei), motor nerve nuclei, and spinal cord. α1B is high in various thalamic nuclei, including specific thalamic nuclei, lateral nucleus of the amygdala, pineal gland, and dorsal and medial raphe nuclei. α1D (formerly α1A or α1A/D) is high in the olfactory bulb, cerebral cortex, hippocampus, lateral nucleus of the amygdala, reticular thalamic nucleus, motor nerve nuclei, inferior olivary nucleus, and spinal cord.

5. Histamine receptor H1

Ligand binding sites by [^{3}H]mepyramine or [^{125}I]iodobolpyramine have revealed a wide distribution of central histamine H1 receptor (46, 335, 408, 514). It is particularly high in areas involving arousal, i.e., thalamus, cerebral cortex, cholinergic neuron groups in the mesopontine tegmentum and basal forebrain, as well as the locus ceruleus and raphe nuclei. In addition, high densities of H1 receptor are present in the limbic system, including hypothalamic nuclei, septum, medial amygdala, and hippocampus. The distribution of ligand binding sites is almost consistent with that of H1 mRNA in the rat brain (303).

6. Orexin receptor OX1R

OX1R mRNA is highly expressed in the hippocampal CA1 and CA2, tenia tecta, ventromedial hypothalamic nucleus, dorsal raphe, and locus ceruleus (518). The ventromedial hypothalamic nucleus coexpresses CB_1 mRNA at high to moderate levels (318, 338), and their functional potentiation and heterodimerization are suggested by transfection to heterologous cells (145, 218).

C. G_q Protein α-Subunit

Among the four members of G_q protein α-subunits ($G\alpha_q$, $G\alpha_{11}$, $G\alpha_{14}$, and $G\alpha_{15/16}$), $G\alpha_q$ and $G\alpha_{11}$ are the major isoforms in the brain (506). Immunoreactivity of $G\alpha_q$/$G\alpha_{11}$, as detected by polyclonal antibody against their common COOH-terminal sequence, shows wide distribution in the brain with higher levels in the telencephalon and cerebellar cortex. Through lipid modification of the NH_2-terminus as well as interaction with hydrophobic $\beta\gamma$-complex, $G\alpha_q$/$G\alpha_{11}$ are tightly attached to the extrasynaptic membrane of somatodendritic neuronal elements in hippocampal pyramidal cells and cerebellar Purkinje cells (506). Furthermore, $G\alpha_q$/$G\alpha_{11}$ display extensive overlap with mGluR1 in Purkinje cells and with mGluR5 in hippocampal pyramidal cells, indicating that they are localized in the right place to transduce signals from mGluR1/mGluR5.

D. Phospholipase Cβ

Each of the four PLCβ isoforms displays distinct, largely nonoverlapping expression in the brain; PLCβ1 in the telencephalon, PLCβ2 in the white matter, PLCβ3 in the caudal cerebellum, and PLCβ4 in the rostral cerebellum, thalamus, and brain stem (444, 447, 507, 556). PLCβ1 is coexpressed with mGluR5 and M_1 in principal or projection-type neurons, including pyramidal cells in the cerebral cortex and hippocampus, granule cells and mossy cells in the dentate gyrus, and medium spiny neurons in the striatum (167a). PLCβ3 is coexpressed with mGluR1 in a subset of Purkinje cells (385). PLCβ4 is also coexpressed with mGluR1 in telencephalic interneurons, neurons in the bed nucleus of anterior commissure, thalamus, substantia nigra, inferior olivary nucleus, unipolar brush cells, and Purkinje cells other than those expressing PLCβ3 (372).

Several fundamental properties are notable in cellular expression and subcellular localization of the PLCβ family in the brain (372, 385, 556). First, single neurons express a single major PLCβ isoform. Second, PLCβ is present in association with the smooth endoplasmic reticulum (sER) or plasma membrane. The association with the cell membrane may represent enzymatic activation by GTP-bound $G\alpha_q$/$G\alpha_{11}$ to hydrolyze PIP_2, while the association with the sER would be beneficial for swift and unfailing Ca^{2+} mobilization following IP_3 production. Third, PLCβ is accumulated at the perisynaptic site of excitatory synapses, and thereby exhibits extensive overlap with mGluR1, mGluR5, and M_1. Therefore, PLCβ1 is a

major effector enzyme downstream to mGluR5 and M_1, while PLCβ3 and PLCβ4 are downstream to mGluR1.

E. Diacylglycerol Lipase

Subcellular distribution of DGLα around synapses has been investigated in cerebellar Purkinje cells, pyramidal cells in the hippocampus and prefrontal cortex, and medium spiny neurons in the striatum (264, 287, 528, 575). In all these neurons, DGLα is selective to somatodendritic elements, being most abundant in dendritic spines. Again here, DGLα is apposed closely to $G_{q/11}$ protein-coupled receptors, and also to PLCβ (575). Thus subcellular arrangement of $G_{q/11}$ protein-coupled receptors, $G_{q/11}$ protein α subunits, PLCβ, and DGLα is well orchestrated at particular synaptic and neuronal surface, and will be the molecular-anatomical basis for Ca^{2+}-assisted RER.

Interestingly, the degree of spine accumulation of DGLα as well as its fine localization within spines varies among neurons. In Purkinje cells, DGLα is excluded from spine head; it is present at the highest density in spine neck and also distributed on the dendritic surface (575) (Fig. 11). In contrast, DGLα is highly concentrated on spine head in hippocampal pyramidal cells, whereas it is low on the dendritic surface (264, 575). In striatal medium spiny neurons, DGLα distributed widely on the somatodendritic membrane in the order of spine $>$ dendritic shaft $>$ and soma (528) (Fig. 12). Expression of CB_1 is generally higher at inhibitory synapses than at excitatory synapses. However, the strength of CB_1 expression and the types of presynaptic elements with high CB_1 expression are variable depending on brain regions (263, 267). Therefore, fine localization of DGLα seems to be unique to each brain region so that the induction threshold of 2-AG-mediated retrograde suppression of excitation and inhibition may be coordinated.

