British Journal of Pharmacology (2008) 154, 369–383

0007–1188/08 $30.00
www.brjpharmacol.org

REVIEW

The endocannabinoid system in brain reward processes

M Solinas[1], SR Goldberg[2] and D Piomelli[3]

[1]*Institut de Physiologie et Biologie Cellulaires, Université de Poitiers, CNRS, Poitiers, France;* [2]*Preclinical Pharmacology Section, Behavioral Neuroscience Research Branch, National Institute on Drug Abuse, National Institutes of Health, Department of Health and Human Services, Baltimore, MD, USA and* [3]*Department of Pharmacology, Center of Drug Discovery, University of California, Irvine, CA, USA*

Food, drugs and brain stimulation can serve as strong rewarding stimuli and are all believed to activate common brain circuits that evolved in mammals to favour fitness and survival. For decades, endogenous dopaminergic and opioid systems have been considered the most important systems in mediating brain reward processes. Recent evidence suggests that the endogenous cannabinoid (endocannabinoid) system also has an important role in signalling of rewarding events. First, CB_1 receptors are found in brain areas involved in reward processes, such as the dopaminergic mesolimbic system. Second, activation of CB_1 receptors by plant-derived, synthetic or endogenous CB_1 receptor agonists stimulates dopaminergic neurotransmission, produces rewarding effects and increases rewarding effects of abused drugs and food. Third, pharmacological or genetic blockade of CB_1 receptors prevents activation of dopaminergic neurotransmission by several addictive drugs and reduces rewarding effects of food and these drugs. Fourth, brain levels of the endocannabinoids anandamide and 2-arachidonoylglycerol are altered by activation of reward processes. However, the intrinsic activity of the endocannabinoid system does not appear to play a facilitatory role in brain stimulation reward and some evidence suggests it may even oppose it. The influence of the endocannabinoid system on brain reward processes may depend on the degree of activation of the different brain areas involved and might represent a mechanism for fine-tuning dopaminergic activity. Although involvement of the various components of the endocannabinoid system may differ depending on the type of rewarding event investigated, this system appears to play a major role in modulating reward processes.
British Journal of Pharmacology (2008) **154,** 369–383; doi:10.1038/bjp.2008.130; published online 14 April 2008

Keywords: addiction; anandamide; 2-arachidonoylglycerol; dopamine; FAAH; cannabinoid receptors; endocannabinoid transport; food; drugs

Abbreviations: 2-AG, 2-arachidonoylglycerol; CB, cannabinoid; FAAH, fatty acid amide hydrolase; NAcc, nucleus accumbens; THC, delta-9-tetrahydrocannabinol; TRPV1, transient receptor potential vanilloid type 1; VTA, ventral tegmental area

Endogenous cannabinoid system

The endogenous cannabinoid (endocannabinoid) system is a signalling system composed of cannabinoid (CB) receptors, endogenous ligands for these receptors and proteins involved in the formation and deactivation of these endogenous ligands (Freund *et al*., 2003; Piomelli, 2003; Di Marzo *et al*., 2004; Fride, 2005). Although the endocannabinoid system is thought to serve multiple functions in the brain and in peripheral tissues (Freund *et al*., 2003; Piomelli, 2003; Di Marzo *et al*., 2004; Fride, 2005), in this review we will focus on its involvement in motivational processes. In particular, we will review the effects of activation and inactivation of the endocannabinoid system on behaviours maintained by natural, electrical and drug rewards and on the activity of the mesolimbic dopamine system, which is the brain neurotransmitter system most clearly involved in reward processes (Koob, 2000; Di Chiara *et al*., 2004; Wise, 2004; Everitt and Robbins, 2005; Gardner, 2005). We refer the reader to recent excellent reviews on the interactions of the endocannabinoid system with other neurotransmitters involved in reward processes such as endoopioids (Manzanares *et al*., 1999; Fattore *et al*., 2005; Gardner, 2005; Vigano *et al*., 2005; Solinas *et al*., 2007c), on the involvement of the endocannabinoid system in neuroplastic changes that may underlie drug addiction (Maldonado *et al*., 2006) and on the role of the endocannabinoid system in relapse (De Vries and Schoffelmeer, 2005).

Correspondence: Dr M Solinas, Institut de Physiologie et Biologie Cellulaires, Université de Poitiers, CNRS, 40 avenue du recteur Pineau, Poitiers F-86022, France.
E-mail: marcello.solinas@univ-poitiers.fr
Received 3 December 2007; revised 21 January 2008; accepted 17 March 2008; published online 14 April 2008

CB receptors

For decades it was believed that the effects of the main active ingredient in cannabis, delta-9-tetrahydrocannabinol (THC), were due to alterations of cellular membrane structure. However, in the late 1980s, due to the availability of new synthetic CB receptor agonists, it was first suggested that specific CB receptors exist (Devane *et al*., 1988; Howlett *et al*., 1990). Soon after, the first CB receptor was sequenced and cloned (Matsuda *et al*., 1990). This receptor, named CB_1, is highly expressed in the brain (Herkenham *et al*., 1990, 1991) and mediates most, if not all, of the psychoactive/central effects of cannabis. A short time later, a second CB receptor, named CB_2, was discovered (Munro *et al*., 1993). Until recently it was thought that CB_2 receptors were present only in the periphery and did not mediate any central effect of CBs, but recent findings suggest that CB_2 receptors are present at low levels in some areas of the brain (Van Sickle *et al*., 2005; Gong *et al*., 2006; Onaivi *et al*., 2006). On the basis of studies showing certain behavioural and pharmacological effects of CB ligands that could not be explained exclusively by CB_1 and CB_2 receptors, it has also been hypothesized that additional non-CB_1 and non-CB_2 receptors might exist (Jarai *et al*., 1999; Zimmer *et al*., 1999; Breivogel *et al*., 2001; Hajos *et al*., 2001; Freund and Hajos, 2003; Fride *et al*., 2003; Begg *et al*., 2005; Ryberg *et al*., 2007). The potential involvement of CB_2 and non-CB_1 and non-CB_2 receptors in central effects of CBs needs further investigation and is not discussed in the present review.

CB_1 receptors are the most abundant G-protein-coupled receptors found in the brain (Howlett *et al*., 2002). They are metabotropic receptors coupled to Gi/o proteins, whose activation results in inhibition of adenylyl cyclase activity and in a consequent decrease in cytosolic cAMP content, closure of Ca^{2+} channels, opening of K^+ channels and stimulation of kinases that phosphorylate tyrosine, serine and threonine residues in proteins (Howlett *et al*., 1998; McAllister and Glass, 2002). CB_1 receptors are localized preferentially at the presynaptic level and, thus, it is believed that they inhibit the release of glutamate, GABA and other neurotransmitters (Schlicker and Kathmann, 2001; Wilson and Nicoll, 2002).

The localization of CB_1 receptors in the brain is consistent with the known central effects of CBs, with highest concentrations in areas involved in memory (for example, hippocampus), motor coordination (for example, the cerebellum) and emotionality (for example, prefrontal cortex) (Herkenham *et al*., 1990; Tsou *et al*., 1998a). In the dopaminergic mesolimbic system, the best known circuit involved in motivational processes (Koob, 2000; Di Chiara *et al*., 2004; Wise, 2004; Everitt and Robbins, 2005), average to high concentrations of CB_1 receptors are found in the terminal region, the striatum, whereas low concentrations of CB_1 receptors are found in the origin, the ventral tegmental area (VTA) (Herkenham *et al*., 1990; Tsou *et al*., 1998a). These relatively low concentrations in the VTA do not necessarily indicate that CBs do not have important actions in this area. Several lines of evidence indicate that CB_1 receptor agonists have strong modulating effects on VTA neuron activity (Szabo *et al*., 2002; Cheer *et al*., 2003; Riegel and Lupica, 2004; Melis *et al*., 2004a, b) and that CBs can produce rewarding effects when directly injected into this structure (Zangen *et al*., 2006).

It should be noted that anandamide, along with a variety of other lipids, can also activate transient receptor potential vanilloid type 1 (TRPV1) vanilloid receptors (Zygmunt *et al*., 1999; Di Marzo *et al*., 2001a). However, the role of these receptor channels in the behavioural and neurochemical effects of anandamide in brain reward processes remains largely undefined (Piomelli, 2003; Solinas *et al*., 2006a, 2007b).

Endogenous ligands for CB receptors

In the early 1990s, anandamide (Devane *et al*., 1992; Di Marzo *et al*., 1994) and 2-arachidonoylglycerol (2-AG) (Mechoulam *et al*., 1995; Sugiura *et al*., 1995; Stella *et al*., 1997) were discovered and characterized as the first endogenous ligands for CB receptors. Subsequently, other possible endocannabinoids have been proposed, such as noladin ether (Hanus *et al*., 2001), virodhamine (Porter *et al*., 2002) and arachidonoyldopamine (Porter *et al*., 2002), but their natural occurrence and their roles are still unclear.

Anandamide and 2-AG have different structures, different biosynthesis and degradation pathways and, in addition, appear to be formed under different conditions and to be differently affected by several manipulations, including pharmacological stimulation, as reviewed elsewhere (Freund *et al*., 2003; Piomelli, 2003; Di Marzo *et al*., 2004; Fride, 2005). In addition, a recent paper has shown that anandamide inhibits the metabolism and the effects of 2-AG levels in the stiratum (Maccarrone *et al*., 2008). Thus, it has been proposed that anandamide and 2-AG might play different roles in physiological and pathophysiological conditions (Piomelli, 2003).

A peculiarity of the endocannabinoids, which makes them an interesting target for the discovery of new drugs, is that they are not present in vesicular stores but instead, are formed 'on demand' and undergo rapid metabolic deactivation, so that drugs that target this system would act predominantly when and where altered levels of endocannabinoids are present (Piomelli, 2003; Piomelli *et al*., 2006).

Synthesis and degradation of endocannabinoids and endocannabinoid transport

Endocannabinoid signalling is terminated by a two-step process consisting of (1) transport inside the cell and (2) metabolic degradation by specific enzymes. Although this has not been established, it is believed that anandamide and 2-AG might use the same intracellular transport as the first step of degradation (Freund *et al*., 2003; Piomelli, 2003; Di Marzo *et al*., 2004; Fride, 2005). In contrast, it has been clearly demonstrated that their metabolic degradation diverges (Freund *et al*., 2003; Piomelli, 2003; Di Marzo *et al*., 2004; Fride, 2005).

Transport. Because of their lipophilic nature, anandamide and 2-AG can diffuse passively through lipid membranes. However, it appears that diffusion is accelerated by a rapid and selective carrier system that would use a facilitated

diffusion mechanism (Beltramo *et al*., 1997; Hillard *et al*., 1997). This carrier might work bi-directionally and could also facilitate the release of endocannabinoids (Hillard *et al*., 1997). Endocannabinoid transport is the target of pharmacological tools such as AM404, VDM11, UCM707 and AM1172 that inhibit it and produce elevations in endocannabinoid levels, CB-like effects, and enhanced and prolonged effects of exogenously administered endocannabinoids (Piomelli *et al*., 1999; De Petrocellis *et al*., 2000; Lopez-Rodriguez *et al*., 2003; Fegley *et al*., 2004; Glaser *et al*., 2005). However, the protein(s) responsible for this transport has not yet been isolated or characterized.

Anandamide degradation. Once taken up into the cell, anandamide is broken down into arachidonic acid and ethanolamine by fatty acid amide hydrolase (FAAH), which has been molecularly cloned and characterized (Desarnaud *et al*., 1995; Hillard *et al*., 1995; Cravatt *et al*., 1996; Bracey *et al*., 2002). FAAH is widely distributed in the CNS and its distribution partially overlaps with that of CB_1 receptors, with FAAH being present mainly at the postsynaptic level and CB_1 receptors at the presynaptic level (Tsou *et al*., 1998b; Egertova *et al*., 2003). The availability of FAAH-deficient mice (Cravatt *et al*., 2001) and of selective FAAH inhibitors (Kathuria *et al*., 2003) has allowed characterization of the critical role played by this enzyme in the degradation of anandamide (Cravatt *et al*., 2001; Kathuria *et al*., 2003; Fegley *et al*., 2005; Solinas *et al*., 2006a, 2007b).

