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SPECIAL ISSUE ARTICLE

THE ENDOCANNABINOID SYSTEM: PHYSIOLOGY AND PHARMACOLOGY

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Abstract — The endogenous cannabinoid system is an ubiquitous lipid signalling system that appeared early in evolution and which has important regulatory functions throughout the body in all vertebrates. The main endocannabinoids (endogenous cannabis-like substances) are small molecules derived from arachidonic acid, anandamide (arachidonoylethanolamide) and 2-arachidonoylglycerol. They bind to a family of G-protein-coupled receptors, of which the cannabinoid CB_1 receptor is densely distributed in areas of the brain related to motor control, cognition, emotional responses, motivated behaviour and homeostasis. Outside the brain, the endocannabinoid system is one of the crucial modulators of the autonomic nervous system, the immune system and microcirculation. Endocannabinoids are released upon demand from lipid precursors in a receptor-dependent manner and serve as retrograde signalling messengers in GABAergic and glutamatergic synapses, as well as modulators of postsynaptic transmission, interacting with other neurotransmitters, including dopamine. Endocannabinoids are transported into cells by a specific uptake system and degraded by two well-characterized enzymes, the fatty acid amide hydrolase and the monoacylglycerol lipase. Recent pharmacological advances have led to the synthesis of cannabinoid receptor agonists and antagonists, anandamide uptake blockers and potent, selective inhibitors of endocannabinoid degradation. These new tools have enabled the study of the physiological roles played by the endocannabinoids and have opened up new strategies in the treatment of pain, obesity, neurological diseases including multiple sclerosis, emotional disturbances such as anxiety and other psychiatric disorders including drug addiction. Recent advances have specifically linked the endogenous cannabinoid system to alcoholism, and cannabinoid receptor antagonism now emerges as a promising therapeutic alternative for alcohol dependence and relapse.

INTRODUCTION

Twenty-four years of pharmacological research separate the identification of the main psychoactive constituent of *Cannabis sativa* preparations, $(-)$-Δ^9-tetrahydrocannabinol (THC) (Gaoni and Mechoulam, 1964; Mechoulam, 1970) from the characterization (Devane *et al*., 1988; Herkenham *et al*., 1991) and molecular cloning (Matsuda *et al*., 1990) of its cellular target, the cannabinoid CB_1 receptor (CB_1). The extensive research on the structure and activity of the natural constituents of *Cannabis* (termed cannabinoids) and the development of synthetic compounds with high potency and stereoselectivity have led to the identification of the main physiological functions that are modulated by this new class of drugs (Howlett *et al*., 1990). The discovery of the cannabinoid receptor and the availability of highly selective and potent cannabimimetics led to the rapid identification of a family of lipid transmitters that serve as natural ligands for the CB_1 receptor: arachidonoylethanolamide (AEA), named anandamide from the Sanskrit 'internal bliss' (Devane *et al*., 1992) and 2-arachidonoylglycerol (2-AG) (Mechoulam *et al*., 1995; Sugiura *et al*., 1995). The pharmacological properties of the endocannabinoids were found to be very similar to those of the synthetic cannabimimetics. The subsequent description of a complex biochemical pathway for the synthesis, release (Di Marzo *et al*., 1994; Cadas *et al*., 1996), transport (Beltramo *et al*., 1997) and degradation (Cravatt *et al*., 1996) of endocannabinoids completed the scaffold of a new signalling system termed the 'endocannabinoid system'. Since the discovery of anandamide, more than 3500 scientific reports have comprehensively explored the main aspects of the endocannabinoid system. This system now appears as a relevant modulator of physiological functions not only in the central nervous system but also in the autonomic nervous system, the endocrine network, the immune system, the gastrointestinal tract, the reproductive system and in microcirculation (Di Marzo *et al*., 1998; Table 1).

The present review gives a general perspective of the endogenous cannabinoid system, including the main pharmacological advances in the development of drugs capable of modulating their dynamics. The review focuses on the role of endocannabinoids as modulators of reward circuits and motivated behaviour that are relevant for drug addiction, including alcoholism. In light of the extensive research over the past 12 years, several specialized reviews wherein the reader will find a more profound analysis of the role played by the endocannabinoid system in selected physiological functions are shown in Table 1.

Biochemistry of the endogenous cannabinoid system

Endocannabinoids. When discovered, the endocannabinoids were found to be derivatives of arachidonic acid, which resembled other lipid transmitters (eicosanoids such as prostaglandins or leukotrienes). Additional studies revealed the existence of other structure-related lipid messengers including palmitylethanolamide or oleoylethanolamide, which are not active at cannabinoid receptors. These messengers will not be included in this review, although they serve important physiological functions in inflammation, pain

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Table 1. A selection of reviews and reports that explore in depth the main aspects of endocannabinoids and their receptors

Topic	References
Biochemistry and molecular biology	Matsuda, 1997; Felder and Glass, 1998; Giuffrida *et al*., 2001; Piomelli, 2003
Signalling	Schlicker and Kathmann, 2001; Wilson and Nicoll, 2002; Freund *et al*., 2003
Anatomy and development	Breivogel and Childers, 1998; Fernandez-Ruiz *et al*., 2000; Elphick and Egertova, 2001
Physiology	Di Marzo *et al*., 1998; De Petrocellis *et al*., 2004
Pharmacology	Howlett *et al*., 2002
Addiction	Maldonado, 2002; Maldonado and Rodríguez de Fonseca, 2002; Tanda and Goldberg, 2003; Martin *et al*., 2004
Pain	Calignano *et al*., 2000; Pertwee, 2001
Behaviour	Rodríguez de Fonseca *et al*., 1998; Chaperon and Thiebot, 1999; Castellano *et al*., 2003
Gastrointestinal system	Izzo *et al*., 2001
Immune system	Cabral, 2001
Cardiovascular system	Kunos *et al*., 2002; Randall *et al*., 2002; Hiley and Ford, 2004
Therapeutic applications	Piomelli *et al*., 2000; Cravatt and Lichtman, 2003; Guzman, 2003; Smith *et al*., 2004

Since the discovery of anandamide in 1992, over 3500 publications have reported new data on the biological role of the endogenous cannabinoid system.

