

# Neuropharmacology of the Endocannabinoid Signaling System-Molecular Mechanisms, Biological Actions and Synaptic Plasticity

Balopal S. Basavarajappa<sup>1,2,3,\*</sup>

<sup>1</sup>Division of Analytical Psychopharmacology, New York State Psychiatric Institute, <sup>2</sup>Department of Psychiatry, College of Physicians & Surgeons, Columbia University, New York, NY 10032, USA; <sup>3</sup>Nathan Kline Institute for Psychiatric Research, Orangeburg, Orangeburg, NY 10962, USA

**Abstract:** The endocannabinoid signaling system is composed of the cannabinoid receptors; their endogenous ligands, the endocannabinoids; the enzymes that produce and inactivate the endocannabinoids; and the endocannabinoid transporters. The endocannabinoids are a new family of lipidic signal mediators, which includes amides, esters, and ethers of long-chain polyunsaturated fatty acids. Endocannabinoids signal through the same cell surface receptors that are targeted by  $\Delta^9$ -tetrahydrocannabinol ( $\Delta^9$ -THC), the active principles of *cannabis sativa* preparations like hashish and marijuana. The biosynthetic pathways for the synthesis and release of endocannabinoids are still rather uncertain. Unlike neurotransmitter molecules that are typically held in vesicles before synaptic release, endocannabinoids are synthesized on demand within the plasma membrane. Once released, they travel in a retrograde direction and transiently suppress presynaptic neurotransmitter release through activation of cannabinoid receptors. The endocannabinoid signaling system is being found to be involved in an increasing number of pathological conditions. In the brain, endocannabinoid signaling is mostly inhibitory and suggests a role for cannabinoids as therapeutic agents in central nervous system (CNS) disease. Their ability to modulate synaptic efficacy has a wide range of functional consequences and provides unique therapeutic possibilities. The present review is focused on new information regarding the endocannabinoid signaling system in the brain. First, the structure, anatomical distribution, and signal transduction mechanisms of cannabinoid receptors are described. Second, the synthetic pathways of endocannabinoids are discussed, along with the putative mechanisms of their release, uptake, and degradation. Finally, the role of the endocannabinoid signaling system in the CNS and its potential as a therapeutic target in various CNS disease conditions, including alcoholism, are discussed.

**Key Words:** Alcoholism, endocannabinoids, CNS, synaptic plasticity, reward, CB1 receptors, alcohol-drinking behavior, therapy.

## INTRODUCTION

The earliest anthropological evidence of *Cannabis* use comes from the oldest known Neolithic culture in China where it was used in the production of hemp for ropes and textiles, and also for its psychotropic effects [159]. An 1848 commentary in the British Pharmacopoeia outlined quite accurately the psychotropic effects of *Cannabis* and pointed out its merits as an antispasmodic and analgesic [59].

The major psychoactive constituent of *Cannabis sativa* is  $\Delta^9$ -tetrahydrocannabinol ( $\Delta^9$ -THC, dronabinol), which is mainly responsible for the pharmacological effects of the *Cannabis* plant [79, 147].  $\Delta^9$ -THC was isolated, stereochemically defined, and synthesized in 1964 [102] and its psychoactive properties were recognized immediately. The hydrophobic nature of  $\Delta^9$ -THC delayed experimentation and suggested that the compound might act by influencing membrane fluidity rather than by binding to a specific receptor. The development of new classes of potent and selective  $\Delta^9$ -THC analogues led to the pharmacological identification of cannabinoid-sensitive sites in the brain [77]. Currently  $\Delta^9$ -THC and its analogs are used for the treatment of nausea and vomiting induced by radiotherapy or chemotherapy, and wasting syndrome in AIDS patients. Cannabinoids are also useful for the treatment of pain, spasticity, glaucoma and other disorders [310]. However, the clinical usefulness of  $\Delta^9$ -THC and its analogs is greatly hampered by their numerous side effects, including the potential for abuse [133, 241]. Research on the molecular and neurobiological bases of the physiological and neurobehavioral effects of marijuana use was slowed by the lack of specific tools and technology for many decades. Over the last one and half decades, advances in our knowledge of the endocannabinoid signaling system have progressed enormously. This review is focussed on our understanding of the endocannabinoid signaling system in the brain. First, the structure, anatomical distribution, and signal transduction mechanisms of cannabinoid receptors are described. Second, the pathways of endocannabinoid synthesis are discussed, along with the putative mechanisms of endocannabinoid release, uptake, and degradation.

Finally, the role of the endocannabinoid signaling system in the central nervous system (CNS) and its potential as a therapeutic target for the treatment of various CNS disease conditions, including alcoholism, are discussed.

## THE ENDOCANNABINOID SIGNALING SYSTEM

### Cannabinoid Receptors

Evidence for the existence of the marijuana receptor has been accumulating since the 1980s [77, 149]. It has now been shown that cannabinoids have two specific G-protein-coupled cannabinoid receptor subtypes, which have been cloned. These are named CB1 and CB2. Evidence for a third type of G-protein-coupled cannabinoid receptor ("CB3" or "Anandamide receptor") in brain and in endothelial tissues is mounting [38, 83, 157, 306]. However, the cloning, expression and characterization of CB3 is yet to come.

The CB1 and CB2 receptors belong to the large superfamily of G protein-coupled receptors (GPCR) (for a recent review see [13, 23]). The human CB1 and CB2 receptors share 44% overall amino acid identity (for details see [222]). The CB2 receptors of human and rat share 81% amino acid identity and those of mouse and rat share 81%.

Although significant progress has been achieved into many aspects of the biology of the endocannabinoid system, and our knowledge of cannabinoid genomics and proteomics is increasing, the regulation of cannabinoid receptor genes is poorly understood. cDNA sequences encoding CB1- or CB2-like receptors have been reported for the rat [193], human [106, 208], mouse [1, 52], cow (Wessner, GeneBank submission, 1997), cat [104] (GeneBank submission, 1997), puffer fish [316], leech [268], zebra finch [266], and newt [267].

The CB1 receptor gene is polymorphic, with implications not only for substance abuse but also for other neuropsychiatric disorders. The CB1 receptor gene is intronless and its structure is similar in mouse, rat, and human. The CB2 receptor gene is also intronless, at least in its coding region [222]. Unlike the CB1 receptor gene, which is highly conserved across the human, rat, and mouse species, the CB2 receptor gene is much more divergent [127]. Like other GPCRs, the primary structures of the cannabinoid receptors are characterized by seven hydrophobic stretches of 20–25 amino acids predicted to form transmembrane  $\alpha$  helices, which are con-

\*Address correspondence to this author at Nathan Kline Institute for Psychiatric Research, 140 Old Orangeburg Rd, Orangeburg, NY-10962, USA; Tel: 845-398-3234 or 5454; Fax: 845-398-5451; E-mail: Basavaraj@nki.rfmh.org

nected by alternating extracellular and intracellular loops. In both the CB1 and CB2 receptors, no cysteines are found within the second extracellular domain, but the third extracellular domain contains two or more cysteines. Cysteine residues appear to stabilize the tertiary structure of the receptor because of their ability to form intra-molecular disulfide bridges. In most GPCRs, these cysteines occur in the extracellular domains that lie between hydrophobic domains 2 and 3 and hydrophobic domains 4 and 5 (i.e., the second and third extracellular domains, assuming that the N-terminal domain is also extracellular). One other deviation from most other GPCRs is that the CB1 and CB2 receptors lack a highly conserved proline residue in hydrophobic domain 5 [192]. The structural features of these proteins that are critical for ligand binding and functional properties have been evaluated *in vivo* and *in vitro* [2, 3]. The consensus sites for *N*-glycosylation are mainly concentrated at the N-terminus of the CB1 proteins. Whether any of these potential *N*-glycosylation sites are naturally glycosylated or even essential for CB1 receptor function is not known.

There are four clusters of potential cAMP-dependent protein kinase and Ca<sup>2+</sup>-calmodulin-dependent protein kinase sites in CB1 receptors. These clusters are conserved across the human, rat, and mouse receptor proteins. There is a single potential protein kinase C site that is also conserved in these CB1 receptors. There is no such site in CB2 receptors. The biological significance of these sites has been shown in non-neuronal cells. For example, in AtT-20 cells transfected with rat cannabinoid receptor (CB1), the activation of an inwardly rectifying potassium current (Kir current) and depression of P/Q-type Ca<sup>2+</sup> channels by cannabinoids were prevented by stimulation of protein kinase C by 100 nM phorbol 12-myristate 13-acetate (PMA) [103]. However, the biological significance of these potential protein phosphorylation sites in these receptor molecules in brain is yet to be determined (for a detailed discussion, see the recent review [23]).

CB1 and CB2 receptor knockout mice have been generated. The CB2 receptor knockout mouse was generated [43] to study the function of CB2 receptors in immune cells and in immunomodulation. Three groups have generated CB1 receptor gene knockout mice independently. The first CB1 knockout mouse were generated on a CD1 background [178], the second knockout mouse was generated on a C57BL background [269, 321], and the third conditional mutant mice that lack expression of the CB1 receptor in principal forebrain neurons but not in adjacent inhibitory interneurons was generated in 2003 [189]. The availability of cannabinoid knockout mice provides an excellent opportunity to study the biological roles of these genes. However, the use of transgenic mouse models to study the effects of over-expression of CB1 or CB2 receptor genes (as opposed to inactivation of the CB1 or CB2 receptor genes as described above) on the regulation and site-specific mechanisms of action of cannabinoids are currently unexplored. Obviously, CB1 or CB2 receptor transgenic animals will provide new *in vivo* systems for studying genetic regulation, development, normal physiology, and apparent dysfunctions associated with the cannabinoid system.

The genomic location of the human cannabinoid receptor gene (CB1) was mapped using genetic linkage studies and chromosomal *in situ* hybridization to chromosome 6q14-q15 [145]. The mouse CB1 and CB2 receptor genes are located in the proximal arm of chromosome 4 [222, 272]. The rat CB1 and CB2 receptor genes both map to chromosome 5. The bovine CB1 receptor gene has been mapped to chromosome 9q22 [230]. As the neurobiological effects of marijuana and other cannabinoids suggest the involvement of the cannabinoid receptor genes in mental and neurological disturbances, the mapping of receptor genes and the identification of allelic variants will undoubtedly enhance our understanding of the links between gene (dys) function and cannabinoid abnormalities.

The CB1 receptor is mainly expressed in the brain and spinal cord and thus is often referred to as the “*brain cannabinoid recep-*

*tor*”. CB1 receptors are among the most abundant G-protein-coupled receptors in the brain, their densities being similar to the levels of  $\gamma$ -aminobutyric acid (GABA)- and glutamate-gated ion channels [139]. The distribution of CB1 receptors is highly heterogeneous in rats, with the highest densities of receptors present in the basal ganglia, substantia nigra, pars reticulata, and globus pallidus. In addition, very high levels of binding are present in the hippocampus, particularly within the dentate gyrus, and also in the molecular layer of the cerebellum. In contrast, there are few CB1 receptors in the brainstem. There is a similar distribution of CB1 receptors in humans [27, 116]. The highest densities are found in association with limbic cortices, with much lower levels within the primary sensory and motor regions, suggesting an important role in motivational (limbic) and cognitive (associative) information processing. CB1 receptors have been shown to be localized presynaptically on GABAergic interneurons and glutamatergic neurons [131, 132, 162, 163]. This would be consistent with the proposed role of endocannabinoid compounds in modulating neurotransmission.

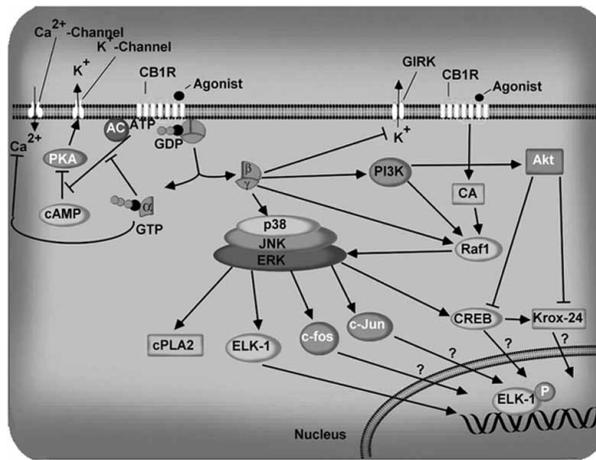
The CB2 receptor is sometimes referred to as the “*peripheral cannabinoid receptor*” because it was thought that CB2 receptors were predominantly present in immune cells in the periphery and absent from the brain. Recent studies suggested that CB2 cannabinoid receptors are functionally expressed in neurons in the brain [92, 121, 140, 221, 299]. CB2 is expressed in peripheral tissues, including white blood cells [94, 208]. CB2 receptor mRNA has been found in the spleen, tonsils, and thymus, which are the major tissues of immune cell production and regulation [see [45, 148] for reviews]. CB2 agonists generally suppress the functions of these cells, but both CB1 and CB2 receptors could contribute to these effects [45].

### The Signal Transduction Mechanism of Cannabinoid Receptors

Activation of a cannabinoid receptor promotes its interaction with G proteins, resulting in guanosine diphosphate/ guanosine triphosphate exchange and subsequent dissociation of the  $\alpha$  and  $\beta\gamma$  subunits. These subunits regulate the activity of multiple effector proteins to bring about biological functions (Fig. 1). Both CB1 and CB2 are coupled with G<sub>i</sub> or G<sub>o</sub> proteins. However, their affinity for G<sub>i</sub> or G<sub>o</sub> proteins may vary, as revealed by several receptor-ligand-stimulated GTP $\gamma$ S binding studies [39, 165]. CB1 receptors differ from many other GPCR proteins in being constitutively active, as they are pre-coupled with G-proteins in the absence of exogenously added agonists [206]. A schematic diagram of a cannabinoid-mediated signal transduction pathway is shown in Fig. (1).

Adenylate cyclase activity was inhibited by anandamide (AEA), R-(+)-methanandamide (MetAEA), and 2-arachi-donylglycerol (2-AG) in N18TG2 cells [58, 150, 231]. In some cases, up-regulation of adenylate cyclase activity was reported without G<sub>i/o</sub> coupling (pertussis toxin sensitive) probably through activation of G<sub>s</sub> proteins [118]. In another study, the *in vitro* expression of specific isoforms of adenylate cyclase (I, III, V, VI, or VIII) with co-expression of CB1 or CB2 was associated with the inhibition of cyclic AMP accumulation. However, the expression of other adenylate cyclase isoforms (II, IV, or VII) with co-expression of the CB1 or CB2 receptor was associated with stimulation of cAMP accumulation [247]. The questions of whether CB1 receptor coupling to G<sub>s</sub> proteins has physiological importance and whether such coupling increases after G<sub>i</sub> or G<sub>o</sub> protein sequestration by co-localized non-cannabinoid G<sub>i/o</sub> protein-coupled receptors have yet to be answered.

Inhibition of N-type voltage-gated channels by cannabinoids has been demonstrated in several neuronal cells using a whole-cell voltage clamp technique and intracellular Ca<sup>2+</sup> measurement [50, 185, 215, 226]. L-type Ca<sup>2+</sup> channels are inhibited by CB1 receptor agonist in cat brain arterial smooth muscle cells, which express mRNA and CB1 receptors [104]. This is blocked by pertussis toxin and SR141716A (rimonabant) [104]. CB1 receptors are also cou-



**Fig. (1). Schematic summary of CB1 receptor signaling.** CB1 receptors are 7-transmembrane domain, G-protein-coupled proteins located in the cell membrane. The  $\text{Ca}^{2+}$  channels inhibited by CB1 receptors include N-, P/Q- and L-type channels. Actions on  $\text{Ca}^{2+}$  channels and adenylyl cyclase (AC) are thought to be mediated by the  $\alpha$  subunits of the G-protein, and those on GIRK and PI3K by the  $\beta\gamma$  subunits. Inhibition of AC and the subsequent decrease in cAMP decreases activation of cAMP-dependent protein kinase A (PKA), which leads to decreased phosphorylation of the  $\text{K}^+$  channels. Stimulatory effects are shown by a ( $\rightarrow$ ) sign and inhibitory effects by a ( $\perp$ ) sign.

pled to ion channels through  $G_{i/o}$  proteins, and activate A-type and inwardly rectifying potassium channels. The coupling to A-type and D-type potassium channels is thought to be through adenylyl cyclase [205]. These are also stimulated by the inhibition of adenylyl cyclase by cannabinoids. Because of the decrease in cAMP accumulation, cAMP-dependent protein kinase (PKA) is inhibited by CB1 receptor activation. Without cannabinoids, PKA phosphorylates the potassium channel protein, resulting in decreased outward potassium current. In the presence of cannabinoids, however, the phosphorylation of the channel by PKA is reduced, which leads to an increased outward potassium current [57]. Cannabinoids inhibit N-type, P/Q-type calcium channels and D-type potassium channels [148, 150]. In addition, cannabinoids can close sodium channels, but whether this effect is receptor-mediated has yet to be proved. Based on these findings, it has been suggested that cannabinoids play a role in regulation of neurotransmitter release. There is also evidence from experiments with rat hippocampal CA1 pyramidal neurons that CB1 receptors are negatively coupled to M-type potassium channels [261]. CB1 receptors may also mobilize arachidonic acid and close  $5\text{HT}_3$  receptor ion channels [229], and under certain conditions couple to Gs proteins to activate adenylyl cyclase [47] to reduce outward potassium K current, possibly through arachidonic acid-mediated stimulation of protein kinase C [134]. CB1 receptors have also been reported to activate phospholipase C through G proteins in COS-7 cells co-transfected with CB1 receptors and G sub-units [144]. CB1 receptors on cultured cerebellar granule neurons can operate through a phospholipase C-sensitive mechanism to increase *N*-methyl-D-aspartate (NMDA)-elicited calcium release from inositol 1,4,5-triphosphate-gated intracellular stores [214]. Activation of CB1 receptors by cannabinoid agonists evokes a rapid, transient increase in intracellular free  $\text{Ca}^{2+}$  in N18TG2 and NG108-15 cells [274, 275, 277].

One of the most interesting research areas is the regulation of neurogenesis, axonal growth and synaptogenesis by cannabinoids. The molecular mechanism involved in this process is not yet clear. The regulation of cellular growth has been usually associated with tyrosine kinase receptors. However, recent studies suggest that GPCRs can stimulate the mitogen-activated protein kinase (MAPK) pathway and thereby induce cellular growth. After the first observation of activation of the MAPK cascade by AEA [309], several *in vivo* and *in vitro* studies have implicated both the cannabinoids and the endocannabinoids in the MAPK pathway. Activation of two isoforms (p42/p44) of MAPK was observed in non-neuronal

U373MG astrocytoma cells and in host cells expressing recombinant CB1 receptors mediated by CB1 receptor and Gi/o protein [37]. Similarly, activation of Gi/o protein by  $\Delta^9$ -THC and HU-210 *via* CB1 receptors activated p42/p44 MAPK in C6 glioma and primary astrocytes cultures [130, 257]. In WI-38 fibroblasts, AEA *via* CB1 receptor and Gi/o proteins promoted tyrosine-phosphorylation of the extracellular signal-regulated kinase 2 (ERK2 or p44) and increased MAPK activity [309]. In some cells, CB1 receptor-mediated activation of MAPK was mediated through the PI3 kinase pathway [37, 309]. AEA, CP,55, 940 and WIN 55,212-2 increased phosphorylation of FAK+ 6,7, a neural isoform of FAK, in hippocampal slices and in cultured neurons [72].  $\Delta^9$ -THC, AEA and 2-AG stimulated phosphorylation of the Tyr-397 residue of FAK in the hippocampus, which is crucial for FAK activation [73]. Cannabinoids increased phosphorylation of p130-Cas, a protein associated with FAK in the hippocampus. Endocannabinoids increased the association of Fyn, but not Src, with FAK+6,7. These effects were mediated through inhibition of a cAMP pathway. CB1 receptor-stimulated FAK-autophosphorylation was shown to be upstream of the Src family kinases [73]. These new mechanisms for cannabinoid regulation of the MAPK pathway might play a role in endocannabinoid-induced modulation of synaptic plasticity, cell migration and neurite remodeling.  $\Delta^9$ -THC promoted phosphorylation of Raf-1 and its subsequent translocation to the membrane in cortical astrocytes [257]. CB1 receptor-mediated release of  $\beta\gamma$  subunits leads to activation of PI3K, resulting in tyrosine phosphorylation and activation of Raf-1 and the phosphorylation of MAPK. Activation of p38 MAPK was observed in CHO cells expressing recombinant CB1 receptors [255] and in human vascular endothelial cells having endogenous CB1 receptors [181].  $\Delta^9$ -THC was shown to induce activation of c-Jun N-terminal kinase (JNK1 and JNK2) in CHO cells expressing recombinant CB1 receptors [255]. The pathway for JNK activation involves Gi/o protein, PI3K and Ras [255].