2-AG degradation. Once taken up into the cell, 2-AG is broken down into arachidonic acid and glycerol, mainly by monoacylglycerol lipase (MGL), which has been cloned and characterized (Dinh *et al*., 2002a, b). MGL is widely distributed in the CNS and its distribution partially parallels that of CB_1 receptors (Dinh *et al*., 2002a). However, both MGL and CB_1 receptors appear to be situated at the presynaptic level (Dinh *et al*., 2002a). This localization, together with the fact that FAAH and MGL only partially overlap, suggests that anandamide and 2-AG play different roles in the modulation of neurotransmission.

Role of CB receptors in brain reward processes

CB_1 receptors appear to play an important role in brain reward processes. One long-standing line of evidence for the role for CB_1 receptors in brain reward processes is that CB_1 receptor agonists, such as the active ingredient in cannabis, THC, have rewarding effects in humans and animals (Maldonado and Rodriguez de Fonseca, 2002; Tanda and Goldberg, 2003; Gardner, 2005; Justinova *et al*., 2005a; Solinas *et al*., 2007c). The reinforcing effects of THC have been extensively reviewed elsewhere (Tanda and Goldberg, 2003; Justinova *et al*., 2005a). Here, we focus on recent evidence for a modulatory role of endocannabinoids on the rewarding effects of drugs of abuse, food and electric brain stimulation.

Effects of CB_1 receptor agonists on reward processes

CB_1 receptor agonists, such as THC, WIN 55,212-2 and HU-210, can facilitate the rewarding effects of drugs. For example, administration of THC or WIN 55,212-2 increases the reinforcing effects of heroin (Solinas *et al*., 2005), nicotine (Valjent *et al*., 2002) and alcohol (Gallate *et al*., 1999; Colombo *et al*., 2002). Concerning psychostimulants, one study in rats has shown that administration of WIN 55,212-2 decreased self-administration of cocaine under a fixed-ratio (FR1) schedule (Fattore *et al*., 1999). However, as a decrease in the number of drug injections self-administered under a FR1 schedule can be interpreted either as a decrease or an increase in reinforcing efficacy (Arnold and Roberts, 1997), definitive conclusions cannot be drawn from these experiments.

In addition to anecdotal evidence that cannabis increases appetite, especially for sweet food, in recreational cannabis smokers (Abel, 1975), several preclinical studies have shown that CB_1 receptor agonists facilitate food reward, in particular, the hedonic response to sweet food that Berridge and Robinson (1998) call 'liking'. For example, THC increases the intake of food (Williams *et al*., 1998; Williams and Kirkham, 2002b) and increases the consumption of sweet solutions (Gallate *et al*., 1999) in rats. In addition, low doses of THC increase hedonic reactions to sucrose and decrease aversive reactions to bitter quinine solutions (Jarrett *et al*., 2005, 2007) and THC increases the palatability of sucrose (Higgs *et al*., 2003) in rats. Also, we recently found that the motivation to respond for food, as measured by break points in responding for food under a progressive-ratio schedule (Arnold and Roberts, 1997), is increased by administration of THC (Solinas and Goldberg, 2005). Interestingly, enhancement of the motivation to respond for food by THC is dependent on actual food consumption (Solinas and Goldberg, 2005), suggesting that both appetitive and consummatory aspects of food reward may involve the endocannabinoid system. Taken together, these findings provide a rationale for the clinical use of CB_1 receptor agonists such as Marinol in anorexic cancer and HIV patients (Croxford and Miller, 2003).

There are contradictory reports on the effects of CBs on brain stimulation reward. Brain stimulation reward or intracranial self-stimulation is an operant procedure where animals have to press a lever to receive a small electrical current in restricted areas of the brain (Wise, 2002; Kornetsky, 2004). Brain stimulation reward is arguably the most robust form of reinforcement and is believed to derive from the ability of electrical currents to activate, probably indirectly, the dopaminergic mesolimbic system (Wise, 2002; Kornetsky, 2004). In support of this hypothesis, drugs that activate the dopaminergic system and increase dopamine levels in the nucleus accumbens (NAcc) (namely drugs of abuse) also facilitate brain stimulation reward (increase thresholds for self-stimulation), whereas drugs that block dopamine receptors (but also opioid antagonists, see Schaefer, 1988) reduce thresholds for self-stimulation (Wise, 2002; Kornetsky, 2004). Concerning, CB_1 receptor agonists, Gardner and colleagues found that THC facilitates brain stimulation reward (Gardner *et al*., 1988; Lepore *et al*., 1996), whereas other investigators found no effects of the synthetic

agonists CP55,940 or AMG-3 (Arnold *et al*., 2001; Antoniou *et al*., 2005) and some investigators have found a reduction of brain stimulation reward with CB_1 receptor agonists (Kucharski *et al*., 1983; Vlachou *et al*., 2005, 2007). These discrepancies, which are likely due to procedural differences, remain to be resolved.

A caveat of all experiments with directly acting CB_1 receptor agonists is that, for several reasons, these drugs do not provide a realistic picture of the physiological role of the endocannabinoids. First, anandamide is a partial agonist (Mackie *et al*., 1993), whereas synthetic CB_1 receptor agonists (not THC) are often full agonists and have higher affinities for CB_1 receptors (Childers, 2006). Second, anandamide and 2-AG have very short half-lives (Deutsch and Chin, 1993; Dinh *et al*., 2002a), whereas THC and synthetic CB_1 receptor agonists have relatively long half-lives. Finally, systemic injections of these compounds result in the activation of all brain areas containing CB_1 receptors, whereas physiological activation of endocannabinoid synthesis and release is likely to be region, neuron or even synapse specific (Piomelli, 2003; Di Marzo *et al*., 2004). The availability of mice genetically deprived of CB_1 receptors in a tissue-specific manner (Marsicano *et al*., 2003; Monory *et al*., 2006, 2007) may help address this possibility.

Effects of CB_1 receptor antagonists on reward processes

Assessment of the roles the endocannabinoid system plays in brain reward processes was greatly facilitated by the discovery of selective CB_1 receptor antagonists/inverse agonists such as rimonabant (SR141716A) (Rinaldi-Carmona *et al*., 1994) and AM251 (Gatley *et al*., 1996). CB_1 receptor antagonists decrease the rewarding effects of a wide variety of abused drugs under certain conditions. For example, the rewarding effects of opioids are generally decreased in both intravenous self-administration (Navarro *et al*., 2001; Caille and Parsons, 2003; De Vries *et al*., 2003; Solinas *et al*., 2003) and conditioned place preference procedures (Chaperon *et al*., 1998; Mas-Nieto *et al*., 2001; Navarro *et al*., 2001; Singh *et al*., 2004). There have also been reports that rimonabant and AM251 reduce the rewarding effects of methamphetamine (Vinklerova *et al*., 2002), alcohol (Arnone *et al*., 1997; Colombo *et al*., 1998; Gallate and McGregor, 1999; Freedland *et al*., 2001; Cippitelli *et al*., 2005; Gessa *et al*., 2005; Economidou *et al*., 2006; Lallemand and De Witte, 2006) and nicotine (Cohen *et al*., 2002; Le Foll and Goldberg, 2004; Forget *et al*., 2005).

The rewarding effects of cocaine are reduced in a very specific way by CB_1 receptor antagonists. CB_1 receptor antagonists do not generally alter self-administration of cocaine under low fixed-ratio schedules (Tanda *et al*., 2000; De Vries *et al*., 2001; Filip *et al*., 2006) or conditioned place preference procedures (Chaperon *et al*., 1998), but AM251 has been found to significantly reduce self-administration of cocaine under progressive-ratio schedules (Xi *et al*., 2007) and rimonabant prevents relapse to cocaine-induced and cue-induced cocaine-seeking behaviour (De Vries *et al*., 2001). This suggests that the appetitive and conditioned effects of cocaine, but not its direct reinforcing effects, depend on CB_1 receptor activation.

The effects of rimonabant on opioid reward may be mediated primarily in the NAcc, as blockade of CB_1 receptors in this area reduces heroin self-administration (Caille and Parsons, 2006). On the other hand, the modulation of ethanol reward by the CB system appears to take place both in the NAcc (Caille *et al*., 2007) and in the prefrontal cortex (Hansson *et al*., 2007). The brain sites where CBs act to alter the rewarding effects of nicotine and psychostimulants are not known at present.

Drugs of abuse share the ability to elevate extracellular levels of dopamine in the shell of the NAcc, as measured by *in vivo* microdialysis, and this effect is believed to play an important role in their reinforcing effects (Koob, 2000; Di Chiara *et al*., 2004; Wise, 2004; Everitt and Robbins, 2005). CB_1 receptor antagonists have been shown to block the elevations of accumbal dopamine levels induced by administration of nicotine or ethanol (Cohen *et al*., 2002), but not by administration of heroin (Tanda *et al*., 1997; Caille and Parsons, 2003), morphine or cocaine (Caille and Parsons, 2006). Transient surges of dopamine in the NAcc, as measured by cyclic voltammetry, are also produced by drugs of abuse (Cheer *et al*., 2007) and are believed to be involved in drug seeking (Phillips *et al*., 2003). Interestingly, the transient increases in dopamine produced by administration of nicotine, ethanol and cocaine in the shell of the NAcc of freely moving rats are all blocked by CB_1 receptor antagonists (Cheer *et al*., 2007).

Consistent with a role for endocannabinoids in the rewarding effects of food and in the regulation of appetite and food intake (Di Marzo and Matias, 2005), blocking endocannabinoid tone with CB_1 receptor antagonists reduces intake of food and sweet solutions (Arnone *et al*., 1997; Colombo *et al*., 1998; Simiand *et al*., 1998; Freedland *et al*., 2001; McLaughlin *et al*., 2003; Thornton-Jones *et al*., 2005, 2006, 2007). Also, injection of rimonabant within 24 h of birth completely prevents milk intake and causes almost 100% mortality in mouse pups (Fride *et al*., 2001, 2003). The motivational effects of food measured by a progressive-ratio schedule of food reinforcement (Solinas and Goldberg, 2005) and the appetitive aspects of food reward (Thornton-Jones *et al*., 2005) are significantly reduced by rimonabant in rats, indicating that some aspects of food intake regulation involve reward and motivational processes. In addition, AM251 decreases hedonic reactions to sucrose and increases aversive reactions to quinine (Jarrett *et al*., 2007). Consistent with these preclinical findings, rimonabant has been found to be effective in the clinical treatment of obesity (Padwal and Majumdar, 2007), although the clinical efficacy of this agent appears to be primarily due to its ability to alter peripheral lipid metabolism, rather than to reduce food intake (Cota *et al*., 2003a, b).

As in the case of CB_1 receptor agonists, the effects of CB_1 receptor antagonists on brain stimulation reward are somewhat controversial. Rimonabant has been shown to increase the threshold for brain stimulation reward in some studies (Deroche-Gamonet *et al*., 2001; De Vry *et al*., 2004) or to produce no change in brain stimulation reward thresholds in other studies (Arnold *et al*., 2001; Vlachou *et al*., 2003, 2005, 2007; Antoniou *et al*., 2005).

It should be noted that both rimonabant and AM251 act as inverse agonists at CB_1 receptors and, therefore, can produce effects that are opposite to those of CB_1 receptor agonists even in the absence of endogenous ligands (Landsman *et al*., 1997; Pan *et al*., 1998). Thus, it is possible that certain effects of these agents might be due to their inverse agonist properties. The development of new antagonists devoid of inverse agonist properties (Pertwee, 2005; Bergman *et al*., 2008) will undoubtedly help to provide a more definitive description of the effects of CB_1 receptor blockade on brain reward processes.

Effects of genetic ablation of CB_1 receptors on brain reward processes

Mice genetically engineered to lack CB_1 receptors (Ledent *et al*., 1999; Zimmer *et al*., 1999; Marsicano *et al*., 2002) do not show dramatic changes in body weight, food consumption or fertility (Ledent *et al*., 1999; Zimmer *et al*., 1999; Marsicano *et al*., 2002), suggesting that CB_1 receptors modulate rather than mediate basic reward functions or that other systems can compensate for their absence.

