Advances in the field of cannabinoid–opioid cross-talk

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ABSTRACT

A remarkable amount of literature has been generated demonstrating the functional similarities between the endogenous opioid and cannabinoid systems. Anatomical, biochemical and molecular data support the existence of reciprocal interactions between these two systems related to several pharmacological responses including reward, cognitive effects, and the development of tolerance and dependence. However, the assessment of the bidirectionality of these effects has been difficult due to their variety and complexity. Reciprocal interactions have been well established for the development of physical dependence. Cross-tolerance and cross-sensitization, although not always bidirectional, are also supported by a number of evidence, while less data have been gathered regarding the relationship of these systems in cognition and emotion. Nevertheless, the most recent advances in cannabinoid–opioid cross-modulation have been made in the area of drug craving and relapse processes. The present review is focused on the latest developments in the cannabinoid–opioid cross-modulation of their behavioural effects and the possible neurobiological substrates involved.

Keywords Emotion, learning, physical dependence, reinforcement, reward, tolerance.

INTRODUCTION

Cannabis sativa derivatives, such as marijuana and hashish, as well as opium derivatives, have been used for thousands of years for recreational and medicinal purposes, and nowadays are two of the most widely consumed illicit drugs. The sites of action of these psychoactive substances are two endogenous neuromodulatory systems termed endogenous cannabinoid system and endogenous opioid system. Both systems have a remarkable level of expression in the central nervous system.

The endogenous cannabinoid system is expressed in the brain as well as in peripheral tissues (Fride & Mechoulam 2003). This modulatory system is composed of membrane receptors, fatty acid-derived neurotransmitters, and enzymes involved in the generation and degradation of these neurotransmitters (Pacher, Batkai & Kunos 2006). Cannabinoid compounds (endogenous and exogenous) induce their pharmacological effects by activating at least two different populations of receptors that have been identified and cloned: the CB1 cannabinoid receptor (also named CNR1), which is highly expressed in the central nervous system (Devane et al. 1988), and the CB2 cannabinoid receptor (also named CNR2), which is mainly localized in peripheral tissues like the immune system (Munro, Thomas & Abu-Shaar 1993), although more recently it was found to be expressed in brainstem, cortex and cerebellar neurons as well (Van Sickle et al. 2005). The existence of a third type of cannabinoid receptor in the brain has been proposed based on pharmacological evidences. This receptor would be sensitive to WIN 55,212-2, anandamide (AEA) and rimonabant, but not to delta9-tetrahydrocannabinol (THC) (Jarai et al. 1999; Breivogel et al. 2001; Monory et al. 2002). Whether this new cannabinoid receptor corresponds to the already cloned G-protein-coupled receptor-55 is under study (Baker et al. 2006). Among the endogenous ligands identified, the fatty acid derivative AEA and 2-arachidonoylglycerol (2-AG) seem to be the most important, although other ligands such as noladin ether and virodhamide have been also detected in the brain (Fride & Mechoulam 2003). These endocannabinoids are mainly synthesized postsynaptically in an activity-dependent manner and act as retrograde messengers regulating the release of a variety of neurotransmitters at the presynaptic level (Wilson & Nicoll 2002).
The ligands of the endogenous opioid system, the endogenous opioid peptides and the opioid receptors are largely distributed within the central nervous system and are also present in several peripheral tissues. Three families of endogenous peptides derived from either proopiomelanocortin, proenkephalin and prodynorphin have been identified and cloned (Kieffer 1995). Three different subtypes of opioid receptors, mu, delta and kappa, have also been identified, cloned and characterized at the molecular, biochemical and pharmacological level (Kieffer 1995; Kieffer & Simonin 2003).

Cannabinoid and opioid receptors belong to the rhodopsin subfamily of G-protein-coupled receptor superfamilies (seven transmembrane domain receptors) that couple to Gi/Go GTP-binding proteins (Cichewicz 2004). Indeed, activation of both types of receptors reduces the cellular levels of cyclic adenosine monophosphate (cAMP) by inhibiting the adenylyl cyclase activity (Sharma, Klee & Nirenberg 1975; Howlett & Fleming 1984). The stimulation of cannabinoid and opioid receptors has been associated with an increase in the activity of mitogen-activated protein kinase pathway (Bouaboula et al. 1995; Fukuda et al. 1996) and the modulation of potassium conductances through protein kinase C signalling (Hampson, Mu & Deadwyler 2000). Thus, opioid (Morita & North 1982; Hescheler et al. 1987) and cannabinoid (Felder et al. 1992) agonists also modify the permeability of several ion channels. Both agonists increase the permeability of potassium channels and inhibit calcium influx through voltage-gated calcium channels. Furthermore, both cannabinoid and opioid receptors are mainly located at presynaptic terminals, and their activation inhibits the release of several neurotransmitters (Schlicker & Kathmann 2001). Anatomical studies have found that CB1 and mu-opioid receptor mRNA have an overlapping distribution in several areas of the central nervous system, including the limbic system, mesencephalon, brain stem and spinal cord (Rodríguez, Mackie & Pickel 2001; Salio et al. 2001). Furthermore, CB1 cannabinoid and mu-opioid receptors co-localize in striatal GABAergic neurons (Hohmann & Herkenham 2000; Rodríguez et al. 2001; Pickel et al. 2004), suggesting potential coupling to similar second messenger systems and raising the possibility of formation of heterodimers between these two receptors (Rios, Gomes & Devi 2006).