Activation of the  $\text{Na}^+/\text{H}^+$  exchanger in CHO cells stably expressing the CB1 receptor [35] was shown to be mediated through MAPK and CB1 receptors. AEA-stimulated activation of MAPK activity was shown to phosphorylate cytoplasmic phospholipase A2 (cPLA2), release of arachidonic acid (AA), and result in the synthesis of prostaglandin E2 in WI-38 cells [309]. MAPK activation by cannabinoids was shown to induce immediate-early gene expression (*krox-24*) in U373MG human astrocytoma cells [36].  $\Delta^9$ -THC induced the expression of *krox-24*, BDNF and *c-Fos* in mouse hippocampus [74]. CB1- and MEK-ERK-mediated activation of *krox-24* is negatively regulated through PI3K-Akt in neuro2a cells

24 is negatively regulated through PI3K-Akt in neuro2a cells [126]. The suppression of prolactin receptor and trk nerve growth factor receptor synthesis by AEA was shown to be associated with a CB1 receptor-mediated decrease in protein kinase A and an increase in MAPK activities [199]. CB1 receptor agonists induced the expression of c-fos and c-Jun in the brain [8, 187, 196]; whether this is mediated by CB1 receptor-activated MAPK is not known.  $\Delta^9$ -THC-induced phosphorylation of the transcription factor Elk-1 is mediated by MAPK/ERK [295]. Intracerebroventricular injection of AEA evoked an increase in *c-Fos* protein in rat brain with a generally similar distribution to that of CB1 receptors [228].  $\Delta^9$ -THC and HU-210 increased glucose metabolism and glycogen synthesis in C6 glioma and astrocytes cultures [130]. The activation of protein kinase B/Akt (isoforms IB) by cannabinoid agonists is mediated by Gi/o and PI3K in U373MG astrocytoma and CHO cells expressing recombinant CB1 receptors [120]. CB1 receptor-mediated gene regulation through the activation of MAPK is an important physiological mechanism by which cannabinoids and endocannabinoids can modulate synaptic plasticity.

### Endocannabinoids

Since  $\Delta^9$ -THC was structurally defined and synthesized more than 30 years ago, there has been considerable speculation as to whether endogenous ligands exist, especially as CB1 is the most abundantly expressed neuronal receptor. The identification of endogenous cannabinoid receptors suggested that the brain must produce its own chemicals that interact with the CB1 receptor during normal brain function.

Beginning in 1992, the first endogenous cannabinoid was identified as anandamide (AEA, arachidonylethanolamide). It was named from the Sanskrit *ananda*, "internal bliss," making reference to its chemical structure (the amide of arachidonic acid and ethanolamine) [78]. Subsequently, another endogenous cannabinoid receptor ligand, 2-arachidonyl-glycerol (2-AG) was discovered and characterized [197, 278] (Fig. 2). The third ether-type endocannabinoid, 2-arachidonylglycerol ether (noladin ether), was isolated from the CNS and shown to display pharmacological properties similar to AEA [137]. The fourth type of endocannabinoid, virodhamine, in contrast to the previously described endocannabinoids, is a partial agonist with *in vivo* antagonist activity at the CB1 receptor [239]. The fifth type of endocannabinoid, N-arachidonyldopamine (NADA), not only binds to CB1 receptor but also stimulates vanilloid receptors (VR1) [152]. Previously, the existence of AEA analogs in chocolate had been demonstrated [88]. It is thought that chocolate and cocoa contain *N*-acylethanolamines (NAEs) and oleamide, but no or very little AEA and no 2-AG [86]. These lipids could mimic cannabinoid ligands either directly by activating cannabinoid receptors or indirectly by increasing AEA levels [40]. These observations indicate that endocannabinoid analogs exist in plants and animals and, further, show the evolutionary conservation of the cannabinoid system in nature.

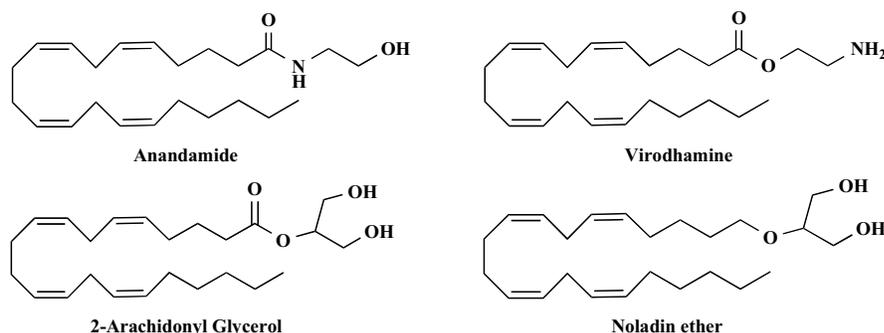
Endocannabinoids are present in peripheral and brain tissues and have recently been found in breast milk [86]. The expression of functional CB1 receptor in the preimplantation embryo and synthesis of AEA in the uterus of pregnant mice suggest that cannabinoid ligand-receptor signaling is operative in the regulation of preimplantation embryo development and implantation [227]. Since the information on noladin ether, virodhamine, and N-arachidonyldopamine is limited, the detailed discussion is limited to AEA and 2-AG.

### Anandamide

AEA has been identified and quantitated throughout the human brain and periphery [95]. Signal transduction and ligand binding studies have suggested that it can act at both the CB1 ( $K_i$  61nM), CB2 ( $K_i$  1930nM) and also act as a full agonist at vanilloid TRPV1 receptors (for references see recent review [253]), although it may be more efficacious at CB1 [97]. AEA resembles  $\Delta^9$ -THC in structural aspects [256, 262]. AEA behaves as an affinity-driven CB1 receptor agonist. Thus its efficacy at CB1 receptors, although higher than that of  $\Delta^9$ -THC, is often found to be lower than that of other cannabinoid agonists [(+)-WIN55212-2 or CP-55940].

AEA was shown to be widely distributed in the brain and peripheral tissues [95, 96]. AEA levels vary by a factor of 4-6 within different regions of the rat brain, with the highest levels in the striatum and brainstem and the lowest levels in the cerebellum and cortex [28, 317]. AEA was found in regions of both the rat and human brain that contain a high density of CB1 receptors (e.g., hippocampus, cerebellum, and striatum) and in a region that is sparse in CB1 receptors, the thalamus [95]. In the rat, the concentration of AEA in the thalamus was approximately twice the concentration measured in the cerebellum. It is clear from these data that for AEA, the relative regional abundance in the brain does not correlate with the distribution of CB1 receptors. AEA levels in the brain are equivalent to those of other neurotransmitters such as dopamine and serotonin, but at least 10-fold lower than those reported for GABA and glutamate. AEA was also found in peripheral tissues such as human and rat spleen, which express high levels of CB2 receptors, suggesting that AEA is an agonist at both CB1 ( $K_i$  = 89± 10 nM) [246] and CB2 receptors ( $K_i$  = 371± 102 nM) [246]. Small amounts of AEA were also found in the human heart and thalamus and also in rat skin, whereas only trace quantities were detected in human serum, plasma, and cerebrospinal fluid [95].

Enzymes responsible for the biosynthesis and degradation of AEA were characterized in various tissues. Unlike classical neurotransmitters and neuropeptides, AEA is not stored in intracellular compartments, but is produced on demand by receptor-stimulated cleavage of lipid precursors [17, 21, 22, 46, 84, 198] and released from neurons immediately afterwards [17, 21, 22, 84, 113, 198]. The postulated biosynthetic pathway for AEA involves the energy-independent condensation of ethanolamine and free arachidonic



**Fig. (2). Molecular structure of endocannabinoids.** These endocannabinoids share a polyunsaturated fatty acid moiety (arachidonic acid) and a polar head group consisting of ethanolamine or glycerol.

acid [75, 294]. The biochemical characteristics and inhibitor kinetics observed for the enzyme catalyzing this condensation reaction were essentially identical to those described for the enzyme catalyzing fatty acid amide (FAA) hydrolysis [fatty acid amide hydrolase (FAAH); see discussion below] [66, 75, 294]. Several lines of evidence derived from both physical and biological data argue that the production of AEA *in vivo* does not occur through the energy-independent condensation of free arachidonic acid and ethanolamine [84, 161, 173]. The AEA precursor is an N-arachidonyl phosphatidylethanolamine (N-ArPE), which is believed to originate from the transfer of arachidonic acid (AA) from the sn-1 position of 1,2-sn-di-arachidonylphosphatidylcholine to phosphatidylethanolamine, catalyzed by a calcium-dependent N-acyltransferase (NAT) (Fig. 3). N-ArPE is then cleaved by a N-acyl phosphatidylethanolamine (NAPE)-specific phospholipase D (PLD) [84, 212, 260], which releases AEA and phosphatidic acid. It is not clear whether the NAT or the NAPE-PLD controls the rate-limiting step of AEA synthesis [81, 135, 273]. The NAT enzyme has been characterized biochemically in both brain and testis microsomal preparations [46, 84, 279, 280]. The NAT has only recently been purified, cloned, and characterized [220]. AEA biosynthesis was unaffected in NAPE-PLD knockout mice suggesting the involvement of other enzymes [179]. Another pathway which involves the conversion of NAPE into 2-lysol-NAPEs *via* the action of secretory PLA<sub>2</sub> has also been reported. 2-Lysol-NAPEs are then converted into N-acyl-ethanolamides, including AEA, *via* a selective lyso phospholipase D (lyso-PLD) [283]. A recent study proposed the existence in mouse brain and RAW264.7 macrophages of a parallel pathway through which AEA is generated from NAPE by a two-step process involving the PLC-catalyzed cleavage of NAPE to yield pAEA, which is subsequently dephosphorylated by protein tyrosine phosphatases (PTPN22) [182] (Fig. 3).

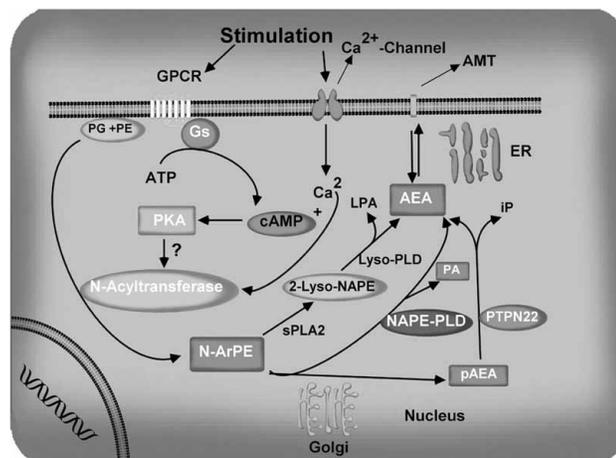
As a putative neuromodulator, AEA that is released into the synaptic cleft is expected to be rapidly inactivated. In general, two mechanisms are known that could remove endocannabinoids from the synaptic cleft to ensure rapid signal inactivation: re-uptake or enzymatic degradation. An enzyme that catalyzes hydrolysis of AEA, forming arachidonic acid and ethanolamine, was detected in rat brain tissue [75] (Fig. 4) (also in recent review [14]) soon after the discovery of AEA and is variously known as anandamide amidase, anandamide hydrolase, anandamide amidohydrolase, or fatty acid amide hydrolase (FAAH). In addition to hydrolysis by FAAH, AEA is metabolized by COX-2, LOX and cytochrome P450. COX-

2 has been shown to metabolize AEA into PGE<sub>2</sub>-ethanolamide (PGE<sub>2</sub>-EA) [254]. 12- and 15-LOX, nonheme iron-containing enzymes convert AEA into 12- and 15-hydroxy-AEA (12- and 15-HAEA) *in vitro*, respectively [170, 184]. Cytochrome P450 also metabolizes AEA into several polar lipids [44]. Recently, in the absence of FAAH, exogenously injected AEA has been shown to be converted into o-phosphorylcholine (PC)-AEA in the brain and spinal cord. The choline-specific phosphodiesterase (NPP6) was found to convert PC-AEA into AEA [207] (Fig. 4). Further research is required to elucidate the exact mechanism and enzymes involved in this pathway of AEA metabolism.

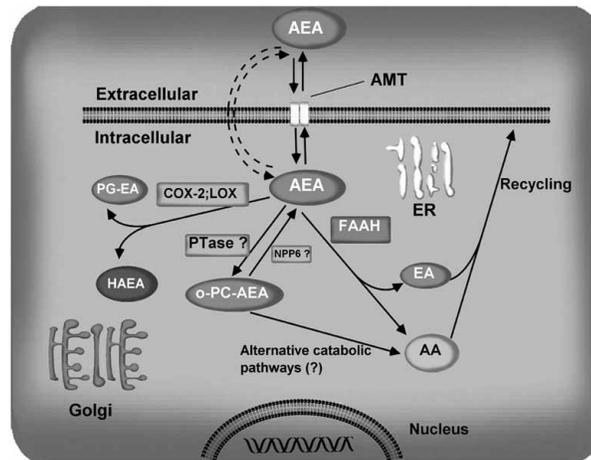
The existence of a membrane transporter that mediates the uptake of AEA has also been investigated (see below). Thus, the ability of brain tissue to enzymatically synthesize and metabolize AEA, and the existence of AEA receptors and of carrier-mediated transport mechanisms essential for the termination of AEA signaling, suggest that AEA may represent a member of a new family of fatty-acid-derived neuromodulators [6].

## 2-Arachidonylglycerol

Soon after the discovery of AEA, the existence of a second phospholipid-derived messenger, 2-arachidonylglycerol (2-AG), was shown [197, 278]. 2-AG has been characterized as a unique molecular species of monoacylglycerol isolated from both the canine gut [197] and the rat brain [281], where it presumably functions as an endogenous cannabinoid receptor ligand. According to its chemical structure, this new putative endocannabinoid is an arachidonyl ester rather than an amide (Fig. 2). 2-AG was found to bind both CB1 (K<sub>i</sub> 2.4 μM) and CB2 receptors, although its CB1 receptor binding activity is 24-times less potent than that of AEA. 2-AG elicited the typical effects of Δ<sup>9</sup>-THC, such as antinociception, immobility, immunomodulation, and inhibition of electrically evoked contractions of the mouse vas deferens [197, 275, 278]. However, further studies are necessary to determine the relative importance of 2-AG in the human body and brain. Information regarding tissue levels of 2-AG is still limited. Brain tissue concentrations of 2-AG are approximately 200-fold higher than those of AEA [28]. The distribution of the two endocannabinoids in the different brain regions is similar. The highest concentrations were found in the brainstem, medulla, limbic forebrain, striatum, and hippocampus and the lowest in the cortex, diencephalons, mesencephalon, hypothalamus, and cerebellum (for review see [273]). However, no correlation was found between 2-AG concentrations



**Fig. (3). The potential pathways of anandamide biosynthesis.** Stimulation of adenylate cyclase and cAMP-dependent protein kinase potentiate the N-acyltransferase (Ca<sup>2+</sup>-dependent transacylase, NAT). A fatty arachidonic acid chain is transferred by NAT from the sn-1 position of phospholipids to the primary amine of phosphatidylethanolamine, in a Ca<sup>2+</sup>-dependent manner, forming an N-arachidonyl phosphatidylethanolamine (N-ArPE). This N-ArPE intermediate is then hydrolyzed by a phospholipase D (PLD)-like enzyme to yield the anandamide (AEA). In addition, possible alternative pathways for AEA formation from N-ArPE are shown. Once synthesized, AEA can be transported to the outside of the cell through a process that has not yet been well characterized. AMT, anandamide membrane transporter.



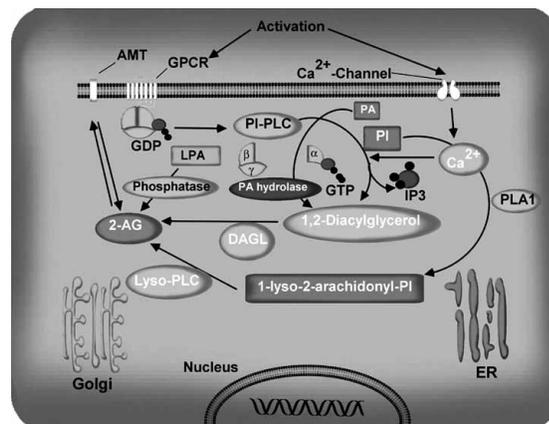
**Fig. (4).** Scheme illustrating the potential mechanism of anandamide uptake and degradation. Anandamide can be internalized by neurons through a yet unidentified transport mechanism, “the endocannabinoid transporter”. Once inside a neuron, AEA is rapidly cleaved by the hydrolytic enzyme fatty acid amide hydrolase (FAAH), releasing arachidonic acid (AA) and ethanolamine (EA). Alternatively, different lipoxygenases (LOXs) and cyclooxygenase-2 (COX-2) can metabolize AEA, generating hydroxyl derivatives of AEA (HAEAs) and prostaglandins-ethanolamides (PG-EAs), respectively. On the other hand, in FAAH (-/-) mice, AEA is converted into PC-AEA by an unidentified biosynthetic pathway, and PC-AEA can in turn be catabolized to AA by choline-specific phosphodiesterase (NPP6). AMT, anandamide membrane transporter.

and CB1 receptor distribution. 2-AG was also detected in the peripheral nervous system, such as in the sciatic nerve, lumbar spinal cord, and lumbar dorsal root ganglion. 2-AG was also detected in the rat retina and bovine retina (for review see [273]).

#### Biosynthesis and Metabolism of 2-AG

Generation of 2-AG was first described in ionomycin-stimulated N18TG2 cells [33], in electrically stimulated rat hippocampal slices, and in ionomycin-stimulated neurons [270]. Rapid generation of 2-AG was also observed in rat brain homogenate during incubation with  $\text{Ca}^{2+}$  [167] and in thrombin- or A23187-stimulated human umbilical vein endothelial cells [276]. Intraperitoneal injection of picrotoxin stimulated 2-AG levels in the rat brain [281]. Generation of 2-AG was also observed in cerebellar granular neurons in culture in response to chronic alcohol exposure [21]. Substantial amounts of this stimulus-induced 2-AG were released from the cells [21, 32, 276]. 2-AG biosynthesis occurs by two possible routes in neurons, which are illustrated in Fig. (5) and also in recent review [14]. Phospholipase C (PLC)-mediated hydrolysis of membrane phospholipids may produce diacylglycerol (DAG), which

may be subsequently converted to 2-AG by diacylglycerol lipase (DAGL) activity [240, 278]. The formation of DAG also involves the hydrolysis of phosphatidic acid through  $\text{Mg}^{2+}$  and  $\text{Ca}^{2+}$ -dependent PA phosphohydrolase activity [31, 48]. Alternatively, phospholipase A1 (PLA1) may generate a lysophospholipid, which may be hydrolyzed to 2-AG by lyso-PLC activity [278]. Various structurally distinct inhibitors of PLC and DAGL activities prevent 2-AG formation in cultures of cortical neurons, suggesting that the PLC/DAGL pathway may play a primary role 2-AG synthesis [270]. The molecular character of the enzymes involved remains to be identified, although the molecular cloning of DAGL $\alpha$  was reported recently [29]. Both DAGL $\alpha$  and  $\beta$  are associated with the cell membrane and are stimulated by  $\text{Ca}^{2+}$  and blocked by DAGL inhibitors. DAGL $\alpha$  appears to be targeted to postsynaptic spines; it is highly enriched at the base of the spine neck and in the adjacent somatodendritic membrane but is excluded from the main body of the spines and the excitatory synapses in cerebellar Purkinje cells. In hippocampal pyramidal cells, DAGL $\alpha$  is distributed at the spine head or neck or at both structures [164, 318]. Under certain conditions, 2-AG can also be synthesized through the conversion of 2-



**Fig. (5).** The biosynthetic pathways of 2-arachidonylglycerol. Intracellular  $\text{Ca}^{2+}$  initiates 2-AG biosynthesis by inducing the formation of diacylglycerol (DAG) in the membrane either by stimulating the phosphatidylinositol-phospholipase C (PI-PLC) pathway, or the formation of phosphatidic acid (PA), and the subsequent activation of phosphatidic acid hydrolase. 2-AG is the product of DAG-lipase acting on DAG. The second pathway involves hydrolysis of PI by phospholipase A1 (PLA<sub>1</sub>) and hydrolysis of the resultant lyso-PI by a specific lyso-PLC. In certain conditions, 2-AG can also be synthesized through the conversion of 2-arachidonyl lysophosphatidic acid (LPA) by phosphatase to yield 2-AG. Once synthesized, 2-AG can be transported to the outside of the cell through a process that has not yet been characterized. AMT, anandamide membrane transporter.

arachidonyl lysophosphatidic acid (LPA) by phosphatase to yield 2-AG [211]. Molecular characterization of these potential pathways remains to be accomplished.