F. *N*-acyl-phosphatidylethanolamine-hydrolyzing Phospholipase D

N-acyl-phosphatidylethanolamine-hydrolyzing phospholipase D (NAPE-PLD) mRNA is expressed at the highest levels in granule cells of the dentate gyrus (141, 390). In addition, low to moderate expression is seen in CA3 pyramidal cells of the hippocampus, superficial layers of the neocortex, piriform cortex, olfactory bulb, and several thalamic and hypothalamic nuclei. Different from DGLα, NAPE-PLD is distributed in presynaptic elements at the highest levels in mossy fibers projecting to CA3 pyramidal cells, although mossy fibers do not express CB_1. Within mossy fibers, NAPE-PLD is localized predominantly on the sER, an intracellular Ca^{2+} store. Intense labeling is also detected in vomeronasal nerves projecting to the accessory olfactory bulb. Since catalytic activity of NAPE-PLD is Ca^{2+}-dependent, Ca^{2+} release from intracellular store may facilitate anandamide synthesis. Indeed, in neurons of the dorsal root ganglion, Ca^{2+} mobilization from intracellular store was reported to trigger anandamide synthesis and result in amplification of Ca^{2+} influx through TRPV1 channels (533). Therefore, the sER-associated distribution of NAPE-PLD may contribute to short-term facilitation of transmitter release (390). Distribution of NAPE-PLD in axons (141, 390) suggests that anandamide and other related *N*-acylethanolamines may be synthesized in presynaptic elements and function as an anterograde messenger. However, in contrast to these reports of exclusive axonal distribution of NAPE-PLD, Cristino et al. (102) reported intense NAPE-PLD labeling in neuronal perikarya and proximal dendrites of hippocampal pyramidal cells and cerebellar Purkinje cells.

G. Monoacylglycerol Lipase

Monoacylglycerol lipase (MGL) mRNA is widely expressed in the brain with higher levels in the cerebral cortex, hippocampal CA3 region, anterior thalamus, and cerebellar granular layer (128). MGL immunoreactivity was shown to be distributed as dense punctate stainings in the neuropil of the hippocampus, amygdala, and cerebellar cortex, which are most likely axon terminals (128, 188). Immunoelectron microscopy confirmed its presence in axon terminals forming asymmetrical and symmetrical synapses onto MGL-negative somata and dendrites of principal neurons and interneurons (188).

In the hippocampus, MGL appears to be expressed in axon terminals with varying amounts (188). Stronger MGL immunoreactivity was seen in pericellular baskets around principal neurons and hilar neurons, and in mossy fiber terminals of dentate gyrus granule cells onto thorny excrescences of CA3 pyramidal cells. In contrast, although terminals of Schaffer collaterals innervating CA1 pyramidal cells express MGL, terminals of CA1 pyramidal cells innervating CA1 interneurons lack MGL. Interestingly, MGL is expressed not only in CCK(+)/CB_1(+) basket cell terminals, but also in parvalbumin(+)/CB_1(−) basket cell terminals. From the presynaptic localization at various terminals, it is conceivable that MGL limits spatial and temporal extents of 2-AG, which is released from postsynaptic neurons to the extracellular space. Hashimotodani et al. (207) have shown in the hippocampus that presynaptic MGL not only hydrolyzes 2-AG released from activated postsynaptic neurons but also contributes to degradation of constitutively produced 2-AG and prevention of its accumulation around presynaptic terminals. Thus the MGL activity determines basal endocannabinoid tone and terminates retrograde endocannabinoid signaling.

H. Fatty Acid Amide Hydrolase

Fatty acid amide hydrolase (FAAH) is widely distributed in the brain showing overlap with CB_1 in many regions (139, 188). However, FAAH is almost absent in the globus pallidus and SNr, where CB_1 is present most abundantly (139). FAAH is selective to somatodendritic elements of principal neurons, but not of interneurons, in various brain regions (102, 188, 521). This expression pattern is complementary to that of CB_1 and MGL, which are abundant in interneurons and expressed in axons and terminals. Immunoelectron microscopy showed that most immunogold particles for FAAH were located intracellularly, being mostly on intracellular Ca^{2+} stores (i.e., mitochondria and sER) and the rest (10%) on the somatodendritic cell membrane (188). The almost complementary distributions of FAAH and MGL, together with distinct distribution of NAPE-PLD and DGL, suggest that anandamide and 2-AG signaling may subserve distinct functions that are spatially segregated.

I. Cyclooxygenase-2

COX-2 mRNA is mainly expressed in the cerebral cortex, hippocampus, and amygdala with low levels in the striatum, thalamus, and hypothalamus (569). Its expression was shown to be enhanced by NMDA receptor-mediated synaptic activity and by high-frequency stimulation associated with LTP induction, and suppressed by glucocorticoids (569). COX-2 is expressed in excitatory neurons and localized in somata, dendrites, and spines (48, 102, 265). The postsynaptic distribution of COX-2 suggests the possibilities that COX-2 determines the basal endocannabinoid tone by degrading postsynaptically produced 2-AG, and that COX-2 restrict spatial and temporal extents of endocannabinoid signaling by degrading presynaptically produced anandamide and other related *N*-acylethanolamines.

J. Organization of 2-AG Signaling Molecules in the Cerebellum, Hippocampus, and Striatum

The histochemical evidence mentioned above highlights a well-orchestrated arrangement of 2-AG signaling molecules, which fits with the role of endocannabinoids in retrograde suppression of transmitter release (285, 314, 394, 564). Furthermore, the molecular arrangement appears to be fine-tuned depending on brain regions (Fig. 13).

At Purkinje cell synapses (Fig. 13*A*), mGluR1 and PLCβ4/3 highly accumulate at the perisynaptic region of the spine head (372, 385, 389), while DGLα is rather excluded from the spine head and concentrated densely at the base of spine neck (575). This molecular arrangement suggests that the base of spine neck is the major site of 2-AG synthesis following activation of excitatory synapses in Purkinje cells. CB_1 accumulation at the perisynaptic portion of PFs (267, 391, 575) as well as MGL distribution in their terminals (128, 188) will be important for increasing the efficiency of 2-AG signaling and for controlling its specificity and spatiotemporal extents. Moreover, higher CB_1 density at inhibitory synapses than at PF synapses (267), together with DGLα expression on dendritic shafts (575), would help retrograde suppression occur at nearby inhibitory synapses that are formed on dendritic shafts apart from the major site of 2-AG synthesis. This structured molecular arrangement is consistent with the electrophysiological data for mGluR1-dependent eCB-STD/LTD at Purkinje cell synapses.