Several pharmacological responses induced by opioid and cannabinoid agonists in vivo are also similar, such as antinociception, hypothermia, sedation, hypotension, inhibition of intestinal motility, motor depression and reward (Manzanares et al. 1999a; Maldonado, Valverde & Berrendero 2006).

Taking into account the abundant biochemical, pharmacological and anatomical evidence supporting the existence of a functional link between cannabinoids and opioids (Cichewicz 2004), the present review will focus on the recent literature concerning the putative cross-talk between the endogenous cannabinoid system and the endogenous opioid system in specific topics, including the development of tolerance and dependence, reward and reinforcement, learning and memory, and emotional-like responses.

DEVELOPMENT OF TOLERANCE

Pharmacological studies

Chronic administration of cannabinoid or opioid agonists results in the development of tolerance to most of their acute pharmacological effects (Maldonado 2002). The interaction between cannabinoids and opioids on the development of tolerance is supported by the presence of asymmetric cross-modulation at several pharmacological responses induced by these compounds, such as antinociception, hypolocomotion, catalepsy and hypothermia (Pertwee, Stevenson & Griffin 1993; Fan et al. 1994; Vigano et al. 2005). Some contradictory results have been reported with regard to the development of tolerance to the antinociceptive effects. Thus, although morphine pre-treatment was shown to induce tolerance to the acute antinociceptive effects of systemic THC administration in mice (Thorat & Bhargava 1994), other studies demonstrated sensitization to the acute antinociceptive effects of the cannabinoid agonist CP 55,940 (Vigano et al. 2005) or THC (Rubino et al. 1997) in morphine-tolerant rats. Chronic treatment with the selective kappa agonists U-50,488H or CI-977 also produced tolerance to the antinociceptive effects of intrathecally administered THC in mice (Smith, Welch & Martin 1994). In agreement, the administration of antisense oligodeoxynucleotides directed against kappa-opioid receptors accelerated the development of tolerance induced by chronic administration of THC, suggesting that a down-regulation of kappa-opioid receptors participates in tolerance to THC antinociceptive effects (Rowen et al. 1998).

In reciprocal experiments, chronic exposure to cannabinoids such as THC (Thorat & Bhargava 1994) or CP 55,940 (Vigano et al. 2005) produced cross-tolerance to the acute antinociceptive effects of morphine. In contrast, the development of tolerance to the antinociceptive effects of the endogenous cannabinoid AEA seems to involve different mechanisms than those implicated in tolerance to exogenous cannabinoid agonists like THC or CP 55,940. Hence, AEA-tolerant mice did not show cross-tolerance to the antinociceptive responses induced by mu-, delta- or kappa-opioid agonists, while THC-tolerant mice exhibited cross-tolerance to dynorphin, U-50,488H and CI-977 (kappa-opioid agonists) (Smith et al. 1994; Welch 1997).
Development of tolerance in knockout mice

The bidirectionality of the interaction between the endocannabinoid and opioid systems in the development of tolerance has been also investigated using knockout mice deficient in CB1 cannabinoid receptors or in the different components of the endogenous opioid system. Thus, a decrease in the development of tolerance to THC antinociceptive effects was observed in knockout mice lacking the pre-proenkephalin gene, showing that this peptide is involved in the antinociceptive responses of THC (Valverde et al. 2000). However, the lack of the CB1 cannabinoid receptors did not influence the development of tolerance to opioid antinociception (Ledent et al. 1999), suggesting an asymmetrical interaction between these neuromodulatory systems.