By using CB_1-null mice, the role of CB_1 receptors in the rewarding effects of drugs of abuse has been confirmed. For example, in these mutant mice morphine is not self-administered (Ledent *et al*., 1999; Cossu *et al*., 2001), does not induce conditioned place preferences (Martin *et al*., 2000) and does not elevate dopamine levels in the NAcc (Mascia *et al*., 1999). Also, the rewarding effects of alcohol are reduced in CB_1-null mice, as demonstrated by data showing that CB_1-null mice do not develop conditioned place preference with this drug (Houchi *et al*., 2005; Thanos *et al*., 2005) and that they do not prefer it over water in a two-bottle free-choice paradigm (Wang *et al*., 2003; Naassila *et al*., 2004; Thanos *et al*., 2005; Lallemand and De Witte, 2006). However, another study (Racz *et al*., 2003) reported that CB1-null mice show slight and short-lasting decreases in preference for ethanol. Development of conditioned place preferences with cocaine is unaltered in CB_1-null mice (Martin *et al*., 2000; Houchi *et al*., 2005). On the other hand, development of cocaine self-administration behaviour under fixed-ratio schedules of reinforcement in CB_1-null mice was reported to be unaltered when mice were restrained (Cossu *et al*., 2001), but was reduced in freely moving mice (Soria *et al*., 2005). In addition, cocaine self-administration was significantly reduced under a progressive-ratio schedule of self-administration in CB_1-null mice (Soria *et al*., 2005). These contrasting results highlight the fact that, when working with genetically modified mice such as CB1-null mice, not only methodological differences but also differences in the genetic background may result in very different and sometimes contrasting behavioural outputs (Bucan and Abel, 2002; Bailey *et al*., 2006).

It is interesting to note that CB_1-null mice show normal elevations in dopamine levels in the NAcc following administration of cocaine (Soria *et al*., 2005), but no elevations following administration of morphine (Mascia *et al*., 1999) or ethanol (Hungund *et al*., 2003), compared with wild-type controls. Finally, CB_1-null mice do not develop conditioned place preferences to nicotine (Castane *et al*., 2002), but they self-administer the drug like wild-type controls (Cossu *et al*., 2001). It remains to be seen whether increasing the effort needed to obtain nicotine, as with cocaine (Soria *et al*., 2005), could reveal a role of CB_1 receptors in some aspects of nicotine reinforcement as suggested by the results with CB_1 receptor antagonists (Cohen *et al*., 2005).

CB_1-null mice have also provided evidence for the involvement of the endocannabinoid system in food reward. For example, CB_1-null mice eat less than their wild-type control littermates after food restriction (Di Marzo *et al*., 2001b). Moreover, CB_1-null mice respond less for sucrose in a two-lever paradigm, have lower break points under progressive-ratio schedules of sucrose delivery (Sanchis-Segura *et al*., 2004) and show less preference for sucrose over water in a two-bottle free-choice procedure (Poncelet *et al*., 2003; Sanchis-Segura *et al*., 2004). Genetic ablation of CB_1 receptors results in small reduction in body weight, reduction in adiposity and resistance to diet-induced obesity (Ravinet Trillou *et al*., 2004). However, as in the case of CB_1 receptor antagonists, these effects appeared to be related more to increased metabolic energy consumption than to differences in rewarding effects of food or hypophagia (Cota *et al*., 2003a, b; Ravinet Trillou *et al*., 2004). Interestingly, in the study by Fride *et al*. (2003), administration of rimonabant in mice pups lacking CB_1 receptors still induced a decrease in milk intake and survival rate, suggesting that some of the effects of CBs on food intake may be mediated by still uncharacterized CB receptors.

To date, no study has investigated the effects of CB_1 receptor deletion on brain stimulation reward. On the other hand, it should be noted that CB_1-null mice show increased anhedonia after chronic mild stress (Martin *et al*., 2002), a measure of reduced activity of the reward system and a model of depression (Willner, 2005), further supporting a role for the endocannabinoid system in brain reward functions.

Role of anandamide in brain reward processes

Effects of systemically administered anandamide on reward processes

We have recently shown that anandamide and the metabolically stable anandamide analogue, methanandamide, can serve as powerful reinforcers of self-administration behaviour in non-human primates when injected intravenously (Justinova *et al*., 2005b) and they both increase extracellular levels of dopamine in the shell of the NAcc when injected intravenously, a neurochemical effect common to many rewarding stimuli (Solinas *et al*., 2006a). In addition, anandamide and methanandamide produce THC-like discriminative effects in two-lever choice drug discrimination procedures (Wiley *et al*., 1997; Solinas *et al*., 2006b, 2007b). Importantly, these effects of anandamide appear to be mediated by CB_1, but not TRPV1, receptors and are potentiated by pharmacological inhibition of FAAH activity, but not by administration of the anandamide transport inhibitor AM404 (Solinas *et al*., 2006a, 2007b).

In addition to its direct reinforcing effects, systemically administered anandamide can potentiate the effects of food reward. Systemic administration of anandamide was found to increase food intake (Hao *et al*., 2000) and induce overeating in sated rats (Williams and Kirkham, 1999, 2002a). Also, hypothalamic (Jamshidi and Taylor, 2001) or intra-shell injections of anandamide into the NAcc increase food intake (Mahler *et al*., 2007). Similarly to THC, anandamide appears to increase food intake, at least in part, by increasing the palatability of food, as measured by changes in patterns of sucrose drinking (Higgs *et al*., 2003). The effects of systemically administered anandamide on food palatability, as measured by hedonic reactions to food, appear to be mediated by the more dorsal part of the shell of the NAcc (Mahler *et al*., 2007).

We are not aware of any study investigating the effects of systemically administered anandamide on brain stimulation reward.

Effects of abused drugs, food or electrical brain stimulation on brain anandamide levels

Although measurements of the effects of systemic or intracranial injections of anandamide provide useful information on the functions of the endocannabinoid system, to provide support for a role of neurally released anandamide in brain reward processes it is also important to measure anandamide released in the brain by different abused drugs, by food and by electrical brain stimulation. Release of neurotransmitters such as dopamine or glutamate can be readily measured by microdialysis techniques, but only a few studies have employed microdialysis techniques to measure extracellular brain levels of endocannabinoids (Giuffrida *et al*., 1999; Caille *et al*., 2007). Thus, most information on the modification of endocannabinoid levels comes from measurements of tissue levels in different brain areas. Measuring tissue level of anandamide has the limitation that only one time point can be established at a time, limiting information on the pattern of endocannabinoid release.

Using tissue levels, it has been demonstrated that chronic administration of several drugs of abuse leads to region-specific increases in anandamide levels. For example, when administered chronically, THC decreases levels of anandamide in the striatum (Di Marzo *et al*., 2000), ethanol decreases levels of anandamide in the midbrain but not in the striatum (Gonzalez *et al*., 2002), nicotine decreases levels of anandamide in the striatum but not in the midbrain (Gonzalez *et al*., 2002), and cocaine and morphine do not alter anandamide levels, either in the striatum or midbrain (Gonzalez *et al*., 2002; Vigano *et al*., 2003). However, it is difficult to determine from these studies whether measured levels of endocannabinoids reflected the consequences of chronically administered drug or withdrawal.

Vigano *et al*. (2004) compared the effects of chronic versus acute administration of morphine on endocannabinoid levels in the brain. They found that acute administration of morphine increased anandamide levels in the striatum, whereas chronic treatment with the drug failed to do so. In addition, they found that chronic treatment with morphine did not alter the ability of a challenge dose of morphine to increase anandamide levels in the striatum; that is, repeated administration of morphine did not induce sensitization or tolerance to this effect (Vigano *et al*., 2004). Thus, chronic administration of drug followed by withdrawal, chronic administration of drug followed by an acute drug challenge and acute administration of drug can lead to very different changes in brain anandamide levels. Such profiles of release may indicate that anandamide is released in response to relevant changes in homoeostasis but not when an adaptive response has already occurred. This suggests that it may be preferable to assess the role of anandamide in drug reward processes by measuring changes in anandamide levels directly produced by administration of the drug rather than changes in anandamide levels produced after chronic drug exposure, when alterations may be opposite to those after acute administration and may reflect biochemical alterations associated with withdrawal. This is supported by a recent study in which Caille *et al*. (2007) employed microdialysis techniques to monitor extracellular endocannabinoid levels in the brain during active self-administration of drugs and found that anandamide levels were elevated during heroin self-administration, consistent with findings by Vigano *et al*. (2004). Interestingly, extracellular levels of anandamide were not altered during cocaine or ethanol self-administration (Caille *et al*., 2007). Differences in the molecular target and mechanism of actions of drugs of abuse could account for the different effects on accumbal levels of endocannabinoids.

There is only limited information on the effects of food reward on brain levels of anandamide. In one study, anandamide levels in the NAcc, but not in the hypothalamus, increased after 24-h food deprivation but not during active feeding (Kirkham *et al*., 2002). This suggests that appetitive aspects of food reward involve release of anandamide, but consummatory aspects of reward do not. However, it is also possible that, as in the case of dopamine (Di Chiara *et al*., 2004), natural rewards produce much lower increases in anandamide release than pharmacological rewards and that increases in anandamide occur with food reward but are lower than the limit of detection with current techniques.

Administration of addictive drugs or exposure to food increases dopamine levels in the NAcc (Koob, 2000; Di Chiara *et al*., 2004; Wise, 2004; Everitt and Robbins, 2005) and activation of dopamine D_2 receptors in this region increases extracellular levels of anandamide (Giuffrida *et al*., 1999). This suggests that increases in anandamide levels in the NAcc after administration of abused drugs or after eating may be secondary to increases in dopamine levels. However, Caille *et al*. (2007) have recently shown that drugs such as cocaine produce large increases in dopamine levels but do not alter anandamide levels in the NAcc.

Finally, to the best of our knowledge, the release of CBs during electrical brain self-stimulation remains to be investigated. However, indirect pharmacological evidence indicates that, at least in the VTA, 2-AG and not anandamide is released after application of brain stimulation that would sustain self-administration in rats (Melis *et al*., 2004a; Pillolla *et al*., 2007).

Effects of inhibiting anandamide degradation by FAAH on reward processes

As anandamide is quickly degraded, studying its normal roles can be facilitated by blocking the mechanisms of degradation and, thus, magnifying and prolonging its actions. Importantly, increases in anandamide levels would presumably be obtained only in those brain regions where anandamide is released. As previously described, the intracellular step of anandamide degradation is mediated mainly by FAAH activity (Freund *et al*., 2003; Piomelli, 2003; Di Marzo *et al*., 2004; Fride, 2005). Thus, the specific contribution of anandamide to reward functions can be more readily investigated when FAAH is inhibited by drugs such as URB597 (Kathuria *et al*., 2003). It should be noted that URB597 has no rewarding effects by itself in conditioned place preference paradigms, does not produce THC-like discriminative effects (Gobbi *et al*., 2005) and does not alter dopamine levels in the shell of NAcc (Solinas *et al*., 2006a, 2007b) in rats.

The effects of FAAH inhibition on drug reward have been the focus of several recent papers. One study found that the directly acting CB receptor agonists such as THC and WIN 55,212-2 increased the reinforcing effects of heroin, but FAAH inhibition by URB597 had no significant effect, suggesting that heroin-induced release of anandamide is not necessary for opiate reward (Solinas *et al*., 2005). Another study found that administration of URB597 increased ethanol preference and intake in a two-bottle free-choice procedure (Blednov *et al*., 2007). In a recent study, we found that nicotine can produce THC-like discriminative effects when given in combination with URB597, indicating that nicotine does release anandamide and that, under conditions of FAAH inhibition, can actually produce THC-like behavioural effects (Solinas *et al*., 2007a). However, it has to be established whether the reinforcing effects of nicotine itself, rather than its ability to produce THC-like discriminative effects, are altered by stimulation of the endocannabinoid system, as another study found that the discriminative effects of nicotine are not altered by administration of URB597 or combinations of URB597 plus anandamide (Zaniewska *et al*., 2006). Finally, one study has found that the effects of ethanol on the mesolimbic dopaminergic system are reduced by URB597 in mutant FAAH-null mice (Perra *et al*., 2005); however, the behavioural relevance of these neurochemical alterations remains to be established as mutant FAAH-null mice show a higher preference for ethanol (Basavarajappa and Hungund, 2005; Blednov *et al*., 2007).

The effects of URB597 on food reward have not yet been systematically studied, but one study investigated the effects of administration of URB597 on brain stimulation reward. FAAH inhibition increased the threshold for intracranial self-stimulation, indicating a decrease in the reinforcing effects of brain stimulation (Vlachou *et al*., 2006). However, these effects were obtained at doses of URB597 3–10 times higher than those required to produce significant inhibition of FAAH (Kathuria *et al*., 2003) and dramatic potentiation of the effects of exogenously administered anandamide (Solinas *et al*., 2006a, 2007b) and, thus, the interpretation of these results is difficult and may be related to loss of selectivity at high doses.