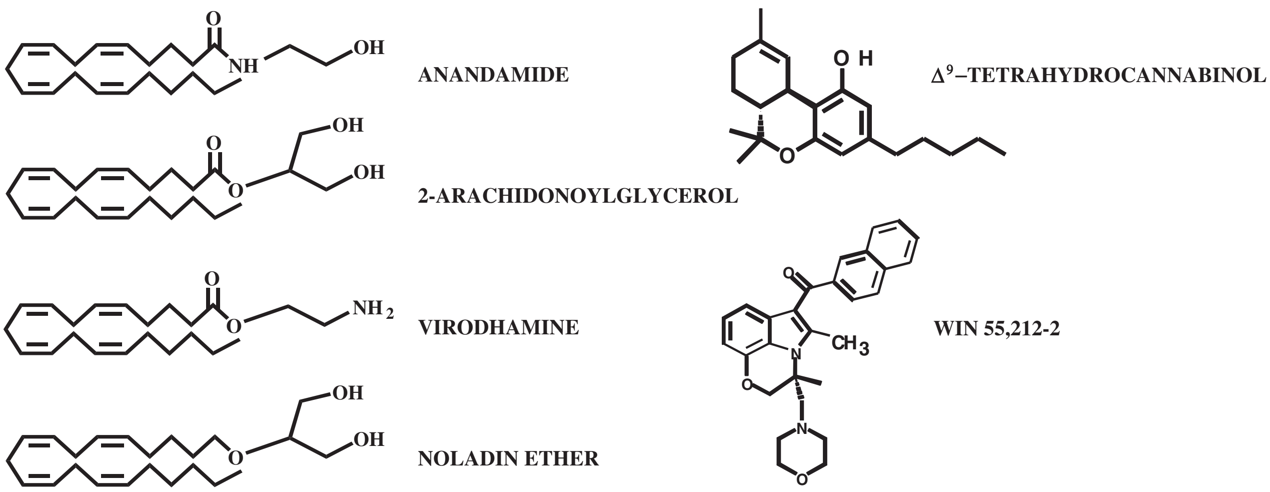


Fig. 1. Cannabinoid receptor agonists. Left, the structure of four arachidonic acid derivatives that have been identified as endogenous ligands for both the cannabinoid CB_1 and CB_2 receptors. Right, the structure of Δ^9-tetrahydrocannabinol (THC), the main cannabinoid receptor agonist present in *Cannabis* preparations and that of the aminoalkylindole WIN-55,2122, a synthetic cannabinoid receptor agonist active at CB_1 and CB_2 receptors.

control, feeding behaviour and lipid metabolism (Calignano *et al.* 1998; Rodríguez de Fonseca *et al.*, 2001; Fu *et al.*, 2003; Piomelli, 2003).

Endocannabinoids are derivatives of arachidonic acid conjugated with ethanolamine or glycerol. Figure 1 depicts the chemical structure of four endocannabinoids, anandamide, 2-arachidonoylglycerol (2-AG), the ester of arachidonic acid and ethanolamine; virodhamine which resembles anandamide (Porter *et al*., 2002), and the 2-arachidonyl glyceryl ether noladin, an analogue of 2-AG (Hanus *et al*., 2001). All these endocannabinoids have been found in the brain, plasma and peripheral tissues, although the relevance of noladin has been questioned recently (Oka *et al*., 2003) because its concentration in the brain is too low for this compound to act as an endogenous cannabinoid receptor ligand. In the brain, the concentration of anandamide is 200-fold lower than that of 2-AG (Sugiura *et al*., 1995; Stella *et al*., 1997). The monoglyceride 2-AG is a metabolic intermediate in lipid metabolism whereas anandamide is the product of the cleavage of a membrane phospholipid. However, after depolarization or receptor stimulation (e.g. dopamine D2 receptor-mediated), the concentration of anandamide can rise up to 5–12 fold in a time-limited fashion (Giuffrida *et al*., 1999; Stella and Piomelli, 2001; Kim *et al*., 2002).

Synthesis and release. Different pathways are involved in the synthesis and release of anandamide and 2-AG. Figure 2 shows the dynamics of formation and degradation of anandamide.

Anandamide is formed by the cleavage of a phospholipid precursor, the *N*-arachidonoyl-phosphatidylethanolamine (NAPE). The precursor is synthesized by the enzyme *N*-acyltransferase (NAT), which catalyses the transfer of arachidonic acid from phosphatidylcholine to the head group of phosphatidylethanolamine. This enzyme requires the presence of Ca^{2+} and is regulated by cAMP, which enhances the activity of NAT by phosphorylation mediated through the cAMP-dependent activity of protein kinase A (Cadas *et al*., 1996; Piomelli, 2003). The release of anandamide from NAPE is catalysed by a specific phospholipase D (PLD), which has been cloned recently (Okamoto *et al*., 2004). This enzyme has no homology with the known PLD enzymes and is classified as a member of the zinc metallohydrolase family. Its presence is highest in the brain, kidneys and testis. The activity of PLD is