At hippocampal pyramidal cell synapses (Fig. 13*B*), the 2-AG synthetic machinery consisting of mGluR5, PLCβ1, and DGLα is concentrated in the spine head (264, 575). On the other hand, CB_1 is expressed at very high levels in inhibitory terminals of CCK-positive basket cells, whereas it is considerably low in excitatory terminals (263, 267, 520). The induction threshold of DSI is much lower than that of DSE in the hippocampus (399). Taking the anatomical and electrophysiological properties into account, 2-AG-mediated suppression of hippocampal synapses is targeted primarily to inhibitory inputs by CCK-positive interneurons, and then to excitatory inputs by pyramidal cells. Furthermore, low CB_1 levels at excitatory synapses may lead to selective induction of DSE at the activated synapse.

In the striatum (Fig. 13*C*), mGluR5, M_1, and DGLα are widely distributed on the somatodendritic surface of MSNs, with the density being in the order of spine $>$ dendrite $>$ soma for mGluR5 and DGLα, and of dendrite $=$ soma $>$ spine for M_1 (376, 528). CB_1 is expressed in three striatal synapses: corticostriatal excitatory synapse, MSN-MSN inhibitory synapse, and parvalbumin interneuron-MSN inhibitory synapse (528). At corticostriatal synapses, coincidental depolarization and mGluR activation are required for eCB-STD/LTD (282, 375), presumably due to low CB_1 levels in corticostriatal afferents (528). Induction of mGluR-enhanced DSE is further facilitated by coactivation of mAChR, although mAChR coactivation alone cannot enhance DSE (528). This DSE facilitation mechanism will lead to the suppression of MSN's hyperactivity. At MSN-MSN and parvalbumin interneuron-MSN synapses, DSI can be induced by depolarization alone (377), due to high CB_1 levels in these inhibitory afferents. Nevertheless, coactivation of mGluR or mAChR robustly enhances DSI (528), which should lead to the increase of MSN's excitability and striatal output.

Thus the detailed histochemical examinations of the cerebellum, hippocampus, and striatum have clarified how receptors and enzymes involved in 2-AG-mediated

retrograde signaling are organized around synapses. Although fine subcellular localizations are different depending on synaptic organization of each brain region, these molecules appear to be arranged so that neuronal excitability can be controlled efficiently by 2-AG-mediated retrograde signaling.

IX. PHYSIOLOGICAL ROLES OF THE ENDOCANNABINOID SYSTEM

In this section, we briefly introduce the behavioral studies that examined physiological roles of the endocannabinoid system in the CNS of living animals. The endocannabinoid system plays important roles in various aspects of neural functions including learning and memory, anxiety, depression, addiction, appetite and feeding behavior, pain, and neuroprotection. We describe *1*) how the neural function is altered when the endocannabinoid system is enhanced or blocked in laboratory animals, *2*) which brain region(s) are primarily responsible for the actions of endocannabinoids, and *3*) what will be the significance of the phenomena from a clinical point of view. For more information, see the specialized reviews cited in each section.

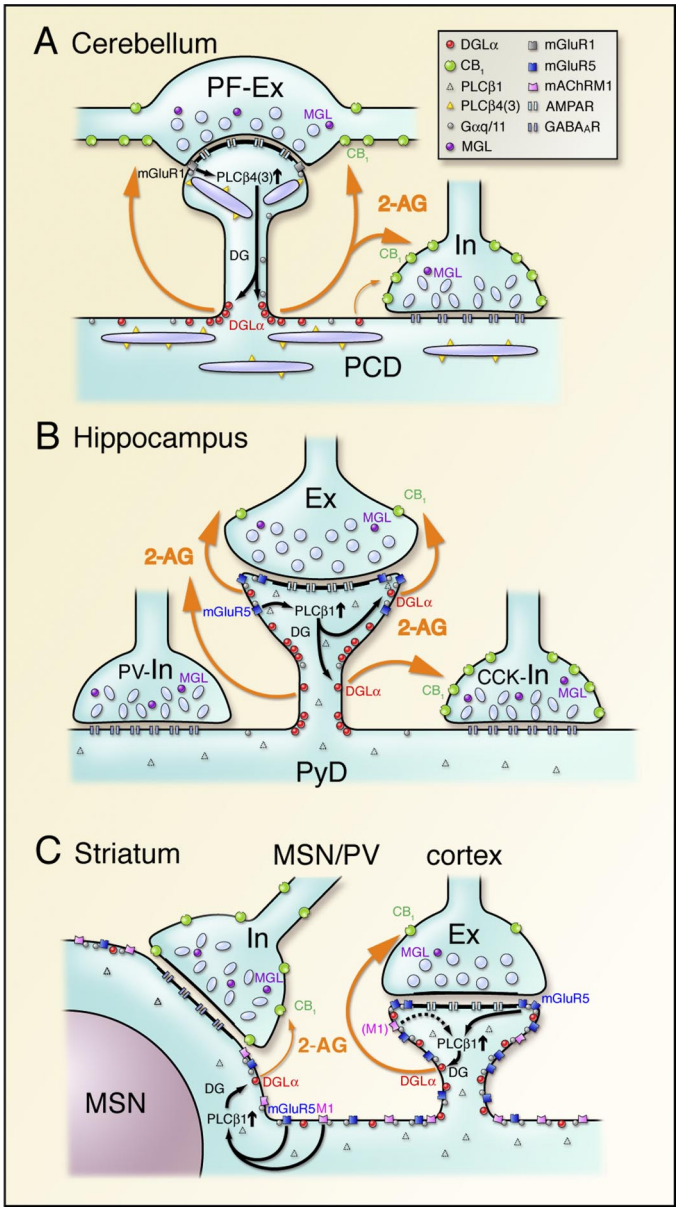


FIG. 13. Schematic diagrams showing the organization of signaling molecules for endocannabinoid-mediated synaptic modulation in the cerebellum, hippocampus, and striatum. *A*: cerebellar Purkinje cell. Following mGluR1 activation, diacylglycerol (DAG) is produced by PLCβ4 and, presumably, PLCβ3 at the spine head and diffuses to the spine neck, where DAG is converted to 2-AG by DGLα. 2-AG is then released, and activates CB_1 receptors that are located on perisynaptic region of parallel fiber terminals (PF-Ex) or nearby inhibitory terminals (In). PCD, Purkinje cell dendrite. *B*: CA1 pyramidal cell. DGLα is distributed in the spine head and neck, where 2-AG is produced and released. 2-AG then activates CB_1 receptors located on excitatory (Ex) or CCK-positive inhibitory terminals (CCK-In). The density of CB_1 receptors is extremely high at CCK-positive inhibitory terminals compared with excitatory terminals. PyD, pyramidal cell dendrite; PV-In, parvalbumin-positive inhibitory terminal. *C*: striatal medium spiny neuron (MSN). 2-AG is produced in the somatodendritic surface of MSN and travels to activate presynaptic CB_1 receptors. The density of CB_1 receptors is low at corticostriatal excitatory terminals and high at inhibitory terminals that are derived from either MSN or PV-containing interneurons. Note that relevant signaling molecules are arranged to modulate the transmission at corticostriatal, MSN-MSN, and PV interneuron-MSN synapses in the striatum. Presynaptic localization and relative levels of MGL in the cerebellum and striatum are based on our unpublished data. [Modified from Yoshida et al. (575) and Uchigashima et al. (528).]