Effects of genetic deletion of FAAH on reward processes

In parallel with results obtained with URB597, the availability of FAAH-null mice (Cravatt *et al*., 2001) has further expanded possibilities of assessing anandamide's role in brain reward processes. As with CB_1-null mice, FAAH-null mice do not show dramatic changes in body weight, food consumption or fertility (Cravatt *et al*., 2001), suggesting, again, that the endocannabinoid system plays only a modulatory role in basic reward functions.

There are only a few studies on the effects of genetic ablation of FAAH on reward processes. Among drugs of abuse, information is available only regarding ethanol. In one study, it was found that ethanol consumption and preference increase in female, but not male, FAAH-null mice (Basavarajappa and Hungund, 2005), whereas in another study it was found that ethanol consumption and preference increase in both female and male FAAH-null mice (Blednov *et al*., 2007). Interestingly, conditioned place preferences were similarly obtained in males, but not in females, when ethanol was injected intraperitoneally (Blednov *et al*., 2007), indicating that the consequences of FAAH deletion on ethanol's rewarding effects may be complex. To date, no study has investigated the effects of FAAH deletion on food or brain stimulation reward. The finding that FAAH-null mice show a slightly antidepressant-like phenotype (Naidu *et al*., 2007) is consistent with a role for anandamide in mood regulation (Kathuria *et al*., 2003; Gobbi *et al*., 2005).

Role of 2-AG in brain reward processes

Although 2-AG was discovered only 3 years after anandamide, much less is known about the role of this endogenous compound in reward processes and most knowledge comes from indirect evidence. Hopefully, the development of new tools such as drugs selectively targeting enzymes involved in 2-AG formation and degradation or genetically modified mice will help to characterize the physiological and physiopathological roles of this endocannabinoid.

Effects of systemically administered 2-AG on reward processes

Only one study has reported behavioural effects related to reward processes for systemically administered 2-AG. Kirkham *et al*. (2002) found that 2-AG directly injected into the shell of the NAcc increased food intake.

Effects of abused drugs, food or electrical brain stimulation on 2-AG levels

The best evidence for a role of 2-AG in brain reward processes comes from studies that measured changes in brain levels of 2-AG produced by drugs, food or brain stimulation (with all the limitations of these measurements described for anandamide, section 'Effects of inhibiting anandamide degradation by FAAH on reward processes').

Chronic administration of many abused drugs alters levels of 2-AG in brain areas involved in reward. For example, it has been shown that chronic administration of (1) THC decreases tissue levels of 2-AG in the striatum (Di Marzo

et al., 2000), (2) ethanol decreases tissue levels of 2-AG in the midbrain but not in the nucleus accumbens (Gonzalez *et al*., 2002), (3) nicotine or (4) cocaine do not alter 2-AG levels either in the midbrain or the striatum (Gonzalez *et al*., 2002) and (5) morphine decreases 2-AG levels in the striatum without altering 2-AG levels in the striatum (Vigano *et al*., 2003). Moreover, Vigano *et al*. (2004) found that acute injection of morphine decreased tissue levels of 2-AG in the striatum more than chronic administration and that a challenge injection of morphine, after a 2-week period of withdrawal, in rats chronically treated with morphine, had effects similar to those of an acute injection. On the other hand, no changes in extracellular levels of 2-AG were found in the NAcc of rats self-administering heroin, but there was an increase in 2-AG levels in rats self-administering ethanol (Caille *et al*., 2007). The general picture that can be drawn from these data is that 2-AG is involved in some consequences of repeated administration of abused drugs but it may be more important for the rewarding effects of ethanol than for the rewarding effects of other abused drugs, such as heroin, cocaine or nicotine.

One study investigated the effects of manipulation of food intake and food deprivation on brain levels of 2-AG and found that tissue levels of 2-AG were increased by fasting but were decreased by feeding (Kirkham *et al*., 2002), indicating that 2-AG would be more important for appetitive aspects than consummatory aspects of food reward.

Finally, no study has investigated brain levels of 2-AG during electrical brain self-stimulation reward. However, one study has shown that electrical stimulation of the medial prefrontal cortex produces a decrease in presynaptic glutamate release that might be explained by release of 2-AG (Melis *et al*., 2004a). Future studies are needed to verify that these neurochemical results have behavioural relevance.

Endocannabinoid transport

As noted in section 'Synthesis and degradation of endocannabinoids and endocannabinoid transport', endocannabinoid transport remains one of the less understood features of the endocannabinoid system. Although endocannabinoid transport was proposed long ago and drugs thought to inhibit the transport of endocannabinoids through the cell membrane have been available for a decade (Beltramo *et al*., 1997; Hillard *et al*., 1997), the molecular entity(ies) mediating this transport is still unknown and the nature of endocannabinoid transport is debated (Glaser *et al*., 2003, 2005; Moore *et al*., 2005). However, drugs such as AM404, VDM11, UCM707 and AM1172 (Piomelli *et al*., 1999; De Petrocellis *et al*., 2000; Lopez-Rodriguez *et al*., 2003; Fegley *et al*., 2004; Glaser *et al*., 2005), which are thought to block endocannabinoid uptake into the cell, can be important tools for investigating the role of the endocannabinoid system in brain reward processes.

Unlike the FAAH inhibitor URB597, the uptake inhibitor AM404 has some rewarding effects by itself using a conditioned place preference procedure, although its rewarding effects measured with this procedure are clearly smaller than those of the direct CB receptor agonist WIN 55,212-2 (Bortolato *et al*., 2006). Interestingly, AM404 induced conditioned place preferences in rats at a dose that did not significantly increase tissue levels of anandamide or 2-AG in the brain areas investigated (Bortolato *et al*., 2006). On the other hand, AM404 does not produce THC-like discriminative effects and does not alter dopamine levels in the shell of NAcc in rats (Solinas *et al*., 2007b). It is, thus, surprising that the effects of AM404 or other uptake inhibitors on drug, food or brain stimulation reward have seldom been studied. To our knowledge, only one study has investigated the effects of uptake inhibition on drug reward and in that study AM404 did not increase heroin self-administration under a progressive-ratio schedule and, instead, produced a small decrease (Solinas *et al*., 2005). In that study, CB_1 receptor agonists such as THC and WIN 55,212-2 potentiated heroin self-administration and URB597 had no effect on heroin self-administration. One interpretation of these results is that enhancement of endocannabinoid tone has either an inhibitory or a neutral effect on opioid reward, whereas broad activation of CB_1 receptors has a facilitatory effect on opioid reward.

The effects of blockade of anandamide transport on food intake and electrical brain stimulation reward have been investigated using the uptake inhibitors VDM-11 and OMDM-2. It was found that VDM-11 does not alter food intake in rats (Chambers *et al*., 2004) and that high doses (30 mg/kg i.p.) of OMDM-2 increase the threshold for electrical brain self-stimulation reward (Vlachou *et al*., 2006).

Obviously, more research is needed to interpret the effects of inhibition of endocannabinoid uptake on brain reward processes and to understand its role in the regulation of the activity of the endocannabinoid system. For example, we have recently found that some effects of anandamide, such as its ability to produce THC-like discriminative effects and to increase dopamine levels in the NAcc, are potentiated by URB597 but not by AM404 (Solinas *et al*., 2007b), suggesting that regional differences may exist in the relative roles of uptake inhibition and FAAH inhibition.

Summary

Involvement of the endocannabinoid system in drug reward

The importance of the endocannabinoid system in drug reward varies greatly depending on the drug studied, with a pronounced role for the endocannabinoid system in opioid and ethanol reward and more subtle roles in nicotine and psychostimulant reward.

It is clear that activity of CB_1 receptors is important for opioids to produce maximal reinforcing effects (Fattore *et al*., 2005; Vigano *et al*., 2005). Although opioids release anandamide in the NAcc (Caille *et al*., 2007), the importance of this release remains unclear given that compounds that increase brain concentrations of anandamide and prolong anandamide's actions do not alter heroin self-administration (Solinas *et al*., 2005). Thus, interactions between CB_1 receptors and mu-opioid receptors (Berrendero *et al*., 2003; Kathmann *et al*., 2006) may be more important than endocannabinoid release in the modulation of opioid effects.

It is also clear that activity of CB_1 receptors is important for ethanol to produce maximal reinforcing effects (Mechoulam and Parker, 2003). Although it has been reported that ethanol self-administration increases 2-AG but not anandamide levels in the NAcc (Caille *et al*., 2007), FAAH-null mice show higher preferences for ethanol but no higher ethanol-induced conditioned place preferences (Blednov *et al*., 2007). Thus, although release of endocannabinoids appears to participate in ethanol reward, the relative importance of 2-AG and anandamide needs to be established.

CB_1 receptors may be critically involved in the rewarding effects of nicotine (Castane *et al*., 2002; Viveros *et al*., 2005). The role of release of endocannabinoids is less clear, however, as we have reported that URB597 unmasks some THC-like discriminative effects of nicotine (Solinas *et al*., 2007a), whereas another study found that URB597 did not potentiate the discriminative effects of nicotine itself (Zaniewska *et al*., 2006). Importantly, unpublished results from company-sponsored clinical trials suggest that blockade of CB_1 receptors may be effective in promoting smoking cessation and abstinence (http://en.sanofi-aventis.com/press/ppc_1960.asp). Finally, the few studies available have not supported a role of CB_1 receptors in the primary reinforcing effects of cocaine. In addition, *in vivo* microdialysis studies have found that neither anandamide nor 2-AG is formed in the shell of the NAcc during active self-administration of cocaine (Caille *et al*., 2007). However, CB_1 receptors appear to be required for the incentive motivational effects of cocaine, as measured by self-administration under progressive-ratio schedules (Soria *et al*., 2005) and by reinstatement of extinguished cocaine self-administration (De Vries *et al*., 2001).

Involvement of the endocannabinoid system in food reward

Food reward also clearly depends on CB_1 receptors and almost every aspect of food reward is affected by activation or inactivation of CB_1 receptors (Di Marzo and Matias, 2005). Again, data from clinical trials appear to support the preclinical findings that CB_1 blockade is effective in promoting weight loss, although the peripheral and hormonal effects of rimonabant may be more important that the central effects on reward (Padwal and Majumdar, 2007). However, the involvement of released endocannabinoids may be limited to appetitive aspects of food reward, as concentrations of both anandamide and 2-AG increase in the NAcc during fasting but not during consumption of food. In addition, inhibitors of anandamide uptake do not increase food intake (Chambers *et al*., 2004), further indicating that endogenous anandamide, at least, may be insufficient to drive food intake.

Involvement of the CB system in electrical brain stimulation reward

Brain stimulation reward remains the most obscure aspect of CB-related reward. In contrast to other neurotransmitter systems involved in brain stimulation reward functions, such as dopaminergic and opioid systems (Kornetsky, 2004), the effects of endocannabinoid system alterations in different studies have been contradictory and, in fact, activation of the endocannabinoid system appears to reduce the rewarding effects of electrical brain stimulation. Further studies are needed, but it is worth noting that in some instances dopaminergic systems and CB systems appear to have opposite antagonistic effects. For example, dopamine D_2 receptor activation in the dorsal striatum releases anandamide, which might act to modulate or counterbalance the effects of dopamine (Giuffrida *et al*., 1999). Also, glutamate release in the VTA activates dopaminergic neurons and, at the same time, leads to the release of 2-AG that in turn reduces glutamate release (Melis *et al*., 2004a, b). On the other hand, CB agonists induce release of dopamine in the shell of the NAcc (Tanda *et al*., 1997; Solinas *et al*., 2006a) and have rewarding effects when administered locally both in the NAcc and the VTA (Zangen *et al*., 2006). Thus, it is possible that, depending on the balance between endocannabinoids and dopamine and the intensity of stimulation of the region, the systems facilitate or oppose each other. This could be a mechanism for fine-tuning of dopaminergic activity. As electrical brain stimulation is a very strong excitatory stimulus, it is possible that the endocannabinoid system acts to counteract and oppose such stimulation.