Biochemical studies

Few studies have evaluated the cross-effects of chronic exposure to cannabinoids or opioids on the density and/or functionality of the reciprocal receptors, and the results do not help to explain the cross-modulation observed at the behavioural level between these two systems. Thus, in one study, chronic morphine slightly decreased CB1 density and functionality measured by [35S]GTPgammaS binding (Vigano et al. 2005). This decrease disagreed with the behavioural results showing no cross-tolerance and even a sensitization to the acute antinociceptive effects of the cannabinoid agonist CP 55,940 in morphine-tolerant rats. On the other hand, other studies show an increase (Rubino et al. 1997; González et al. 2002) or no change in CB1 density after chronic morphine administration (Thorat & Bhargava 1994; Romero et al. 1998). Nevertheless, at the spinal level, chronic intrathecal administration of morphine increased the expression of CB1 and CB2 cannabinoid receptors (Lim, Wang & Mao 2005), which may explain the sensitization to the acute antinociceptive effects of CP 55,490 observed in morphine-tolerant animals in the tail-flick test (Vigano et al. 2005).

Reciprocally, only a few studies have examined changes in opioid receptor function in animals tolerant to cannabinoids. In this sense, cannabinoid tolerance seems to be associated with a slight increase in the mu-opioid receptor density in the limbic system and brain areas involved in the supraspinal control of pain such as the periaqueductal grey matter (PAG) and thalamus (Corchero, Manzanares & Fuentes 2004; Vigano et al. 2005). This increase in mu-opioid receptor binding is difficult to reconcile with the cross-tolerance to the acute antinociceptive effect of morphine shown by chronic treatment with cannabinoids (Thorat & Bhargava 1994; Vigano et al. 2005).

The downstream events associated with both cannabinoid and opioid receptor activation have also been evaluated. Thus, chronic morphine did not change the sensitivity of CP-55,940 in inhibiting the forskolin-stimulated cAMP production in the caudate putamen and dorsal mesencephalon (Vigano et al. 2005). On the other hand, chronic CP-55,490 desensitized the inhibitory effect on forskolin-stimulated cAMP production of the opioid agonist DAMGO. These results show a differential cross-talk between the two systems at the level of cAMP regulation, supporting the asymmetric interaction between these systems shown at the behavioural level.

At the spinal level, an important modulator of opioid-induced tolerance is the calcitonin gene-related peptide (CGRP) (Menard et al. 1996). This peptide is present in nociceptive primary afferents and its expression increases markedly in the dorsal horn of the spinal cord after chronic exposure to morphine in correlation with the development of morphine antinociceptive tolerance (Menard et al. 1996; Powell et al. 2000). Co-administration of morphine with the selective CB1 cannabinoid antagonist/inverse agonist AM 251 inhibited the development of tolerance to the antinociceptive effects of morphine and concomitantly reduced the over-activity of CGRP (Trang, Sutak & Jhamandas 2007). Hence, this peptide may represent another possible mechanism underlying cross-talk between these two systems in terms of tolerance development.

DEVELOPMENT OF DEPENDENCE AND EXPRESSION OF WITHDRAWAL

Several studies have revealed clear evidence for a reciprocal relationship between the endogenous cannabinoid and opioid systems in the development of dependence. Indeed, similar biochemical changes have been observed during withdrawal to cannabinoids and opioids. Thus, an inhibition of mesolimbic dopamine (DA) activity, an elevation in extracellular levels of corticotropin-releasing factor and increased Fos immunoreactivity in the amygdala have been related to the dysphoric consequences of cannabinoid and opioid withdrawal (Rodriguez de Fonseca et al. 1997; Valverde, Robledo & Maldonado 2004). Similar to opioid abstinence, cannabinoid withdrawal is also characterized by an increase in adenylyl cyclase activity (Hutcheson et al. 1998), which seems directly related to the somatic expression of the abstinence symptoms. However, these biochemical changes occur in different brain structures during cannabinoid and opioid withdrawal in agreement with the different involvement of these brain structures in the somatic manifestations of withdrawal to these two drugs. Brainstem areas such as the locus coeruleus and the PAG (Maldonado et al. 1992) are related to the somatic
signs of opioid withdrawal, while the cerebellum seems to be the most relevant structure involved in the somatic expression of cannabinoid abstinence symptoms (Hutcheson et al. 1998; Tzavara et al. 2000). A recent study has shown that in addition to the cerebellum, the hippocampus and the amygdala also play a significant role in this cannabinoid abstinence (Castañé, Maldonado & Valverde 2004).