2-AG is inactivated by reuptake [25, 30] via a membrane transport molecule, the 'AEA membrane transporter' (AMT) (see below for details) [22, 25, 26, 111, 141, 142, 184], and subsequent intracellular enzymatic degradation [68, 76, 84] by monoacylglycerol (MAGL) lipase (Fig. 6), like other monoacylglycerols [168]. Similarly, 2-AG is metabolized by MAGL lipase from porcine brain cytosol and particulate fractions [125]. FAAH has also been shown to metabolize 2-AG [82, 125]. 2-AG may be degraded by FAAH in addition to monoacylglycerol lipase under some circumstances (Fig. 6). Recently, the gene encoding MAGL lipase was cloned from both mouse adipose tissue and the rat brain [160]. Rat MAGL lipase contains 303 amino acids and is expressed in various regions of the rat brain; the highest levels of expression are in regions where the CB1 receptor is abundant, such as the hippocampus, cortex, and cerebellum. Interestingly, MAGL is expressed in presynaptic terminals [90, 129], suggesting it has a role in terminating retrograde signaling at presynaptic neurons [271]. 2-AG is metabolized to 2-arachidonyl LPA through the action of monoacyl glycerol kinase(s). 2-Arachidonyl LPA is then converted into 1-steroyl-2-arachidonyl PA [265]. 1-steroyl-2-arachidonyl PA is further utilized in the "PI cycle" or is used in the *de novo* synthesis of PC and PE. Furthermore, 2-AG is metabolized by enzymatic oxygenation of 2-AG by COX-2 into PGH2 glycerol esters. The biological activity and the role of oxygenated 2-AG are yet to be determined.

### Endocannabinoid Uptake

AEA appears to be inactivated by a two-step process involving the transport of this lipid into cells [26] (Figs. 4 and 6) followed by intracellular hydrolysis by the integral membrane enzyme FAAH. However, with respect to the inactivation of AEA, only FAAH has been molecularly characterized and structurally studied. Indeed, the actual mechanism of AEA uptake or, more generally, the movement of this fatty acid amide not only through biological membranes but also through aqueous media (extracellular and cytoplasmic) remains an enigmatic and controversial subject. Some authors have suggested that the uptake is due to a facilitated diffusion process [22, 25, 26, 141], whereas others have described it as a passive process driven by the AEA-metabolizing enzyme FAAH [68, 76]. For a current discussion of these two points of view, see the recent articles [114] and [143].

### Physiological Roles of Endocannabinoids in the CNS

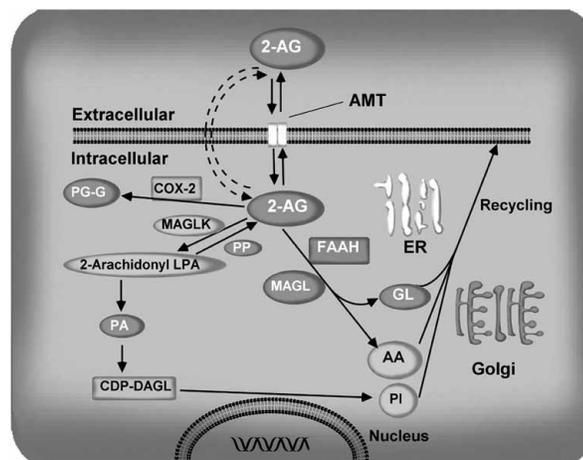
The discovery of endocannabinoids such as AEA and 2-AG and the widespread localization of CB1 receptors in the brain have stimulated considerable excitement over the previously unrecognized cannabinoid system and questions about the function of this ubiquitous network in the nervous system. There is now overwhelming evidence that AEA and 2-AG interact with CB1 receptors and share some of the biological properties of other cannabinoids, but with significant differences. These significant differential effects involve other non-CB1 receptors and/or postulated CB3 receptors as described. In recent years, the functions of endocannabinoids at the synaptic and network levels have been elucidated. Therefore, in the following, an attempt has been made to provide a comprehensive review of the many pathophysiological roles of endocannabinoids in the CNS, including in alcohol addiction.

### ENDOCANNABINOIDS AND SYNAPTIC PLASTICITY

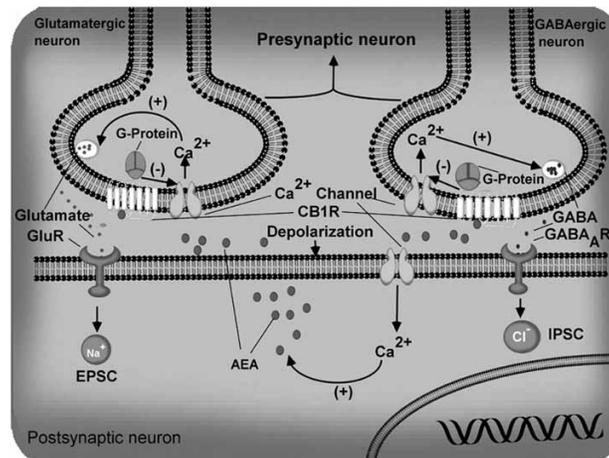
#### Regulation of Short-Term Synaptic Plasticity

Changes in the strength and number of synaptic connections between neurons are believed to be one of the major mechanisms underlying learning and memory and mediating other physiological functions of the CNS. This phenomenon is called *synaptic plasticity*. When neuronal cells (postsynaptic neurons and possibly presynaptic terminals as well) are activated, they produce endocannabinoids, which travel in a retrograde direction and transiently (<1 min) suppress presynaptic neurotransmitter release by activating CB1 receptor-mediated inhibition of voltage-gated  $Ca^{2+}$  channels. Such a negative feedback mechanism should be effective in calming stimulated neurons after excitation.

It was reported that brief activation of CA1 pyramidal cells in the hippocampus [4, 5, 218, 235-237] or Purkinje cells in the cerebellum [183, 303-305] (Fig. 7) caused a reduction in the amplitude of GABAergic inhibitory postsynaptic currents (IPSCs). This phenomenon, called depolarization-induced suppression of inhibition (DSI), is initiated postsynaptically by the voltage-dependent influx of  $Ca^{2+}$  into the soma and dendrites of the neuron, but is expressed presynaptically through inhibition of transmitter release from axon terminals of GABA interneurons. This suggests that a chemical messenger generated during depolarization of the pyramidal neurons must travel backwards across the synapse to induce DSI. DSI has been observed in both excitatory and inhibitory neurons in hippocampal cell culture [217], in CA3 pyramidal cells [203], in den-



**Fig. (6). Schematic summary of 2-AG uptake and degradation.** 2-arachidonylglycerol can be internalized by neurons through a yet unidentified transport mechanism, "the endocannabinoid transporter". Once inside neurons, 2-AG can be hydrolyzed by hydrolytic enzymes, fatty acid amide hydrolase (FAAH) or monoacylglycerol (MAGL) lipase. Alternatively, 2-AG can be metabolized to 2-arachidonyl LPA (2-AG-LPA) through the action of monoacyl glycerol kinase(s) (MAGLK). 2-AG-LPA is then converted into 1-steroyl-2-arachidonyl PA. 1-steroyl-2-arachidonyl PA is further utilized in the *de novo* synthesis of PC and PE. Furthermore, 2AG is metabolized by enzymatic oxygenation of 2-AG by COX-2 into PGH2 glycerol esters (PG-G). AMT, anandamide membrane transporter.



**Fig. (7).** Schematic diagram to illustrate the role of AEA and CB1 receptors on excitatory and inhibitory neurotransmission. Depolarization of a postsynaptic neuron leads to Ca<sup>2+</sup> influx through voltage-gated channels and causes the generation and the release of endocannabinoids such as anandamide (AEA). The mechanism through which Ca<sup>2+</sup> promotes endocannabinoid release is not yet known. The released endocannabinoids then activate the CB1 receptors (CB1R) at presynaptic terminals and suppress the release of glutamate (left) or GABA (right) by inhibiting Ca<sup>2+</sup> channels [146, 151, 264]. GluR, Glutamate receptors.

tate gyrus granule cells [4], and in neocortical pyramidal cells [320].

The retrograde messenger in DSI remained unknown until recent investigations by Wilson and Nicoll [311-313] and by Ohno-Shosaku *et al.* [216] indicated that in hippocampal cells the messenger was likely to be an endocannabinoid. Shortly thereafter, cerebellar DSI was also reported to be mediated by an endocannabinoid [89, 171, 319]. Furthermore, it was reported that CB1 receptor agonists selectively reduced IPSCs in both the hippocampus [131, 146, 163] and cerebellum [288]. There is strong evidence that this retrograde signaling process involves an endocannabinoid. (a) CB1 receptor antagonists selectively blocked DSI whereas agonists enhanced it [216, 312]. (b) DSI is absent in CB1 receptor knockout mice [311, 319]. (c) The GABA interneurons that are implicated in DSI express high levels of CB1 receptors, which are localized to their axon terminals [163]. (d) Neuronal activity and Ca<sup>2+</sup> entry stimulate the synthesis of 2-AG in hippocampal neurons and AEA and 2-AG in other neuronal cells [17, 21, 22]. Recently, DSI mediated by 2-AG was shown in the mouse substantia nigra pars reticulata and rat cerebellum [286]. It remains to be established that endocannabinoid-mediated DSI is present in other brain regions such as the ventromedial medulla [302], amygdala [163], and striatum [284], in which exogenously applied CB1 receptor agonists are known to suppress IPSCs. These reports convincingly established that endocannabinoids are important mediators of short-term plasticity.

Metabotropic glutamate receptors (mGluRs) are G-protein-coupled receptors distributed throughout the CNS that modulate multiple CNS functions, including neuronal excitability [7, 63] and neurotransmitter release [49]. Recent data from several investigators have begun to uncover an entirely novel signaling mechanism for mGluRs, namely the production and subsequent release of endocannabinoids. Some of the effects (short- and long-term forms of synaptic plasticity) previously attributed directly to mGluR activation are in fact indirectly mediated by signaling through the endocannabinoid system [91]. Recent studies suggest that mGluR/endocannabinoid signaling is a widespread feature of neuronal circuitry [91], given the widespread expression of postsynaptic group I mGluRs throughout the CNS and a similar extensive expression of CB1 receptors. Activation of group I mGluRs can cause the release of endocannabinoids in the cerebellum [186] and hippocampus [300]. Synaptic depression in the cerebellum has been observed at glutamatergic synapses. A similar mechanism operates in the hippocampus. As in the cerebellum, activation of postsynaptic group I

mGluRs depresses neurotransmitter release through endocannabinoid-mediated retrograde activation of presynaptic CB1 receptors (Fig. 7). In contrast to the cerebellum, however, synaptic depression in the hippocampus has been observed at GABAergic rather than at glutamatergic synapses.

The neurons in the hippocampus and cerebellum use endocannabinoids to carry out a signaling process that is analogous in mechanism but opposite in sign to DSI, called depolarization-induced suppression of excitation (DSE). Like DSI, DSE is induced by neuronal depolarization; it consists of a transient depression in neurotransmitter release, and it requires a retrograde endocannabinoid messenger. But unlike DSI, DSE targets glutamatergic rather than GABA axon terminals and therefore it reduces the excitatory input to the affected cell [4, 232]. DSE is mimicked and blocked by agonists and antagonists of CB1 receptors respectively [172, 186] and it is absent in the CB1 receptor knockout mouse [219]. CB1 receptor agonists suppress EPSCs in other areas of the brain, evidently through presynaptic actions. For instance, similar DSE was reported in the ventral tegmental area (VTA) as a Ca<sup>2+</sup>-dependent phenomenon, blocked by both AM281 and rimonabant, and enhanced by WIN55212-2 [201]. Importantly, DSE was partially blocked by the D<sub>2</sub> DA antagonist eticlopride and enhanced by the D<sub>2</sub> DA agonist quinpirole without changing the presynaptic cannabinoid activity [201]. These observations indicate that activation of D<sub>2</sub> DA receptors in the VTA significantly enhances the depolarization-induced release of endocannabinoids, which are responsible for the inhibition of glutamate transmission in the VTA [201]. The synchronous release of mEPSCs in Sr<sup>2+</sup>-substituted extracellular solution was found to be reduced by endocannabinoids in the prefrontal cortex and striatum [10, 107]. Recently, it was shown that 2-AG is the retrograde messenger for train-induced suppression of excitation at the VTA-DA synapses [200]. It remains to be demonstrated whether or not DSE is present in the striatum [107], substantia nigra [287], periaqueductal gray [301], and spinal cord [204].

Several studies addressed the issue of how endocannabinoids affect transmitter release from presynaptic neurons [4]. It is clearly established that the  $\beta\gamma$ -subunits of heterotrimeric G-proteins directly interact with and inhibit the high-voltage-activated Ca<sup>2+</sup> channels that mediate transmitter release at most synapses (Fig. 7). CB1 receptors are pertussis-sensitive G-protein-coupled receptors, and their activation reduces N-type Ca<sup>2+</sup> current [50, 51, 185, 226, 292]. In some cells, it appears that the CB1 receptor could reduce GABA release from at least some nerve terminals through a mechanism that is independent of N-/P/Q-type Ca<sup>2+</sup> channels [107,

288, 300, 301], perhaps by direct action on the transmitter release machinery.

### Regulation of Long-Term Synaptic Plasticity

As in endocannabinoid-mediated short-term plasticity, all the studies to date suggest that endocannabinoid-mediated long-term plasticity takes the form of depression of neurotransmission. It was observed that striatal long-term depression (LTD) was absent in CB1 receptor knockout mice, reduced or eliminated by rimonabant, and enhanced by HU-210 in the striatum. These data suggested that striatal LTD was mediated by an endocannabinoid [109]. As in the striatum, LTD in the nucleus accumbens (NAc) is prevented by CB1 receptor antagonists, enhanced by CB1 receptor agonists, and absent in CB1 receptor knockout mice [249]. Importantly, once NAc LTD was induced, the antagonist did not affect it, demonstrating that the LTD was not maintained by a continual release of endocannabinoids, but rather represented a persistent effect of transient CB1 receptor activation. The endocannabinoid that mediated LTD was evidently released as a retrograde messenger, because LTD was prevented by chelating postsynaptic  $\text{Ca}^{2+}$  (with 20mM BAPTA) in the recorded cell [249]. CB1 knockout mice showed strongly impaired short-term and long-term extinction in auditory fear-conditioning tests, with unaffected memory acquisition and consolidation. Treatment of wild-type mice with rimonabant mimicked the phenotype of CB1 knockout mice, suggesting that CB1 is required at the moment of memory extinction [190]. Although, the mechanism needs to be elucidated, these studies suggest that endocannabinoid actions are important for the induction, but not the expression, phase of LTD. Activity-dependent modifications of synaptic strength in the dorsal striatum [158, 233] and NAc [213, 251, 290] are thought to underlie neuronal adaptations in the animal behaviors mediated by these structures. CB1 receptors are present on glutamatergic terminals in the prefrontal cortex [138], and activation of the CB1 receptor by agonists suppresses glutamate EPSCs in layer V slices of rat cortex, evidently by acting at a presynaptic site [10]; it remains to be determined whether CB1 receptors mediate LTD in the cortex as well.

Long-term potentiation (LTP) and LTD of CA3-CA1 synaptic transmission are two *in vitro* models for learning and memory. It has been shown that CB1 receptor activation inhibits both LTP and LTD induction in the hippocampus [270, 282]. Elucidation of the mechanism(s) by which cannabinoids inhibit LTP and LTD may provide clues to the cellular and molecular processes underlying some of the cannabinoid-induced learning and memory impairments. Another important finding is the enhanced LTP found in CB1 receptor knockout mice [34]. These data could not be mimicked by application of CB1 receptor antagonists to wild-type mice [191], suggesting some other factor is involved in the enhancement of LTP. Compensatory up-regulation of other genes in the CB1 receptor knockout mice or altered neurophysiological responses might be responsible. It has been shown that synaptic-driven release of endocannabinoids within the dendritic compartment of CA1 pyramidal cells in a highly localized and efficient process was responsible for facilitating subsequent induction of LTP at nearby excitatory synapses [55, 56]. Recently, it was shown that endocannabinoid-mediated LTD contributes to experience-induced changes in psychomotor behaviors by affecting the information flow in the dorsal striatum and NAc [108]. These early investigations are just beginning to address the effects of endocannabinoids on the neurophysiology of the brain, and further studies are necessary before the roles of endocannabinoids in short- and long-term plasticity are fully elucidated.

### The Endocannabinoid Signaling System and Diseases of the CNS

There is mounting evidence that the endocannabinoid signaling system modulates the activity of most neurotransmitters in the CNS. The neurophysiological consequences of the activation of

CB1 receptors depend on the localization of these receptors in various brain regions and the excitatory or inhibitory pathways being stimulated. Hence, the clinical potential of cannabinoid drugs in neurological disorders is vast. Reduced levels of endocannabinoids, CB1 receptors, and CB1 receptor mRNA have been reported in Huntington's disease [71, 117, 119, 176, 177]. While the mechanism and the significance of the loss of endocannabinoid function is not clear at present, these observations may indicate that the endocannabinoid signaling system has a central role in the progression of neurodegeneration in Huntington's disease, and that cannabinoid agonists could be of significant therapeutic benefit in Huntington's disease because of their anhyperkinetic and neuroprotective effects [176]. A recent study showed a loss of CB1 receptors in progenitor cells in the adult human brain subependymal layer in Huntington's disease and suggested the possibility that these cells could be a suitable endogenous source for the replacement of cells lost due to neurodegenerative disease [67].

Increased cannabinoid tone in the globus pallidus has been reported to be responsible for the production of Parkinsonian symptomatology [188]. A recent study demonstrated increased 2-AG in the globus pallidus of rats treated with reserpine, which is a rodent model of Parkinson's disease (PD) [85]. Endocannabinoid signaling was shown to be involved in the pathophysiology of parkinsonism and LID in 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-lesioned, non-human primate models of Parkinson's disease [296]. The deficiency in endocannabinoid transmission may contribute to levodopa-induced dyskinesias; these complications may be alleviated by activation of CB1 receptors [98]. Increased levels of AEA were reported in rat models of PD [85, 128]. Recently, it was found that cannabinoid CB1 receptor binding and the activation of G proteins by cannabinoid agonists were significantly increased in the postmortem basal ganglia of humans affected by PD [175]. The increase in CB1 receptors was also seen in MPTP-treated marmosets, a primate PD model [175]. A recent study found high levels of endocannabinoids in the cerebrospinal fluid of untreated PD patients [234]. Low doses of rimonabant partially attenuated the hypokinesia shown by a rat model of Parkinson's disease [123]. Further studies to understand the functional interaction between dopamine and the endocannabinoid system should bring new perspectives on the treatment of PD.

Several lines of evidence suggest a role for endocannabinoid signaling in schizophrenia [115]. The highest densities of CB1 receptors are found in regions of the human brain implicated in schizophrenia, including the prefrontal cortex, basal ganglia, hippocampus, and the anterior cingulate cortex [115]. Increased binding of [ $^3\text{H}$ ]CP-55,940 to CB1 receptors in the dorsolateral prefrontal cortex of schizophrenia patients compared to controls was shown [70]. In addition, Leweke *et al.* [180] reported significant twofold elevations of AEA levels in the cerebrospinal fluid (CSF) of patients with schizophrenia compared to age-matched controls. Finally, a recent study also indicates that rimonabant reverses ketamine-induced impairment in prepulse inhibition of the acoustic startle reflex, an animal model of the deficient sensorimotor gating observed in schizophrenia [209]. It was recently found that CSF AEA levels are eightfold higher in antipsychotic-naive first-episode paranoid schizophrenics than in healthy controls, dementia patients or affective disorder patients. This alteration is absent in schizophrenics treated with 'typical' antipsychotics, which antagonize dopamine D2-like receptors, but not in those treated with 'atypical' antipsychotics, which preferentially antagonize 5HT(2A) receptors [112]. Recent data suggest that dysregulated striatal endocannabinoid neurotransmission is associated with a hyperdopaminergic state in dopamine transporter knockout mice [293]. AEA release in the dorsal striatum is stimulated by activation of D2 dopamine receptors [98]. The amounts of AEA were significantly increased in the blood of patients with acute schizophrenia than in healthy volunteers [69].