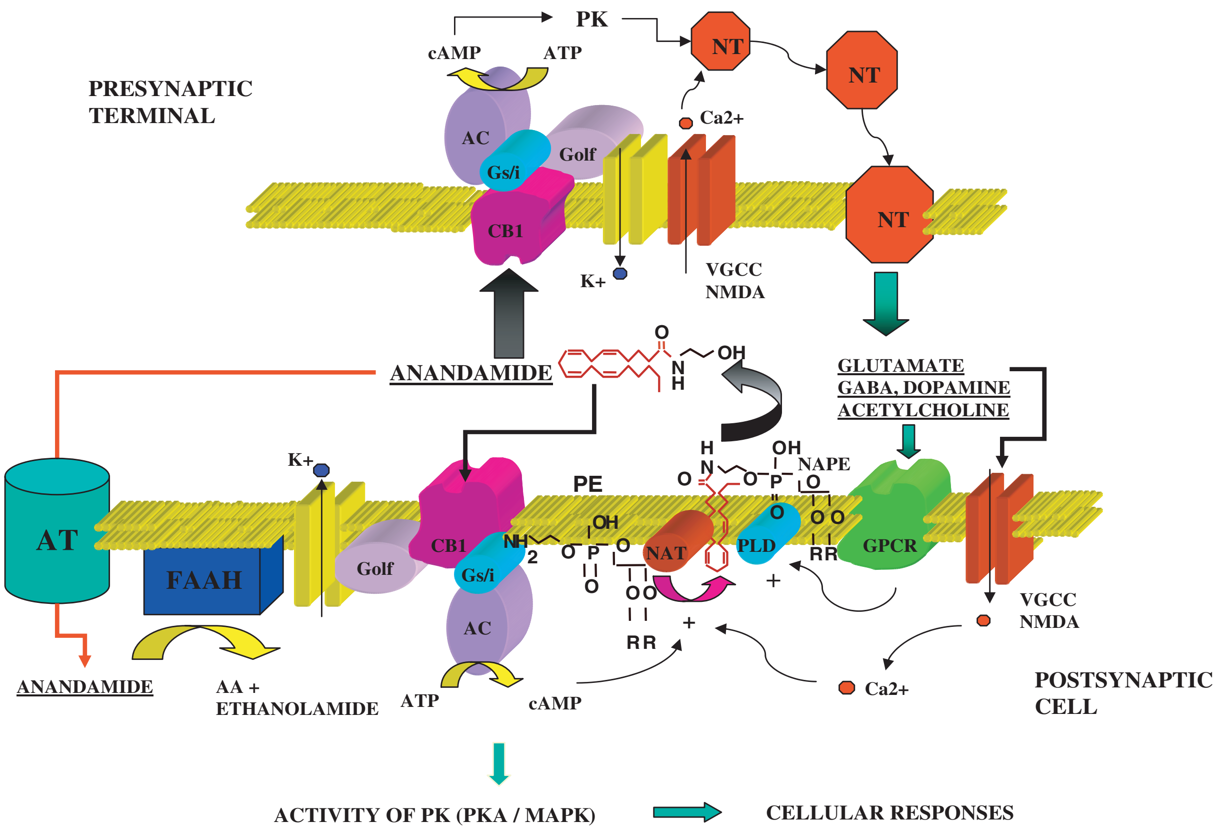


Fig. 2. Overview of the biochemical pathways for synthesis, degradation and cellular actions of the endogenous cannabinoid anandamide. Anandamide is released from a membrane lipid precursor (*N*-arachidonoyl-phosphatidylethanolamine, NAPE) by the action of a specific phospholipase D (PLD) activated by depolarization or G-protein-coupled receptor (GPCR) stimulation. NAPE biosynthesis is catalysed by a membrane enzyme, *N*-acyltransferase (NAT) activated by calcium (Ca^{2+}) and cAMP. Anandamide acts as a retrograde messenger at presynaptic cannabinoid receptors (CB_1), where it regulates neurotransmitter release (NT) through its second transduction systems [mainly Ca^{2+} incorporated through voltage-gated calcium channels (VGCC) or glutamate NMDA (*N*-methyl-*D*-aspartate) receptors]. Anandamide also acts as a neuromodulator of major transmitter systems, including dopamine, at postsynaptic cells, where it regulates excitability and synaptic plasticity through its modulation of potassium (K^+) channels, and the regulation of a broad spectrum of protein kinases (PK) including protein kinase A and mitogen-activated protein kinases (MAPK). Anandamide action is terminated through a two-step process, which includes, first, its cellular uptake through a specific anandamide transporter (AT) and second, degradation by enzymatic cleavage to arachidonic acid (AA) and ethanolamide by the membrane-bound enzyme fatty acid amidohydrolase (FAAH).

regulated by depolarization or by activation of the ionotropic glutamate *N*-methyl-*D*-Aspartate (NMDA) receptors or nicotinic α7 neuronal receptors (Stella and Piomelli, 2001; Piomelli, 2003) or stimulation of the metabotropic receptors of major neurotransmitters including dopamine, glutamate and acetylcholine (Giuffrida *et al*., 1999; Varma *et al*., 2001; Kim *et al*., 2002).

The synthesis and release of 2-AG is different from that of anandamide. Because 2-AG is a monoglyceride, its formation is closely associated with the metabolism of triacylglycerol, mainly by the receptor-dependent activation of phosphatidylinositol-specific phospholipase C (PLC). The standard model proposes that activation of metabotropic receptors coupled to the PLC and diacylglycerol (DG) lipase pathway will systematically lead to increases in 2-AG production (Stella *et al*., 1997; Piomelli, 2003). Cloning of the enzyme 1,2-diacylglycerol lipase (Bisogno *et al*., 2003) has confirmed this hypothesis, as well as the contribution of ionotropic purinergic receptors such as P2XT, which boosts 2-AG formation (Witting *et al*., 2004). Although 2-AG formation is dependent on Ca^{2+}, its regulation is independent of anandamide synthesis and release. Once anandamide and 2-AG are formed, they target the CB_1 receptors in the same cell where they were formed, via diffusion within the plasmalemma, or they can be released to the extracellular fluid where they reach distant targets (i.e. presynaptic terminals) with the apparent help of protein carriers such as lipocalins or albumin (Piomelli, 2003).

Uptake and degradation. Endocannabinoid signalling is terminated by a two-step process that includes transport into cells and hydrolysis by two specific enzymatic systems. Both steps exert a tight control of endocannabinoid levels in tissues, rapidly eliminating these signalling molecules. Endocannabinoid uptake is mediated by a transporter (Beltramo *et al*., 1997), which is widely distributed throughout the brain (Giuffrida *et al*., 2001). The transporter is an elusive molecule which works in a manner that is similar to other lipid carriers: it facilitates the uptake of both anandamide and 2-AG in an energy-independent fashion (Beltramo *et al*., 1997). The anandamide transporter is saturable, displays substrate specificity and can be blocked by specific drugs such as

AM 404 (Fig. 4). A major issue of debate has been the potential coupling of endocannabinoid transport and degradation: it is possible that the energy for the uptake process is obtained by its coupling to the enzymatic hydrolysis of anandamide. However, a recent report seems to confirm that transport and degradation are independent processes (Fegley *et al.*, 2004). The degradation of endocannabinoids is performed by two specific enzymatic systems: the fatty acid amide hydrolase (FAAH) (Cravatt *et al.*, 1996) and the monoacylglyceride lipase (MAGL) (Dinh *et al.*, 2002). FAAH is a membrane enzyme that belongs to the serine–hydrolase family. FAAH is widely distributed throughout the body, with high concentrations in the brain and liver. FAAH can degrade many fatty acid amides, including acylethanolamides such as anandamide and the sleep factor oleamide. Although FAAH can inactivate 2-AG, the main enzyme responsible for the inactivation of this monoglyceride is MAGL (Dinh *et al.*, 2002). This enzyme is also a serine hydrolase and its distribution in the nerve terminals of specific brain neurons has been determined recently (Gulyas *et al.,* 2004).

Receptors. Two major cannabinoid receptors have been cloned, both of which belong to the superfamily of G-protein-coupled receptors. The first receptor described was named the CB_1 receptor and it is mainly located in the terminals of nerve cells (central and peripheral neurons and glial cells), the reproductive system (i.e. testis), some glandular systems and the microcirculation (Devane *et al.*, 1988; Howlett *et al.,* 1990; Herkenham *et al.*, 1991; Wagner *et al.*, 1997; Batkai *et al.*, 2001). The CB_2 cannabinoid receptor was found initially in multiple lymphoid organs with the highest expression detected in B lymphocytes, moderate expression in monocytes and polymorphonuclear neutrophils and the lowest expression in T lymphocytes, although subsequent studies identified it in microglial cells as well (Munro *et al.*, 1993; Galiègue *et al.*, 1995; Piomelli, 2003). An interesting aspect of cannabinoid receptors is their expression during development of the brain, where they control cell differentiation (Rueda *et al.*, 2002), and their presence in tumour cells derived from glial cells and the main epithelia (Galve-Roperh *et al.*, 2000; Sanchez et al., 2001; Casanova *et al.*, 2003). Pharmacological studies revealed the existence of other endocannabinoid targets including the vanilloid receptor (Zygmunt *et al.*, 1999) and at least two non-CB_1 non-CB_2 'CB-like' receptors, one in the vascular bed and the other in glutamatergic axon terminals (Hajos *et al.*, 2001; Howlett *et al.*, 2002; Kunos *et al.*, 2002). The existence of these and other putative cannabinoid receptors, and their role in endocannabinoid physiology can be clarified only after their molecular characterization. Cannabinoid receptors, especially the CB_1 receptor, display unique properties. The most relevant property is their preservation throughout evolution: e.g. human, rat and mouse CB_1 receptors have 97–99% amino acid sequence identity. The preservation of this ancient signalling system in vertebrates and several invertebrate phyla reflects the important functions played by the endocannabinoids in cell and system physiology. A second remarkable characteristic of the CB_1 receptors is their high expression in the brain. The CB_1 receptor is the most abundant G-protein-coupled receptor, with densities 10–50 fold above those of classical transmitters such as dopamine or opioid receptors (Howlett *et al.*, 1990; Herkenham *et al.*, 1991). Another important characteristic is the low efficiency of CB_1 receptor coupling to its transduction system: e.g. when compared with opioid receptors, CB_1 receptors are 7-fold less efficient in their ability to couple to G proteins (Breivogel *et al.*, 1998; Felder and Glass, 1998; Manzanares *et al.*, 1999).