Future directions

The endocannabinoid field has been in active expansion since the early 1990s and promises to continue expanding at a rapid pace. The intellectual and financial investments in the field are providing new tools that will allow scientists working in the field to understand better the physiological and pathophysiological roles of the endocannabinoids. Among the innovations that will improve our knowledge are (1) improvement of tools already present, such as more selective FAAH and anandamide transport blockers, compounds that do not pass the blood–brain barrier and conditional and region-specific CB_1- and FAAH-null mice; (2) the molecular identification of the components comprising the endocannabinoid transport system; (3) availability of potent and selective MGL blockers that are active *in vivo* and of MGL-null mice and (4) availability of pharmacological and genetic tools to regulate the synthesis of endocannabinoids.

Conclusions

The involvement of the endocannabinoid system in brain reward processes can be inferred from at least four lines of evidence: (1) CB_1 receptors are highly expressed in areas of the brain involved in reward functions such as the mesolimbic dopaminergic system; (2) CB_1 receptor agonists such as THC produce reinforcing effects; (3) pharmacological or genetic activation of CB_1 receptors facilitates, whereas pharmacological or genetic inactivation of CB_1 receptors antagonizes, several types of brain reward functions and (4) levels of endocannabinoids in brain areas such as the mesolimbic dopamine system are altered following manipulations that involve reward processes. The involvement of various components of the endocannabinoid system may

differ from one type of rewarding stimulus to another. Notwithstanding these differences and the need for further investigations, the data available strongly suggest that the endocannabinoid system plays an important role in brain reward processes.

Acknowledgements

This study was supported by the Centra National de la Recherche Scientifique, by the Intramural Research Program of the National Institute on Drug Abuse (NIDA), National Institutes of Health, Department of Health and Human Services and by grants from NIDA DA12413, DA14447 and DA07318 to DP.

References

Abel EL (1975). Cannabis: effects on hunger and thirst. *Behav Biol* **15**: 255–281.

Antoniou K, Galanopoulos A, Vlachou S, Kourouli T, Nahmias V, Thermos K *et al*. (2005). Behavioral pharmacological properties of a novel cannabinoid 1′,1′-dithiolane delta8-THC analog, AMG-3. *Behav Pharmacol* **16**: 499–510.

Arnold JC, Hunt GE, McGregor IS (2001). Effects of the cannabinoid receptor agonist CP 55,940 and the cannabinoid receptor antagonist SR 141716 on intracranial self-stimulation in Lewis rats. *Life Sci* **70**: 97–108.

Arnold JM, Roberts DC (1997). A critique of fixed and progressive ratio schedules used to examine the neural substrates of drug reinforcement. *Pharmacol Biochem Behav* **57**: 441–447.

Arnone M, Maruani J, Chaperon F, Thiebot MH, Poncelet M, Soubrie P *et al*. (1997). Selective inhibition of sucrose and ethanol intake by SR 141716, an antagonist of central cannabinoid (CB1) receptors. *Psychopharmacology (Berl)* **132**: 104–106.

Bailey KR, Rustay NR, Crawley JN (2006). Behavioral phenotyping of transgenic and knockout mice: practical concerns and potential pitfalls. *ILAR J* **47**: 124–131.

Basavarajappa BS, Hungund BL (2005). Role of the endocannabinoid system in the development of tolerance to alcohol. *Alcohol Alcohol* **40**: 15–24.

Begg M, Pacher P, Batkai S, Osei-Hyiaman D, Offertaler L, Mo FM *et al*. (2005). Evidence for novel cannabinoid receptors. *Pharmacol Ther* **106**: 133–145.

Beltramo M, Stella N, Calignano A, Lin SY, Makriyannis A, Piomelli D (1997). Functional role of high-affinity anandamide transport, as revealed by selective inhibition. *Science* **277**: 1094–1097.

Bergman J, Delatte MS, Paronis CA, Vemuri K, Thakur GA, Makriyannis A (2008). Some effects of CB1 antagonists with inverse agonist and neutral biochemical properties. *Physiol Behav* **93**: 666–670.

Berrendero F, Mendizabal V, Murtra P, Kieffer BL, Maldonado R (2003). Cannabinoid receptor and WIN 55 212-2-stimulated [35S]-GTPgammaS binding in the brain of mu-, delta- and kappa-opioid receptor knockout mice. *Eur J Neurosci* **18**: 2197–2202.

Berridge KC, Robinson TE (1998). What is the role of dopamine in reward: hedonic impact, reward learning, or incentive salience? *Brain Res Brain Res Rev* **28**: 309–369.

Blednov YA, Cravatt BF, Boehm II SL, Walker D, Harris RA (2007). Role of endocannabinoids in alcohol consumption and intoxication: studies of mice lacking fatty acid amide hydrolase. *Neuropsychopharmacology* **32**: 1570–1582.

Bortolato M, Campolongo P, Mangieri RA, Scattoni ML, Frau R, Trezza V *et al*. (2006). Anxiolytic-like properties of the anandamide transport inhibitor AM404. *Neuropsychopharmacology* **31**: 2652–2659.

Bracey MH, Hanson MA, Masuda KR, Stevens RC, Cravatt BF (2002). Structural adaptations in a membrane enzyme that terminates endocannabinoid signaling. *Science* **298**: 1793–1796.

Breivogel CS, Griffin G, Di Marzo V, Martin BR (2001). Evidence for a new G protein-coupled cannabinoid receptor in mouse brain. *Mol Pharmacol* **60**: 155–163.

Bucan M, Abel T (2002). The mouse: genetics meets behaviour. *Nat Rev Genet* **3**: 114–123.

Caille S, Alvarez-Jaimes L, Polis I, Stouffer DG, Parsons LH (2007). Specific alterations of extracellular endocannabinoid levels in the nucleus accumbens by ethanol, heroin, and cocaine self-administration. *J Neurosci* **27**: 3695–3702.

Caille S, Parsons LH (2003). SR141716A reduces the reinforcing properties of heroin but not heroin-induced increases in nucleus accumbens dopamine in rats. *Eur J Neurosci* **18**: 3145–3149.

Caille S, Parsons LH (2006). Cannabinoid modulation of opiate reinforcement through the ventral striatopallidal pathway. *Neuropsychopharmacology* **31**: 804–813.

Castane A, Valjent E, Ledent C, Parmentier M, Maldonado R, Valverde O (2002). Lack of CB1 cannabinoid receptors modifies nicotine behavioural responses, but not nicotine abstinence. *Neuropharmacology* **43**: 857–867.

Chambers AP, Sharkey KA, Koopmans HS (2004). Cannabinoid (CB)1 receptor antagonist, AM 251, causes a sustained reduction of daily food intake in the rat. *Physiol Behav* **82**: 863–869.

Chaperon F, Soubrie P, Puech AJ, Thiebot MH (1998). Involvement of central cannabinoid (CB1) receptors in the establishment of place conditioning in rats. *Psychopharmacology (Berl)* **135**: 324–332.

Cheer JF, Kendall DA, Mason R, Marsden CA (2003). Differential cannabinoid-induced electrophysiological effects in rat ventral tegmentum. *Neuropharmacology* **44**: 633–641.

Cheer JF, Wassum KM, Sombers LA, Heien ML, Ariansen JL, Aragona BJ *et al*. (2007). Phasic dopamine release evoked by abused substances requires cannabinoid receptor activation. *J Neurosci* **27**: 791–795.

Childers SR (2006). Activation of G-proteins in brain by endogenous and exogenous cannabinoids. *AAPS J* **8**: E112–E117.

Cippitelli A, Bilbao A, Hansson AC, del Arco I, Sommer W, Heilig M *et al*. (2005). Cannabinoid CB1 receptor antagonism reduces conditioned reinstatement of ethanol-seeking behavior in rats. *Eur J Neurosci* **21**: 2243–2251.

Cohen C, Perrault G, Griebel G, Soubrie P (2005). Nicotine-associated cues maintain nicotine-seeking behavior in rats several weeks after nicotine withdrawal: reversal by the cannabinoid (CB1) receptor antagonist, rimonabant (SR141716). *Neuropsychopharmacology* **30**: 145–155.

Cohen C, Perrault G, Voltz C, Steinberg R, Soubrie P (2002). SR141716, a central cannabinoid (CB(1)) receptor antagonist, blocks the motivational and dopamine-releasing effects of nicotine in rats. *Behav Pharmacol* **13**: 451–463.

Colombo G, Agabio R, Fa M, Guano L, Lobina C, Loche A *et al*. (1998). Reduction of voluntary ethanol intake in ethanol-preferring sP rats by the cannabinoid antagonist SR-141716. *Alcohol Alcohol* **33**: 126–130.

Colombo G, Serra S, Brunetti G, Gomez R, Melis S, Vacca G *et al*. (2002). Stimulation of voluntary ethanol intake by cannabinoid receptor agonists in ethanol-preferring sP rats. *Psychopharmacology (Berl)* **159**: 181–187.

Cossu G, Ledent C, Fattore L, Imperato A, Bohme GA, Parmentier M *et al*. (2001). Cannabinoid CB1 receptor knockout mice fail to self-administer morphine but not other drugs of abuse. *Behav Brain Res* **118**: 61–65.

Cota D, Marsicano G, Lutz B, Vicennati V, Stalla GK, Pasquali R *et al*. (2003a). Endogenous cannabinoid system as a modulator of food intake. *Int J Obes Relat Metab Disord* **27**: 289–301.

Cota D, Marsicano G, Tschop M, Grubler Y, Flachskamm C, Schubert M *et al*. (2003b). The endogenous cannabinoid system affects energy balance via central orexigenic drive and peripheral lipogenesis. *J Clin Invest* **112**: 423–431.

Cravatt BF, Demarest K, Patricelli MP, Bracey MH, Giang DK, Martin BR *et al*. (2001). Supersensitivity to anandamide and enhanced endogenous cannabinoid signaling in mice lacking fatty acid amide hydrolase. *Proc Natl Acad Sci USA* **98**: 9371–9376.

Cravatt BF, Giang DK, Mayfield SP, Boger DL, Lerner RA, Gilula NB (1996). Molecular characterization of an enzyme that degrades neuromodulatory fatty-acid amides. *Nature* **384**: 83–87.

Croxford JL, Miller SD (2003). Immunoregulation of a viral model of multiple sclerosis using the synthetic cannabinoid R+WIN55,212. *J Clin Invest* **111**: 1231–1240.

De Petrocellis L, Bisogno T, Davis JB, Pertwee RG, Di Marzo V (2000). Overlap between the ligand recognition properties of the anandamide transporter and the VR1 vanilloid receptor: inhibitors of anandamide uptake with negligible capsaicin-like activity. *FEBS Lett* **483**: 52–56.

De Vries TJ, Homberg JR, Binnekade R, Raaso H, Schoffelmeer AN (2003). Cannabinoid modulation of the reinforcing and motivational properties of heroin and heroin-associated cues in rats. *Psychopharmacology (Berl)* **168**: 164–169.

De Vries TJ, Schoffelmeer AN (2005). Cannabinoid CB1 receptors control conditioned drug seeking. *Trends Pharmacol Sci* **26**: 420–426.

De Vries TJ, Shaham Y, Homberg JR, Crombag H, Schuurman K, Dieben J *et al.* (2001). A cannabinoid mechanism in relapse to cocaine seeking. *Nat Med* **7**: 1151–1154.

De Vry J, Schreiber R, Eckel G, Jentzsch KR (2004). Behavioral mechanisms underlying inhibition of food-maintained responding by the cannabinoid receptor antagonist/inverse agonist SR141716A. *Eur J Pharmacol* **483**: 55–63.

Deroche-Gamonet V, Le Moal M, Piazza PV, Soubrie P (2001). SR141716, a CB1 receptor antagonist, decreases the sensitivity to the reinforcing effects of electrical brain stimulation in rats. *Psychopharmacology (Berl)* **157**: 254–259.

Desarnaud F, Cadas H, Piomelli D (1995). Anandamide amidohydrolase activity in rat brain microsomes. Identification and partial characterization. *J Biol Chem* **270**: 6030–6035.

Deutsch DG, Chin SA (1993). Enzymatic synthesis and degradation of anandamide, a cannabinoid receptor agonist. *Biochem Pharmacol* **46**: 791–796.

Devane WA, Dysarz III FA, Johnson MR, Melvin LS, Howlett AC (1988). Determination and characterization of a cannabinoid receptor in rat brain. *Mol Pharmacol* **34**: 605–613.

Devane WA, Hanus L, Breuer A, Pertwee RG, Stevenson LA, Griffin G *et al.* (1992). Isolation and structure of a brain constituent that binds to the cannabinoid receptor. *Science* **258**: 1946–1949.