Involvement of the endogenous opioid system in cannabinoid dependence

The role of the endogenous opioid system in cannabinoid dependence has been widely demonstrated by different studies. The opioid antagonist naloxone precipitates behavioural signs of abstinence in rats chronically treated with cannabinoid agonists (Kaymakcalan, Ayhan & Tulunay 1977; Navarro et al. 1998). In addition, the expression of the cannabinoid withdrawal syndrome is attenuated in mice lacking the pre-proenkephalin gene (Valverde et al. 2000), suggesting that the endogenous enkephalin system is involved in the expression of cannabinoid abstinence. Nevertheless, conflicting results have been obtained by using mu-opioid receptor knockout mice. Thus, while no modifications in the severity of cannabinoid withdrawal were found in some studies (Ghozland et al. 2002), a decrease of this syndrome was observed in others using different doses of THC (Lichtman et al. 2001). The somatic manifestations of cannabinoid withdrawal have been reported to decrease in double-knockout mice deficient in mu- and delta-opioid receptors (Castañé et al. 2003), suggesting that a cooperative action between these two opioid receptors is essential for the entire expression of cannabinoid dependence.

Involvement of the endogenous cannabinoid system in opioid dependence

Reciprocally, a large body of literature points to a role of the endogenous cannabinoid system in opioid dependence. Thus, the administration of the CB1 cannabinoid antagonist rimonabant can also precipitate behavioural and biochemical manifestations of withdrawal in morphine-dependent rats (Navarro et al. 1998), although a later study did not report such an expression of morphine abstinence in mice (Lichtman et al. 2001). On the other hand, AEA decreases the somatic signs of naloxone-precipitated withdrawal in morphine-dependent mice (Vela, Ruiz-Gayo & Fuentes 1995). In agreement, the inhibitor of AEA uptake AM404 also attenuates the manifestations of the spontaneous opioid withdrawal syndrome (Del Arco et al. 2002), although this compound did not modify the severity of morphine abstinence precipitated by naloxone. In the same line, prolonged pre-treatment with THC before starting chronic morphine administration attenuated the somatic manifestations of naloxone-precipitated morphine withdrawal (Valverde et al. 2001). Taken together, these studies suggest an alleviatory effect of cannabinoid agonists on the expression of opioid withdrawal syndrome. On the other hand, the participation of CB1 cannabinoid receptors in the development of opioid dependence has been demonstrated in studies showing a robust decrease in the severity of the somatic signs of morphine withdrawal in knockout mice lacking these receptors (Ledent et al. 1999). In agreement, pharmacological studies show that chronic treatment with the CB1 antagonist rimonabant reduced the intensity of naloxone-precipitated withdrawal in morphine pellet-implanted rats (Rubino et al. 2000). In addition, the co-administration of rimonabant and morphine for 5 days attenuated the incidence of morphine withdrawal manifestations (Mas-Nieto et al. 2001). These data indicate that cannabinoid antagonists could be useful in preventing the development of opioid dependence. Accordingly, changes in the density of CB1 cannabinoid receptors have been observed in the brain of morphine-dependent rats in several areas related to the manifestations of drug dependence and withdrawal, such as the midbrain, the cerebral cortex and the brainstem (González et al. 2003a). Interestingly, a recent study indicates that the endocannabinoid system, through the activation of CB1 receptors, contributes to the development of opioid physical dependence by modulating the spinal expression of CGRP (Trang et al. 2006). Thus, the blockade of CB1 receptors during repeated morphine administration prevents the changes in the expression of CGRP and attenuates the manifestations of naloxone-precipitated morphine withdrawal (Trang et al. 2006).

REWARD AND REINFORCEMENT

Common neurobiological substrate

The endogenous opioid and cannabinoid systems induce rewarding/reinforcing and motivational effects through their action at several common neurochemical substrates. One of the major common links is the mesolimbic dopaminergic system, where cross-talk between opioids and cannabinoids have been widely described. Hence, an early study demonstrated that the activation of DA outflow from the nucleus accumbens produced by THC was blocked by naloxone (Chen et al. 1990). A few years later, Tanda, Pontieri & Di Chiara (1997) confirmed the involvement of the opioid system in the cannabinoid-induced DA activation. Thus, THC and heroin increased extracellular DA concentrations selectively in the shell of the nucleus accumbens, and naloxone administered systemically, or naloxonazine, a selective mu-opioid antagonist infused into the ventral tegmental area, prevented...
the action of cannabinoids and heroin on DA transmission. However, the bidirectionality of the interaction between cannabinoids and opioids on DA transmission was not corroborated in that study because the CB1 cannabinoid antagonist rimonabant prevented the effects of THC but not those of heroin (Tanda et al. 1997). In agreement with this lack of reciprocity, electrophysiological studies revealed that THC dose-dependently increased DA cell firing in the ventral tegmental area, and this effect was inhibited by rimonabant, but not by naloxone (French 1997). Additional support for the divergent modulation of DA by cannabinoids and opioids was put forward in a study demonstrating that THC and morphine activate dopaminergic neurons through distinct mechanisms (Melis, Gessa & Diana 2000). Thus, the THC-induced activation was antagonized by rimonabant, but not by naloxone, while the morphine-induced stimulation was blocked by naloxone but not by rimonabant. Moreover, morphine inhibited GABAergic substantia nigra pars reticulata neurons, while THC did not. The authors concluded that morphine would enhance DA transmission by removing the inhibitory input to the ventral tegmental area through its action on mu-opioid receptors, while THC would activate DA neurons by acting, at least in part, on CB1 receptors not functionally linked to opioid activity (Melis et al. 2000).