Several studies have demonstrated the ability of cannabinoids to provide neuroprotection against  $\beta$ -amyloid peptide (A $\beta$ ; a key pathological marker of Alzheimer disease) toxicity [156, 202, 243]. Stereotaxic injection of A $\beta$  into the rat cortex, caused a neuronal damage in the hippocampus and increased 2-AG, but not AEA levels. Further, inhibition of endocannabinoid cellular reuptake concomitantly reversed hippocampal damage in rats, and loss of memory retention in the passive avoidance test in mice, but only when administered from the 3rd day after A $\beta$  injection [297]. These observations suggest that pharmacological enhancement of brain endocannabinoid levels through the inhibition of endocannabinoid metabolism or uptake inhibitors might have a therapeutic value in the protection against A $\beta$ -induced neurodegeneration [297]. Blockade of CB1 receptors by rimonabant lessens the amnesia induced by a  $\beta$ -amyloid fragment in mice, suggesting that the endocannabinoid system may be involved in cognitive impairment in Alzheimer's disease [195]. A recent study provides evidence that  $\Delta^9$ -THC inhibits the enzyme acetylcholinesterase (AChE) as well as prevents AChE-induced A $\beta$  aggregation.  $\Delta^9$ -THC binds in the peripheral anionic site of AChE, the critical region involved in amyloidogenesis [93].

The compounds such as AEA and other NAEs present in chocolate [88] may function as a "cannabinoid mimics" [87] in the purported rewarding properties of cocoa [86] suggesting that the endocannabinoid system participate in the control of food intake. Transient inhibition of food intake and reduction in fat mass were observed following treatment of mice and rats with rimonabant [244]. CB1 receptor knockout mice on a high-fat diet were shown to have a lower susceptibility to obesity [245]. To date, data obtained from clinical trials (RIO North America, RIO Europe and RIO Lipid) indicate that rimonabant may have clinical benefits in relation to its anti-obesity properties and as a novel candidate for the treatment of metabolic and cardiovascular disorders associated with overweight and obesity [166, 291]. These studies also suggested that the drug has a reasonable safety profile. Treatment with rimonabant is also associated with favorable changes in serum lipid levels and an improvement in glycemic control in type 2 diabetic patients [105]. Following 1 year of treatment, rimonabant 20 mg/day produced significant increases in the number cigarette smokers who quit smoking compared with a placebo [259]. In another recent clinical study, orlistat, which specifically inhibit the critical enzymes (PLC and DAGL) involved in the biosynthetic pathway of 2-AG [286], reduced weight by 2.7 kg on average and decreased the incidence of type 2 diabetes from 9.0% to 6.2% [225]. In the same study, rimonabant significantly reduced weight by 4.6 kg (95% CI 4.3–5.0), reduced waist circumference, and improved triglyceride and HDL cholesterol profiles [225]. These observations suggest that the CB1 receptor may have a role in both obesity and cessation of smoking. Psychopathological disorders and depression in particular are strongly linked to eating attitude in obese patients. The identification of cannabinoid CB1 receptors (CB1Rs) in areas of the CNS that have been implicated in regulation of mood and food intake suggests that these receptors may mediate such a behavioral link.

In recent years, experimental data overwhelmingly suggested that the endocannabinoid system is a major player among the neurotransmitter systems involved in the regulation of different alcohol-related phenomena, including tolerance, vulnerability, reinforcement, and consumption (for details, see the recent reviews [12, 13, 15]). It was shown that AEA and 2-AG synthesis is increased by chronic alcohol exposure. Chronic alcohol treatment led to a significant increase in the brain levels of AEA and a significant reduction in N-ArPE, an immediate precursor for AEA synthesis [154]. Chronic alcohol exposure of rats caused a decrease in the content of both AEA and 2-AG in the midbrain, while AEA content increased in the limbic forebrain, a key area for the reinforcing

properties of habit-forming drugs, including alcohol [122]. It was observed that, in the limbic forebrain, 2-AG content was decreased after 48 h of alcohol deprivation. There was a further decrease in 2-AG content when rats were allowed to relapse to alcohol consumption [124]. These observations indicate the involvement of the endocannabinoids in alcohol-induced neuroadaptive changes in the brain and that activation of endocannabinoid-mediated neurotransmission may be responsible for the activation of the reward system by alcohol. It was found that chronic exposure to alcohol leads to an increase in extracellular AEA by inhibiting the uptake of AEA. This effect was independent of the CB1 receptor, since CB1 receptor knockout mice have normal uptake activity [22]. After prolonged exposure to alcohol, cells become tolerant to these effect such that AEA uptake is no longer inhibited by acute alcohol [22]. Chronic alcohol did not show any direct inhibition of FAAH activity in these neurons. These data suggest that alcohol-induced inhibition of AEA uptake may in part be responsible for the alcohol-induced increase in extracellular AEA.

Evidence demonstrating the down-regulation of CB1 receptor numbers and function in chronic alcohol-exposed mouse brain has recently been reported. The results of this study indicate that chronic alcohol exposure decreases the number of CB1 receptors [16]. These observations are consistent with another study which found that forced consumption of high levels of alcohol significantly decreased CB1 receptor gene expression in the caudate putamen (Cpu), ventromedial nucleus of the hypothalamus (VMN), and the CA1 and CA2 fields of the hippocampus [224]. Studies to examine if the chronic alcohol-mediated down-regulation of brain CB1 receptors has any functional effect on CB1 receptor-activated G-proteins revealed that the net binding of [ $^{35}$ S] GTP $\gamma$ S was reduced significantly without any significant changes in the G-protein affinity in chronic alcohol-exposed mice [18]. These observations support the participation of the endocannabinoid signaling mechanism in mediating some of the pharmacological and behavioral effects of alcohol, and hence the CB1 receptor may constitute an important target for therapeutic intervention in alcohol-related behaviors.

The dopaminergic system that projects from the VTA of the midbrain to the NAc, and to other forebrain sites, including the dorsal striatum, is the major substrate of reward and reinforcement produced by most drugs of abuse, including alcohol [80, 250, 314, 315]. It is well established that cannabinoids activate dopaminergic neurons in the VTA [80, 110, 250, 289, 314, 315], resulting in the release of DA in the NAc [285]. Moreover, the acute alcohol-induced release of DA in the NAc is in fact mediated by CB1 receptors [155]. The acute alcohol-induced increase in DA in NAc dialysates in C57BL/6 mice was completely inhibited by pretreatment with rimonabant or in CB1 receptor knockout mice [155]. These data suggest that activation of the limbic DA system is important for the reinforcing effects of alcohol and further suggest an interaction between the cannabinoidergic and dopaminergic systems in the reinforcing properties of alcohol abuse.

Studies from various laboratories have shown the inhibition of voluntary alcohol intake by rimonabant in rodents. Rimonabant has been shown to decrease voluntary alcohol intake in alcohol-preferring C57BL/6 mice [9], in Sardinian alcohol-preferring (sP) rats [61], in alcohol self-administering Long Evans rats [99], and in alcohol-preferring congenic B6.Cb4i5- $\beta$ /13C/Vad and B6.Cb4i5- $\beta$ 14/Vad mouse strains [154]. The acute administration of rimonabant suppressed alcohol self-administration in chronic alcohol-exposed Wistar rats [252]. A significant increase in alcohol preference (free-choice) was observed when Wistar rats were treated with an acute dose of rimonabant during chronic alcohol treatment [174]. The administration of rimonabant after chronic alcoholization significantly decreased the preference for alcohol. Alcohol withdrawal symptoms were also decreased by administration of rimonabant in

these studies. Furthermore, acute administration of the CB1 receptor agonist CP55,940 increased the motivation to consume alcohol in Wistar rats, and this effect was completely prevented by pretreatment with rimonabant [100, 101]. An acute dose of rimonabant completely abolished the alcohol deprivation effect (i.e., the temporary increase in alcohol intake after a period of alcohol withdrawal) in sP rats [263]. Acute administration of CP55,940 or another CB1 receptor agonist, WIN 55,212-2, significantly stimulated voluntary alcohol consumption in alcohol-preferring sP rats and this was completely prevented by rimonabant [62]. The existing evidence taken together suggests that activation of the CB1 receptor by a CB1 receptor agonist may involve the release of DA in the NAc and inactivation of the CB1 receptor by rimonabant may involve the inhibition of DA release. This in turn may regulate voluntary alcohol intake in these animals.

The first biochemical evidence to show the participation of the cannabinoid system in alcohol-drinking behavior is derived from the observed differences in CB1 receptor function in two genetic strains of alcohol-preferring C57BL/6 and alcohol-avoiding DBA/2 mice. In this study, it was found that C57BL/6 mice have a significantly lower level of CB1 receptor binding sites and higher affinity for [<sup>3</sup>H]CP-55,940 than DBA/2 mice [153]. Interestingly, the significantly higher levels of CB1 receptors found in DBA/2 mice are less coupled to G-proteins (as shown by a GTPγS binding assay) compared to those of C57BL/6 mouse strains [19], suggesting the involvement of these receptors in controlling voluntary alcohol consumption. Another study reported lower regional levels of CB1 receptor function and lower CB1 receptor gene expression in the brain of Fawn Hooded rats (alcohol-preferring) compared to Wistar rats (alcohol-nonpreferring) [223]. These results further strengthen the hypothesis that the inactivation or down-regulation of CB1 receptors blocks voluntary alcohol consumption. It is therefore quite possible that the animals with a genetic predisposition to high alcohol consumption have inherited a greater sensitivity of the endogenous cannabinoid system to alcohol. Several investigators using genetically modified CB1 receptor knockout mice further examined this notion. CB1 receptor knockout mice exhibited dramatically reduced voluntary alcohol consumption [155]. There was also a remarkable gender difference, with female wild-type mice consuming significantly more alcohol than male wild-type mice, whereas this gender difference was nonexistent in male and female CB1 knockout mice [155]. It was shown that young wild-type mice exhibited a significantly higher alcohol preference and voluntary alcohol intake compared to their CB1 knockout littermates [308]. Furthermore, rimonabant has been shown to reduce voluntary alcohol intake in wild-type but not in CB1 receptor knockout mice [308]. Similarly, administration of rimonabant significantly reduced alcohol and sucrose intake in C57BL/6x129/Ola mice and had no effect in CB1 receptor knockout C57BL/6x129/Ola mice [238]. Alcohol and sucrose intake were significantly reduced in CB1 receptor knockout C57BL/6x129/Ola mice [238]. The age-dependent decline in the appetite for both alcohol and food was shown using rimonabant and CB1 receptor knockout mice [308].

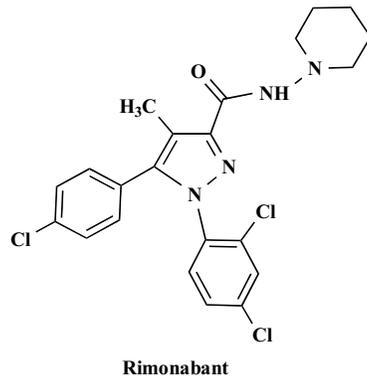
Another study not only provides evidence for the participation of the CB1 receptor in the regulation of voluntary alcohol consumption, but also for some of the acute intoxicating effects caused by administration of alcohol [210]. Alcohol consumption and preference are decreased, whereas alcohol sensitivity and withdrawal severity are increased, in CB1 receptor knockout mice [210]. CB1 receptor knockout mice showed an increase in alcohol withdrawal-induced convulsions, suggesting that alcohol consumption is also inversely related to alcohol withdrawal severity. In another study, CB1 knockout mice (CD1 background) were more sensitive to the hypothermic and sedative/hypnotic effects of alcohol than wild-type mice [210]. CB1 knockout mice displayed a significant decrease in locomotor activity following injection of alcohol (1-2.5g/kg) [210].

Alcohol self-administrations and alcohol withdrawal severity have been shown to have an inverse genetic relationship [64]. Rodents selectively bred for low alcohol intake showed greater alcohol withdrawal severity than did rodents selectively bred for high alcohol intake [53, 54, 65, 169]. Importantly, analysis of recombinant inbred strains for alcohol withdrawal severity led to identification of a quantitative trait locus on chromosome 4 in close proximity to the CB1 receptor gene [41, 42]. Furthermore, CB1 receptor expression has been shown to be increased in the rat medial frontal cortex after intermittent exposure to alcohol [248]. However, chronic alcohol exposure has been shown to reduce CB1 receptor gene expression in the rat caudate-putamen, ventromedial nucleus of the hypothalamus, and the CA1 and CA2 fields of the hippocampus; conversely, chronic alcohol exposure increases CB1 receptor gene expression in the dentate gyrus [224]. Alcohol withdrawal symptoms observed in CB1 receptor wild-type mice were abolished in CB1 receptor knockout mice [242]. A decreased expression and activity of FAAH was found in the prefrontal cortex (PFC) of alcohol-preferring rats with a compensatory down-regulation of CB1 signaling [136]. Furthermore, intra-PFC injections of the FAAH inhibitor (URB597) increased alcohol self-administration in Wistar rats [136]. Recently, increased alcohol consumption and preference and decreased alcohol sensitivity were observed only in female mice but not in male FAAH knockout mice [24]. These results suggest that impaired FAAH function may present a phenotype of high voluntary alcohol consumption, and identify FAAH both as a regulator of endocannabinoid function and a possible therapeutic target for alcohol-related disorders. These data collectively indicate that the endocannabinoid system could be important for alcohol's reinforcing effects. These findings are significant for the development of potential therapeutic strategies for the treatment of alcoholism and addiction in general.

### Therapeutic Opportunity

Even though the detailed pathophysiology of the endocannabinoid system is not yet fully understood, there is already overwhelming evidence indicating that a pharmacological modulation of the endogenous cannabinoid system could provide new tools for a number of disease states, including drug addiction. In terms of drug development, the CB1 receptor antagonist rimonabant (Fig. 8) has progressed furthest and is in late phase III trials for the treatment of obesity and as an aid for smoking cessation [60, 298]. An NIAAA clinical study of the efficacy of rimonabant to reduce voluntary alcohol drinking is in phase I trials. Pending the results of the clinical trials, rimonabant could become an important addition to the limited arsenal of effective treatments for alcoholism. During disease or drug abuse, including alcohol abuse, there are changes in endocannabinoid levels in various regions of the brain [11, 20, 258, 307]. Therefore, drugs or agents which regulate the level of endocannabinoids by inhibiting their metabolism (FAAH inhibitors such as URB597) or uptake (AM404) or synthesis (orlistat) could locally target sites while limiting the effects on uninvolved cognitive areas, and would thus be expected to have a higher therapeutic value [24, 136]. Recent evidence suggests that the blockade of CB1 receptors with rimonabant might be beneficial to alleviate motor inhibition typical of Parkinson's disease (PD) [123]. In addition to rimonabant, several specific endocannabinoid transport inhibitors, FAAH and MAGL inhibitors which regulate brain endocannabinoid levels might have a therapeutic value in the protection against Aβ-induced neurodegeneration [297] and memory deficit in rodents [194]. Endocannabinoid interactions with the dopamine system have been offered as a possible mechanism for some of the therapeutic potential of cannabinoid-based drugs in alcoholism. A recent study provides evidence of the ability of rimonabant to mitigate alcohol-withdrawal symptoms and block the formation of physical dependency by inhibiting alcohol intake. Recent data on the role of CB1 receptors in alcohol drinking behavior, including alcohol tolerance,

as discussed above collectively suggest that agents such as CB1 receptor antagonists, including rimonabant, will be promising therapeutic agents for the treatment of alcohol abuse.



**Fig. (8).** Molecular structure of CB1 receptor-selective antagonist/inverse agonist, rimonabant. Rimonabant (SR141716A) is highly potent and selective CB1 receptor ligand that readily prevents and reverses CB1 mediated effects both *in vitro* and *in vivo* [148].

#### ACKNOWLEDGEMENTS

The author acknowledges support in part by grants from the National Institutes of Health (NIH AA11031) and the New York State Psychiatric Institute.