A. Learning and Memory

It has long been recognized that Δ^9-THC intake causes memory impairment in humans. In laboratory animals, effects of exogenously applied cannabinoid agonists on learning and memory have been intensively investigated using various behavioral paradigms (109, 301). These studies have revealed that in cannabinoid-treated animals, certain aspects of memory are impaired, while other aspects are largely intact. In general, short-term memory or working memory is highly sensitive, whereas retrieval of previously learned information is resistant to cannabinoids. Disruptive effects of cannabinoid agonists have been reported in various behavioral paradigms (301), including the Morris water maze, fear conditioning, and eyeblink conditioning, which are discussed in the following subsections.

1. Spatial memory

The Morris water maze is one of the most widely used spatial learning tasks, and known to be sensitive to disruptions of hippocampal functions. In this task, the subjects (rats or mice) are required to navigate in a water

pool to locate a hidden platform by learning its position relative to visual cues. In a standard version, the hidden platform remains in a fixed location between trials. In a working memory version, the location of the platform is changed before each session. Systemic administration of cannabinoid agonists impairs the learning in both the fixed hidden platform task (156) and the working memory version (539), the latter being more sensitive to cannabinoid agonists. The doses of Δ^9-THC required for disruption of the performance are much lower for the working memory version than for the fixed platform version (539).

Effects of CB_1 blockade on spatial memory have been examined by using genetic and pharmacological tools. The performance in the fixed hidden platform task was intact in CB_1-knockout mice (540) and in the wild-type mice that were treated with the CB_1 antagonist SR141716 (538). However, when the platform was moved to a new place after the mice had acquired the task, marked differences in behavior became evident between the wild-type and the CB_1 knockout mice. When the platform was moved to the opposite side of the pool, the wild-type mice gradually ceased returning to the previous platform location and readily learned the new location. In contrast, the CB_1-knockout mice continued to return to the previous location and exhibited a significant deficit in learning the new location, suggesting the impairment of extinction process (540). In a later study by the same group, extinction of spatial learning was examined by removing the platform, and compared between the control and the CB_1-disrupted mice (538). The data showed that both the CB_1-knockout mice and SR141716-treated wild-type mice exhibited deficits in extinction processes when a mild extinction procedure was used.

These data suggest that the endocannabinoid system is involved in the extinction of spatial memory. Contribution of the endocannabinoid system in the hippocampus is supported by a recent study (438). From a clinical aspect, these studies give a warning to the use of cannabinoid agonists and antagonists to human patients, since it may have adverse side effects on hippocampal functions.

2. Aversive memory

Fear conditioning is widely used to study aversive memory in laboratory animals. In this paradigm, a conditioned stimulus (e.g., context or tone) is paired with a punishment (e.g., foot shock). After conditioning, the animal shows fear response such as freezing when reexposed to the conditioned stimulus, indicating acquisition of aversive memory (acquisition of aversive memory). The fear response is extinguished gradually when the conditioned stimulus is applied repeatedly without the punishment (extinction of aversive memory).

Systemic administration of the cannabinoid agonist WIN55,212-2 (2.5 and 5 mg/kg) impaired the acquisition of contextual, but not auditory, fear conditioning in rats (410). While auditory fear conditioning requires the basolateral amygdala, contextual fear conditioning is known to depend on the hippocampus (10). Therefore, this study suggests that WIN55,212-2 selectively affects acquisition of the hippocampus-dependent aversive memory in rat. The same group found that administration of WIN55,212-2 facilitated the extinction of contextual fear conditioning at a low dose (0.25 mg/kg), but disrupted it at a higher dose (2.5 mg/kg) in rats (409). As for blocking endocannabinoid signaling, studies with pharmacological or genetic disruption of CB_1 have consistently demonstrated impaired extinction of aversive memory. In an auditory fear conditioning paradigm, CB_1-knouckout mice showed strongly impaired extinction, with normal acquisition of the fear memory (330). The CB_1 antagonist SR141716A (3 mg/kg) similarly impaired the extinction in wild-type mice when injected subcutaneously just before the first extinction trial, whereas SR141716A failed to affect the acquisition as well as extinction when applied before conditioning. This result indicates that CB_1 receptors are required at the moment of memory extinction. The extinction of contextual fear conditioning in mice was also suppressed by systemic administration of SR141716A (1–10 mg/kg ip) (501). In fear conditioning with light, systemic administration of SR141716A (1.5–5 mg/kg ip) just before extinction trials impaired the extinction in rats (87). All these studies support that the endocannabinoid system is crucially involved in the extinction of conditioned fear. However, it is not well understood how CB_1 receptors mediate the extinction. Extinction of aversive memory involves at least two distinct processes, i.e., learning of the association between the conditioned stimulus and the absence of punishment ("associative safety learning") and habituation to a repeatedly presented stimulus. Involvement of CB_1 receptors in habituation-like processes, rather than associative safety learning, are suggested by the study using auditory fear conditioning paradigm in mice (255).

The basolateral amygdala is known to control extinction of conditioned fear. Therefore, it is most likely that the endocannabinoid system in the basolateral amygdala itself is involved in this process. Several lines of evidence support this possibility. First, the CB_1 receptor is highly expressed in the basolateral amygdala (87, 261, 329). Second, a conditioned stimulus (tone) during extinction trials elevates levels of endocannabinoids (anandamide and 2-AG) in the basolateral amygdala (330). Third, endocannabinoid-mediated synaptic plasticity can be induced by neural activity in the basolateral amygdala (330).

From a clinical point of view, these studies suggest that drugs activating the endocannabinoid system may be useful for the treatments of psychiatric disorders related to retrieval of fear memories, including panic disorders, phobias, and posttraumatic stress disorder (PTSD). Strategies to activate the endocannabinoid system in the brain

include administration of exogenous cannabinoid agonists and elevating endocannabinoid levels in the brain by inhibiting endocannabinoid degradation and uptake. In fact, administration of the anandamide transport inhibitor AM404 (10 mg/kg) before extinction trials facilitated the extinction of light-conditioned fear in rats (87). It is tempting to speculate that drugs with similar action to that of AM404 but with no serious side effects will be developed and used for the treatments of psychiatric disorders.