Two other important targets for the interaction between cannabinoids and opioids related to reward and motivation are the glutamatergic and GABAergic systems. Both cannabinoids and opioids modulate these neurotransmitters in areas involved in addictive behaviours (Pistis et al. 2002; Melis et al. 2004; Margolis et al. 2005; Nugent, Penick & Kauer 2007). In addition, it has been proposed that CB1 and mu-opioid receptors, functionally linked in the nucleus accumbens (Pickel et al. 2004), may form heterodimers, which when activated produce a synergistic release of GABA or a non-additive release of glutamate (Schoffelmeer et al. 2006). However, here also, there is some discrepant data as to the bidirectionality of cannabinoid and opioid effects on inhibitory and excitatory neurotransmission in the nucleus accumbens (Hoffman & Lupica 2001). The interaction between cannabinoids and opioids in terms of GABAergic neurotransmission was also described in the ventral pallidum, a brain structure intimately related to the nucleus accumbens and other limbic areas involved in the rewarding effects of drugs (Caillé & Parsons 2006). In that study, the CB1 receptor agonist WIN 55,212-2 reduced GABA efflux in a manner similar to morphine, and this effect was reversed by naloxone (Caillé & Parsons 2006).

Chronic intake of cannabinoids and opioids produce enduring adaptive changes in brain reward circuits, which can be central to the development of addictive processes. In this regard, the density of CB1 receptors was increased in the medial caudate-putamen and in the nucleus accumbens, but reduced in the basolateral amygdala following repeated exposure to morphine (González et al. 2003a). Furthermore, a reciprocal modulation of CB1 cannabinoid and mu-opioid receptors has been reported in several limbic structures in rats following self-administration of WIN 55,212-2 or heroin (Fattore et al. 2007a). However, this bidirectional interaction appeared to be asymmetric because the observed increase in CB1 receptors following heroin self-administration was greater than the increase in mu-opioid receptors produced by WIN 55,212-2 self-administration (Fattore et al. 2007a). Adaptive changes in brain endocannabinoids (AEA and 2-AG) have also been observed following the development and expression of behavioural sensitization to opioids (Vigano et al. 2004), and following self-administration of heroin (Caillé et al. 2007). Interestingly, long-lasting cross-tolerance to morphine was developed by adolescent rats treated chronically with WIN 55,212-2 in terms of the responsiveness of mesoaccumbens DA neurons. However, this effect was not observed in adult rats treated chronically with WIN 55,212-2, indicating age-dependent effects in this interaction (Pistis et al. 2004).

**Behavioural models of reward/reinforcement**

Reciprocal interactions between the cannabinoid and opioid systems have been widely revealed in experimental models of reward and reinforcement. In terms of the effects of cannabinoids on opioid-induced reward, studies using genetically modified mice showed that morphine-induced conditioned place preference (CPP) (Martin et al. 2000) and intravenous self-administration (Ledent et al. 1999) were abolished in knockout mice lacking CB1 cannabinoid receptors. In accord, rimonabant reduced opioid self-administration and CPP in rodents (Navarro et al. 2001; De Vries et al. 2003; Singh et al. 2004). Notably, the attenuating effects of rimonabant were found to be greater when heroin’s reinforcing properties were tested in a progressive ratio schedule rather than under fixed ratio schedules (Solinas et al. 2003), pointing to the particular involvement of the endocannabinoid system in the motivational aspects of opioid reward. More recently, it was shown that pre-treatment with a sub-threshold dose of the cannabinoid agonist WIN 55,212-2 induced CPP of a non-effective dose of morphine, and this effect was blocked by rimonabant (Manzaneo et al. 2004).