#### REFERENCES

- [1] Abood, M.E., Ditto, K.E., Noel, M.A., Showalter, V.M., Tao, Q. (1997) Isolation and expression of a mouse CB1 cannabinoid receptor gene. Comparison of binding properties with those of native CB1 receptors in mouse brain and N18TG2 neuroblastoma cells. *Biochem. Pharmacol.*, **53**, 207-214.
- [2] Akinshola, B.E., Chakrabarti, A., Onaivi, E.S. (1999) *In-vitro* and *in-vivo* action of cannabinoids. *Neurochem. Res.*, **24**, 1233-1240.
- [3] Akinshola, B.E., Taylor, R.E., Ogunseitan, A.B., Onaivi, E.S. (1999) Anandamide inhibition of recombinant AMPA receptor subunits in *Xenopus* oocytes is increased by forskolin and 8-bromo-cyclic AMP. *Naunyn Schmiedeberg Arch. Pharmacol.*, **360**, 242-248.
- [4] Alger, B.E. (2002) Retrograde signaling in the regulation of synaptic transmission: focus on endocannabinoids. *Prog. Neurobiol.*, **68**, 247-286.
- [5] Alger, B.E., Pitler, T.A., Wagner, J.J., Martin, L.A., Morishita, W., Kirov, S.A., Lenz, R.A. (1996) Retrograde signalling in depolarization-induced suppression of inhibition in rat hippocampal CA1 cells. *J. Physiol.*, **496**, 197-209.
- [6] Ameri, A. (1999) The effects of cannabinoids on the brain. *Prog. Neurobiol.*, **58**, 315-348.
- [7] Anwyl, R. (1999) Metabotropic glutamate receptors: electrophysiological properties and role in plasticity. *Brain Res. Brain Res. Rev.*, **29**, 83-120.
- [8] Arnold, J.C., Toppole, A.N., Mallet, P.E., Hunt, G.E., McGregor, I.S. (2001) The distribution of cannabinoid-induced Fos expression in rat brain: differences between the Lewis and Wistar strain. *Brain Res.*, **921**, 240-255.
- [9] Arnone, M., Maruani, J., Chaperon, F., Thiebot, M., Poncelet, M., Soubrie, P., Le Fur, G. (1997) Selective inhibition of sucrose and ethanol intake by SR 141716, an antagonist of central cannabinoid (CB1) receptors. *Psychopharmacology*, **132**, 104-106.
- [10] Auclair, N., Otani, S., Soubrie, P., Crepel, F. (2000) Cannabinoids modulate synaptic strength and plasticity at glutamatergic synapses of rat prefrontal cortex pyramidal neurons. *J. Neurophysiol.*, **83**, 3287-3293.
- [11] Baker, D., Pryce, G., Croxford, J.L., Brown, P., Pertwee, R.G., Makriyannis, A., Khanolkar, A., Layward, L., Fezza, F., Bisogno, T., Di Marzo, V. (2001) Endocannabinoids control spasticity in a multiple sclerosis model. *FASEB J.*, **15**, 300-302.
- [12] Basavarajappa, B.S. (2005) Endocannabinoid system in the development of tolerance to alcohol. *Klinik Forschung*, **11**, 16-19.
- [13] Basavarajappa, B.S. (2006) Endocannabinoid Signaling and Alcohol Addiction. In: Baye, D.R. Ed, *New Research on Alcoholism* New York, Nova Science Publishers, Inc. p. (In Press).
- [14] Basavarajappa, B.S. (2007) Critical enzymes involved in endocannabinoid metabolism. *Protein Pept. Lett.*, **14**, 237-246.
- [15] Basavarajappa, B.S. (2007) The endocannabinoid signaling system: a potential target for next-generation therapeutics for alcoholism. *Mini Rev. Med. Chem.*, In Press.
- [16] Basavarajappa, B.S., Cooper, T.B., Hungund, B.L. (1998) Chronic ethanol administration down-regulates cannabinoid receptors in mouse brain synaptic plasma membrane. *Brain Res.*, **793**, 212-218.
- [17] Basavarajappa, B.S., Hungund, B.L. (1999) Chronic ethanol increases the cannabinoid receptor agonist, anandamide and its precursor N-arachidonyl phosphatidyl ethanolamine in SK-N-SH cells. *J. Neurochem.*, **72**, 522-528.
- [18] Basavarajappa, B.S., Hungund, B.L. (1999) Down-regulation of cannabinoid receptor agonist-stimulated [<sup>35</sup>S] GTPγS binding in synaptic plasma membrane from chronic ethanol exposed mouse. *Brain Res.*, **815**, 89-97.
- [19] Basavarajappa, B.S., Hungund, B.L. (2001) Cannabinoid receptor agonist-stimulated [<sup>35</sup>S]Guanosine Triphosphate binding in the brain of C57BL/6 and DBA/2 mice. *J. Neurosci. Res.*, **64**, 429-436.
- [20] Basavarajappa, B.S., Hungund, B.L. (2002) Neuromodulatory role of the endocannabinoid signaling system in alcoholism: an overview. *Prostaglandins Leukot. Essent. Fatty Acids*, **66**, 287-299.
- [21] Basavarajappa, B.S., Saito, M., Cooper, T.B., Hungund, B.L. (2000) Stimulation of cannabinoid receptor agonist 2-arachidonylglycerol by chronic ethanol and its modulation by specific neuromodulators in cerebellar granule neurons. *Biochem. Biophys. Acta*, **1535**, 78-86.
- [22] Basavarajappa, B.S., Saito, M., Cooper, T.B., Hungund, B.L. (2003) Chronic ethanol inhibits the anandamide transport and increases extracellular anandamide levels in cerebellar granule neurons. *Eur. J. Pharmacol.*, **466**, 73-83.
- [23] Basavarajappa, B.S., Yalamanchili, R., Cooper, T.B., Hungund, B.L. (2007) Endocannabinoid System. In: Hamon, M., Sylvestre, V.E. Eds, *Handbook of Neurochemistry and Molecular Neurobiology*. NY, Springer.
- [24] Basavarajappa, B.S., Yalamanchili, R., Cravatt, B.F., Cooper, T.B., Hungund, B.L. (2006) Increased ethanol consumption and preference and decreased ethanol sensitivity in female FAAH knockout mice. *Neuropharmacology*, **50**, 834-844.
- [25] Beltramo, M., Piomelli, D. (2000) Carrier-mediated transport and enzymatic hydrolysis of the endogenous cannabinoid 2-arachidonylglycerol. *Neuroreport*, **11**, 1231-1235.
- [26] Beltramo, M., Stella, N., Calignano, A., Lin, S.Y., Makriyannis, A., Piomelli, D. (1997) Functional role of high-affinity anandamide transport, as revealed by selective inhibition. *Science*, **277**, 1094-1097.
- [27] Biegon, A., Kerman, I.A. (2001) Autoradiographic study of pre- and postnatal distribution of cannabinoid receptors in human brain. *Neuroimage*, **14**, 1463-1468.
- [28] Bisogno, T., Berrendero, F., Ambrosino, G., Cebeira, M., Ramos, J.A., Fernandez-Ruiz, J.J., Di Marzo, V. (1999) Brain regional distribution of endocannabinoids: implications for their biosynthesis and biological function. *Biochem. Biophys. Res. Commun.*, **256**, 377-380.
- [29] Bisogno, T., Howell, F., Williams, G., Minassi, A., Cascio, M.G., Ligresti, A., Matias, I., Schiano-Moriello, A., Paul, P., Williams, E.J., Gangadharan, U., Hobbs, C., Di Marzo, V., Doherty, P. (2003) Cloning of the first sn1-DAG lipases points to the spatial and temporal regulation of endocannabinoid signaling in the brain. *J. Cell Biol.*, **163**, 463-468.
- [30] Bisogno, T., MacCarrone, M., De Petrocellis, L., Jarrhian, A., Finazzi-Agro, A., Hillard, C., Di Marzo, V. (2001) The uptake by cells of 2-arachidonylglycerol, an endogenous agonist of cannabinoid receptors. *Eur. J. Biochem.*, **268**, 1982-1989.
- [31] Bisogno, T., Melck, D., De Petrocellis, L., Di Marzo, V. (1999) Phosphatidic acid as the biosynthetic precursor of the endocannabinoid 2-arachidonylglycerol in intact mouse neuroblastoma cells stimulated with ionomycin. *J. Neurochem.*, **72**, 2113-2119.
- [32] Bisogno, T., Sepe, N., De Petrocellis, L., Di Marzo, V. (1997) Biosynthesis of 2-arachidonyl-glycerol, a novel cannabinimetic eicosanoid, in mouse neuroblastoma cells. *Adv. Exp. Med. Biol.*, **433**, 201-204.
- [33] Bisogno, T., Sepe, N., Melck, D., Maurelli, S., De Petrocellis, L., Di Marzo, V. (1997) Biosynthesis, release and degradation of the novel endogenous cannabinimetic metabolite 2-arachidonylglycerol in mouse neuroblastoma cells. *Biochem. J.*, **322**, 671-677.
- [34] Bohme, G.A., Laville, M., Ledent, C., Parmentier, M., Imperato, A. (2000) Enhanced long-term potentiation in mice lacking cannabinoid CB1 receptors. *Neuroscience*, **95**, 5-7.
- [35] Bouaboula, M., Bianchini, L., McKenzie, F.R., Pouyssegur, J., Casellas, P. (1999) Cannabinoid receptor CB1 activates the Na<sup>+</sup>/H<sup>+</sup> exchanger NHE-1 isoform via Gi-mediated mitogen activated protein kinase signaling transduction pathways. *FEBS Lett.*, **449**, 61-65.
- [36] Bouaboula, M., Bourrie, B., Rinaldi-Carmona, M., Shire, D., Le Fur, G., Casellas, P. (1995) Stimulation of cannabinoid receptor CB1 induces krox-24 expression in human astrocytoma cells. *J. Biol. Chem.*, **270**, 13973-13980.
- [37] Bouaboula, M., Pointot-Chazel, C., Bourrie, B., Canat, X., Calandra, B., Rinaldi-Carmona, M., Le Fur, G., Casellas, P. (1995) Activation of mitogen-activated protein kinases by stimulation of the central cannabinoid receptor CB1. *Biochem. J.*, **312**, 637-641.
- [38] Breivogel, C.S., Griffin, G., Di Marzo, V., Martin, B.R. (2001) Evidence for a new G protein-coupled cannabinoid receptor in mouse brain. *Mol. Pharmacol.*, **60**, 155-163.
- [39] Breivogel, C.S., Sim, L.J., Childers, S.R. (1997) Regional differences in cannabinoid receptor/G-protein coupling in rat brain. *J. Pharmacol. Exp. Ther.*, **282**, 1632-1642.
- [40] Bruinisma, K., Taren, D.L. (1999) Chocolate: food or drug? *J. Am. Diet. Assoc.*, **99**, 1249-1256.
- [41] Buck, K.J. (1998) Recent progress toward the identification of genes related to risk for alcoholism. *Mamm. Genome*, **9**, 927-928.
- [42] Buck, K.J., Metten, P., Belknap, J.K., Crabbe, J.C. (1997) Quantitative trait loci involved in genetic predisposition to acute alcohol withdrawal in mice. *J. Neurosci.*, **17**, 3946-3955.
- [43] Buckley, N.E., Mezey, E., Bonner, T., Zimmer, A., Felder, C.C., Glass, M., Zimmer, A. (1997) Development of a CB2 knockout mouse. In: *Proceedings of the Symposium on the Cannabinoids*. International Cannabinoid Research Society, Burlington, Vermont.
- [44] Burstein, S.H., Rossetti, R.G., Yagen, B., Zurier, R.B. (2000) Oxidative metabolism of anandamide. *Prostaglandins Other Lipid Mediat.*, **61**, 29-41.

- [45] Cabral, G.A., Dove Pettit, D.A. (1998) Drugs and immunity: cannabinoids and their role in decreased resistance to infectious disease. *J. Neuroimmunol.*, **83**, 116-123.
- [46] Cadas, H., di Tomaso, E., Piomelli, D. (1997) Occurrence and biosynthesis of endogenous cannabinoid precursor, N-arachidonoyl phosphatidylethanolamine, in rat brain. *J. Neurosci.*, **17**, 1226-1242.
- [47] Calandra, B., Portier, M., Kemeis, A., Delpuch, M., Carillon, C., Le Fur, G., Ferrara, P., Shire, D. (1999) Dual intracellular signaling pathways mediated by the human cannabinoid CB1 receptor. *Eur. J. Pharmacol.*, **374**, 445-455.
- [48] Carrier, E.J., Kearns, C.S., Barkmeier, A.J., Breese, N.M., Yang, W., Nithipatikom, K., Pfister, S.L., Campbell, W.B., Hillard, C.J. (2004) Cultured rat microglial cells synthesize the endocannabinoid 2-arachidonoylglycerol, which increases proliferation via a CB2 receptor-dependent mechanism. *Mol. Pharmacol.*, **65**, 999-1007.
- [49] Cartmell, J., Schoepp, D.D. (2000) Regulation of neurotransmitter release by metabotropic glutamate receptors. *J. Neurochem.*, **75**, 889-907.
- [50] Caulfield, M.P., Brown, D.A. (1992) Cannabinoid receptor agonists inhibit Ca current in NG108-15 neuroblastoma cells via a pertussis toxin-sensitive mechanism. *Br. J. Pharmacol.*, **106**, 231-232.
- [51] Caulfield, M.P., Robbins, J., Brown, D.A. (1992) Neurotransmitters inhibit the omega-conotoxin-sensitive component of Ca current in neuroblastoma x glioma hybrid (NG 108-15) cells, not the nifedipine-sensitive component. *Pflugers Arch.*, **420**, 486-492.
- [52] Chakrabarti, A., Onaivi, E.S., Chaudhuri, G. (1995) Cloning and sequencing of a cDNA encoding the mouse brain-type cannabinoid receptor protein. *DNA Seq.*, **6**, 385-388.
- [53] Chester, J.A., Blose, A.M., Froehlich, J.C. (2003) Further evidence of an inverse genetic relationship between innate differences in alcohol preference and alcohol withdrawal magnitude in multiple selectively bred rat lines. *Alcohol Clin. Exp. Res.*, **27**, 377-387.
- [54] Chester, J.A., Risinger, F.O., Cunningham, C.L. (1998) Ethanol reward and aversion in mice bred for sensitivity to ethanol withdrawal. *Alcohol Clin. Exp. Res.*, **22**, 468-473.
- [55] Chevalerey, V., Castillo, P.E. (2004) Endocannabinoid-mediated metaplasticity in the hippocampus. *Neuron*, **43**, 871-881.
- [56] Chevalerey, V., Takahashi, K.A., Castillo, P.E. (2006) Endocannabinoid-Mediated Synaptic Plasticity in the CNS. *Annu. Rev. Neurosci.*, **29**, 37-76.
- [57] Childers, S.R., Deadwyler, S.A. (1996) Role of cyclic AMP in the actions of cannabinoid receptors. *Biochem. Pharmacol.*, **52**, 819-827.
- [58] Childers, S.R., Sexton, T., Roy, M.B. (1994) Effects of anandamide on cannabinoid receptors in rat brain membranes. *Biochem. Pharmacol.*, **47**, 711-715.
- [59] Christison, R. (1848) In A Dispensatory or Commentary on the Pharmacopoeias of Great Britain (and the United States). Philadelphia, Lea and Blanchard. pp. 971-974.
- [60] Cleland, J.G., Ghosh, J., Freemantle, N., Kaye, G.C., Nasir, M., Clark, A.L., Coletta, A.P. (2004) Clinical trials update and cumulative meta-analyses from the American College of Cardiology: WATCH, SCD-HeFT, DINA-MIT, CASINO, INSPIRE, STRATUS-US, RIO-Lipids and cardiac resynchronization therapy in heart failure. *Eur. J. Heart Fail.*, **6**, 501-508.
- [61] Colombo, G., Agabio, R., Fa, M., Guano, L., Lobina, C., Loche, A., Reali, R., Gessa, G. (1998) Reduction of voluntary ethanol intake in ethanol-preferring sP rats by the cannabinoid antagonist SR-141716. *Alcohol Alcohol.*, **33**, 126-130.
- [62] Colombo, G., Serra, S., Brunetti, G., Gomez, R., Melis, S., Vacca, G., Carai, M.M., Gessa, L. (2002) Stimulation of voluntary ethanol intake by cannabinoid receptor agonists in ethanol-preferring sP rats. *Psychopharmacology (Berl.)*, **159**, 181-187.
- [63] Conn, P.J., Pin, J.P. (1997) Pharmacology and functions of metabotropic glutamate receptors. *Annu. Rev. Pharmacol. Toxicol.*, **37**, 205-237.
- [64] Crabbe, J.C., Belknap, J.K., Metten, P., Grisel, J.E., Buck, K.J. (1998) Quantitative trait loci: mapping drug and alcohol-related genes. *Adv. Pharmacol.*, **42**, 1033-1037.
- [65] Crabbe, J.C., Gallaher, E.S., Phillips, T.J., Belknap, J.K. (1994) Genetic determinants of sensitivity to ethanol in inbred mice. *Behav. Neurosci.*, **108**, 186-195.
- [66] Cravatt, B.F., Giang, D.K., Mayfield, S.P., Boger, D.L., Lerner, R.A., Gilula, N.B. (1996) Molecular characterization of an enzyme that degrades neuro-modulatory fatty-acid amides. *Nature*, **384**, 83-87.
- [67] Curtis, M.A., Faull, R.L., Glass, M. (2006) A novel population of progenitor cells expressing cannabinoid receptors in the subependymal layer of the adult normal and Huntington's disease human brain. *J. Chem. Neuroanat.*, **31**, 210-215.
- [68] Day, T.A., Rakhshan, F., Deutsch, D.G., Barker, E.L. (2001) Role of fatty acid amide hydrolase in the transport of the endogenous cannabinoid anandamide. *Mol. Pharmacol.*, **59**, 1369-1375.
- [69] De Marchi, N., De Petrocellis, L., Orlando, P., Daniele, F., Fezza, F., Di Marzo, V. (2003) Endocannabinoid signalling in the blood of patients with schizophrenia. *Lipids Health Dis.*, **2**, 5.
- [70] Dean, B., Sundram, S., Bradbury, R., Scarr, E., Copolov, D. (2001) Studies on [3H]CP-55940 binding in the human central nervous system: regional specific changes in density of cannabinoid-1 receptors associated with schizophrenia and cannabis use. *Neuroscience*, **103**, 9-15.
- [71] Denovan-Wright, E.M., Robertson, H.A. (2000) Cannabinoid receptor messenger RNA levels decrease in a subset of neurons of the lateral striatum, cortex and hippocampus of transgenic Huntington's disease mice. *Neuroscience*, **98**, 705-713.
- [72] Derkinderen, P., Toutant, M., Burgaya, F., Le Bert, M., Siciliano, J.C., de Francis, V., Gelman, M., Girault, J.A. (1996) Regulation of a neuronal form of focal adhesion kinase by anandamide. *Science*, **273**, 1719-1722.
- [73] Derkinderen, P., Toutant, M., Kadare, G., Ledent, C., Parmentier, M., Girault, J.A. (2001) Dual role of Fyn in the regulation of FAK+6,7 by cannabinoids in hippocampus. *J. Biol. Chem.*, **276**, 38289-38296.
- [74] Derkinderen, P., Valjent, E., Toutant, M., Corvol, J.C., Enslin, H., Ledent, C., Trzaskos, J., Caboche, J., Girault, J.A. (2003) Regulation of extracellular signal-regulated kinase by cannabinoids in hippocampus. *J. Neurosci.*, **23**, 2371-2382.
- [75] Deutsch, D.G., Chin, S.A. (1993) Enzymatic synthesis and degradation of anandamide, a cannabinoid receptor agonist. *Biochem. Pharmacol.*, **46**, 791-796.
- [76] Deutsch, D.G., Glaser, S.T., Howell, J.M., Kunz, J.S., Puffenberger, R.A., Hillard, C.J., Abumrad, N. (2001) The cellular uptake of anandamide is coupled to its breakdown by fatty-acid amide hydrolase. *J. Biol. Chem.*, **276**, 6967-6973.
- [77] Devane, W.A., Dysarz, F.A.I., Johnson, M.R., Melvin, L.S., Howlett, A.C. (1988) Determination and characterization of a cannabinoid receptor in rat brain. *Mol. Pharmacol.*, **34**, 605-613.
- [78] Devane, W.A., Hanus, L., Breuer, A., Pertwee, R.G., Stevenson, L.A., Griffin, G., Gibson, D., Mandelbaum, A., Etinger, A., Mechoulam, R. (1992) Isolation and structure of a brain constituent that binds to the cannabinoid receptor. *Science*, **258**, 1946-1949.
- [79] Dewey, W.L. (1986) Cannabinoid pharmacology. *Pharmacol. Rev.*, **38**, 151-178.
- [80] Di Chiara, G., Imperato, A. (1988) Drugs abused by humans preferentially increase synaptic dopamine concentrations in the mesolimbic system of freely moving rats. *Proc. Natl. Acad. Sci. USA*, **85**, 5274-5278.
- [81] Di Marzo, V. (1998) 'Endocannabinoids' and other fatty acid derivatives with cannabinimetic properties: biochemistry and possible. *Biochim. Biophys. Acta*, **1392**, 153-175.
- [82] Di Marzo, V., Bisogno, T., Sugiura, T., Melck, D., De Petrocellis, L. (1998) The novel endogenous cannabinoid 2-arachidonoylglycerol is inactivated by neuronal- and basophil-like cells: connections with anandamide. *Biochem. J.*, **331**, 15-19.
- [83] Di Marzo, V., Breivogel, C.S., Tao, Q., Bridgen, D.T., Razdan, R.K., Zimmer, A.M., Zimmer, A., Martin, B.R. (2000) Levels, metabolism, and pharmacological activity of anandamide in CB(1) cannabinoid receptor knockout mice: evidence for non-CB(1), non-CB(2) receptor-mediated actions of anandamide in mouse brain. *J. Neurochem.*, **75**, 2434-2444.
- [84] Di Marzo, V., Fontana, A., Cadas, H., Schinelli, S., Cimino, G., Schwartz, J.C., Piomelli, D. (1994) Formation and inactivation of endogenous cannabinoid anandamide in central neurons. *Nature*, **372**, 686-691.
- [85] Di Marzo, V., Hill, M.P., Bisogno, T., Crossman, A.R., Brotchie, J.M. (2000) Enhanced levels of endogenous cannabinoids in the globus pallidus are associated with a reduction in movement in an animal model of Parkinson's disease. *FASEB J.*, **14**, 1432-1438.
- [86] Di Marzo, V., Sepe, N., De Petrocellis, L., Berger, A., Crozier, G., Fride, E., Mechoulam, R. (1998) Trick or treat from food endocannabinoids? *Nature*, **396**, 636-637.
- [87] James, J.S. (1996) Marijuana and chocolate. *Aids Treat. News*, **257**, 3-4.
- [88] Di Tomaso, E., Beltramo, M., Piomelli, D. (1996) Brain cannabinoids in chocolate. *Nature*, **382**, 677-678.
- [89] Diana, M.A., Levenes, C., Mackie, K., Marty, A. (2002) Short-term retrograde inhibition of GABAergic synaptic currents in rat Purkinje cells is mediated by endogenous cannabinoids. *J. Neurosci.*, **22**, 200-208.
- [90] Dinh, T.P., Carpenter, D., Leslie, F.M., Freund, T.F., Katona, I., Sensi, S.L., Kathuria, S., Piomelli, D. (2002) Brain monoglyceride lipase participating in endocannabinoid inactivation. *Proc. Natl. Acad. Sci. USA*, **99**, 10819-10824.
- [91] Doherty, J., Dingleline, R. (2003) Functional interactions between cannabinoid and metabotropic glutamate receptors in the central nervous system. *Curr. Opin. Pharmacol.*, **3**, 46-53.
- [92] Ellert-Miklaszewska, A., Grajkowska, W., Gabrusiewicz, K., Kaminska, B., Konarska, L. (2006) Distinctive pattern of cannabinoid receptor type II (CB2) expression in adult and pediatric brain tumors. *Brain Res.*, **1137**, 161-9.
- [93] Eubanks, L.M., Rogers, C.J., Beuscher IV, A.E., Koob, G.F., Olson, A.J., Dickerson, T.J., Janda, K.D. (2006) A molecular link between the active component of Marijuana and Alzheimer's Disease pathology. *Mol. Pharm.*, **3**, 773-7.
- [94] Facci, L., Dal Toso, R., Romanello, S., Burianni, A., Skaper, S.D., Leon, A. (1995) Mast cells express a peripheral cannabinoid receptor with differential sensitivity to anandamide and palmitoylethanolamide. *Proc. Natl. Acad. Sci. USA*, **92**, 3376-3380.
- [95] Felder, C., Nielsen, A., Briley, E., Palkovits, M., Priller, J., Axelrod, J., Nguyen, D., Richardson, J., RM., R., Koppel, G., Paul, S., Becker, G. (1996) Isolation and measurement of the endogenous cannabinoid receptor agonist, anandamide, in brain and peripheral tissues of human and rat. *FEBS Lett.*, **393**, 231-235.
- [96] Felder, C.C., Briley, E.M., Axelrod, J., Simpson, J.T., Mackie, K., Devane, W.A. (1993) Anandamide, an endogenous cannabinimetic eicosanoid, binds to the cloned human cannabinoid receptor and stimulates receptor-mediated signal transduction. *Proc. Natl. Acad. Sci. USA*, **90**, 7656-7660.
- [97] Felder, C.C., Joyce, K.E., Briley, E.M., Mansouri, J., Mackie, K., Blond, O., Lai, Y., Ma, A.L., Mitchell, R.L. (1995) Comparison of the pharmacology and signal transduction of the human cannabinoid CB1 and CB2 receptors. *Mol. Pharmacol.*, **48**, 443-450.
- [98] Ferrer, B., Asbrock, N., Kathuria, S., Piomelli, D., Giuffrida, A. (2003) Effects of levodopa on endocannabinoid levels in rat basal ganglia: implications for the treatment of levodopa-induced dyskinesias. *Eur. J. Neurosci.*, **18**, 1607-1614.
- [99] Freedland, C.S., Sharpe, A.L., Samson, H.H., Porrino, L.J. (2001) Effects of SR141716A on ethanol and sucrose self-administration. *Alcohol Clin. Exp. Res.*, **25**, 277-282.
- [100] Gallate, J.E., McGregor, I.S. (1999) The motivation for beer in rats: effects of ritanserin, naloxone and SR 141716. *Psychopharmacology*, **142**, 302-308.