3. Eyeblink conditioning

Contribution of the endocannabinoid system to motor learning was demonstrated by using CB_1-knockout mice and CB_1 antagonists (277). Classical eyeblink conditioning can be categorized into two types: delay and trace paradigms. The delay paradigm is a test for cerebellum-dependent discrete motor learning (511), whereas the trace paradigm is a form of hippocampus-dependent associative learning (277, 560). In the delay paradigm, a brief periorbital electrical shock (unconditioned stimulus, US) is applied during a tone with a longer duration (as conditioned stimulus, CS) such that the two stimuli terminate simultaneously. In the trace paradigm, the US is started 500–750 ms after termination of the CS. The study demonstrated that the delay paradigm of eyeblink conditioning, but not the trace version, is severely impaired in CB_1-knockout mice. Systemic administration of SR141716A (3 mg/kg ip) 20 min before the daily training caused severe impairment in acquisition but not extinction of the delay eyeblink conditioning.

These results are in line with the electrophysiological data that cerebellar LTD at PF-Purkinje cell synapses, which plays a crucial role in discrete motor learning (244), is dependent on the endocannabinoid system (455). It is most likely that the impaired delay eyeblink conditioning in CB_1-knockout mice is attributable to the deficiency in cerebellar LTD. In consistency with this possibility, many animal models with deficient cerebellar LTD, including mGluR1-knockout mice (5, 275) and PLCβ4-knockout mice (276, 358), display impairment of delay eyeblink conditioning.

B. Anxiety

Anxiety is an emotional response to dangerous situations. Transient anxiety elicits an appropriate response such as escape and is of fundamental importance for survival. Anxious states are controlled by a complex system consisting of inhibitory and facilitatory mechanisms. Many neurotransmitters and modulators are involved in the control of anxiety responses, and agents acting on GABAergic and serotonergic systems are currently used for treating anxiety disorders (355). There is an increasing interest in the relationship between cannabinoids and anxiety. Recent studies with experimental animals and humans have suggested the involvement of the endocannabinoid system in the regulation of anxious states (547).

Effects of cannabinoid agonists on anxiety have been examined in rats and mice, by using several different paradigms including the elevated plus-maze, light-dark crossing, vocalization, and social interaction tests. The obtained results are complex and often contradictory. Effects of cannabinoids are dependent on the environmental context, but generally anxiolytic at low doses and anxiogenic at high doses (547). Possible involvement of the opioid system in these effects is suggested. In the light-dark crossing test, anxiolytic effects of Δ^9-THC, which was shown to be CB_1 dependent, was abolished by the μ-opioid antagonist β-funaltrexamine and the δ-opioid antagonist naltrindole, but not by the κ-opioid antagonist nor-binaltorphimine (34). In contrast, anxiogenic effects of CP55,940 in the plus-maze test were abolished by the κ-opioid antagonist nor-binaltorphimine, but not by either the μ-opioid antagonist cyprodime or the δ-opioid antagonist naltrindole (326). Thus it is likely that the anxiolytic and anxiogenic effects of cannabinoids are mediated by distinct mechanisms.

Physiological roles of the endocannabinoid system in the regulation of anxiety have been studied by using CB_1 antagonists and CB_1-knockout mice. The CB_1 antagonist SR141716 produced anxiogenic effects in the elevated plus-maze and vocalization tests (12, 349, 379). Similarly, the CB_1-knockout mice showed an anxiogenic-like behavior in the elevated plus-maze, light-dark crossing, and social interaction tests (197, 334, 531). These results provide evidence for the presence of endogenous anxiolytic cannabinoid tone. Interestingly, actions of anxiolytic drugs such as bromazepam and buspirone were impaired in the CB_1-knockout mice (531). The impaired actions of buspirone, a partial agonist for the 5-HT_{1A} receptor, suggest the interaction between the cannabinoid and serotonergic systems for regulating anxiety (324).

How the endocannabinoid system regulates anxious states is not clearly determined. It was suggested that endocannabinoids, especially anandamide, might be generated in the amygdala during anxiety and regulate emotional states by influencing amygdala outputs (169). This hypothesis is supported by the finding that endocannabinoid level in the basolateral amygdala was elevated in response to anxiogenic situations (330). In accordance with this hypothesis, pharmacological blockade of FAAH by URB597 and URB532, which elevated brain anandamide levels, produced anxiolytic effects in a CB_1-depenent manner (260). Thus agents acting on FAAH and other molecules involved in the endocannabinoid system may have a therapeutic potential for anxiety-related disorders.

C. Depression

Depression is a prevalent neuropsychiatric disorder and is a high-risk factor of suicide (381). Monoamine neurotransmitters are known to be associated with depression, and antidepressants that have the ability to enhance the monoamine systems are widely used for the treatment of depression (381). There is accumulating evidence showing the relationship between the endocannabinoid system and depressive disorders. For example, depressive disorder in Parkinson's disease was reported to be related to polymorphisms of the CB_1 gene (20). Moreover, many pharmacological studies in laboratory animal have revealed the importance of the endocannabinoid system in depression-like responses, which has been reviewed in detail (546, 565).

Activation of CB_1 receptors exhibits an antidepressant activity. In the forced-swim test (FST) applied to the rat, the CB_1 agonist HU210 (5–25 μg/kg ip) and the anandamide-transporter inhibitor AM404 (5 mg/kg) mimicked the effect of desipramine (a positive antidepressant control), which reduced immobility duration (220). This antidepressant effect of HU210 and AM404 was reversed by the CB_1 antagonist AM251. In this study, administration of AM251 (1–5 mg/kg) alone had no significant effect on immobility. The antidepressant response caused by the enhancement of CB_1 signaling might be related to the results that activation of CB_1 receptors by WIN55,212-2 or Δ^9-THC elevated norepinephrine (405) and dopamine (424) levels in the frontal cortex, and also increased the firing activity of locus coeruleus noradrenergic neurons (370).