Both cannabinoid receptors are coupled to similar transduction systems. Cannabinoid receptor activation was initially reported to inhibit cAMP formation through its coupling to Gi proteins (Devane *et al.*, 1988; Howlett *et al.*, 1990), resulting in a decrease of the protein kinase A-dependent phosphorylation processes as well. However, additional studies found that the cannabinoid receptors were also coupled to ion channels through the Golf protein, resulting in the inhibition of Ca^{2+} influx through N (Mackie and Hille, 1992), P/Q (Twitchell *et al.*, 1997) and L (Gebremedhin *et al.*, 1999) type calcium channels, as well as the activation of inwardly rectifying potassium conductance and A currents (Mackie *et al.*, 1995; Childers and Deadwyler, 1996). These actions are relevant to the role of cannabinoids as modulators of neurotransmitter release (Schlicker and Kathmann, 2001) and short-term synaptic plasticity (Wilson and Nicoll, 2001), as discussed below. Further research also described the coupling of CB_1 and CB_2 receptors to the mitogen-activated protein kinase cascade, to the phosphatidylinositol 3-kinase, to the focal adhesion kinase, to ceramide signalling and to nitric oxide production (Derkinderen *et al.*, 1996; Bouaboula *et al.*, 1997; Molina-Holgado *et al.*, 1997; Galve-Roperh, 2000; Howlett *et al.*, 2002). Finally, recent studies revealed that under certain conditions, the CB_1 receptors can stimulate formation of cAMP by coupling to the Gs protein (Felder *et al.*, 1998).

Endocannabinoids exhibit different binding properties and intrinsic activity at CB_1 and CB_2 receptors. Anandamide behaves as a partial agonist at both CB_1 and CB_2 receptors, but has higher affinity for the CB_1 receptor (Hillard *et al.*, 1999; Howlett *et al.*, 2002). The intrinsic activity of anandamide at CB_1 receptors is 4–30 fold higher than at CB_2 receptors. However, 2-AG is a complete agonist at both CB_1 and CB_2 receptors and it exhibits less affinity than anandamide for both CB_1 and CB_2 receptors (Stella *et al.*, 1997; Howlett *et al.*, 2002).

Functional neuroanatomy of the endogenous cannabinoid system

As described above, the endogenous cannabinoid system is widely distributed throughout the body. In the peripheral tissues the localization of the elements of the endogenous cannabinoid system reflects the distribution of the cell types where they are located (e.g. B lymphocytes in spleen and lymph nodes). However, in the nervous system the distribution is much more complex and structured, and clearly reflects the importance of this system in synaptic transmission. In some regions, such as the hippocampus, there is a complementary distribution of cannabinoid receptors, endocannabinoid transporters and degradation enzymes. However, in other areas of the brain, for instance the thalamus, there are discrepancies (i.e. transport activity and MAGL expression in the absence of a relevant presence of the CB_1 receptors) in its distribution, which reflects the gaps in our knowledge of the composition of the endocannabinoid system.

Receptors. From the early work of Herkenham *et al.* (1991) it was clear that the CB_1 receptor distribution was unique among G-protein-coupled receptors, not only because of the very high densities of cannabinoid binding sites but also because of the dynamics of CB_1 receptor synthesis and transport. Binding studies and *in situ* hybridization analysis showed that the cannabinoid receptors are synthesized in somata and the protein transported to axon terminals (Herkenham *et al*., 1991; Matsuda *et al*., 1993). The phenotype of the CB_1 receptor-expressing neurons corresponds mainly to GABAergic neurons including cholecystokinin-containing neocortical, amygdalar and hippocampal neurons and dynorphin- and substance P-expressing medium spiny neurons of the outflow nuclei of basal ganglia (Tsou *et al*., 1999; Julian *et al*., 2003). Several glutamatergic and cholinergic telencephalic and cerebellar neurons also express the CB_1 receptors (Piomelli, 2003). In the peripheral nervous system, the CB_1 receptors are located in sensory neurons of the dorsal root ganglia. Figure 3 shows how the CB_1 receptors are synthesized in medium spiny neurons of the caudate-putamen and the protein transported to the axon terminals in the globus pallidus and substantia nigra. The dense presence of CB_1 binding sites in the cerebellum, hippocampus, striatum, globus pallidum and substantia nigra clearly reflects this biological characteristic of CB_1 receptors.

Enzymes. Fatty acid amide hydrolase is present in large principal neurons, such as the pyramidal cells of the cerebral cortex, the pyramidal cells of the hippocampus, the Purkinje cells of the cerebellar cortex and the mitral cells of the olfactory bulb.

Immunocytochemical analysis of these brain regions revealed a complementary pattern of FAAH and CB_1 expression with CB_1 immunoreactivity occurring in fibres surrounding FAAH-immunoreactive cell bodies and/or dendrites (Egertova *et al*., 2003). This complementary distribution suggests that FAAH closely controls the duration of cannabinoid effects, although there are sites where this association does not occur, such as the outflow nuclei of basal ganglia. Monoglyceride lipase is located mainly in the hippocampus, cortex, cerebellum and anterior thalamus, with moderate expression in the extended amygdala, including the shell of the nucleus accumbens (Dinh *et al*., 2002). Comparison of the distribution of FAAH and MAGL at the cellular level shows that FAAH is primarily a postsynaptic enzyme, whereas MAGL is presynaptic. The spatial segregation of the two enzymes suggests that anandamide and 2-AG signalling may subserve functional roles that also involve spatial segregation, raising a controversy with respect