Related data referring to the effects of opioids on the rewarding properties of cannabinoids in rats show that naltrexone attenuates CPP induced by the cannabinoid agonist CP 55,940 (Braida et al. 2001a) and intracerebral self-administration of CP 55,940 (Braida et al. 2001b).
Moreover, CPP induced by THC was suppressed in mu-opioid receptor knockout mice (Ghozland et al. 2002), and THC self-administration was attenuated by naltrexone in monkeys (Justinova et al. 2004), while no modifications in THC-induced CPP was evidenced in mice lacking delta or kappa-opioid receptors (Ghozland et al. 2002) or in mice lacking the pre-proenkephalin gene (Valverde et al. 2000). These results suggested that mu, but not delta or kappa-opioid receptors were specifically involved in the rewarding properties of cannabinoids. The involvement of mu-opioid receptors in the rewarding properties of THC was confirmed in a study showing that THC-induced CPP was completely abolished in double-knockout mice lacking both mu- and delta-opioid receptors (Castañé et al. 2003).

On the other hand, the aversive effects induced by a high dose of THC (5 mg/kg) were slightly attenuated in mu knockout mice, and completely blocked in mice lacking kappa-opioid receptors (Ghozland et al. 2002). In agreement with these data, place aversion induced by THC was also abolished in prodynorphin knockout mice (Zimmer et al. 2001). Finally, a recent study demonstrated that mice acquired WIN 52,212-2 self-administration if the drug’s dysphoric effects were avoided by either a previous injection of the drug in the home cage or by administration of the kappa antagonist, nor-binaltorphimine. In addition, the reinforcing effects of WIN 52,212-2 were facilitated in prodynorphin knockout mice (Mendizábal, Zimmer & Maldonado 2006). These data corroborate the crucial role for the kappa–dynorphin opioid system in the aversive properties of cannabinoids.

**Sensitization, craving and relapse**

An experimental model widely used to reveal behavioural sensitization is the long-lasting increase in locomotor activity induced after repeated exposure to a stimulant drug (see review by Vanderschuren & Kalivas 2000). Repeated administration of cannabinoids (Cadoni et al. 2001; Rubino et al. 2001) as well as opioids (Vanderschuren & Kalivas 2000) has been shown to produce locomotor sensitization in rodents, and cross-sensitization has been observed between these two systems. In fact, chronic treatment with THC increased the locomotor response induced by acute heroin in rats (Lamarque, Taghzouti & Simon 2001), and repeated administration of the CB1 receptor agonist CP 55,940 increased behavioural sensitization to morphine in Lewis rats (Norwood et al. 2003). Furthermore, heroin (0.5 mg/kg) induced locomotor activation in animals pre-treated repeatedly with WIN 55,212-2, while hypoactivity was observed in vehicle-treated rats (Pontieri et al. 2001a). Additionally, the acute administration of WIN 55,212-2 enhanced the locomotor effects produced by repeated heroin administration in rats. These effects were blocked by both the cannabinoid antagonist SR141716A and the opioid antagonist naloxone (Pontieri et al. 2001b). Another study investigating cross-sensitization effects showed that repeated exposure to THC induced behavioural sensitization to morphine. This cross-sensitization was symmetrical because rats sensitized to morphine were also sensitized to THC and WIN 55, 212-2, and these latter effects were blocked by rimonabant (Cadoni et al. 2001).

Although sensitization to chronic morphine administration was blocked in mice lacking CB1 receptors (Martin et al. 2000), this blockade was not observed in Wistar rats receiving the CB1 antagonist, rimonabant (Singh et al. 2004), implying either species differences or discrepancies between pharmacological and genetic models.

Sensitization of the mesolimbic DA activity has been identified as the neural substrate underlying the phenomenon of behavioural sensitization, and it has been proposed to mediate the behavioural changes induced by chronic use of drugs including craving and relapse (Robinson & Berridge 1993). A role for cannabinoid–opioid interactions in the control of relapse to drug-seeking behaviour has recently been put forward (De Vries & Schoffelmeer 2005; Fattore et al. 2005, 2007b). Thus, the cannabinoid receptor agonists HU-210 (De Vries et al. 2003), WIN 55,212-2 and CP 55,940 (Fattore et al. 2003) reinstated previously extinguished heroin-seeking behaviour, and rimonabant attenuated the reinstatement of heroin-seeking by a priming injection of heroin (De Vries et al. 2003; Fattore et al. 2003) or by heroin-associated cues (De Vries et al. 2003). Reciprocally, heroin reinstated drug-seeking behaviour in rats trained to self-administer WIN 55, 212-2 following 3 weeks of extinction (Spano et al. 2004). Supporting the reciprocal nature of cannabinoid–opioid interactions in craving and relapse processes, rimonabant and naloxone were shown to prevent cannabinoid-seeking behaviour induced by heroin and WIN administration, respectively (Spano et al. 2004; Fattore et al. 2005).