Interestingly, microdialysis studies demonstrated that the administration of CB_1 antagonists also increased the levels of monoamines including 5-HT, dopamine, and norepinephrine in the prefrontal cortex (525). As expected from these neurochemical effects, CB_1 antagonists exerted antidepressant-like behavioral effects in animal models. In tail-suspension test (TST) and FST applied to the mouse, which are sensitive to antidepressant compounds, AM251 significantly reduced immobility at 10 mg/kg in the TST and at 1–10 mg/kg in the FST (467). Effects of AM251 in the FST were absent in CB_1-knockout mice. In the FST applied to the rat, SR141716A (3 mg/kg ip) decreased immobility (525).

These results suggest that antidepressant-like effects of CB_1 antagonists and agonists may be associated with changes in the activity of monoaminergic pathways. Further exploration of functional relationships between the endocannabinoid and monoamine systems will be essential for understanding the pathophysilogy of depressive disorders. Importantly, the neurochemical and behavioral effects of CB_1 agonists are not opposite to those of CB_1 antagonists in animal studies. One possibility is that effects of these agents might be biphasic, depending on the dose and experimental conditions, as observed in the case of anxiety.

In humans, the CB_1 antagonist SR141716A (rimonabant) was reported to increase the incidence of depression and suicide. At clinical levels, rimonabant has been approved in several countries as an antiobesity agent. A meta-analysis of four double-blind, randomized controlled trials (including 4105 participants) reported that patients given rimonabant (20 mg/day) were 2.5 times more likely to discontinue the treatment because of depressive mood disorders than those given placebo (91). Moreover, the United States Food and Drug Administration reported an increased risk of suicide attempts or suicidal ideation in participants given rimonabant (20 mg/day) compared with placebo (odds ratio 1.9) (356). Further preclinical and clinical studies are needed to evaluate these adverse effects.

D. Addiction

Numerous studies have suggested the involvement of the endocannabinoid system in addiction. There are a number of excellent reviews on this issue (112, 150, 290, 323). Drug addiction is characterized by long-lasting motivational disturbances leading to compulsive drug seeking and drug craving. Compounds that lead to addictive behavior include alcohol, nicotine, opioids, psychostimulants, and cannabinoids. These compounds interact with common neural circuits in the brain and cause dysregulation of brain motivational and reward pathways. Major components of the brain reward circuit are the VTA, which contains cell bodies of dopaminergic neurons, and their terminal regions in the basal forebrain, which include the NAc, amygdala, and prefrontal and limbic cortices.

In animal models, the endocannabinoid system has been shown to be crucial for rewarding effects of some addictive compounds, including nicotine, ethanol, and morphine. In a conditioned place preference paradigm, nicotine (0.5 mg/kg sc) produced a significant rewarding effect in wild-type mice, but not in CB_1-knockout mice (69). The CB_1 antagonist SR141716A reduced nicotine self-administration at 0.3–1 mg/kg (93) and nicotine-induced conditioned place preference at 1–3 mg/kg (291) in rats. In a two-bottle free-choice paradigm, ethanol preference of young wild-type mice was reduced by SR141716A (3 mg/kg ip) to the level observed in their CB_1-knockout littermates (554). Morphine self-administration was abolished in CB_1-knockout mice (292). All these studies indicate that the endocannabinoid system is involved in rewarding effects of these addictive compounds.

Mechanisms of action of psychostimulants might differ from those of other drugs. Effects of CB_1 blockade on rewarding properties of cocaine were different in differ-

ent paradigms (323). Cocaine-induced conditioned place preference was not modified in CB_1-knockout mice. Moreover, cocaine self-administration was not modified in CB_1-knockout mice and the rats treated with SR141716A. When the effort required to obtain a cocaine infusion was enhanced, however, acquisition of an operant response to self-administrable cocaine was impaired in CB_1-knockout mice (482). One possible explanation is that the endocannabinoid system does not participate in the primary reinforcing effects of psychostimulants, but is important for maintaining psychostimulant-seeking behavior.

It is unclear how the endocannabinoid system contributes to drug-rewarding effects. One possibility is that it might modulate the activity of mesolimbic dopaminergic pathway. This possibility is supported by the findings that elevation of extracellular dopamine level in the NAc by nicotine or alcohol was blocked in SR141716A-treated rats (93) and CB_1-knockout mice (240).

Clinical and preclinical studies have suggested that CB_1 antagonists might be useful for the treatment of addictive behaviors. The pooled data from three randomized controlled trials showed that the rate of quitting smoking at 1 year was higher in participants given rimonabant (20 mg/day) compared with placebo (odds ratio 1.61) (62). These results suggest that CB_1 receptors may be a new target for treating tobacco addiction.

E. Appetite and Feeding Behavior

Appetite stimulation is known to be one of the most notable effects of cannabis in humans. In laboratory animals, cannabinoids increase food intake in a CB_1-dependent manner. In these studies, cannabinoids were applied at low doses so that they did not elicit sedation that might suppress feeding behavior (407). Conversely, CB_1 antagonists reduced food intake in wild-type and genetically obese animals (407), but not in CB_1-knockout mice (120). These results indicate that the endocannabinoid system is involved in control of feeding behavior through activation of CB_1 receptors (121).

Although precise mechanisms are not fully understood, it has been proposed that the endocannabinoid system is involved in control of food intake at multiple levels. The endocannabinoid system may function at the mesolimbic level to modulate the motivation for food intake. It may interact with orexigenic (e.g., orexins) and anorexigenic (e.g., leptin) mediators at the hypothalamic level. This hypothesis is supported by the following results. Fasting increased endocannabinoid levels in the limbic forebrain and hypothalamus (274), and administration of 2-AG into the NAc stimulated feeding behavior (274). Feeding behavior induced by Δ^9-THC was attenuated by a D_1 antagonist (543). Leptin decreased endocannabinoid levels in the hypothalamus (120), and administration of anandamide into the ventromedial hypothalamus induced hyperphagia in a CB_1-dependent manner (246). In addition to these cannabinoid actions at central levels, the peripheral endocannabinoid system may also play a role in controlling feeding behavior. The CB_1 receptor is present in several peripheral organs, including the thyroid gland, adrenal gland, adipocytes, and gastrointestinal tract (407). These peripheral CB_1 receptors may participate in the regulation of body weight through controlling feeding behavior and food intake-independent metabolic functions (407).