- [101] Gallate, J.E., Saharov, T., Mallet, P.E., McGregor, I.S. (1999) Increased motivation for beer in rats following administration of a cannabinoid CB1 receptor agonist. *Eur. J. Pharmacol.*, **370**, 233-240.
- [102] Gaoni, Y., Mechoulam, R. (1964) Isolation, structure and partial synthesis of an active constituent of hashish. *J. Am. Chem. Soc.*, **86**, 1646-1647.
- [103] Garcia, D.E., Brown, S., Hille, B., Mackie, K. (1998) Protein kinase C disrupts cannabinoid actions by phosphorylation of the CB1 cannabinoid receptor. *J. Neurosci.*, **18**, 2834-2841.
- [104] Gebremedhin, D., Lange, A.R., Campbell, W.B., Hillard, C.J., Harder, D.R. (1999) Cannabinoid CB1 receptor of cat cerebral arterial muscle functions to inhibit L-type Ca<sup>2+</sup> channel current. *Am. J. Physiol.*, **276**, H2085-2093.
- [105] Gelfand, E.V., Cannon, C.P. (2006) Rimonabant: a selective blocker of the cannabinoid CB1 receptors for the management of obesity, smoking cessation and cardiometabolic risk factors. *Expert Opin. Invest. Drugs*, **15**, 307-315.
- [106] Gerard, C.M., Mollereau, C., Vassart, G., Parmentier, M. (1991) Molecular cloning of a human cannabinoid receptor which is also expressed in testis. *Biochem. J.*, **279**, 129-134.
- [107] Gerdeman, G., Lovinger, D.M. (2001) CB1 cannabinoid receptor inhibits synaptic release of glutamate in rat dorsolateral striatum. *J. Neurophysiol.*, **85**, 468-471.
- [108] Gerdeman, G.L., Partridge, J.G., Lupica, C.R., Lovinger, D.M. (2003) It could be habit forming: drugs of abuse and striatal synaptic plasticity. *Trends Neurosci.*, **26**, 184-192.
- [109] Gerdeman, G.L., Ronesi, J., Lovinger, D.M. (2002) Postsynaptic endocannabinoid release is critical to long-term depression in the striatum. *Nat. Neurosci.*, **5**, 446-451.
- [110] Gessa, G.L., Melis, M., Muntoni, A.L., Diana, M. (1998) Cannabinoids activate mesolimbic dopamine neurons by an action on cannabinoid CB1 receptors. *Eur. J. Pharmacol.*, **341**, 39-44.
- [111] Giuffrida, A., Beltramo, M., Piomelli, D. (2001) Mechanisms of endocannabinoid inactivation: biochemistry and pharmacology. *J. Pharmacol. Exp. Ther.*, **298**, 7-14.
- [112] Giuffrida, A., Leweke, F.M., Gerth, C.W., Schreiber, D., Koethe, D., Faulhaber, J., Klosterkotter, J., Piomelli, D. (2004) Cerebrospinal anandamide levels are elevated in acute schizophrenia and are inversely correlated with psychotic symptoms. *Neuropsychopharmacology*, **29**, 2108-2114.
- [113] Giuffrida, A., Parsons, L.H., Kerr, T.M., Rodriguez de Fonseca, F., Navarro, M., Piomelli, D. (1999) Dopamine activation of endogenous cannabinoid signaling in dorsal striatum. *Nat. Neurosci.*, **2**, 358-363.
- [114] Glaser, S.T., Abumrad, N.A., Fatade, F., Kaczocha, M., Studholme, K.M., Deusch, D.G. (2003) Evidence against the presence of an anandamide transporter. *Proc. Natl. Acad. Sci. USA*, **100**, 4269-4274.
- [115] Glass, M. (2001) The role of cannabinoids in neurodegenerative diseases. *Prog. Neuropsychopharmacol. Biol. Psychiatry*, **25**, 743-765.
- [116] Glass, M., Dragunow, M., Faull, R.L. (1997) Cannabinoid receptors in the human brain: a detailed anatomical and quantitative autoradiographic study in the fetal, neonatal and adult human brain. *Neuroscience*, **77**, 299-318.
- [117] Glass, M., Dragunow, M., Faull, R.L. (2000) The pattern of neurodegeneration in Huntington's disease: a comparative study of cannabinoid, dopamine, adenosine and GABA(A) receptor alterations in the human basal ganglia in Huntington's disease. *Neuroscience*, **97**, 505-519.
- [118] Glass, M., Felder, C.C. (1997) Concurrent stimulation of cannabinoid CB1 and dopamine D2 receptors augments cAMP accumulation in striatal neurons: evidence for a Gs linkage to the CB1 receptor. *J. Neurosci.*, **17**, 5327-5333.
- [119] Glass, M., van Dellen, A., Blakemore, C., Hannan, A.J., Faull, R.L. (2004) Delayed onset of huntington's disease in mice in an enriched environment correlates with delayed loss of cannabinoid CB1 receptors. *Neuroscience*, **123**, 207-212.
- [120] Gomez del Pulgar, T., Velasco, G., Guzman, M. (2000) The CB1 cannabinoid receptor is coupled to the activation of protein kinase B/Akt. *Biochem. J.*, **347**, 369-373.
- [121] Gong, J.P., Onaivi, E.S., Ishiguro, H., Liu, Q.R., Tagliaferro, P.A., Brusco, A., Uhl, G.R. (2006) Cannabinoid CB2 receptors: immunohistochemical localization in rat brain. *Brain Res.*, **1071**, 10-23.
- [122] Gonzalez, S., Grazia Cascio, M., Fernandez-Ruiz, J., Fezza, F., Di Marzo, V., Ramos, J.A. (2002) Changes in endocannabinoid contents in the brain of rats chronically exposed to nicotine, ethanol or cocaine. *Brain Res.*, **954**, 73.
- [123] Gonzalez, S., Scorticati, C., Garcia-Arencibia, M., de Miguel, R., Ramos, J.A., Fernandez-Ruiz, J. (2006) Effects of rimonabant, a selective cannabinoid CB1 receptor antagonist, in a rat model of Parkinson's disease. *Brain Res.*, **1073-1074**, 209-219.
- [124] Gonzalez, S., Valenti, M., De Miguel, R., Fezza, F., Fernandez-Ruiz, J., Di Marzo, V., Ramos, J.A. (2004) Changes in endocannabinoid contents in reward-related brain regions of alcohol-exposed rats, and their possible relevance to alcohol relapse. *Br. J. Pharmacol.*, **143**, 455-464.
- [125] Goparaju, S.K., Ueda, N., Yamaguchi, H., Yamamoto, S. (1998) Anandamide amidohydrolase reacting with 2-arachidonoylglycerol, another cannabinoid receptor ligand. *FEBS Lett.*, **422**, 69-73.
- [126] Graham, E.S., Ball, N., Scotter, E.L., Narayan, P., Dragunow, M., Glass, M. (2006) Induction of KROX-24 by endogenous CB1 receptors in neuro2A cells is mediated by the MEK-ERK MAPK pathway and is suppressed by the PI3K pathway. *J. Biol. Chem.*, **281**, 29085-95.
- [127] Griffin, G., Tao, Q., Abood, M.E. (2000) Cloning and pharmacological characterization of the rat CB(2) cannabinoid receptor. *J. Pharmacol. Exp. Ther.*, **292**, 886-894.
- [128] Gubellini, P., Picconi, B., Bari, M., Battista, N., Calabresi, P., Centonze, D., Bernardi, G., Finazzi-Agro, A., Maccarrone, M. (2002) Experimental parkinsonism alters endocannabinoid degradation: implications for striatal glutamatergic transmission. *J. Neurosci.*, **22**, 6900-6907.
- [129] Gulyas, A.I., Cravatt, B.F., Bracey, M.H., Dinh, T.P., Piomelli, D., Boschia, F., Freund, T.F. (2004) Segregation of two endocannabinoid-hydrolyzing enzymes into pre- and postsynaptic compartments in the rat hippocampus, cerebellum and amygdala. *Eur. J. Neurosci.*, **20**, 441-458.
- [130] Guzman, M., Sanchez, C. (1999) Effects of cannabinoids on energy metabolism. *Life Sci.*, **65**, 657-664.
- [131] Hajos, N., Katona, I., Naiem, S.S., MacKie, K., Ledent, C., Mody, I., Freund, T.F. (2000) Cannabinoids inhibit hippocampal GABAergic transmission and network oscillations. *Eur. J. Neurosci.*, **12**, 3239-3249.
- [132] Hajos, N., Ledent, C., Freund, T.F. (2001) Novel cannabinoid-sensitive receptor mediates inhibition of glutamatergic synaptic transmission in the hippocampus. *Neuroscience*, **106**, 1-4.
- [133] Hall, W., Solowij, N. (1998) Adverse effects of cannabis. *Lancet*, **352**, 1611-1616.
- [134] Hampson, R.E., Mu, J., Deadwyler, S.A. (2000) Cannabinoid and kappa opioid receptors reduce potassium K current via activation of G(s) proteins in cultured hippocampal neurons. *J. Neurophysiol.*, **84**, 2356-2364.
- [135] Hansen, H.H., Hansen, S.H., Schousboe, A., Hansen, H.S. (2000) Determination of the phospholipid precursor of anandamide and other N-acyl ethanolamine phospholipids before and after sodium azide-induced toxicity in cultured neocortical neurons. *J. Neurochem.*, **75**, 861-871.
- [136] Hansson, A.C., Bermudez-Silva, F.J., Malinen, H., Hyytia, P., Sanchez-Vera, I., Rimondini, R., Rodriguez de Fonseca, F., Kunos, G., Sommer, W.H., Heilig, M. (2006) Genetic impairment of frontocortical endocannabinoid degradation and high alcohol preference. *Neuropsychopharmacology*, **32**, 117-26.
- [137] Hanus, L., Abu-Lafi, S., Frider, E., Breuer, A., Vogel, Z., Shalev, D.E., Kustanovich, I., Mechoulam, R. (2001) 2-arachidonoyl glyceryl ether, an endogenous agonist of the cannabinoid CB1 receptor. *Proc. Natl. Acad. Sci. USA*, **98**, 3662-3665.
- [138] Herkenham, M., Lynn, A.B., Little, M.D., Johnson, M.R., Melvin, L.S., de Costa, B.R., Rice, K.C. (1990) Cannabinoid receptor localization in brain. *Proc. Natl. Acad. Sci. USA*, **87**, 1932-1936.
- [139] Herkenham, M., Lynn, A.B., Johnson, M.R., Melvin, L.S., de Cost, B.R., Rice, K.C. (1991) Characterization and localization of cannabinoid receptors in rat brain: a quantitative *in vitro* autoradiographic study. *J. Neurosci.*, **16**, 8057-8066.
- [140] Hill, E.L., Gallopin, T., Ferezou, I., Cauli, B., Rossier, J., Schweitzer, P., Lambolez, B. (2007) Functional CB1 receptors are broadly expressed in neocortical GABAergic and glutamatergic neurons. *J. Neurophysiol.*, [Epub ahead of print].
- [141] Hillard, C.J., Edgemond, W.S., Jarrhian, A., Campbell, W.B. (1997) Accumulation of N-arachidonylethanolamine (anandamide) into cerebellar granule cells occurs via facilitated diffusion. *J. Neurochem.*, **69**, 631-638.
- [142] Hillard, C.J., Jarrhian, A. (2000) The movement of N-arachidonylethanolamine (anandamide) across cellular membranes. *Chem. Phys. Lipids*, **108**, 123-134.
- [143] Hillard, C.J., Jarrhian, A. (2003) Cellular accumulation of anandamide: consensus and controversy. *Br. J. Pharmacol.*, **140**, 802-808.
- [144] Ho, B.Y., Uezono, Y., Takada, S., Takase, I., Izumi, F. (1999) Coupling of the expressed cannabinoid CB1 and CB2 receptors to phospholipase C and G protein-coupled inwardly rectifying K<sup>+</sup> channels. *Recept. Channels*, **6**, 363-374.
- [145] Hoehle, M.R., Caenazzo, L., Martinez, M.M., Hsieh, W.T., Modi, W.S., Gershon, E.S., Bonner, T.I. (1991) Genetic and physical mapping of the human cannabinoid receptor gene to chromosome 6q14-q15. *New Biol.*, **3**, 880-885.
- [146] Hoffman, A.F., Lupica, C.R. (2000) Mechanisms of cannabinoid inhibition of GABA(A) synaptic transmission in the hippocampus. *J. Neurosci.*, **20**, 2470-2479.
- [147] Hollister, L.E. (1986) Health aspects of cannabis. *Pharmacol. Rev.*, **38**, 1-20.
- [148] Howlett, A.C., Barth, F., Bonner, T.I., Cabral, G., Casellas, P., Devane, W.A., Felder, C.C., Herkenham, M., Mackie, K.R., Martin, B.R., Mechoulam, R., Pertwee, R.G. (2002) International Union of Pharmacology. XXVII. Classification of cannabinoid receptors. *Pharmacol. Rev.*, **54**, 161-202.
- [149] Howlett, A.C., Johnson, M.R., Melvin, L.S., Milne, G.M. (1988) Nonclassical cannabinoid analgesics inhibit adenylate cyclase: development of a cannabinoid receptor model. *Mol. Pharmacol.*, **33**, 297-302.
- [150] Howlett, A.C., Mukhopadhyay, S. (2000) Cellular signal transduction by anandamide and 2-arachidonoylglycerol. *Chem. Phys. Lipids*, **108**, 53-70.
- [151] Huang, C.C., Lo, S.W., Hsu, K.S. (2001) Presynaptic mechanisms underlying cannabinoid inhibition of excitatory synaptic transmission in rat striatal neurons. *J. Physiol.*, **532**(Pt3), 731-748.
- [152] Huang, S.M., Bisogno, T., Trevisani, M., Al-Hayani, A., De Petrocellis, L., Fezza, F., Tognetto, M., Petros, T.J., Krej, J.F., Chu, C.J., Miller, J.D., Davies, S.N., Geppetti, P., Walker, J.M., Di Marzo, V. (2002) An endogenous capsaicin-like substance with high potency at recombinant and native vanilloid VR1 receptors. *Proc. Natl. Acad. Sci. USA*, **99**, 8400-8405.
- [153] Hungund, B.L., Basavarajappa, B.S. (2000) Distinct differences in the cannabinoid receptor binding in the brain of C57BL/6 and DBA/2 mice, selected for their differences in voluntary ethanol consumption. *J. Neurosci. Res.*, **60**, 122-128.
- [154] Hungund, B.L., Basavarajappa, B.S., Vadasz, C., Kunos, G., Rodriguez de Fonseca, F., Colombo, G., Serra, S., Parsons, L., Koob, G.F. (2002) Ethanol, endocannabinoids and cannabinoidergic signaling system. *Alcoholism Clin. Exp. Res.*, **26**, 565-574.
- [155] Hungund, B.L., Szakall, I., Adam, A., Basavarajappa, B.S., Vadasz, C. (2003) Cannabinoid CB1 receptor knockout mice exhibit markedly reduced voluntary alcohol consumption and lack alcohol-induced dopamine release in the nucleus accumbens. *J. Neurochem.*, **84**, 698-704.
- [156] Iuvone, T., Esposito, G., Esposito, R., Santamaria, R., Di Rosa, M., Izzo, A.A. (2004) Neuroprotective effect of cannabidiol, a non-psychoactive component from Cannabis sativa, on beta-amyloid-induced toxicity in PC12 cells. *J. Neurochem.*, **89**, 134-141.
- [157] Jarai, Z., Wagner, J.A., Varga, K., Lake, K.D., Compton, D.R., Martin, B.R., Zimmer, A.M., Bonner, T.I., Buckley, N.E., Mezey, E., Razdan, R.K., Zim-