Di Chiara G, Bassareo V, Fenu S, De Luca MA, Spina L, Cadoni C *et al.* (2004). Dopamine and drug addiction: the nucleus accumbens shell connection. *Neuropharmacology* **47** (Suppl 1): 227–241.

Di Marzo V, Berrendero F, Bisogno T, Gonzalez S, Cavaliere P, Romero J *et al.* (2000). Enhancement of anandamide formation in the limbic forebrain and reduction of endocannabinoid contents in the striatum of delta9-tetrahydrocannabinol-tolerant rats. *J Neurochem* **74**: 1627–1635.

Di Marzo V, Bifulco M, De Petrocellis L (2004). The endocannabinoid system and its therapeutic exploitation. *Nat Rev Drug Discov* **3**: 771–784.

Di Marzo V, Bisogno T, De Petrocellis L (2001a). Anandamide: some like it hot. *Trends Pharmacol Sci* **22**: 346–349.

Di Marzo V, Fontana A, Cadas H, Schinelli S, Cimino G, Schwartz JC *et al.* (1994). Formation and inactivation of endogenous cannabinoid anandamide in central neurons. *Nature* **372**: 686–691.

Di Marzo V, Goparaju SK, Wang L, Liu J, Batkai S, Jarai Z *et al.* (2001b). Leptin-regulated endocannabinoids are involved in maintaining food intake. *Nature* **410**: 822–825.

Di Marzo V, Matias I (2005). Endocannabinoid control of food intake and energy balance. *Nat Neurosci* **8**: 585–589.

Dinh TP, Carpenter D, Leslie FM, Freund TF, Katona I, Sensi SL *et al.* (2002a). Brain monoglyceride lipase participating in endocannabinoid inactivation. *Proc Natl Acad Sci USA* **99**: 10819–10824.

Dinh TP, Freund TF, Piomelli D (2002b). A role for monoglyceride lipase in 2-arachidonoylglycerol inactivation. *Chem Phys Lipids* **121**: 149–158.

Economidou D, Mattioli L, Cifani C, Perfumi M, Massi M, Cuomo V *et al.* (2006). Effect of the cannabinoid CB1 receptor antagonist SR-141716A on ethanol self-administration and ethanol-seeking behaviour in rats. *Psychopharmacology (Berl)* **183**: 394–403.

Egertova M, Cravatt BF, Elphick MR (2003). Comparative analysis of fatty acid amide hydrolase and cb(1) cannabinoid receptor expression in the mouse brain: evidence of a widespread role for fatty acid amide hydrolase in regulation of endocannabinoid signaling. *Neuroscience* **119**: 481–496.

Everitt BJ, Robbins TW (2005). Neural systems of reinforcement for drug addiction: from actions to habits to compulsion. *Nat Neurosci* **8**: 1481–1489.

Fattore L, Deiana S, Spano SM, Cossu G, Fadda P, Scherma M *et al.* (2005). Endocannabinoid system and opioid addiction: behavioural aspects. *Pharmacol Biochem Behav* **81**: 343–359.

Fattore L, Martellotta MC, Cossu G, Mascia MS, Fratta W (1999). CB1 cannabinoid receptor agonist WIN 55,212-2 decreases intravenous cocaine self-administration in rats. *Behav Brain Res* **104**: 141–146.

Fegley D, Gaetani S, Duranti A, Tontini A, Mor M, Tarzia G *et al.* (2005). Characterization of the fatty acid amide hydrolase inhibitor cyclohexyl carbamic acid 3′-carbamoyl-biphenyl-3-yl ester (URB597): effects on anandamide and oleoylethanolamide deactivation. *J Pharmacol Exp Ther* **313**: 352–358.

Fegley D, Kathuria S, Mercier R, Li C, Goutopoulos A, Makriyannis A *et al.* (2004). Anandamide transport is independent of fatty-acid amide hydrolase activity and is blocked by the hydrolysis-resistant inhibitor AM1172. *Proc Natl Acad Sci USA* **101**: 8756–8761.

Filip M, Golda A, Zaniewska M, McCreary AC, Nowak E, Kolasiewicz W *et al.* (2006). Involvement of cannabinoid CB(1) receptors in drug addiction: effects of rimonabant on behavioral responses induced by cocaine. *Pharmacol Rep* **58**: 806–819.

Forget B, Hamon M, Thiebot MH (2005). Cannabinoid CB1 receptors are involved in motivational effects of nicotine in rats. *Psychopharmacology (Berl)* **181**: 722–734.

Freedland CS, Sharpe AL, Samson HH, Porrino LJ (2001). Effects of SR141716A on ethanol and sucrose self-administration. *Alcohol Clin Exp Res* **25**: 277–282.

Freund TF, Hajos N (2003). Excitement reduces inhibition via endocannabinoids. *Neuron* **38**: 362–365.

Freund TF, Katona I, Piomelli D (2003). Role of endogenous cannabinoids in synaptic signaling. *Physiol Rev* **83**: 1017–1066.

Fride E (2005). Endocannabinoids in the central nervous system: from neuronal networks to behavior. *Curr Drug Targets CNS Neurol Disord* **4**: 633–642.

Fride E, Foox A, Rosenberg E, Faigenboim M, Cohen V, Barda L *et al.* (2003). Milk intake and survival in newborn cannabinoid CB1 receptor knockout mice: evidence for a 'CB3' receptor. *Eur J Pharmacol* **461**: 27–34.

Fride E, Ginzburg Y, Breuer A, Bisogno T, Di Marzo V, Mechoulam R (2001). Critical role of the endogenous cannabinoid system in mouse pup suckling and growth. *Eur J Pharmacol* **419**: 207–214.

Gallate JE, McGregor IS (1999). The motivation for beer in rats: effects of ritanserin, naloxone and SR 141716. *Psychopharmacology (Berl)* **142**: 302–308.

Gallate JE, Saharov T, Mallet PE, McGregor IS (1999). Increased motivation for beer in rats following administration of a cannabinoid CB1 receptor agonist. *Eur J Pharmacol* **370**: 233–240.

Gardner EL (2005). Endocannabinoid signaling system and brain reward: emphasis on dopamine. *Pharmacol Biochem Behav* **81**: 263–284.

Gardner EL, Paredes W, Smith D, Donner A, Milling C, Cohen D *et al.* (1988). Facilitation of brain stimulation reward by delta 9-tetrahydrocannabinol. *Psychopharmacology (Berl)* **96**: 142–144.

Gatley SJ, Gifford AN, Volkow ND, Lan R, Makriyannis A (1996). 123I-labeled AM251: a radioiodinated ligand which binds *in vivo* to mouse brain cannabinoid CB1 receptors. *Eur J Pharmacol* **307**: 331–338.

Gessa GL, Serra S, Vacca G, Carai MA, Colombo G (2005). Suppressing effect of the cannabinoid CB1 receptor antagonist, SR147778, on alcohol intake and motivational properties of alcohol in alcohol-preferring sP rats. *Alcohol Alcohol* **40**: 46–53.

Giuffrida A, Parsons LH, Kerr TM, Rodriguez de Fonseca F, Navarro M, Piomelli D (1999). Dopamine activation of endogenous cannabinoid signaling in dorsal striatum. *Nat Neurosci* **2**: 358–363.

Glaser ST, Abumrad NA, Fatade F, Kaczocha M, Studholme KM, Deutsch DG (2003). Evidence against the presence of an anandamide transporter. *Proc Natl Acad Sci USA* **100**: 4269–4274.

Glaser ST, Kaczocha M, Deutsch DG (2005). Anandamide transport: a critical review. *Life Sci* **77**: 1584–1604.

Gobbi G, Bambico FR, Mangieri R, Bortolato M, Campolongo P, Solinas M *et al*. (2005). Antidepressant-like activity and modulation of brain monoaminergic transmission by blockade of anandamide hydrolysis. *Proc Natl Acad Sci USA* **102**: 18620–18625.

Gong JP, Onaivi ES, Ishiguro H, Liu QR, Tagliaferro PA, Brusco A *et al*. (2006). Cannabinoid CB2 receptors: immunohistochemical localization in rat brain. *Brain Res* **1071**: 10–23.

Gonzalez S, Cascio MG, Fernandez-Ruiz J, Fezza F, Di Marzo V, Ramos JA (2002). Changes in endocannabinoid contents in the brain of rats chronically exposed to nicotine, ethanol or cocaine. *Brain Res* **954**: 73–81.

Hajos N, Ledent C, Freund TF (2001). Novel cannabinoid-sensitive receptor mediates inhibition of glutamatergic synaptic transmission in the hippocampus. *Neuroscience* **106**: 1–4.

Hansson AC, Bermudez-Silva FJ, Malinen H, Hyytia P, Sanchez-Vera I, Rimondini R *et al*. (2007). Genetic impairment of frontocortical endocannabinoid degradation and high alcohol preference. *Neuropsychopharmacology* **32**: 117–126.

Hanus L, Abu-Lafi S, Fride E, Breuer A, Vogel Z, Shalev DE *et al*. (2001). 2-Arachidonyl glyceryl ether, an endogenous agonist of the cannabinoid CB1 receptor. *Proc Natl Acad Sci USA* **98**: 3662–3665.

Hao S, Avraham Y, Mechoulam R, Berry EM (2000). Low dose anandamide affects food intake, cognitive function, neurotransmitter and corticosterone levels in diet-restricted mice. *Eur J Pharmacol* **392**: 147–156.

Herkenham M, Lynn AB, Johnson MR, Melvin LS, de Costa BR, Rice KC (1991). Characterization and localization of cannabinoid receptors in rat brain: a quantitative *in vitro* autoradiographic study. *J Neurosci* **11**: 563–583.

Herkenham M, Lynn AB, Little MD, Johnson MR, Melvin LS, de Costa BR *et al*. (1990). Cannabinoid receptor localization in brain. *Proc Natl Acad Sci USA* **87**: 1932–1936.

Higgs S, Williams CM, Kirkham TC (2003). Cannabinoid influences on palatability: microstructural analysis of sucrose drinking after delta(9)-tetrahydrocannabinol, anandamide, 2-arachidonoyl glycerol and SR141716. *Psychopharmacology (Berl)* **165**: 370–377.

Hillard CJ, Edgemond WS, Jarrahian A, Campbell WB (1997). Accumulation of *N*-arachidonoylethanolamine (anandamide) into cerebellar granule cells occurs via facilitated diffusion. *J Neurochem* **69**: 631–638.

Hillard CJ, Wilkison DM, Edgemond WS, Campbell WB (1995). Characterization of the kinetics and distribution of *N*-arachidonylethanolamine (anandamide) hydrolysis by rat brain. *Biochim Biophys Acta* **1257**: 249–256.

Houchi H, Babovic D, Pierrefiche O, Ledent C, Daoust M, Naassila M (2005). CB1 receptor knockout mice display reduced ethanol-induced conditioned place preference and increased striatal dopamine D2 receptors. *Neuropsychopharmacology* **30**: 339–349.

Howlett AC, Barth F, Bonner TI, Cabral G, Casellas P, Devane WA *et al*. (2002). International Union of Pharmacology. XXVII. Classification of cannabinoid receptors. *Pharmacol Rev* **54**: 161–202.

Howlett AC, Bidaut-Russell M, Devane WA, Melvin LS, Johnson MR, Herkenham M (1990). The cannabinoid receptor: biochemical, anatomical and behavioral characterization. *Trends Neurosci* **13**: 420–423.

Howlett AC, Song C, Berglund BA, Wilken GH, Pigg JJ (1998). Characterization of CB1 cannabinoid receptors using receptor peptide fragments and site-directed antibodies. *Mol Pharmacol* **53**: 504–510.

Hungund BL, Szakall I, Adam A, Basavarajappa BS, 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.

Jamshidi N, Taylor DA (2001). Anandamide administration into the ventromedial hypothalamus stimulates appetite in rats. *Br J Pharmacol* **134**: 1151–1154.

Jarai Z, Wagner JA, Varga K, Lake KD, Compton DR, Martin BR *et al*. (1999). Cannabinoid-induced mesenteric vasodilation through an endothelial site distinct from CB1 or CB2 receptors. *Proc Natl Acad Sci USA* **96**: 14136–14141.