**Adaptive motivational changes in early development**

Several studies have reported contradictory results regarding the effects produced by the exposure to cannabinoids in early stages of development on opioid reward-related processing in adult rats. Hence, maternal exposure to THC facilitated morphine self-administration in adult female offspring (Vela et al. 1998). However, in a recent study, where pregnant rats were treated intravenously with THC from gestational day 5 to postnatal day 2, male adult offspring learned to self-administer heroin on a fixed-ratio schedule of reinforcement as vehicle-treated controls (Spano et al. 2007). Similar
contradictory results have been reported in animals perinatally exposed to THC. Thus, perinatal administration of THC did not increase the vulnerability of male or female rats to acquire morphine self-administration in adulthood (González et al. 2003b), but potentiated CPP induced by heroin in male rats (Singh, McGregor & Mallet 2006). These data can be contrasted with those obtained in adolescent rats pre-treated with THC showing an increase in heroin self-administration in the later stages of acquisition consistent with the hypothesis that early THC exposure modifies the hedonic value of opioids in adulthood (Ellgren, Spano & Hurd 2007). Further investigations will be needed to clarify these contradictory results and to evaluate whether prenatal or perinatal administration of opioids can also influence the motivational properties of cannabinoids in later life.

LEARNING AND MEMORY

Few behavioural studies have investigated the reciprocal interactions between the opioid and cannabinoid systems in learning and memory. Indeed, such interactions have only been demonstrated in mice studies using the passive avoidance paradigm to evaluate the consolidation of an aversive memory (Costanzi et al. 2003, 2004; Zarrindast et al. 2006). Thus, in a one-trial inhibitory avoidance task, immobilization stress exacerbated the memory impairment produced by AEA, and this effect was reversed by naltrexone (Costanzi et al. 2003). Conversely, using the same paradigm, an ineffective dose of AEA exacerbated the memory impairment induced by morphine (Costanzi et al. 2004). In addition, using the morphine state-dependent memory paradigm, it has been shown that intracerebroventricular administration of the CB₁ cannabinoid antagonist AM 251 prevents the morphine-induced improvement of aversive memory recall. Moreover, WIN 55,212-2 mimicked morphine state-dependent aversive learning, an effect that was reversed by naloxone (Zarrindast et al. 2006).

Despite the lack of functional studies, there are anatomical and biochemical data substantiating the potential for cross-talk between these systems related to cognitive processes. In this sense, early studies have reported that opioid and cannabinoid receptors are both localized in various brain areas involved in the control of learning and memory, such as the hippocampus, neocortex and amygdala (Herkenham et al. 1990; Howlett et al. 1990), and are co-expressed in other areas that may also contribute to some aspects of learning, including the nucleus accumbens (Pickel et al. 2004) and caudate nucleus (Rodríguez et al. 2001). In the hippocampus and cortex, both CB₁ cannabinoid and mu-opioid receptors are present in GABAergic interneurons (Mansour et al. 1995; Katona et al. 1999). By inhibiting GABAergic neurotransmission, these systems can activate excitatory neurotransmission and synaptic plasticity promoting long-term potentiation (LTP). On the other hand, the disruptive effects of cannabinoids on spatial memory and sensory processing may be due to the indirect activation of GABAergic function in these structures (Hoffman & Lupica 2000). However, confirmation of a bidirectional interaction between cannabinoids and opioids in the hippocampus modulating inhibitory or excitatory transmission has proven elusive. In fact, an electrophysiological study has shown that inhibition of GABA release in the hippocampus by WIN 55,212-2 was not blocked by the opioid antagonist, naloxone, suggesting that CB₁ receptors do not act through direct activation of opioid receptors or through the stimulation of endogenous opioid release in this brain structure (Hoffman & Lupica 2000).

Finally, both THC and morphine can activate extracellular signal-regulated kinase (ERK) in the prefrontal cortex, amygdala and nucleus accumbens, and this activation is essential for the induction of immediate early genes (Valjent et al. 2004). Because the ERK pathway plays an important role in synaptic plasticity, this intracellular signalling system may constitute a common molecular substrate for cannabinoid-opioid interactions in terms of learning and memory processing. However, the functional correlates of this common molecular ground need to be studied further.