- mer, A., Kunos, G. (1999) Cannabinoid-induced mesenteric vasodilation through an endothelial site distinct from CB1 or CB2 receptors. *Proc. Natl. Acad. Sci. USA*, **96**, 14136-14141.
- [158] Jog, M.S., Kubota, Y., Connolly, C.I., Hillegaart, V., Graybiel, A.M. (1999) Building neural representations of habits. *Science*, **286**, 1745-1749.
- [159] Kabelik, J., Krejci, Z., Santavy, F. (1960) Cannabis as a Medicant. *Bull. Narc.*, **12**, 5-23.
- [160] Karlsson, M., Contreras, J.A., Hellman, U., Tornqvist, H., Holm, C. (1997) cDNA cloning, tissue distribution, and identification of the catalytic triad of monoglyceride lipase. Evolutionary relationship to esterases, lysophospholipases, and haloperoxidases. *J. Biol. Chem.*, **272**, 27218-27223.
- [161] Katayama, K., Ueda, N., Katoh, I., Yamamoto, S. (1999) Equilibrium in the hydrolysis and synthesis of cannabinomimetic anandamide demonstrated by a purified enzyme. *Biochim. Biophys. Acta*, **1440**, 205-214.
- [162] Katona, I., Rancz, E.A., Acsády, L., Ledent, C., Mackie, K., Hajos, N., Freund, T.F. (2001) Distribution of CB1 cannabinoid receptors in the amygdala and their role in the control of GABAergic transmission. *J. Neurosci.*, **21**, 9506-9518.
- [163] Katona, I., Sperlagh, B., Sik, A., Kafalvi, A., Vizi, E.S., Mackie, K., Freund, T.F. (1999) Presynaptically located CB1 cannabinoid receptors regulate GABA release from axon terminals of specific hippocampal interneurons. *J. Neurosci.*, **19**, 4544-4558.
- [164] Katona, I., Urban, G.M., Wallace, M., Ledent, C., Jung, K.M., Piomelli, D., Mackie, K., Freund, T.F. (2006) Molecular composition of the endocannabinoid system at glutamatergic synapses. *J. Neurosci.*, **26**, 5628-5637.
- [165] Keam, C.S., Greenberg, M.J., DiCamelli, R., Kurzawa, K., Hillard, C.J. (1999) Relationships between ligand affinities for the cerebellar cannabinoid receptor CB1 and the induction of GDP/GTP exchange. *J. Neurochem.*, **72**, 2379-2387.
- [166] Kirkham, T.C., Tucci, S.A. (2006) Endocannabinoids in appetite control and the treatment of obesity. *CNS Neurol. Disord. Drug Targets*, **5**, 272-292.
- [167] Kondo, S., Kondo, H., Nakane, S., Kodaka, T., Tokumura, A., Waku, K., Sugiura, T. (1998) 2-Arachidonoylglycerol, an endogenous cannabinoid receptor agonist: identification as one of the major species of monoacylglycerols in various rat tissues, and evidence for its generation through CA2-independent and -independent mechanisms. *FEBS Lett.*, **429**, 152-156.
- [168] Konrad, R.J., Major, C.D., Wolf, B.A. (1994) Diacylglycerol hydrolysis to arachidonic acid is necessary for insulin secretion from isolated pancreatic islets: sequential actions of diacylglycerol and monoacylglycerol lipases. *Biochemistry*, **33**, 13284-13294.
- [169] Kosobud, A., Bodor, A.S., Crabbe, J.C. (1988) Voluntary consumption of ethanol in WSP, WSC and WSR selectively bred mouse lines. *Pharmacol. Biochem. Behav.*, **29**, 601-607.
- [170] Kozak, K.R., Crews, B.C., Morrow, J.D., Wang, L.H., Ma, Y.H., Weinander, R., Jakobsson, P.J., Marnett, L.J. (2002) Metabolism of the endocannabinoids, 2-arachidonoylglycerol and anandamide, into prostaglandin, thromboxane, and prostacyclin glycerol esters and ethanolamides. *J. Biol. Chem.*, **277**, 44877-44885.
- [171] Kreitzer, A.C., Regehr, W.G. (2001) Cerebellar depolarization-induced suppression of inhibition is mediated by endogenous cannabinoids. *J. Neurosci.*, **21**, RC174.
- [172] Kreitzer, A.C., Regehr, W.G. (2001) Retrograde inhibition of presynaptic calcium influx by endogenous cannabinoids at excitatory synapses onto Purkinje cells. *Neuron*, **29**, 717-727.
- [173] Kurahashi, Y., Ueda, N., Suzuki, H., Suzuki, M., Yamamoto, S. (1997) Reversible hydrolysis and synthesis of anandamide demonstrated by recombinant rat fatty-acid amide hydrolase. *Biochem. Biophys. Res. Commun.*, **237**, 512-515.
- [174] Lallemand, F., Soubrie, P.H., De Witte, P.H. (2001) Effects of CB1 cannabinoid receptor blockade on ethanol preference after chronic ethanol administration. *Alcohol Clin. Exp. Res.*, **25**, 1317-1323.
- [175] Lastres-Becker, I., Cebeira, M., de Ceballos, M.L., Zeng, B.Y., Jenner, P., Ramos, J.A., Fernandez-Ruiz, J.J. (2001) Increased cannabinoid CB1 receptor binding and activation of GTP-binding proteins in the basal ganglia of patients with Parkinson's syndrome and of MPTP-treated marmosets. *Eur. J. Neurosci.*, **14**, 1827-1832.
- [176] Lastres-Becker, I., De Miguel, R., Fernandez-Ruiz, J.J. (2003) The endocannabinoid system and Huntington's disease. *Curr. Drug Targets CNS Neurol. Disord.*, **2**, 335-347.
- [177] Lastres-Becker, I., Fezza, F., Cebeira, M., Bisogno, T., Ramos, J.A., Milone, A., Fernandez-Ruiz, J., Di Marzo, V. (2001) Changes in endocannabinoid transmission in the basal ganglia in a rat model of Huntington's disease. *Neuroreport*, **12**, 2125-2129.
- [178] Ledent, C., Valverde, O., Cossu, G., Petitot, F., Aubert, J.F., Beslot, F., Bohme, G.A., Imperato, A., Pedrazzini, T., Roques, B.P., Vassart, G., Fratta, W., Parmentier, M. (1999) Unresponsiveness to cannabinoids and reduced addictive effects of opiates in CB1 receptor knockout mice. *Science*, **283**, 401-404.
- [179] Leung, D., Saghatelian, A., Simon, G.M., Cravatt, B.F. (2006) Inactivation of N-acyl phosphatidylethanolamine phospholipase D reveals multiple mechanisms for the biosynthesis of endocannabinoids. *Biochemistry*, **45**, 4720-4726.
- [180] Leweke, F.M., Schneider, U., Thies, M., Munte, T.F., Emrich, H.M. (1999) Effects of synthetic Delta(9)-tetrahydrocannabinol on binocular depth inversion of natural and artificial objects in man. *Psychopharmacology*, **142**, 230-235.
- [181] Liu, J., Gao, B., Mirshahi, F., Sanyal, A.J., Khanolkar, A.D., Makriyannis, A., Kunos, G. (2000) Functional CB1 cannabinoid receptors in human vascular endothelial cells. *Biochem. J.*, **346**(Pt 3), 835-40.
- [182] Liu, J., Wang, L., Harvey-White, J., Osei-Hyiaman, D., Razdan, R., Gong, Q., Chan, A.C., Zhou, Z., Huang, B.X., Kim, H.Y., Kunos, G. (2006) A biosynthetic pathway for anandamide. *Proc. Natl. Acad. Sci. USA*, **103**, 13345-13350.
- [183] Llano, I., Leresche, N., Marty, A. (1991) Calcium entry increases the sensitivity of cerebellar Purkinje cells to applied GABA and decreases inhibitory synaptic currents. *Neuron*, **6**, 565-574.
- [184] Maccarrone, M., van der Stelt, M., Rossi, A., Veldink, G.A., Vliegthart, J.F., Agro, A.F. (1998) Anandamide hydrolysis by human cells in culture and brain. *J. Biol. Chem.*, **273**, 32332-32339.
- [185] Mackie, K., Hille, B. (1992) Cannabinoids inhibit N-type calcium channels in neuroblastoma-glioma cells. *Proc. Natl. Acad. Sci. USA*, **89**, 3825-3829.
- [186] Maejima, T., Ohno-Shosaku, T., Kano, M. (2001) Endogenous cannabinoid as a retrograde messenger from depolarized postsynaptic neurons to presynaptic terminals. *Neurosci. Res.*, **40**, 205-210.
- [187] Maillieux, P., Verslype, M., Preud'homme, X., Vanderhaeghen, J.J. (1994) Activation of multiple transcription factor genes by tetrahydrocannabinol in rat forebrain. *Neuroreport*, **5**, 1265-1268.
- [188] Maneuf, Y.P., Nash, J.E., Crossman, A.R., Brotchie, J.M. (1996) Activation of the cannabinoid receptor by delta 9-tetrahydrocannabinol reduces gamma-aminobutyric acid uptake in the globus pallidus. *Eur. J. Pharmacol.*, **308**, 161-164.
- [189] Marsicano, G., Goodenough, S., Monory, K., Hermann, H., Eder, M., Cannich, A., Azad, S.C., Cascio, M.G., Gutierrez, S.O., van der Stelt, M., Lopez-Rodriguez, M.L., Casanova, E., Schutz, G., Ziegglansberger, W., Di Marzo, V., Behl, C., Lutz, B. (2003) CB1 cannabinoid receptors and on-demand defense against excitotoxicity. *Science*, **302**, 84-88.
- [190] Marsicano, G., Wotjak, C.T., Azad, S.C., Bisogno, T., Rammes, G., Cascio, M.G., Hermann, H., Tang, J., Hofmann, C., Ziegglansberger, W., Di Marzo, V., Lutz, B. (2002) The endogenous cannabinoid system controls extinction of aversive memories. *Nature*, **418**, 530-534.
- [191] Marsicano, G., Wotjak, C.T., Azad, S.C., Bisogno, T., Rammes, G., Cascio, M.G., Hermann, H., Tang, J., Hofmann, C., Ziegglansberger, W., Di Marzo, V., Lutz, B. (2002) The endogenous cannabinoid system controls extinction of aversive memories. *Nature*, **418**, 530-534.
- [192] Matsuda, L.A. (1997) Molecular aspects of cannabinoid receptors. *Crit. Rev. Neurobiol.*, **11**, 143-166.
- [193] Matsuda, L.A., Bonner, T.I., Lolait, S.J. (1993) Localization of cannabinoid receptor mRNA in rat brain. *J. Comp. Neurol.*, **327**, 535-550.
- [194] Mazzola, C., Micale, V., Drago, F. (2003) Amnesia induced by beta-amyloid fragments is counteracted by cannabinoid CB1 receptor blockade. *Eur. J. Pharmacol.*, **477**, 219-225.
- [195] Mazzola, C., Micale, V., Drago, F. (2003) Amnesia induced by beta-amyloid fragments is counteracted by cannabinoid CB1 receptor blockade. *Eur. J. Pharmacol.*, **477**, 219-225.
- [196] McGregor, I.S., Arnold, J.C., Weber, M.F., Topple, A.N., Hunt, G.E. (1998) A comparison of delta 9-THC and anandamide induced c-fos expression in the rat forebrain. *Brain Res.*, **802**, 19-26.
- [197] Mechoulam, R., Ben-Shabat, S., Hanus, L., Ligumsky, M., Kaminski, N.E., Schatz, A.R., Gopher, A., Almog, S., Martin, B.R., Compton, D.R., Pertwee, R.G., Griffin, G., Bayewitch, M., Barg, J., Vogel, Z. (1995) Identification of an endogenous 2-monoglyceride, present in canine gut, that binds to cannabinoid receptors. *Biochem. Pharmacol.*, **50**, 83-90.
- [198] Mechoulam, R., Fride, E., Di Marzo, V. (1998) Endocannabinoids. *Eur. J. Pharmacol.*, **359**, 1-18.
- [199] Melck, D., Rueda, D., Galve-Roperh, I., De Petrocellis, L., Guzman, M., Di Marzo, V. (1999) Involvement of the cAMP/protein kinase A pathway and of mitogen-activated protein kinase in the anti-proliferative effects of anandamide in human breast cancer cells. *FEBS Lett.*, **463**, 235-240.
- [200] Melis, M., Perra, S., Muntoni, A.L., Pillolla, G., Lutz, B., Marsicano, G., Di Marzo, V., Gessa, G.L., Pistis, M. (2004) Prefrontal cortex stimulation induces 2-arachidonoyl-glycerol-mediated suppression of excitation in dopamine neurons. *J. Neurosci.*, **24**, 10707-10715.
- [201] Melis, M., Pistis, M., Perra, S., Muntoni, A.L., Pillolla, G., Gessa, G.L. (2004) Endocannabinoids mediate presynaptic inhibition of glutamatergic transmission in rat ventral tegmental area dopamine neurons through activation of CB1 receptors. *J. Neurosci.*, **24**, 53-62.
- [202] Milton, N.G. (2002) Anandamide and noladin ether prevent neurotoxicity of the human amyloid-beta peptide. *Neurosci. Lett.*, **332**, 127-130.
- [203] Morishita, W., Alger, B.E. (2000) Differential effects of the group II mGluR agonist, DCG-IV, on depolarization-induced suppression of inhibition in hippocampal CA1 and CA3 neurons. *Hippocampus*, **10**, 261-268.
- [204] Morisset, V., Urban, L. (2001) Cannabinoid-induced presynaptic inhibition of glutamatergic EPSCs in substantia gelatinosa neurons of the rat spinal cord. *J. Neurophysiol.*, **86**, 40-48.
- [205] Mu, J., Zhuang, S.Y., Kirby, M.T., Hampson, R.E., Deadwyler, S.A. (1999) Cannabinoid receptors differentially modulate potassium A and D currents in hippocampal neurons in culture. *J. Pharmacol. Exp. Ther.*, **291**, 893-902.
- [206] Mukhopadhyay, S., McIntosh, H.H., Houston, D.B., Howlett, A.C. (2000) The CB(1) cannabinoid receptor juxtamembrane C-terminal peptide confers activation to specific G proteins in brain. *Mol. Pharmacol.*, **57**, 162-170.
- [207] Mulder, A.M., Cravatt, B.F. (2006) Endocannabinoid Metabolism in the absence of Fatty Acid Amide Hydrolase (FAAH): Discovery of phosphorylcholine derivatives of N-Acyl ethanolamines. *Biochemistry*, **45**, 11267-11277.
- [208] Munro, S., Thomas, K.L., Abu-Shaar, M. (1993) Molecular characterization of a peripheral receptor for cannabinoids. *Nature*, **365**, 61-65.
- [209] Musty, R.E., Deyo, R.A., Baer, J.L., Darrow, S.M., Coleman, B. (2000) Effects of SR141716 on animal models of schizophrenia. In: Symposium on the Cannabinoids, International Cannabinoid Research Society. Burlington, Vermont.
- [210] Naassila, M., Pierrefiche, O., Ledent, C., Daoust, M. (2004) Decreased alcohol self-administration and increased alcohol sensitivity and withdrawal in CB1 receptor knockout mice. *Neuropharmacology*, **46**, 243-253.
- [211] Nakane, S., Oka, S., Arai, S., Waku, K., Ishima, Y., Tokumura, A., Sugiura, T. (2002) 2-Arachidonoyl-sn-glycero-3-phosphate, an arachidonic acid-containing lysophosphatidic acid: occurrence and rapid enzymatic conversion to 2-arachidonoyl-sn-glycerol, a cannabinoid receptor ligand, in rat brain. *Arch. Biochem. Biophys.*, **402**, 51-58.

- [212] Natarajan, V., Reddy, P.V., Schmid, P.C., Schmid, H.H. (1981) On the biosynthesis and metabolism of N-acyl ethanolamine phospholipids in infarcted dog heart. *Biochim. Biophys. Acta*, **664**, 445-448.
- [213] Nestler, E.J. (2001) Molecular neurobiology of addiction. *Am. J. Addict.*, **10**, 201-217.
- [214] Netzeband, J.G., Conroy, S.M., Parsons, K.L., Gruol, D.L. (1999) Cannabinoids enhance NMDA-elicited Ca<sup>2+</sup> signals in cerebellar granule neurons in culture. *J. Neurosci.*, **19**, 8765-8777.
- [215] Nogueron, M.I., Porgilsson, B., Schneider, W.E., Stucky, C.L., Hillard, C.J. (2001) Cannabinoid receptor agonists inhibit depolarization-induced calcium influx in cerebellar granule neurons. *J. Neurochem.*, **79**, 371-381.
- [216] Ohno-Shosaku, T., Maejima, T., Kano, M. (2001) Endogenous cannabinoids mediate retrograde signals from depolarized postsynaptic neurons to presynaptic terminal. *Neuron*, **29**, 729-738.
- [217] Ohno-Shosaku, T., Sawada, S., Kano, M. (2000) Heterosynaptic expression of depolarization-induced suppression of inhibition (DSI) in rat hippocampal cultures. *Neurosci. Res.*, **36**, 67-71.
- [218] Ohno-Shosaku, T., Sawada, S., Yamamoto, C. (1998) Properties of depolarization-induced suppression of inhibitory transmission in cultured rat hippocampal neurons. *Pflugers Arch.*, **435**, 273-279.
- [219] Ohno-Shosaku, T., Tsubokawa, H., Mizushima, I., Yoneda, N., Zimmer, A., Kano, M. (2002) Presynaptic cannabinoid sensitivity is a major determinant of depolarization-induced retrograde suppression at hippocampal synapses. *J. Neurosci.*, **22**, 3864-3872.
- [220] Okamoto, Y., Morishita, J., Tsuboi, K., Tonai, T., Ueda, N. (2004) Molecular characterization of a phospholipase D generating anandamide and its congeners. *J. Biol. Chem.*, **279**, 5298-5305.
- [221] Onaivi, E.S., Ishiguro, H., Gong, J.P., Patel, S., Perchuk, A., Meozzi, P.A., Myers, L., Mora, Z., Tagliaferro, P., Gardner, E., Brusco, A., Akinshola, B.E., Liu, Q.R., Hope, B., Iwasaki, S., Arinami, T., Teasent, L., Uhl, G.R. (2006) Discovery of the presence and functional expression of cannabinoid CB2 receptors in brain. *Ann. N. Y. Acad. Sci.*, **1074**, 514-536.
- [222] Onaivi, E.S., Leonard, C.M., Ishiguro, H., Zhang, P.W., Lin, Z., Akinshola, B.E., Uhl, G.R. (2002) Endocannabinoids and cannabinoid receptor genetics. *Prog. Neurobiol.*, **66**, 307-344.
- [223] Ortiz, S., Oliva, J.M., Perez-Rial, S., Palomo, T., Manzanares, J. (2004) Differences in basal cannabinoid CB1 receptor function in selective brain areas and vulnerability to voluntary alcohol consumption in Fawn Hooded and Wistar rats. *Alcohol Alcohol*, **39**, 297-302.
- [224] Ortiz, S., Oliva, J.M., Perez, S., Palomo, T., Manzanares, J. (2004) Chronic ethanol consumption regulates cannabinoid CB1 receptor gene expression in selected regions of rat brain. *Alcohol Alcohol*, **39**, 88-92.
- [225] Padwal, R.S., Majumdar, S.R. (2007) Drug treatments for obesity: orlistat, sibutramine, and rimonabant. *Lancet*, **369**, 71-77.
- [226] Pan, X., Ikeda, S.R., Lewis, D.L. (1996) Rat brain cannabinoid receptor modulates N-type Ca<sup>2+</sup> channels in a neuronal expression system. *Mol. Pharmacol.*, **49**, 707-714.
- [227] Paria, B.C., Dey, S.K. (2000) Ligand-receptor signaling with endocannabinoids in preimplantation embryo development and implantation. *Chem. Phys. Lipids*, **108**, 211-220.
- [228] Patel, N.A., Moldow, R.L., Patel, J.A., Wu, G., Chang, S.L. (1998) Arachidonyl ethanolamide (AEA) activation of FOS proto-oncogene protein immunoreactivity in the rat brain. *Brain Res.*, **797**, 225-233.
- [229] Pertwee, R.G. (1997) Pharmacology of cannabinoid CB1 and CB2 receptors. *Pharmacol. Ther.*, **74**, 129-180.
- [230] Pfister-Genskow, M., Weesner, G.D., Hayes, H., Eggen, A., Bishop, M.D. (1997) Physical and genetic localization of the bovine cannabinoid receptor (CNRI) gene to bovine chromosome 9. *Mamm. Genome*, **8**, 301-302.
- [231] Pinto, J.C., Potie, F., Rice, K.C., Boring, D., Johnson, M.R., Evans, D.M., Wilken, G.H., Cantrell, C.H., Howlett, A.C. (1994) Cannabinoid receptor binding and agonist activity of amides and esters of arachidonic acid. *Mol. Pharmacol.*, **46**, 516-522.
- [232] Piomelli, D. (2003) The molecular logic of endocannabinoid signalling. *Nat. Rev. Neurosci.*, **4**, 873-884.
- [233] Pisani, A., Centonze, D., Bernardi, G., Calabresi, P. (2005) Striatal synaptic plasticity: implications for motor learning and Parkinson's disease. *Mov. Disord.*, **20**, 395-402.
- [234] Pisani, A., Fezza, F., Galati, S., Battista, N., Napolitano, S., Finazzi-Agro, A., Bernardi, G., Brusa, L., Pierantozzi, M., Stanzione, P., Maccarrone, M. (2005) High endogenous cannabinoid levels in the cerebrospinal fluid of untreated Parkinson's disease patients. *Ann. Neurol.*, **57**, 777-779.
- [235] Pitler, T.A., Alger, B.E. (1990) Activation of the pharmacologically defined M3 muscarinic receptor depolarizes hippocampal pyramidal cells. *Brain Res.*, **534**, 257-262.
- [236] Pitler, T.A., Alger, B.E. (1992) Postsynaptic spike firing reduces synaptic GABA responses in hippocampal pyramidal cells. *J. Neurosci.*, **12**, 4122-4132.
- [237] Pitler, T.A., Alger, B.E. (1994) Depolarization-induced suppression of GABAergic inhibition in rat hippocampal pyramidal cells: G protein involvement in a presynaptic mechanism. *Neuron*, **13**, 1447-1455.
- [238] Poncelet, M., Maruani, J., Calassi, R., Soubrie, P. (2003) Overeating, alcohol and sucrose consumption decrease in CB1 receptor deleted mice. *Neurosci. Lett.*, **343**, 216-218.
- [239] Porter, A.C., Sauer, J.M., Knierner, M.D., Becker, G.W., Berna, M.J., Bao, J., Nomikos, G.G., Carter, P., Bymaster, F.P., Leese, A.B., Felder, C.C. (2002) Characterization of a novel endocannabinoid, virodhamine, with antagonist activity at the CB1 receptor. *J. Pharmacol. Exp. Ther.*, **301**, 1020-1024.
- [240] Prescott, S.M., Majerus, P.W. (1983) Characterization of 1,2-diacylglycerol hydrolysis in human platelets. Demonstration of an arachidonoyl-monoacylglycerol intermediate. *J. Biol. Chem.*, **258**, 764-769.
- [241] Pryce, G., Baker, D. (2005) Emerging properties of cannabinoid medicines in management of multiple sclerosis. *Trends Neurosci.*, **28**, 272-276.
- [242] Racz, I., Bilkei-Gorzó, A., Toth, Z.E., Michel, K., Palkovits, M., Zimmer, A. (2003) A critical role for the cannabinoid CB1 receptors in alcohol dependence and stress-stimulated ethanol drinking. *J. Neurosci.*, **23**, 2453-2458.
- [243] Ramirez, B.G., Blazquez, C., Gomez del Pulgar, T., Guzman, M., de Ceballos, M.L. (2005) Prevention of Alzheimer's disease pathology by cannabinoids: neuroprotection mediated by blockade of microglial activation. *J. Neurosci.*, **25**, 1904-1913.
- [244] Ravinet Trillou, C., Arnone, M., Delgorge, C., Gonalons, N., Keane, P., Maffrand, J.P., Soubrie, P. (2003) Anti-obesity effect of SR141716, a CB1 receptor antagonist, in diet-induced obese mice. *Am. J. Physiol. Regul. Integr. Comp. Physiol.*, **284**, R345-353.
- [245] Ravinet Trillou, C., Delgorge, C., Menet, C., Arnone, M., Soubrie, P. (2004) CB1 cannabinoid receptor knockout in mice leads to leanness, resistance to diet-induced obesity and enhanced leptin sensitivity. *Int. J. Obes. Relat. Metab. Disord.*, **28**, 640-648.
- [246] Reggio, P.H. (2002) Endocannabinoid structure-activity relationships for interaction at the cannabinoid receptors. *Prostaglandins Leukot. Essent. Fatty Acids*, **66**, 143-160.
- [247] Rhee, M.H., Bayewitch, M., Avidor-Reiss, T., Levy, R., Vogel, Z. (1998) Cannabinoid receptor activation differentially regulates the various adenylyl cyclase isozymes. *J. Neurochem.*, **71**, 1525-1534.
- [248] Rimondini, R., Arlinda, C., Sommer, W., Heilig, M. (2002) Long-lasting increase in voluntary ethanol consumption and transcriptional regulation in the rat brain after intermittent exposure to alcohol. *FASEB J.*, **16**, 27-35.
- [249] Robbe, D., Kopf, M., Remaury, A., Bockaert, J., Manzoni, O.J. (2002) Endogenous cannabinoids mediate long-term synaptic depression in the nucleus accumbens. *Proc. Natl. Acad. Sci. USA*, **99**, 8384-8388.
- [250] Robbins, T.W., Everitt, B.J. (1996) Neurobehavioural mechanisms of reward and motivation. *Curr. Opin. Neurobiol.*, **6**, 228-236.
- [251] Robbins, T.W., Everitt, B.J. (2002) Limbic-striatal memory systems and drug addiction. *Neurobiol. Learn. Mem.*, **78**, 625-636.
- [252] Rodriguez de Fonseca, F., Roberts, A.J., Bilbao, A., Koob, G.F., Navarro, M. (1999) Cannabinoid receptor antagonist SR141716A decreases operant ethanol self administration in rats exposed to ethanol-vapor chambers. *Acta Pharmacol. Sin.*, **20**, 1109-1114.
- [253] Ross, R.A. (2003) Anandamide and vanilloid TRPV1 receptors. *Br. J. Pharmacol.*, **140**, 790-801.
- [254] Ross, R.A., Craib, S.J., Stevenson, L.A., Pertwee, R.G., Henderson, A., Toole, J., Ellington, H.C. (2002) Pharmacological characterization of the anandamide cyclooxygenase metabolite: prostaglandin E2 ethanolamide. *J. Pharmacol. Exp. Ther.*, **301**, 900-907.
- [255] Rueda, D., Galve-Roperh, I., Haro, A., Guzman, M. (2002) The CB1 cannabinoid receptor is coupled to the activation of c-Jun N-terminal kinase. *Mol. Pharmacol.*, **58**, 814-820.
- [256] Ryan, W.J., Banner, W.K., Wiley, J.L., Martin, B.R., Razdan, R.K. (1997) Potent anandamide analogs: the effect of changing the length and branching of the end pentyl chain. *J. Med. Chem.*, **40**, 3617-3625.
- [257] Sanchez, C., Galve-Roperh, I., Rueda, D., Guzman, M. (1998) Involvement of sphingomyelin hydrolysis and the mitogen-activated protein kinase cascade in the Delta9-tetrahydrocannabinol-induced stimulation of glucose metabolism in primary astrocytes. *Mol. Pharmacol.*, **54**, 834-843.
- [258] Schabitz, W.R., Giuffrida, A., Berger, C., Aschoff, A., Schwaninger, M., Schwab, S., Piomelli, D. (2002) Release of fatty acid amides in a patient with hemispheric stroke: a microdialysis study. *Stroke*, **33**, 2112-2114.
- [259] Scheen, A.J., Finer, N., Hollander, P., Jensen, M.D., Van Gaal, L.F. (2006) Efficacy and tolerability of rimonabant in overweight or obese patients with type 2 diabetes: a randomised controlled study. *Lancet*, **368**, 1660-1672.
- [260] Schmid, P.C., Reddy, P.V., Natarajan, V., Schmid, H.H. (1983) Metabolism of N-acyl ethanolamine phospholipids by a mammalian phosphodiesterase of the phospholipase D type. *J. Biol. Chem.*, **258**, 9302-9306.
- [261] Schweitzer, P. (2000) Cannabinoids decrease the K(+) M-current in hippocampal CA1 neurons. *J. Neurosci.*, **20**, 51-58.
- [262] Seltzman, H.H., Fleming, D.N., Thomas, B.F., Gilliam, A.F., McCallion, D.S., Pertwee, R.G., Compton, D.R., Martin, B.R. (1997) Synthesis and pharmacological comparison of dimethylheptyl and pentyl analogs of anandamide. *J. Med. Chem.*, **40**, 3626-3634.
- [263] Serra, S., Brunetti, G., Pani, M., Vacca, G., Carai, M.A., Gessa, G.L., Colombo, G. (2002) Blockade by the cannabinoid CB1 receptor antagonist, SR 141716, of alcohol deprivation effect in alcohol-preferring rats. *Eur. J. Pharmacol.*, **443**, 95-97.
- [264] Shen, M., Thayer, S.A. (1998) The cannabinoid agonist Win55,212-2 inhibits calcium channels by receptor-mediated and direct pathways in cultured rat hippocampal neurons. *Brain Res.*, **783**, 77-84.
- [265] Simpson, C.M., Itabe, H., Reynolds, C.N., King, W.C., Glomset, J.A. (1991) Swiss 3T3 cells preferentially incorporate sn-2-arachidonoyl monoacylglycerol into sn-1-stearoyl-2-arachidonoyl phosphatidylinositol. *J. Biol. Chem.*, **266**, 15902-15909.
- [266] Soderstrom, K., Johnson, F. (2000) CB1 cannabinoid receptor expression in brain regions associated with zebra finch song control. *Brain Res.*, **857**, 151-157.
- [267] Soderstrom, K., Leid, M., Moore, F.L., Murray, T.F. (2000) Behavioral, pharmacological, and molecular characterization of an amphibian cannabinoid receptor. *J. Neurochem.*, **75**, 413-423.
- [268] Stefano, G.B., Salzet, B., Salzet, M. (1997) Identification and characterization of the leech CNS cannabinoid receptor: coupling to nitric oxide release. *Brain Res.*, **753**, 219-224.
- [269] Steiner, H., Bonner, T.I., Zimmer, A.M., Kitai, S.T., Zimmer, A. (1999) Altered gene expression in striatal projection neurons in CB1 cannabinoid receptor knockout mice. *Proc. Natl. Acad. Sci. USA*, **96**, 5786-5790.