Jarrett MM, Limebeer CL, Parker LA (2005). Effect of delta9-tetrahydrocannabinol on sucrose palatability as measured by the taste reactivity test. *Physiol Behav* **86**: 475–479.

Jarrett MM, Scantlebury J, Parker LA (2007). Effect of delta9-tetrahydrocannabinol on quinine palatability and AM251 on sucrose and quinine palatability using the taste reactivity test. *Physiol Behav* **90**: 425–430.

Justinova Z, Goldberg SR, Heishman SJ, Tanda G (2005a). Self-administration of cannabinoids by experimental animals and human marijuana smokers. *Pharmacol Biochem Behav* **81**: 285–299.

Justinova Z, Solinas M, Tanda G, Redhi GH, Goldberg SR (2005b). The endogenous cannabinoid anandamide and its synthetic analog *R*(+)-methanandamide are intravenously self-administered by squirrel monkeys. *J Neurosci* **25**: 5645–5650.

Kathmann M, Flau K, Redmer A, Trankle C, Schlicker E (2006). Cannabidiol is an allosteric modulator at mu- and delta-opioid receptors. *Naunyn Schmiedebergs Arch Pharmacol* **372**: 354–361.

Kathuria S, Gaetani S, Fegley D, Valino F, Duranti A, Tontini A *et al*. (2003). Modulation of anxiety through blockade of anandamide hydrolysis. *Nat Med* **9**: 76–81.

Kirkham TC, Williams CM, Fezza F, Di Marzo V (2002). Endocannabinoid levels in rat limbic forebrain and hypothalamus in relation to fasting, feeding and satiation: stimulation of eating by 2-arachidonoyl glycerol. *Br J Pharmacol* **136**: 550–557.

Koob GF (2000). Neurobiology of addiction. Toward the development of new therapies. *Ann NY Acad Sci* **909**: 170–185.

Kornetsky C (2004). Brain-stimulation reward, morphine-induced oral stereotypy, and sensitization: implications for abuse. *Neurosci Biobehav Rev* **27**: 777–786.

Kucharski LT, Williams JE, Kornetsky C (1983). The effects of levonantradol on rewarding brain stimulation thresholds in the rat. *Pharmacol Biochem Behav* **19**: 149–151.

Lallemand F, De Witte P (2006). SR147778, a CB1 cannabinoid receptor antagonist, suppresses ethanol preference in chronically alcoholized Wistar rats. *Alcohol* **39**: 125–134.

Landsman RS, Burkey TH, Consroe P, Roeske WR, Yamamura HI (1997). SR141716A is an inverse agonist at the human cannabinoid CB1 receptor. *Eur J Pharmacol* **334**: R1–R2.

Le Foll B, Goldberg SR (2004). Rimonabant, a CB1 antagonist, blocks nicotine-conditioned place preferences. *Neuroreport* **15**: 2139–2143.

Ledent C, Valverde O, Cossu G, Petitet F, Aubert JF, Beslot F *et al*. (1999). Unresponsiveness to cannabinoids and reduced addictive effects of opiates in CB1 receptor knockout mice. *Science* **283**: 401–404.

Lepore M, Liu X, Savage V, Matalon D, Gardner EL (1996). Genetic differences in delta 9-tetrahydrocannabinol-induced facilitation of brain stimulation reward as measured by a rate-frequency curve-shift electrical brain stimulation paradigm in three different rat strains. *Life Sci* **58**: PL365–PL372.

Lopez-Rodriguez ML, Viso A, Ortega-Gutierrez S, Fowler CJ, Tiger G, de Lago E *et al*. (2003). Design, synthesis and biological evaluation of new endocannabinoid transporter inhibitors. *Eur J Med Chem* **38**: 403–412.

Maccarrone M, Rossi S, Bari M, De Chiara V, Fezza F, Musella A *et al*. (2008). Anandamide inhibits metabolism and physiological actions of 2-arachidonoylglycerol in the striatum. *Nat Neurosci* **11**: 152–159.

Mackie K, Devane WA, Hille B (1993). Anandamide, an endogenous cannabinoid, inhibits calcium currents as a partial agonist in N18 neuroblastoma cells. *Mol Pharmacol* **44**: 498–503.

Mahler SV, Smith KS, Berridge KC (2007). Endocannabinoid hedonic hotspot for sensory pleasure: anandamide in nucleus accumbens shell enhances 'liking' of a sweet reward. *Neuropsychopharmacology* **32**: 2267–2278.

Maldonado R, Rodriguez de Fonseca F (2002). Cannabinoid addiction: behavioral models and neural correlates. *J Neurosci* **22**: 3326–3331.

Maldonado R, Valverde O, Berrendero F (2006). Involvement of the endocannabinoid system in drug addiction. *Trends Neurosci* **29**: 225–232.

Manzanares J, Corchero J, Romero J, Fernandez-Ruiz JJ, Ramos JA, Fuentes JA (1999). Pharmacological and biochemical interactions between opioids and cannabinoids. *Trends Pharmacol Sci* **20**: 287–294.

Marsicano G, Goodenough S, Monory K, Hermann H, Eder M, Cannich A *et al*. (2003). CB1 cannabinoid receptors and on-demand defense against excitotoxicity. *Science* **302**: 84–88.

Marsicano G, Wotjak CT, Azad SC, Bisogno T, Rammes G, Cascio MG *et al*. (2002). The endogenous cannabinoid system controls extinction of aversive memories. *Nature* **418**: 530–534.

Martin M, Ledent C, Parmentier M, Maldonado R, Valverde O (2000). Cocaine, but not morphine, induces conditioned place preference and sensitization to locomotor responses in CB1 knockout mice. *Eur J Neurosci* **12**: 4038–4046.

Martin M, Ledent C, Parmentier M, Maldonado R, Valverde O (2002). Involvement of CB1 cannabinoid receptors in emotional behaviour. *Psychopharmacology (Berl)* **159**: 379–387.

Mascia MS, Obinu MC, Ledent C, Parmentier M, Bohme GA, Imperato A *et al*. (1999). Lack of morphine-induced dopamine release in the nucleus accumbens of cannabinoid CB(1) receptor knockout mice. *Eur J Pharmacol* **383**: R1–R2.

Mas-Nieto M, Pommier B, Tzavara ET, Caneparo A, Da Nascimento S, Le Fur G *et al*. (2001). Reduction of opioid dependence by the CB(1) antagonist SR141716A in mice: evaluation of the interest in pharmacotherapy of opioid addiction. *Br J Pharmacol* **132**: 1809–1816.

Matsuda LA, Lolait SJ, Brownstein MJ, Young AC, Bonner TI (1990). Structure of a cannabinoid receptor and functional expression of the cloned cDNA. *Nature* **346**: 561–564.

McAllister SD, Glass M (2002). CB(1) and CB(2) receptor-mediated signalling: a focus on endocannabinoids. *Prostaglandins Leukot Essent Fatty Acids* **66**: 161–171.

McLaughlin PJ, Winston K, Swezey L, Wisniecki A, Aberman J, Tardif DJ *et al*. (2003). The cannabinoid CB1 antagonists SR 141716A and AM 251 suppress food intake and food-reinforced behavior in a variety of tasks in rats. *Behav Pharmacol* **14**: 583–588.

Mechoulam R, Ben-Shabat S, Hanus L, Ligumsky M, Kaminski NE, Schatz AR *et al*. (1995). Identification of an endogenous 2-monoglyceride, present in canine gut, that binds to cannabinoid receptors. *Biochem Pharmacol* **50**: 83–90.

Mechoulam R, Parker L (2003). Cannabis and alcohol—a close friendship. *Trends Pharmacol Sci* **24**: 266–268.

Melis M, Perra S, Muntoni AL, Pillolla G, Lutz B, Marsicano G *et al*. (2004a). Prefrontal cortex stimulation induces 2-arachidonoylglycerol-mediated suppression of excitation in dopamine neurons. *J Neurosci* **24**: 10707–10715.

Melis M, Pistis M, Perra S, Muntoni AL, Pillolla G, Gessa GL (2004b). Endocannabinoids mediate presynaptic inhibition of glutamatergic transmission in rat ventral tegmental area dopamine neurons through activation of CB1 receptors. *J Neurosci* **24**: 53–62.

Monory K, Blaudzun H, Massa F, Kaiser N, Lemberger T, Schutz G *et al*. (2007). Genetic dissection of behavioural and autonomic effects of delta(9)-tetrahydrocannabinol in mice. *PLoS Biol* **5**: e269.

Monory K, Massa F, Egertova M, Eder M, Blaudzun H, Westenbroek R *et al*. (2006). The endocannabinoid system controls key epileptogenic circuits in the hippocampus. *Neuron* **51**: 455–466.

Moore SA, Nomikos GG, Dickason-Chesterfield AK, Schober DA, Schaus JM, Ying BP *et al*. (2005). Identification of a high-affinity binding site involved in the transport of endocannabinoids. *Proc Natl Acad Sci USA* **102**: 17852–17857.

Munro S, Thomas KL, Abu-Shaar M (1993). Molecular characterization of a peripheral receptor for cannabinoids. *Nature* **365**: 61–65.

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.

Naidu PS, Varvel SA, Ahn K, Cravatt BF, Martin BR, Lichtman AH (2007). Evaluation of fatty acid amide hydrolase inhibition in murine models of emotionality. *Psychopharmacology (Berl)* **192**: 61–70.

Navarro M, Carrera MR, Fratta W, Valverde O, Cossu G, Fattore L *et al*. (2001). Functional interaction between opioid and cannabinoid receptors in drug self-administration. *J Neurosci* **21**: 5344–5350.

Onaivi ES, Ishiguro H, Gong JP, Patel S, Perchuk A, Meozzi PA *et al*. (2006). Discovery of the presence and functional expression of cannabinoid CB2 receptors in brain. *Ann N Y Acad Sci* **1074**: 514–536.

Padwal RS, Majumdar SR (2007). Drug treatments for obesity: orlistat, sibutramine, and rimonabant. *Lancet* **369**: 71–77.

Pan X, Ikeda SR, Lewis DL (1998). SR 141716A acts as an inverse agonist to increase neuronal voltage-dependent Ca^{2+} currents by reversal of tonic CB1 cannabinoid receptor activity. *Mol Pharmacol* **54**: 1064–1072.

Perra S, Pillolla G, Melis M, Muntoni AL, Gessa GL, Pistis M (2005). Involvement of the endogenous cannabinoid system in the effects of alcohol in the mesolimbic reward circuit: electrophysiological evidence *in vivo*. *Psychopharmacology (Berl)* **183**: 368–377.

Pertwee RG (2005). Inverse agonism and neutral antagonism at cannabinoid CB1 receptors. *Life Sci* **76**: 1307–1324.

Phillips PE, Stuber GD, Heien ML, Wightman RM, Carelli RM (2003). Subsecond dopamine release promotes cocaine seeking. *Nature* **422**: 614–618.

Pillolla G, Melis M, Perra S, Muntoni AL, Gessa GL, Pistis M (2007). Medial forebrain bundle stimulation evokes endocannabinoid-mediated modulation of ventral tegmental area dopamine neuron firing *in vivo*. *Psychopharmacology (Berl)* **191**: 843–853.

Piomelli D (2003). The molecular logic of endocannabinoid signalling. *Nat Rev Neurosci* **4**: 873–884.

Piomelli D, Beltramo M, Glasnapp S, Lin SY, Goutopoulos A, Xie XQ *et al*. (1999). Structural determinants for recognition and translocation by the anandamide transporter. *Proc Natl Acad Sci USA* **96**: 5802–5807.

Piomelli D, Tarzia G, Duranti A, Tontini A, Mor M, Compton TR *et al*. (2006). Pharmacological profile of the selective FAAH inhibitor KDS-4103 (URB597). *CNS Drug Rev* **12**: 21–38.

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.

Porter AC, Sauer JM, Knierman MD, Becker GW, Berna MJ, Bao J *et al*. (2002). Characterization of a novel endocannabinoid, virodhamine, with antagonist activity at the CB1 receptor. *J Pharmacol Exp Ther* **301**: 1020–1024.

Racz I, Bilkei-Gorzo A, Toth ZE, 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.

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.

Riegel AC, Lupica CR (2004). Independent presynaptic and postsynaptic mechanisms regulate endocannabinoid signaling at multiple synapses in the ventral tegmental area. *J Neurosci* **24**: 11070–11078.