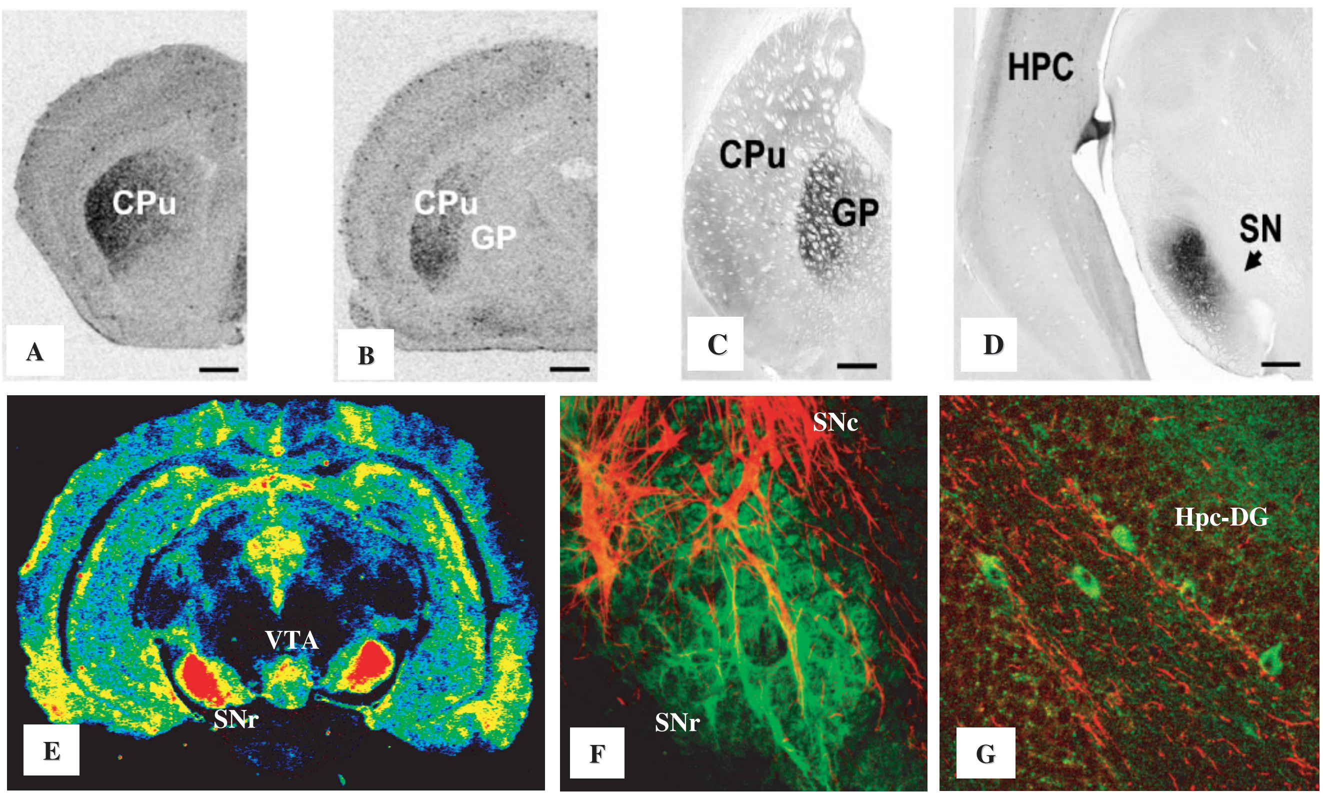


Fig. 3. Imaging cannabinoid CB_1 receptor in circuits of the rat brain reward system. Cannabinoid receptors are mainly located at presynaptic axon terminals. In the basal ganglia, CB_1 receptor mRNA expression (panels A and B) is located mainly in GABAergic projecting neurons of the caudate-putamen (CPu), but not in the target nuclei, the globus pallidus or the substantia nigra (GP and SN). However, the protein is mainly detected by immunohistochemistry (panels C and D) in the axon terminals innervating both outflow nuclei of the basal ganglia. Panel E shows the dense presence of CB_1 receptors in the substantia nigra and ventral tegmental area (VTA) as mapped by CB_1 receptor agonist-stimulated GTP-γ-S incorporation. In these areas, CB_1 receptors are not located in dopaminergic neurons (Panel F): confocal imaging using specific antibodies against CB_1 receptors (green) and tyrosine hydroxilase (red) shows the compartmentalization of CB_1 receptors in GABAergic afferents to the substantia nigra pars reticulata (SNr), whereas dopaminergic cells are restricted to the pars compacta (SNc). The segregation of CB_1 receptors and catecholaminergic transmission is also observed in the hippocampus-dentate gyrus (Hpc-DG, panel G).

Table 2. Targeting the endogenous cannabinoid system: synthetic drugs of reference for cannabinoid CB_1 and CB_2 receptors, anandamide transporter (AT) and endocannabinoid degradation enzyme, fatty acid amidohydrolase (FAAH)

Name	Target	Action	Ki/IC50 (nM)	Reference
ACEA	CB_1	Agonist	1.4	Hillard *et al*., 1999
SR141716A	CB_1	Antagonist	5.6	Rinaldi-Carmona *et al*., 1994
HU-308	CB_2	Agonist	22.7	Hanus *et al*., 1999
SR 144528	CB_2	Antagonist	0.60	Rinaldi-Carmona *et al*., 1998
UCM 707	AT	Blocker	800	Lopez-Rodriguez *et al*., 2001
OL-135	FAAH	Inhibitor (reversible)	2.1	Lichtman *et al*., 2004
URB 597	FAAH	Inhibitor (irreversible)	4.6	Kathuria *et al*., 2003

to the nature and function of the retrograde endocannabinoid signal (Gulyas *et al*., 2004).

Transporter. The distribution of the anandamide transporter has been only partially characterized because the transporter has not been cloned. The distribution of transport activity is highest in areas expressing CB_1 receptors, such as the hippocampus, the amygdala, the striatum and the somatosensory, motor and limbic areas of the cortex. Transport activity is also present in areas with low expression of the CB_1 receptor, such as the thalamus and the hypothalamus (Beltramo *et al*., 1997; Giuffrida *et al*., 2001).

Pharmacology of the endogenous cannabinoid system

During the last twenty years, and especially after the discovery of the CB_1 receptor and anandamide, an intense research effort has yielded numerous series of drugs that interact with most of the main elements of the endogenous cannabinoid system. Today we have drugs that bind to the CB_1 receptor as agonists or antagonists, drugs that block the endocannabinoid transport and drugs that inhibit the activity of FAAH. We lack specific NAT, PLD, sn1-DAGL and MAGL inhibitors. Both *in vitro* and *in vivo* bioassays have been used to evaluate the activity of the new compounds. Prior to the availability of radioligand cannabinoid receptors, *in vitro* assays included the inhibition of forskolin-stimulated cAMP production and the inhibition of electrically evoked contractions of isolated smooth muscle preparations. Smooth muscle preparations most often used for the bioassay of cannabinoids are the mouse-isolated vas deferens and the myenteric plexus-longitudinal muscle preparation from the guinea pig small intestine. These bioassays, which are particularly sensitive, rely on the ability of cannabinoid receptor agonists to act via the CB_1 receptors to inhibit electrically evoked contractions. *In vivo* bioassays include behavioural tests for analgesia and locomotion. A cluster of four effects (analgesia, hypothermia, immobility and catalepsy) in mice constituting the 'mouse tetrad', is classically considered as a signature of cannabimimetic activity. The recent availability of mouse knockouts for the cannabinoid receptors and FAAH (Ledent *et al*., 1999; Cravatt *et al*., 2001) has facilitated these studies, offering a reliable model in the search for selective compounds.

What is the logic of a cannabinoid approach to pharmacotherapeutics? Cannabinoid receptor agonists may be designed to mimic the signalling processes mediated by anandamide and 2-AG, mainly in pathological situations where a boost in cannabinoid receptor stimulation might be needed. Cannabinoid receptor antagonism might be the approach selected in conditions with enhanced endocannabinoid signalling. Transport inhibition and inhibition of degradation are more sophisticated approaches, both oriented towards magnifying the tonic actions of endocannabinoids. A rational use of these therapeutic strategies requires the identification and evaluation of the functional status of endocannabinoid signalling in reference disorders. Thus, a deficit of anandamide signalling during conditions of stress might be counteracted by the blockade of anandamide degradation (Kathuria *et al*., 2003).

As a summary of cannabinoid pharmacology, Table 2 shows the reference compound for each molecular target, indicating Ki in the case of ligand–receptor interaction or IC50 in the case of enzymatic inhibitors.

Cannabinoid receptor agonists. According to the International Union of Pharmacology (reviewed in Howlett *et al*., 2002), cannabinoid agonists can be divided into classical cannabinoids, non-classical cannabinoids, aminoalkylindoles and eicosanoids. New series of compounds have been recently described, including diarylether sulfonylesters (Mauler *et al*., 2002) and pyrrole derivatives (Tarzia *et al*., 2003b).