- [270] Stella, N., Schweitzer, P., Piomelli, D. (1997) A second endogenous cannabinoid that modulates long-term potentiation. *Nature*, **388**, 773-778.
- [271] Straiker, A., Mackie, K. (2005) Depolarization-induced suppression of excitation in murine autaptic hippocampal neurons. *J. Physiol.*, **569**, 501-517.
- [272] Stubbs, L., Chittenden, L., Chakrabarti, A., Onaivi, E. (1996) The gene encoding the central cannabinoid receptor is located in proximal mouse Chromosome 4. *Mamm. Genome*, **7**, 165-166.
- [273] Sugiura, T., Kobayashi, Y., Oka, S., Waku, K. (2002) Biosynthesis and degradation of anandamide and 2-arachidonoylglycerol and their possible physiological significance. *Prostaglandins Leukot. Essent. Fatty Acids*, **66**, 173-192.
- [274] Sugiura, T., Kodaka, T., Kondo, S., Nakane, S., Kondo, H., Waku, K., Ishima, Y., Watanabe, K., Yamamoto, I. (1997) Is the cannabinoid CB1 receptor a 2-arachidonoylglycerol receptor? Structural requirements for triggering a Ca<sup>2+</sup> transient in NG108-15 cells. *J. Biochem. (Tokyo)*, **122**, 890-895.
- [275] Sugiura, T., Kodaka, T., Kondo, S., Tonegawa, T., Nakane, S., Kishimoto, S., Yamashita, A., K., W. (1996) 2-Arachidonoylglycerol, a putative endogenous cannabinoid receptor ligand, induces rapid, transient elevation of intracellular free Ca<sup>2+</sup> in neuroblastoma x glioma hybrid NG108-15 cells. *Biochem. Biophys. Res. Commun.*, **229**, 58-64.
- [276] Sugiura, T., Kodaka, T., Nakane, S., Kishimoto, S., Kondo, S., Waku, K. (1998) Detection of an endogenous cannabinoid-like molecule, 2-arachidonoylglycerol, and cannabinoid CB1 receptor mRNA in human vascular cells: is 2-arachidonoylglycerol a possible vasomodulator? *Biochem. Biophys. Res. Commun.*, **243**, 838-843.
- [277] Sugiura, T., Kodaka, T., Nakane, S., Miyashita, T., Kondo, S., Suhara, Y., Takayama, H., Waku, K., Seki, C., Baba, N., Ishima, Y. (1999) Evidence that the cannabinoid CB1 receptor is a 2-arachidonoylglycerol receptor. *J. Biol. Chem.*, **274**, 2794-2801.
- [278] Sugiura, T., Kondo, S., Sukagawa, A., Nakane, S., Shinoda, A., Itoh, K., Yamashita, A., Waku, K. (1995) 2-Arachidonoylglycerol: a possible endogenous cannabinoid receptor ligand in brain. *Biochem. Biophys. Res. Commun.*, **215**, 89-97.
- [279] Sugiura, T., Kondo, S., Sukagawa, A., Tonegawa, T., Nakane, S., Yamashita, A., Waku, K. (1996) Enzymatic synthesis of anandamide, an endogenous cannabinoid receptor ligand, through N-acylphosphatidylethanolamine pathway in testis: involvement of Ca(2+)-dependent transacylase and phosphodiesterase activities. *Biochem. Biophys. Res. Commun.*, **218**, 113-117.
- [280] Sugiura, T., Kondo, S., Sukagawa, A., Tonegawa, T., Nakane, S., Yamashita, A., Waku, K. (1996) N-arachidonoyl ethanolamine (anandamide), an endogenous cannabinoid receptor ligand, and related lipid molecules in the nervous tissues. *Lipid Mediat. Cell Signal.*, **14**, 51-56.
- [281] Sugiura, T., Yoshinaga, N., Kondo, S., Waku, K., Ishima, Y. (2000) Generation of 2-arachidonoylglycerol, an endogenous cannabinoid receptor ligand, in picrotoxinin-administered rat brain. *Biochem. Biophys. Res. Commun.*, **271**, 654-658.
- [282] Sullivan, J.M. (2000) Cellular and molecular mechanisms underlying learning and memory impairments produced by cannabinoids. *Learn Mem.*, **7**, 132-139.
- [283] Sun, Y.X., Tsuboi, K., Okamoto, Y., Tonai, T., Murakami, M., Kudo, I., Ueda, N. (2004) Biosynthesis of anandamide and N-palmitoylethanolamine by sequential actions of phospholipase A2 and lysophospholipase D. *Biochem. J.*, **380**, 749-756.
- [284] Szabo, B., Dorner, L., Pfreundtner, C., Norenberg, W., Starke, K. (1998) Inhibition of GABAergic inhibitory postsynaptic currents by cannabinoids in rat corpus striatum. *Neuroscience*, **85**, 395-403.
- [285] Szabo, B., Muller, T., Koch, H. (1999) Effects of cannabinoids on dopamine release in the corpus striatum and the nucleus accumbens *in vitro*. *J. Neurochem.*, **73**, 1084-1089.
- [286] Szabo, B., Urbanski, M.J., Bisogno, T., Di Marzo, V., Mendiguren, A., Baer, W.U., Freiman, I. (2006) Depolarization-induced retrograde synaptic inhibition in the mouse cerebellar cortex is mediated by 2-arachidonoylglycerol. *J. Physiol.*, **577**, 263-280.
- [287] Szabo, B., Wallmichrath, I., Mathonia, P., Pfreundtner, C. (2000) Cannabinoids inhibit excitatory neurotransmission in the substantia nigra pars reticulata. *Neuroscience*, **97**, 89-97.
- [288] Takahashi, K.A., Linden, D.J. (2000) Cannabinoid receptor modulation of synapses received by cerebellar Purkinje cells. *J. Neurophysiol.*, **83**, 1167-1180.
- [289] Tanda, G., Pontieri, F.E., Di Chiara, G. (1997) Cannabinoid and heroin activation of mesolimbic dopamine transmission by a common mu1 opioid receptor mechanism. *Science*, **276**, 2048-2050.
- [290] Thomas, M.J., Malenka, R.C. (2003) Synaptic plasticity in the mesolimbic dopamine system. *Philos. Trans. R. Soc. Lond. B. Biol. Sci.*, **358**, 815-819.
- [291] Tucci, S.A., Halford, J.C., Harrold, J.A., Kirkham, T.C. (2006) Therapeutic potential of targeting the endocannabinoids: implications for the treatment of obesity, metabolic syndrome, drug abuse and smoking cessation. *Curr. Med. Chem.*, **13**, 2669-2680.
- [292] Twitchell, W., Brown, S., Mackie, K. (1997) Cannabinoids inhibit N- and P/Q-type calcium channels in cultured rat hippocampal neurons. *J. Neurophysiol.*, **78**, 43-50.
- [293] Tzavara, E.T., Li, D.L., Moutsimilli, L., Bisogno, T., Di Marzo, V., Phebus, L.A., Nomikos, G.G., Giros, B. (2006) Endocannabinoids activate transient receptor potential vanilloid 1 receptors to reduce hyperdopaminergic-related hyperactivity: therapeutic implications. *Biol. Psychiatry*, **59**, 508-515.
- [294] Ueda, N., Kurahashi, Y., Yamamoto, S., Tokunaga, T. (1995) Partial purification and characterization of the porcine brain enzyme hydrolyzing and synthesizing anandamide. *J. Biol. Chem.*, **270**, 23823-23827.
- [295] Valjent, E., Caboche, J., Vanhoutte, P. (2001) Mitogen-activated protein kinase/extracellular signal-regulated kinase induced gene regulation in brain: a molecular substrate for learning and memory? *Mol. Neurobiol.*, **23**, 83-99.
- [296] van der Stelt, M., Fox, S.H., Hill, M., Crossman, A.R., Petrosino, S., Di Marzo, V., Brotchie, J.M. (2005) A role for endocannabinoids in the generation of parkinsonism and levodopa-induced dyskinesia in MPTP-lesioned non-human primate models of Parkinson's disease. *FASEB J.*, **19**, 1140-1142.
- [297] van der Stelt, M., Mazzola, C., Esposito, G., Matias, I., Petrosino, S., De Filippis, D., Micala, V., Steardo, L., Drago, F., Iuvone, T., Di Marzo, V. (2006) Endocannabinoids and beta-amyloid-induced neurotoxicity *in vivo*: effect of pharmacological elevation of endocannabinoid levels. *Cell Mol. Life Sci.*, **63**, 1410-1424.
- [298] Van Gaal, L.F., Rissanen, A.M., Scheen, A.J., Ziegler, O., Rossner, S. (2005) Effects of the cannabinoid-1 receptor blocker rimonabant on weight reduction and cardiovascular risk factors in overweight patients: 1-year experience from the RIO-Europe study. *Lancet*, **365**, 1389-1397.
- [299] Van Sickle, M.D., Duncan, M., Kingsley, P.J., Mouihate, A., Urbani, P., Mackie, K., Stella, N., Makriyannis, A., Piomelli, D., Davison, J.S., Marnett, L.J., Di Marzo, V., Pittman, Q.J., Patel, K.D., Sharkey, K.A. (2005) Identification and functional characterization of brainstem cannabinoid CB2 receptors. *Science*, **310**, 329-332.
- [300] Varma, N., Carlson, G.C., Ledent, C., Alger, B.E. (2001) Metabotropic glutamate receptors drive the endocannabinoid system in hippocampus. *J. Neurosci.*, **21**, RC188.
- [301] Vaughan, C.W., Connor, M., Bagley, E.E., Christie, M.J. (2000) Actions of cannabinoids on membrane properties and synaptic transmission in rat periaqueductal gray neurons *in vitro*. *Mol. Pharmacol.*, **57**, 288-295.
- [302] Vaughan, C.W., McGregor, I.S., Christie, M.J. (1999) Cannabinoid receptor activation inhibits GABAergic neurotransmission in rostral ventromedial medulla neurons *in vitro*. *Br. J. Pharmacol.*, **127**, 935-940.
- [303] Vincent, P., Armstrong, C.M., Marty, A. (1992) Inhibitory synaptic currents in rat cerebellar Purkinje cells: modulation by postsynaptic depolarization. *J. Physiol.*, **456**, 453-471.
- [304] Vincent, P., Marty, A. (1993) Neighboring cerebellar Purkinje cells communicate via retrograde inhibition of common presynaptic interneurons. *Neuron*, **11**, 885-893.
- [305] Vincent, P., Marty, A. (1996) Fluctuations of inhibitory postsynaptic currents in Purkinje cells from rat cerebellar slices. *J. Physiol.*, **494**(Pt 1), 183-199.
- [306] Wagner, J.A., Varga, K., Jarai, Z., Kunos, G. (1999) Mesenteric vasodilation mediated by endothelial anandamide receptors. *Hypertension*, **33**, 429-434.
- [307] Walker, J.M., Huang, S.M. (2002) Endocannabinoids in pain modulation. *Prostaglandins Leukot. Essent. Fatty Acids*, **66**, 235-242.
- [308] Wang, L., Liu, J., Harvey-white, J., Zimmer, A., Kunos, G. (2003) Endocannabinoid signaling via CB1 receptors is involved in ethanol preference and its age-dependent decline in mice. *Proc. Natl. Acad. Sci. USA*, **100**, 1393-1398.
- [309] Wartmann, M., Campbell, D., Subramanian, A., Burstein, S.H., Davis, R.J. (1995) The MAP kinase signal transduction pathway is activated by the endogenous cannabinoid anandamide. *FEBS Lett.*, **2-3**, 133-136.
- [310] Watson, S.J., Benson, J.A., Jr., Joy, J.E. (2000) Marijuana and medicine: assessing the science base: a summary of the 1999 Institute of Medicine report. *Arch. Gen. Psychiatry*, **57**, 547-552.
- [311] Wilson, R.I., Kunos, G., Nicoll, R.A. (2001) Presynaptic specificity of endocannabinoid signaling in the hippocampus. *Neuron*, **31**, 453-462.
- [312] Wilson, R.I., Nicoll, R.A. (2001) Endogenous cannabinoids mediate retrograde signalling at hippocampal synapses. *Nature*, **410**, 588-592.
- [313] Wilson, R.I., Nicoll, R.A. (2002) Endocannabinoid signaling in the brain. *Science*, **296**, 678-682.
- [314] Wise, R.A. (1996) Addictive drugs and brain stimulation reward. *Annu. Rev. Neurosci.*, **19**, 319-340.
- [315] Wise, R.A., Bozarth, M.A. (1987) A psychomotor stimulant theory of addiction. *Psychol. Rev.*, **94**, 469-492.
- [316] Yamaguchi, F., Macrae, A.D., Brenner, S. (1996) Molecular cloning of two cannabinoid type 1-like receptor genes from the puffer fish *Fugu rubripes*. *Genomics*, **35**, 603-605.
- [317] Yang, H.Y., Karoum, F., Felder, C., Badger, H., Wang, T.C., Markey, S.P. (1999) GC/MS analysis of anandamide and quantification of N-arachidonoylphosphatidylethanolamides in various brain regions, spinal cord, testis, and spleen of the rat. *J. Neurochem.*, **72**, 1959-1968.
- [318] Yoshida, T., Fukaya, M., Uchigashima, M., Miura, E., Kamiya, H., Kano, M., Watanabe, M. (2006) Localization of diacylglycerol lipase- $\alpha$  around postsynaptic spine suggests close proximity between production site of an endocannabinoid, 2-arachidonoyl-glycerol, and presynaptic cannabinoid CB1 receptor. *J. Neurosci.*, **26**, 4740-4751.
- [319] Yoshida, T., Hashimoto, K., Zimmer, A., Maejima, T., Araiishi, K., Kano, M. (2002) The cannabinoid CB1 receptor mediates retrograde signals for depolarization-induced suppression of inhibition in cerebellar purkinje cells. *J. Neurosci.*, **22**, 1690-1697.
- [320] Zilberter, Y. (2000) Dendritic release of glutamate suppresses synaptic inhibition of pyramidal neurons in rat neocortex. *J. Physiol.*, **528**(Pt 3), 489-496.
- [321] Zimmer, A., Zimmer, A.M., Hohmann, A.G., Herkenham, M., Bonner, T.I. (1999) Increased mortality, hypoactivity, and hypoalgesia in cannabinoid CB1 receptor knockout mice. *Proc. Natl. Acad. Sci. USA*, **96**, 5780-5785.