Rinaldi-Carmona M, Barth F, Heaulme M, Shire D, Calandra B, Congy C *et al*. (1994). SR141716A, a potent and selective antagonist of the brain cannabinoid receptor. *FEBS Lett* **350**: 240–244.

Ryberg E, Larsson N, Sjogren S, Hjorth S, Hermansson NO, Leonova J *et al*. (2007). The orphan receptor GPR55 is a novel cannabinoid receptor. *Br J Pharmacol* **152**: 1092–1101.

Sanchis-Segura C, Cline BH, Marsicano G, Lutz B, Spanagel R (2004). Reduced sensitivity to reward in CB1 knockout mice. *Psychopharmacology (Berl)* **176**: 223–232.

Schaefer GJ (1988). Opiate antagonists and rewarding brain stimulation. *Neurosci Biobehav Rev* **12**: 1–17.

Schlicker E, Kathmann M (2001). Modulation of transmitter release via presynaptic cannabinoid receptors. *Trends Pharmacol Sci* **22**: 565–572.

Simiand J, Keane M, Keane PE, Soubrie P (1998). SR 141716, a CB1 cannabinoid receptor antagonist, selectively reduces sweet food intake in marmoset. *Behav Pharmacol* **9**: 179–181.

Singh ME, Verty AN, McGregor IS, Mallet PE (2004). A cannabinoid receptor antagonist attenuates conditioned place preference but not behavioural sensitization to morphine. *Brain Res* **1026**: 244–253.

Solinas M, Goldberg SR (2005). Motivational effects of cannabinoids and opioids on food reinforcement depend on simultaneous activation of cannabinoid and opioid systems. *Neuropsychopharmacology* **30**: 2035–2045.

Solinas M, Justinova Z, Goldberg SR, Tanda G (2006a). Anandamide administration alone and after inhibition of fatty acid amide

hydrolase (FAAH) increases dopamine levels in the nucleus accumbens shell in rats. *J Neurochem* **98**: 408–419.

Solinas M, Panlilio LV, Antoniou K, Pappas LA, Goldberg SR (2003). The cannabinoid CB1 antagonist *N*-piperidinyl-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methylpyrazole-3-carboxamide (SR-141716A) differentially alters the reinforcing effects of heroin under continuous reinforcement, fixed ratio, and progressive ratio schedules of drug self-administration in rats. *J Pharmacol Exp Ther* **306**: 93–102.

Solinas M, Panlilio LV, Justinova Z, Yasar S, Goldberg SR (2006b). Using drug-discrimination techniques to study the abuse-related effects of psychoactive drugs in rats. *Nat Protoc* **1**: 1194–1206.

Solinas M, Panlilio LV, Tanda G, Makriyannis A, Matthews SA, Goldberg SR (2005). Cannabinoid agonists but not inhibitors of endogenous cannabinoid transport or metabolism enhance the reinforcing efficacy of heroin in rats. *Neuropsychopharmacology* **30**: 2046–2057.

Solinas M, Scherma M, Tanda G, Wertheim CE, Fratta W, Goldberg SR (2007a). Nicotinic facilitation of delta9-tetrahydrocannabinol discrimination involves endogenous anandamide. *J Pharmacol Exp Ther* **321**: 1127–1134.

Solinas M, Tanda G, Justinova Z, Wertheim CE, Yasar S, Piomelli D *et al*. (2007b). The endogenous cannabinoid anandamide produces delta-9-tetrahydrocannabinol-like discriminative and neurochemical effects that are enhanced by inhibition of fatty acid amide hydrolase but not by inhibition of anandamide transport. *J Pharmacol Exp Ther* **321**: 370–380.

Solinas M, Yasar S, Goldberg SR (2007c). Endocannabinoid system involvement in brain reward processes related to drug abuse. *Pharmacol Res* **56**: 393–405.

Soria G, Mendizabal V, Tourino C, Robledo P, Ledent C, Parmentier M *et al*. (2005). Lack of CB1 cannabinoid receptor impairs cocaine self-administration. *Neuropsychopharmacology* **30**: 1670–1680.

Stella N, Schweitzer P, Piomelli D (1997). A second endogenous cannabinoid that modulates long-term potentiation. *Nature* **388**: 773–778.

Sugiura T, Kondo S, Sukagawa A, Nakane S, Shinoda A, Itoh K *et al*. (1995). 2-Arachidonoylglycerol: a possible endogenous cannabinoid receptor ligand in brain. *Biochem Biophys Res Commun* **215**: 89–97.

Szabo B, Siemes S, Wallmichrath I (2002). Inhibition of GABAergic neurotransmission in the ventral tegmental area by cannabinoids. *Eur J Neurosci* **15**: 2057–2061.

Tanda G, Goldberg SR (2003). Cannabinoids: reward, dependence, and underlying neurochemical mechanisms—a review of recent preclinical data. *Psychopharmacology (Berl)* **169**: 115–134.

Tanda G, Munzar P, Goldberg SR (2000). Self-administration behavior is maintained by the psychoactive ingredient of marijuana in squirrel monkeys. *Nat Neurosci* **3**: 1073–1074.

Tanda G, Pontieri FE, Di Chiara G (1997). Cannabinoid and heroin activation of mesolimbic dopamine transmission by a common mu1 opioid receptor mechanism. *Science* **276**: 2048–2050.

Thanos PK, Dimitrakakis ES, Rice O, Gifford A, Volkow ND (2005). Ethanol self-administration and ethanol conditioned place preference are reduced in mice lacking cannabinoid CB1 receptors. *Behav Brain Res* **164**: 206–213.

Thornton-Jones ZD, Kennett GA, Benwell KR, Revell DF, Misra A, Sellwood DM *et al*. (2006). The cannabinoid CB1 receptor inverse agonist, rimonabant, modifies body weight and adiponectin function in diet-induced obese rats as a consequence of reduced food intake. *Pharmacol Biochem Behav* **84**: 353–359.

Thornton-Jones ZD, Kennett GA, Vickers SP, Clifton PG (2007). A comparison of the effects of the CB(1) receptor antagonist SR141716A, pre-feeding and changed palatability on the microstructure of ingestive behaviour. *Psychopharmacology (Berl)* **193**: 1–9.

Thornton-Jones ZD, Vickers SP, Clifton PG (2005). The cannabinoid CB1 receptor antagonist SR141716A reduces appetitive and consummatory responses for food. *Psychopharmacology (Berl)* **179**: 452–460.

Tsou K, Brown S, Sanudo-Pena MC, Mackie K, Walker JM (1998a). Immunohistochemical distribution of cannabinoid CB1 receptors in the rat central nervous system. *Neuroscience* **83**: 393–411.

Tsou K, Nogueron MI, Muthian S, Sanudo-Pena MC, Hillard CJ, Deutsch DG *et al*. (1998b). Fatty acid amide hydrolase is located preferentially in large neurons in the rat central nervous system as revealed by immunohistochemistry. *Neurosci Lett* **254**: 137–140.

Valjent E, Mitchell JM, Besson MJ, Caboche J, Maldonado R (2002). Behavioural and biochemical evidence for interactions between delta 9-tetrahydrocannabinol and nicotine. *Br J Pharmacol* **135**: 564–578.

Van Sickle MD, Duncan M, Kingsley PJ, Mouihate A, Urbani P, Mackie K *et al*. (2005). Identification and functional characterization of brainstem cannabinoid CB2 receptors. *Science* **310**: 329–332.

Vigano D, Grazia Cascio M, Rubino T, Fezza F, Vaccani A, Di Marzo V *et al*. (2003). Chronic morphine modulates the contents of the endocannabinoid, 2-arachidonoyl glycerol, in rat brain. *Neuropsychopharmacology* **28**: 1160–1167.

Vigano D, Rubino T, Parolaro D (2005). Molecular and cellular basis of cannabinoid and opioid interactions. *Pharmacol Biochem Behav* **81**: 360–368.

Vigano D, Valenti M, Cascio MG, Di Marzo V, Parolaro D, Rubino T (2004). Changes in endocannabinoid levels in a rat model of behavioural sensitization to morphine. *Eur J Neurosci* **20**: 1849–1857.

Vinklerova J, Novakova J, Sulcova A (2002). Inhibition of methamphetamine self-administration in rats by cannabinoid receptor antagonist AM 251. *J Psychopharmacol* **16**: 139–143.

Viveros MP, Llorente R, Moreno E, Marco EM (2005). Behavioural and neuroendocrine effects of cannabinoids in critical developmental periods. *Behav Pharmacol* **16**: 353–362.

Vlachou S, Nomikos GG, Panagis G (2003). WIN 55,212-2 decreases the reinforcing actions of cocaine through CB1 cannabinoid receptor stimulation. *Behav Brain Res* **141**: 215–222.

Vlachou S, Nomikos GG, Panagis G (2005). CB1 cannabinoid receptor agonists increase intracranial self-stimulation thresholds in the rat. *Psychopharmacology (Berl)* **179**: 498–508.

Vlachou S, Nomikos GG, Panagis G (2006). Effects of endocannabinoid neurotransmission modulators on brain stimulation reward. *Psychopharmacology (Berl)* **188**: 293–305.

Vlachou S, Nomikos GG, Stephens DN, Panagis G (2007). Lack of evidence for appetitive effects of delta9-tetrahydrocannabinol in the intracranial self-stimulation and conditioned place preference procedures in rodents. *Behav Pharmacol* **18**: 311–319.

Wang L, Liu J, Harvey-White J, Zimmer A, Kunos G (2003). Endocannabinoid signaling via cannabinoid receptor 1 is involved in ethanol preference and its age-dependent decline in mice. *Proc Natl Acad Sci USA* **100**: 1393–1398.

Wiley JL, Golden KM, Ryan WJ, Balster RL, Razdan RK, Martin BR (1997). Evaluation of cannabimimetic discriminative stimulus effects of anandamide and methylated fluoroanandamide in rhesus monkeys. *Pharmacol Biochem Behav* **58**: 1139–1143.

Williams CM, Kirkham TC (1999). Anandamide induces overeating: mediation by central cannabinoid (CB1) receptors. *Psychopharmacology (Berl)* **143**: 315–317.

Williams CM, Kirkham TC (2002a). Observational analysis of feeding induced by delta9-THC and anandamide. *Physiol Behav* **76**: 241–250.

Williams CM, Kirkham TC (2002b). Reversal of delta 9-THC hyperphagia by SR141716 and naloxone but not dexfenfluramine. *Pharmacol Biochem Behav* **71**: 333–340.

Williams CM, Rogers PJ, Kirkham TC (1998). Hyperphagia in pre-fed rats following oral delta9-THC. *Physiol Behav* **65**: 343–346.

Willner P (2005). Chronic mild stress (CMS) revisited: consistency and behavioural–neurobiological concordance in the effects of CMS. *Neuropsychobiology* **52**: 90–110.

Wilson RI, Nicoll RA (2002). Endocannabinoid signaling in the brain. *Science* **296**: 678–682.

Wise RA (2002). Brain reward circuitry: insights from unsensed incentives. *Neuron* **36**: 229–240.

Wise RA (2004). Dopamine, learning and motivation. *Nat Rev Neurosci* **5**: 483–494.

Xi ZX, Spiller K, Pak AC, Gilbert J, Dillon C, Li X *et al*. (2007). Cannabinoid CB1 receptor antagonists attenuate cocaine's rewarding effects: experiments with self-administration and brain-stimulation reward in rats. *Neuropsychopharmacology* advance online publication, doi:10.1038/sj.npp.1301552.

Zangen A, Solinas M, Ikemoto S, Goldberg SR, Wise RA (2006). Two brain sites for cannabinoid reward. *J Neurosci* **26**: 4901–4907.

Zaniewska M, McCreary AC, Przegalinski E, Filip M (2006). Evaluation of the role of nicotinic acetylcholine receptor subtypes and cannabinoid system in the discriminative stimulus effects of nicotine in rats. *Eur J Pharmacol* **540**: 96–106.

Zimmer A, Zimmer AM, Hohmann AG, Herkenham M, Bonner TI (1999). Increased mortality, hypoactivity, and hypoalgesia in cannabinoid CB1 receptor knockout mice. *Proc Natl Acad Sci USA* **96**: 5780–5785.

Zygmunt PM, Petersson J, Andersson DA, Chuang H, Sorgard M, Di Marzo V *et al*. (1999). Vanilloid receptors on sensory nerves mediate the vasodilator action of anandamide. *Nature* **400**: 452–457.