Classical cannabinoids are tricyclic dibenzopyran derivatives that are either compounds occurring naturally in the plant *C. sativa*, or synthetic analogues of these compounds. The most representative forms are Δ^9-THC (Fig. 1), a partial agonist at both the CB_1 and CB_2 receptors and the main psychoactive constituent of *Cannabis*, along with 11-hydroxy-Δ^8-THC-dimethylheptyl (HU-210), a synthetic compound that displays the highest potency at the CB_1 receptor (Howlett *et al*., 2002). Classical cannabinoids are usually CB_1/CB_2 agonists, although changes in the THC molecule have led to the synthesis of selective CB_2 receptor agonists such as HU-308 (Hanus *et al*., 1999).

Non-classical cannabinoids are synthetic THC analogues that lack the dihydropyran ring. The most representative form is the Pfizer compound CP-55 940, a potent and complete agonist at both the CB_1 and CB_2 receptors, which was used to characterize the CB_1 receptor for the first time (Devane *et al*., 1988; Herkenham *et al*., 1991).

Aminoalkylindoles were the first non-cannabinoid molecules that displayed cannabimimetic activity (Pacheco *et al*., 1991).

R-(+)-WIN-55,212–2 (Fig. 1) is the most representative form, and it behaves as a complete agonist at both the CB_1 and CB_2 receptors, with higher intrinsic activity at the CB_2 receptor.

Eicosanoids are the prototypic endocannabinoids (Fig. 1), of which anandamide (a partial agonist at both the cannabinoid receptors) and 2-AG (a complete agonist at both the CB_1 and CB_2 receptors) are the most representative compounds. Based on the structure of anandamide, minor chemical changes have led to the development of the first generation of CB_1-selective agonists, of which R(+)-methanandamide and arachidonyl-2'-chloroethylamide (ACEA) (Table 2) are the most representative forms (Hillard forms *et al*., 1999).

Cannabinoid receptor antagonists. Several series of compounds have been developed as CB_1 receptor antagonists. The most representative are diarylpyrazoles, substituted benzofuranes, aminoalkylindoles and triazole derivatives.

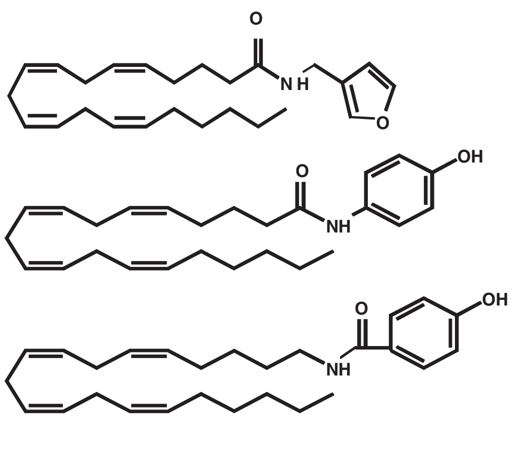
Diarylpyrazoles include both the first CB_1 receptor antagonist synthesized (SR 141716A, Rinaldi-Carmona *et al*., 1994) and the first CB_2 receptor antagonist (SR 144528). They were synthesized by Sanofi and are considered the reference antagonists. However, they are not neutral antagonists since they display significant inverse agonist properties. Modification of the SR 141716A molecule has yielded other CB_1 receptor antagonists with improved properties, including SR 147778 and AM 281 (Howlett *et al*., 2002; Rinaldi-Carmona *et al*., 2004). Diarylpyrazoles are orally active and are currently under clinical trials for the treatment of obesity.

Substituted benzofuranes include LY 320135, a CB_1 receptor antagonist with affinity at serotonin and muscarinic receptors (Felder *et al*., 1998).

Aminoalkylindoles include a CB_2 receptor antagonist, AM 630, which also displays activity as a low-affinity partial CB_1 agonist (Howlett *et al*., 2002).

Triazole derivatives include LH-21 (Jagerovic *et al*., 2004), an *in vivo* CB_1 antagonist with a paradoxic low affinity *in vitro* for CB_1 receptors and devoid of inverse agonist properties.

Uptake blockers. Based on the structure of anandamide, a series of eicosanoid derivatives that have the ability to block anandamide transport have been synthesized. The molecular structures of the three prototypical uptake blockers are depicted in Fig. 4. The first and best studied transport inhibitor is AM 404 (Beltramo *et al*., 1997). The administration of AM 404 results in the accumulation of anandamide and potentiates the effects of exogenously administered anandamide. The compound AM 404 can be degraded by FAAH and behaves as an agonist of vanilloid receptors. A second series of compounds is represented by UCM 707, which displays a higher affinity at the transporter than AM 404 (Lopez-Rodraíguez *et al*., 2001; De Lago *et al*., 2002). A latest addition is AM 1172, a FAAH-resistant transport inhibitor that allows the study of anandamide uptake processes without interference in FAAH activity (Fegley *et al*., 2004). However the IC50 of AM 1172 (2000 nM) is lower than that reported for UCM 707 (800 nM).



UCM 707

AM 404

AM 1172

Fig. 4. Structure of three anandamide uptake blockers. UCM 707 is the compound with the highest affinity at the anandamide transporter. AM 404 was the first blocker designed and has been extensively described. Both molecules, however, had a significant impact on the activity of the fatty acid amidohydrolase (FAAH), the enzyme that degrades anandamide. AM 1172 is a recently described compound without inhibitory action at FAAH, which has been used to demonstrate the independence of anandamide transport and degradation processes.

Inhibitors of fatty acid amide hydrolase. As in the case of the cannabinoid receptors, different lines of research have led to the discovery of chemically heterogeneous FAAH inhibitors. The earlier inhibitors described consisted of reversible electrophilic carbonyl inhibitors (trifluoromethyl ketones, alpha-keto esters and amides, and aldehydes) or irreversible inhibitors (sulfonyl fluorides and fluorophosphonates) incorporated into the fatty acid structures. Based on the structure of alpha-trifluoromethyl ketones a series of potent inhibitors were developed. Of these, alpha-keto N_4-oxazolopyridine provides inhibitors that are 10^2–10^3 times more potent than the corresponding trifluoromethyl ketones (Boger *et al*., 2000). A recent series of alpha heterocycles has been shown to possess very high potency and selectivity to reversibly inhibit FAAH activity *in vivo* and *in vitro*. The most potent of these new compounds is OL-135, which exhibits IC50 in the low nanomolar range (Lichtman *et al*., 2004). A different strategy has been selected by the group of Piomelli *et al*., who have developed exceptionally potent irreversible FAAH inhibitors, which exhibit a promising anxiolytic profile (Kathuria *et al*., 2003; Tarzia *et al*., 2003a). These new classes of inhibitors are carbamate derivatives capable of directly interacting with the serine nucleophile of FAAH. However, these new inhibitors, although extremely potent, are not selective because they may potentially inactivate other serine hydrolases such as heart triacylglycerol hydrolase (Lichtman *et al*., 2004).