Preclinical studies with animal models and clinical trials have confirmed that the CB_1 receptor is a promising target for treating appetitive disorders and obesity. Δ^9-THC or its synthetic analog has been approved as antinausea and antiemetic medications for patients treated with anticancer drugs and as an appetite stimulant for patients with human immunodeficiency virus (HIV)-induced wasting syndrome or patients suffering from Alzheimer's disease in some countries. Comprehensive studies on HIV patients with wasting syndrome have shown that the drug consistently promotes appetite and energy intake in association with a marked improvement in mood (407). Several selective CB_1 antagonists are currently in advanced preclinical or clinical trials, and rimonabant (SR141716A) has been approved as a weight-management drug in some markets. Multiple, large, randomized, and placebo-controlled international trials in Europe and North America provide clear evidence that rimonabant facilitates weight loss at a therapeutic dose (91, 541).

F. Pain

Antinociceptive effects of cannabinoids have been widely described and reviewed (97, 231, 248, 550). With the use of different types of noxious stimulation, it has been demonstrated in animal models that cannabinoids are comparable to opiates both in potency and efficacy. The systemic administration of cannabinoids profoundly suppresses behavioral reactions to acute noxious stimuli, inflammatory pain, and nerve injury, mostly through activation of CB_1 receptors. The suppression of pain behavior is not attributable to motor dysfunction but to the suppression of nociceptive transmission itself, which is supported by electrophysiological and neurochemical studies. Exogenously administered cannabinoids could elicit antinociceptive effects at peripheral, spinal, and supraspinal levels. Local injections of cannabinoid agonists to various brain regions have been used to identify supraspinal sites of cannabinoid-induced antinociception. In the tail-flick test or Formalin-evoked pain-related behavior, antinociception was induced by the microinjection into the brain regions including the dorsolateral periaqueductal gray (PAG), dorsal raphe nucleus, rostral ventromedial medulla (RVM), and amygdala (231).

Participation of the endocannabinoid system in endogenous pain modulation has been demonstrated by the studies with pharmacological methods. The CB_1 antagonist SR141716A induces hyperalgesia in the Formalin test (66, 491) and hot plate test (432) and blocks the analgesia produced by electrical stimulation of the dorsal PAG (549), indicating the contribution of endocannabinoids to analgesia. Involvement of the endocannabinoid system was also demonstrated in a certain form of stress-induced analgesia (SIA) (232). SIA is an adaptive response to stress, and expressed as suppression of pain sensation. It depends on brain pathways from the amygdala to PAG, RVM, and the dorsal horn of the spinal cord (231). SIA includes opioid-dependent and -independent forms, which are induced by different stressor parameters. Opioid antagonists blocked the SIA when foot shock was delivered intermittently for 30 min, but not when a continuous foot shock for 3 min was used (300). This nonopioid SIA was blocked by the CB_1 antagonist SR141716A but not by a CB_2 antagonist (232). Several experimental data suggest that the endocannabinoid system in the PAG plays a pivotal role in the nonopioid SIA. First, the microinjection of SR141716A into the dorsolateral PAG produced the greatest suppression of SIA among all the sites tested. Second, endocannabinoid levels were elevated in dorsal midbrain fragments containing the PAG during the SIA. Third, microinjection of the MGL inhibitor URB602 or the FAAH inhibitor URB597 into the PAG elevated the corresponding endocannabinoid level in this region, and the microinjection enhanced SIA in a CB_1-dependent manner (232). Although the PAG plays a major role in the SIA as well as in the descending control of pain, the coordinated release of endocannabinoids in the RVM and spinal cord was also suggested to contribute to the nonopioid SIA (231). In animal models of neuropathic pain, the endocannabinoid levels in the PAG, RVM, and spinal cord were reported to increase, suggesting that the endocannabinoid system in these regions may play important roles in endogenous pain modulation (248).

Recent studies have clarified the antinociceptive role of the endocannabinoid system, similar to the opioid system. Therefore, the molecules involved in endocannabinoid signaling, such as CB_1 receptor, FAAH, MGL, and COX-2 may be promising targets for developing antinociceptive drugs. Clinical trials of cannabis-based medicines have suggested that cannabinoid agonists are effective in reducing pain in humans (14, 441). Inhibiting endocannabinoid degradation is another approach, which can maximize the effects of endocannabinoids only in the regions where they are produced and released, and thereby minimize the side effects associated with global CB_1 activation (231). The FAAH inhibitor URB597, which has been studied most intensively, was shown to induce analgesia without toxicity in preclinical safety studies with rats and monkeys (451).

Importantly, possible involvement of cannabinoid receptors in the actions of clinically used drugs has been suggested (163). Nonsteroidal anti-inflammatory drugs have antinociceptive effects, which cannot be completely explained by inhibition of prostaglandin synthesis. In the Formalin test, indomethacin-induced spinal antinociception was blocked by coadministration of the CB_1 antagonist AM251 and was absent in CB_1-knockout mice (187). The antinociceptive effect of intrathecally injected flurbiprofen, another nonsteroidal anti-inflammatory drug, was also reversed by coadministration of AM251 (15). These results suggest that endocannabinoids may play a major role in mediating antinociception induced by nonsteroidal anti-inflammatory drugs at the spinal level. In a rat model of neuropathic pain, the peripheral antinociceptive effects of paracetamol were inhibited by both the CB_1 antagonist AM251 and the CB_2 antagonist AM630 (105). In the hot plate test, the antinociceptive effect of intraperitoneally injected cocaine was completely reversed by the CB_1 antagonist SR141716A (159). Mechanisms of these cannabinoid-dependent actions of drugs remain to be elucidated.

G. Neuroprotection

Exogenous and endogenous cannabinoids have been shown to exert neuroprotection in a variety of in vitro and in vivo models of neurodegeneration (533). In transient global or permanent focal cerebral ischemia of adult rats, the cannabinoid agonist WIN55,212-2 (0.1–1 mg/kg) decreased hippocampal neuronal loss or infarct volume. These protective effects were reversed by the CB_1 antagonist SR141716A (1 mg/kg), which had no effect when administered alone (371). In a rat model of neonatal hypoxic-ischemic encephalopathy, administration of WIN55,212-2 (0.1 mg/kg) after the hypoxia-ischemia procedure reduced the final necrotic area. This protective effect was reversed by coadministration of either the CB_1 antagonist SR141716A (3 mg/kg) or the CB_2 antagonist SR141588 (2 mg/kg) (155). In closed head injury of mice, exogenously applied 2-AG (0.1–10 mg/kg) reduced brain edema, infarct volume, and hippocampal death and improved clinical recovery (412). The protective effects of 2-AG were attenuated by SR141716A (20 mg/kg) (412), and absent in CB_1-knockout mice (411). The spontaneous recovery from behavioral deficits after closed head injury was extremely slow in CB_1-knockout mice, compared with wild-type mice, indicating a role of the endocannabinoid system in the recovery after closed head injury (411). The threshold of kainic acid (KA)-induced excitotoxicity was also elevated in CB_1-knockout mice (328).

What are the mechanisms through which exogenous and endogenous cannabinoids protect neural cells from various insults? In a mouse model of KA-induced excito-

toxicity, the neuron types that contributed to neuroprotection through activation of their CB_1 receptors have been determined. By conditional deletion of the CB_1 gene either in cortical glutamatergic neurons or in forebrain GABAergic neurons as well as by virally induced deletion of the CB_1 gene in the hippocampus, Monory et al. (359) demonstrated that the presence of CB_1 receptors in glutamatergic hippocampal neurons is both necessary and sufficient to protect neurons against KA-induced seizures. Therefore, suppression of glutamate release through activation of presynaptic CB_1 receptors seems to be a major action of cannabinoids for neuroprotection against KA-induced seizures. Other mechanisms have also been suggested to be involved in other pathological conditions. The possible mechanisms include the regulation of PI3K/Akt/GSK-3 signaling pathway (406), an enhancement of brain microcirculation (182), and control of microglial function (144).

Preclinical studies with animal models have suggested the therapeutic potential of cannabinoids for the treatment of neurodegenerative diseases, including multiple sclerosis (103), traumatic brain injury (144, 155), Huntington's disease (354), and Alzheimer's disease (354).

X. CONCLUSIONS

Since the discovery of the first cannabinoid receptor in 1990, our understanding of the endocannabinoid system has been markedly expanded. Biochemical studies have identified anandamide and 2-AG as major endocannabinoids and characterized the enzymes involved in generation and degradation of endocannabinoids, some of which have been cloned successfully. Electrophysiological studies with slice and culture preparations have revealed functional roles of endocannbinoids in short-term and long-term forms of synaptic plasticity. Notably, the endocannabinoid 2-AG is perhaps the best-characterized retrograde messenger at synapses in terms of its production, mode of action, and degradation. There are three modes of 2-AG release from neurons, namely, CaER, basal RER, and Ca^{2+}-assisted RER. CaER is triggered by Ca^{2+} influx through either voltage-gated Ca^{2+} channels or NMDA receptors, involves yet unidentified PLC-like enzymes, and leads to 2-AG production by DGL. Basal RER and Ca^{2+}-assisted RER are triggered by activation of $G_{q/11}$-coupled receptors; involve $G\alpha_q/G\alpha_{11}$, PLCβ, and DGL; and lead to 2-AG production. Importantly, PLCβ activity is dependent on Ca^{2+} levels so that it can function as a coincidence detecter of receptor activation and Ca^{2+} elevation in Ca^{2+}-assisted RER. Among the three modes of 2-AG release, Ca^{2+}-assisted RER is perhaps the most physiologically relevant mechanism of 2-AG release. Synaptically driven 2-AG release mostly depends on Ca^{2+}-assisted RER in several central synapses. After released from postsynaptic neurons, 2-AG acts retrogradely onto CB_1 receptors on presynaptic terminals and reduces transmitter release mainly by inhibiting voltage-gated Ca^{2+} channels. Termination of the 2-AG action is facilitated by its degradation by MGL localized in presynaptic terminals and axons. MGL also regulates the ambient level of extracellular 2-AG and thus controls basal endocannabinoid tone. On the other hand, COX-2 is localized in the postsynaptic side and may also contribute to degradation of 2-AG. Anatomical studies have demonstrated cellular and subcellular distributions in the brain of the molecules involved in 2-AG signaling, including CB_1, mGluR1/5, M_1/M_3, $G\alpha_q/G\alpha_{11}$, PLCβ, DGLα, MGL, and COX-2. In general, these molecules are colocalized around excitatory and inhibitory synapses. However, their fine subcellular distributions are unique to each brain region so that the induction threshold of 2-AG-mediated retrograde suppression of excitation and inhibition can be coordiated. Based on these electrophysiological and anatomical studies, we now understand how the neural activity generates 2-AG signaling and how 2-AG signaling modulates the synaptic transmission and neuronal function.

When compared with the well-characterized actions of 2-AG as a retrograde messenger, roles of another major endocannabinoid, anandamide, in modulation of synaptic transmission are less clear. Anatomical studies indicate that the anandamide-producing enzyme NAPE-PLD is localized in presynaptic elements, particularly in mossy fibers in the hippocampus, and is associated with Ca^{2+} stores. On the other hand, the anandamide-degrading enzyme FAAH is localized at the postsynaptic side. These results suggest that anamdamide might function as an anterograde messenger at certain central synapses.

Many behavioral studies have clarified the roles of the endocannabinoid system in various brain functions, including learning and memory, anxiety, depression, drug addiction, appetite, feeding behavior, and pain. Manipulation of the endocannabinoid system exerts complex effects on animals' behaviors, and the results from behavioral studies are not necessarily consistent. However, a consensus is that the endocannabinoid system is important for acquisition and/or extinction of certain forms of memory, regulation of anxious states, antidepressant effects, rewarding effects of some addictive compounds, promotion of appetite, and relieving pain. Neural mechanisms underlying these behavioral effects of the endocannabinoid system are not well understood. In particular, how the endocannabinoid-mediated synaptic plasticity contributes to these brain functions remain to be elucidated.

At the clinical level, the endocannabinoid system is recognized as a promising target for new therapies to treat a variety of neurological and psychiatric disorders. Clinical and preclinical studies using CB_1 agonists and

antagonists have suggested their therapeutic potential for the treatments of some disorders. It should be noted, however, that the endocannabinoid system is involved in a varitety of neural functions, and therefore its global enhancement as well as global suppression might be of advantage to certain functions, but of disadvantage to others. To minimize adverse side effects, it is important to develop strategies that enable us to control the endocannabinoid system in a region- and/or function-specific manner. For this purpose, it is important to clarify properties of the endocannabinoid system involved in individual neural functions, for example, relative contribution of 2-AG and anandamide, rate-limiting step that determines the endocannabinoid tone, and modulations of activity of the endocannabinoid system. A more comprehensive knowledge is required, and in this regard, we are still at the beginning of a revolution in cannabinoid research.

NOTE ADDED IN PROOF

During the editorial process, several new papers on eCB signaling have appeared, which report on eCB-STD in the inferior olive (34a) and PAG (287a), eCB-LTD in the hippocampus (212a) and visual cortex (239a), and immunohistochemical localization of PLCβ1 in the CNS (167a).

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