Physiology of the endogenous cannabinoid system

The ubiquitous presence of the endogenous cannabinoid system correlates with its role as a modulator of multiple physiological processes. A comprehensive analysis of all the functions of the endocannabinoids is beyond the scope of the present review. The reader will find an extensive list of recent reviews that explore the physiological relevance of the endogenous cannabinoid system, as depicted in Table 1. In this section, we focus on the cellular and system physiological events mediated by endocannabinoids that are relevant to our understanding of the contribution of the endogenous cannabinoid system in alcoholism.

Cellular physiology. As described in the section on biochemistry of the endogenous cannabinoid system, endocannabinoids are released upon demand after cellular depolarization or receptor stimulation in a calcium-dependent manner. Once produced, they act on the cannabinoid receptors located in the cells surrounding the site of production. This property indicates that endocannabinoids are local mediators similar to the autacoids (e.g. prostaglandins). In the CNS, the highly organized distribution of endocannabinoid signalling elements in GABAergic and glutamatergic synapses and their preservation throughout evolution suggests a pivotal role in synaptic transmission. Because of the inhibitory effects on adenyl cyclase, the activation of K^+ currents and the inhibition of Ca^{2+} entry into cells, the net effect of the CB_1 receptor stimulation is a local hyperpolarization that leads to the general inhibitory effects described. If endocannabinoids act post-synaptically they will counteract the activatory inputs entering the postsynaptic cells. This mechanism has been proposed for postsynaptic interactions with dopaminergic transmission (Felder *et al*., 1998; Rodríguez de Fonseca *et al*., 1998; Giuffrida *et al*., 1999). Despite its importance, this effect is secondary to the important presynaptic actions whose existence is supported by two facts: (i) the concentration of the CB_1 receptors in presynaptic terminals and (ii) the well-documented inhibitory effects of the CB_1 receptor agonists on the release of GABA, glutamate, acetylcholine and noradrenaline (Schlicker and Kathmann, 2001; Piomelli, 2003). This inhibitory effect has been demonstrated for neuropeptides such as corticotrophin-releasing factor and cholecystokinin as well (Rodríguez de Fonseca *et al*., 1997; Beinfeld and Connolly, 2001). Presynaptic inhibition of neurotransmitter release is associated with the inhibitory action of endocannabinoids on Ca^{2+} presynaptic calcium channels via the activation of CB_1 receptors. Presynaptic inhibition of transmitter release by endocannabinoids may adopt two different forms of short-term synaptic plasticity, depending on the involvement of GABA or glutamate transmission, respectively: depolarization-induced suppression of inhibition (DSI) and depolarization-induced suppression of excitation (DSE) (Wilson and Nicoll, 2002; Diana and Marty, 2004). Both forms of synaptic plasticity involve the initial activation of a postsynaptic large projecting neuron (pyramidal or Purkinje cells) that sends a retrograde messenger to a presynaptic GABA terminal (DSI) or a presynaptic glutamate terminal (DSE), inducing a transient suppression of either the presynaptic inhibitory or the presynaptic excitatory input. The contribution of endocannabinoids to these forms of short-term synaptic plasticity has been described in the hippocampus (Wilson and Nicoll, 2001; Wilson *et al*., 2001) and the cerebellum (Diana *et al*., 2002). The nature of the endocannabinoid system acting as a retrograde messenger is still unknown. The role of endocannabinoid-induced DSI or DSE seems to be the coordination of neural networks within the hippocampus and the cerebellum that are involved in relevant physiological processes, such as memory or motor coordination.

Additional forms of endocannabinoid modulation of synaptic transmission involve the induction of long-term synaptic plasticity, namely long-term potentiation (LTP) and long-term depression (LTD). Both forms of synaptic plasticity involve long-term changes in the efficacy of synaptic transmission in glutamatergic neurons, which have a major impact on consolidation and remodelling of the synapsis. Activation of the cannabinoid receptors prevents the induction of LTP in the hippocampal synapses (Stella *et al*., 1997) and a facilitation of LTD in the striatum (Gerdeman *et al*., 2002) and the nucleus accumbens (Robbe *et al*., 2002). In the hippocampus, the endocannabinoid messengers regulate a form of LTD that affects inhibitory GABAergic neurons (Chevaleyre and Castillo, 2003).

Overall, endocannabinoids act as local messengers that adjust synaptic weight and contribute significantly to the elimination of information flow through specific synapses in a wide range of time frames. The fact that cannabinoid receptor stimulation has a major impact on second messengers involved not only in synaptic remodelling (Derkinderen *et al*., 1996; Piomelli, 2003) but also in neuronal differentiation (Rueda *et al*., 2002) and neuronal survival (Panikashvili *et al*., 2001; Marsicano *et al*., 2003) indicates that the signalling system is a major homeostatic mechanism that guarantees a fine adjustment of information processing in the brain and provides counterregulatory mechanisms aimed at preserving the structure and function of major brain circuits. Both processes are relevant for homeostatic behaviour such as motivated behaviour (feeding, reproduction, relaxation, sleep) and emotions, as well as for cognition, since learning and memory require dynamic functional and morphologic changes in brain circuits. An experimental confirmation of this hypothetical role of the endogenous cannabinoid system was the demonstration of its role in the control of the extinction of aversive memories (Marsicano *et al*., 2002; Terranova *et al*., 1996).

System physiology. The cellular effects of endogenous cannabinoids have a profound impact on the main physiological systems that control body functions (Table 1). Despite the peripheral modulation of the immune system, vascular beds, reproductive organs, gastrointestinal motility and metabolism, the endogenous cannabinoid system tightly regulates perception processes including nociception (cannabinoids are potent analgetics, Martin and Litchman, 1998) and visual processing in the retina (Straiker *et al*., 1999). Additional functions exerted by the endogenous cannabinoid system involve the regulation of basal ganglia and cerebellar circuits, where it is involved in the modulation of implicit learning of motor routines (Rodriguez de Fonseca *et al*., 1998).

Among the varied functions in which the endogenous cannabinoid system is engaged, the homeostatic control of emotions and the regulation of motivated behaviour merit special attention because of its impact on human diseases, including addiction. The endogenous cannabinoid system controls the motivation for appetite stimuli, including food and drugs (Di Marzo *et al*., 1998, 2001; Navarro *et al*., 2001; Gomez *et al*., 2002). The positive effects of endocannabinoids on motivation seem to be mediated not only by the peripheral sensory systems in which cannabinoid receptors are present (i.e. the promotion of feeding induced by cannabinoid CB_1 receptor agonists, Gomez *et al*., 2002), but also by the action of endocannabinoids on the reward system, a set of in-series circuits that link the brain stem, the extended amygdala and the frontal executive cortex. The endogenous cannabinoid system is widely distributed in the extended amygdala, a set of telencephalic nuclei located in medial septal neurons, the nucleus accumbens shell and amygdalar complex, and are involved in the control of motivated behaviour, conditioned responses and gating-associated emotional responses. This hypothesis is supported by two facts: the inhibition of motivated behaviour observed after administration of a cannabinoid antagonist (Colombo *et al*., 1998; Navarro *et al*., 2001) and the reward deficits observed in the CB_1 receptor knockout mice (Ledent *et al*., 1999; Maldonado and Rodríguez de Fonseca, 2002; Sanchis-Segura *et al*., 2004). Research on the neurobiological basis of endocannabinoid effects on motivated behaviour has focused on endocannabinoid–dopamine interaction as well as on the role of the endocannabinoid system in habit learning and conditioning. The extended amygdala is the target of the ascending mesocorticolimbic projections of the ventral tegmental area (VTA) dopaminergic neurons, a subset of mesencephalic neurons that display a consistent response to drugs of major abuse, which appear to be a common substrate for the reward properties of drugs of dependence (Maldonado and Rodríguez de Fonseca, 2002). Most drugs of dependence activate the VTA dopaminergic neurons, as monitored by the dopamine release in terminal areas, especially in the nucleus accumbens and prefrontal cortex, or by the firing rates of VTA dopaminergic neurons. THC and other CB_1 receptor agonists increase dopamine efflux in the nucleus accumbens and prefrontal cortex and increase the dopaminergic cell firing in the VTA (for review see Gardner and Vorel, 1998). This effect is not caused by the direct activation of dopaminergic neurons because they do not express CB_1 receptors (Julian *et al*., 2003). Although the effects of cannabinoid agonists on dopamine release in the projecting areas (i.e. nucleus accumbens) can be blocked by the opioid antagonist naloxone, the increase in VTA dopaminergic cell firing cannot be blocked. This discrepancy may suggest the existence of a differential role for endogenous opioid systems as the modulators of cannabinoid actions in dopamine cell bodies with respect to their axon terminals. Cannabinoid effects might also involve glutamatergic and GABAergic inputs to the nucleus accumbens and VTA, because presynaptic CB_1 receptors regulate glutamate and GABA release in these areas, inducing LTD (Schlicker and Kathmann, 2001; Robbe *et al*., 2002). In agreement with these actions of cannabinoids in brain reward circuits, repeated cannabinoid exposure can induce behavioural sensitization similar to that produced by other drugs of dependence. Chronic cannabinoid administration also produces cross-sensitization to the locomotor effects of psychostimulants (Maldonado and Rodríguez de Fonseca, 2002). Because endocannabinoids induce LTD in the nucleus accumbens (which affect glutamatergic inputs coming from the prefrontal cortex), they probably regulate the acquisition of habit learning and conditioned responses relevant to the progressive loss of control that characterize drug addiction (Maldonado and Rodríguez de Fonseca, 2002). Interestingly, administration of a CB_1 receptor antagonist blocks cue-induced reinstatement to heroin and cocaine self-administration (De Vries *et al*., 2001, 2003). The importance of the endogenous cannabinoid system in the control of motivated behaviour goes far beyond the control of processing ongoing reward signals. The CB_1 receptors are apparently involved in the control of reward homeostasis (Sanchis-Segura *et al*., 2004). Moreover, when cannabinoid homeostatic mechanisms are not adequate to restore the lost equilibrium in reward control derived from continuous uncontrolled exposure to a reinforcer (e.g. opiates or alcohol), allostatic changes involving CB_1 receptors are set in motion to counteract the spiralling distress imposed on the reward circuit. This has been demonstrated in rodents exposed to cycles of dependence–abstinence to alcohol and morphine (Navarro *et al*., 2001; Rimondini *et al*., 2002). In this model, a history of dependence is associated with a permanent upregulation of the expression of CB_1 receptors in reward-related areas and with an enhanced sensitivity to reward disruption induced by cannabinoid receptor antagonists (Rodríguez de Fonseca *et al*., 1999; Rimondini *et al*., 2002). Whether these allostatic changes occur in other models of motivated behaviour (i.e. feeding) remains to be determined.

Cannabinoid receptors are not only associated with motivational disturbances, but also related to emotional processing. A key station for the endocannabinoid regulation of emotions is the amygdalar complex. Endocannabinoids are able to depress the release of glutamate and corticotropin-releasing factor, reducing the amygdalar output and the activity of basolateral inhibitory GABA projections to the central nucleus of the amygdala, thereby activating the amygdalofugal pathway (Rodríguez de Fonseca *et al*., 1996, 1997; Navarro *et al*., 1997; Marsicano *et al*., 2002; Piomelli, 2003). The final balance will lead to anxiety or anxiolysis, depending on the rate of activation of descending projections of the central nucleus of the amygdala to the hypothalamus (endocrine responses) and brain stem (behavioural and autonomic responses). However, recent studies indicate that anxiolysis is the normal response to enhanced cannabinoid transmission in the limbic system, as reflected by the phenotype of FAAH knockout mice and the effects of FAAH inhibitors (Cravatt *et al*., 2003; Kathuria *et al*., 2003). The induction of anxiety by cannabinoid receptor antagonists (Navarro *et al*., 1997) supports this notion as well.

A practical approach: role for the endocannabinoid system in alcoholism

The presence of the endogenous cannabinoid system in reward circuits and its role in motivational and emotional homeostasis suggests that drugs which modulate cannabinoid signalling might serve as therapeutic tools in drug addiction. In accordance with this rationale, the CB_1 receptor antagonists are able to modulate opioid self-administration in rodents (Navarro *et al*., 2001). Extending this hypothesis, converging research lines have established a role for both anandamide and the CB_1 receptor in alcohol dependence (Hungund and Basaravajappa, 2000; Hungund *et al*., 2002; Mechoulam and Parker, 2003). The administration of CB_1 receptor agonists promotes alcohol intake (Colombo *et al*., 2002), whereas the administration of a CB_1 receptor antagonist decreases alcohol self-administration, especially in animals with a history of alcohol dependence (Rodríguez de Fonseca *et al*., 1999) or in alcohol-preferring rat lines (Colombo *et al*., 1998). Molecular studies have shown that chronic alcohol administration is associated with an increased formation of both anandamide and its membrane precursor NAPE (Basavarajappa and Hungund, 1999). Chronic alcohol exposure also resulted in the stimulation of a second endocannabinoid, 2-AG (Basavarajappa *et al*., 2000). Animal studies also revealed that chronic exposure to alcohol downregulated the CB_1 receptors in the brain (Basavarajappa *et al*., 1998). Finally, a recent gene screening study has identified the CB_1 receptor as one of the genes whose expression is permanently affected by serial cycles of alcohol dependence and withdrawal (Rimondini *et al*., 2002). These data indicate a role for the endogenous cannabinoid system as a relevant contributor to alcoholism. Human gene studies support this experimental hypothesis, since a linkage between clinical forms of alcoholism and polymorphisms and/or mutations of the genes encoding either the CB_1 receptor (Comings *et al*., 1997; Schmidt *et al*., 2002) or the FAAH (Sipe *et al*., 2002), the enzyme responsible for AEA inactivation (Cravatt *et al*., 1996), have been described. In the present issue, the reader will find additional experimental approaches to the role of the endogenous cannabinoid system in alcoholism.

CONCLUSION

Since the discovery of anandamide, the increasing information on the physiological roles played by the endogenous cannabinoid system and its contribution to pathology have led to this signalling system becoming more important in neurobiology. The intense pharmacological research based on this information has yielded, in a very short time, potent, selective drugs targeting the endogenous cannabinoid system that have opened up new avenues for the understanding and treatment of major diseases including cancer, pain, neurodegeneration, anxiety and addiction. This is a very promising starting point for a new age that takes over from the ancient use of *Cannabis* as a medicine. Now is the time for clinical trials aimed at evaluating the efficacy of cannabinoid drugs in disorders lacking effective therapeutic approaches, such as alcoholism.

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