EMOTIONAL-LIKE RESPONSES

Both opioids and cannabinoids are known to participate in the regulation of emotional behaviour (Maldonado & Valverde 2003). The distribution of CB₁ and opioid receptors in limbic areas that are engaged in emotional processes is consistent with this function (Corchero et al. 2004). Cannabinoids can induce both anxiolytic- and anxiogenic-like responses in rodents depending on the dose and the familiarity of the environment (Rodríguez de Fonseca et al. 1996). Thus, low doses of cannabinoids produce anxiolytic-like effects while higher doses result in anxiogenic-like responses (Onaivi, Green & Martin 1990; Rodríguez de Fonseca et al. 1996; Valjent et al. 2002). The neurobiological mechanisms involved in the anxiogenic-like effects of cannabinoids are closely related to the corticotropin-releasing factor (Rodríguez de Fonseca et al. 1996; Arévalo, de Miguel & Hernández-Tristán 2001). However, the possible mechanisms involved in the anxiolytic-like responses induced by cannabinoids have not been clearly elucidated.

Few studies have examined the possible involvement of the endogenous opioid system in the regulation of anxiety by cannabinoids. In this sense, the mu-opioid receptor antagonist beta-funaltrexamine, and the delta antagonist naltindole, but not the kappa-opioid...
antagonist nor-binaltorphimine, blocked the anxiolytic-like effects induced by THC in the lit-dark box in mice (Berrendero & Maldonado 2002). In line with these data, nor-binaltorphimine, but not mu- or delta-opioid receptor antagonists, abolished the anxiogenic-like effects induced by CP 55,940 (Marín et al. 2003). These results suggest that the anxiolytic-like effects of cannabinoids are mediated by mu- and delta-opioid receptors, whereas their anxiogenic-like effects are mainly related to kappa-opioid receptor activity. However, while THC-induced increase of the stress hormones corticosterone and adrenocorticotropic hormone was attenuated by naloxone (Manzanares, Corchero & Fuentes 1999b), nor-binaltorphimine did not reverse the activation elicited by CP 55,940 on the adrenocortical activity (Marín et al. 2003).

The possible role of the endogenous cannabinoid system in the emotional-like responses induced by opioids has been poorly studied. A recent study has evaluated the effects of the inhibitor of enkephalin-degrading enzymes RB101 on anxiety- and depressive-like responses of mice lacking CB1 cannabinoid receptors. The antidepressant- and anxiolytic-like effects of RB101 were not modified in CB1 knockout mice (Jurdinaud et al. 2005), indicating a lack of reciprocity in the interaction between these two systems in the control of emotional-like responses. Nevertheless, it cannot be excluded that the absence of physiological interaction in this study could be related to compensatory changes that occurred during knockout mice development. Indeed, a previous study suggests an interaction between these two systems in an animal model used to select antidepressant drugs, the conditioned suppression of motility test. Although THC given alone did not elicit any effect in this test, it facilitated the antidepressant-like effects induced by a non-effective dose of the inhibitor of enkephalin catabolism RB 101. This response was mediated by CB2 receptors given that it was antagonized by the CB1 antagonist rimonabant (Valverde et al. 2001).

CONCLUDING REMARKS

Anatomical, biochemical and molecular studies support the existence of reciprocal interactions between the endocannabinoid and opioid systems. These interactions have been clearly revealed on the development of tolerance to the pharmacological responses produced by cannabinoids and opioids. Several studies using pharmacological tools and genetically modified mice have shown an asymmetric cross-modulation between these two systems on the development of tolerance. A clear evidence for a reciprocal relationship between the cannabinoid and opioid systems in the development of dependence has also been revealed in multiple studies using pharmacological and genetic tools. This interaction is supported by the presence of similar biochemical changes during withdrawal to these two drugs including an inhibition of mesolimbic DA activity, an elevation in corticotropin-releasing factor and Fos immunoreactivity in the amygdala, and an increase in adenyl cyclase activity in different brain structures. Common neurochemical substrates have also been reported for the rewarding and reinforcing effects induced by cannabinoids and opioids. The mesolimbic DA pathway represents the main common link, where cross-talk between these two systems have been widely described, although the glutamatergic and GABAergic systems are also important targets for this interaction. These cross-interactions have been revealed in multiple behavioural models of sensitization, reward and reinforcement. Opioid and cannabinoids cross-talk related to cognitive processes are supported by their similar anatomical distribution and functional role in various brain areas involved in the control of learning and memory such as the hippocampus, neocortex and amygdala. However, at the present moment few behavioural studies have investigated the reciprocal interactions between these two systems in learning and memory. The distribution of cannabinoid and opioid receptors in limbic areas is consistent with their role in regulating emotional processes. Some studies have already revealed the presence of functional interactions between these two systems in several behavioural models of anxiety- and depressive-like responses. The mechanism participating in the cannabinoid–opioid interactions could take place at the level of their receptors, the downstream events associated or a common release of several neurotransmitters.

Acknowledgements

This work was supported by the National Institute on Drug Abuse–National Institutes of Health Grant 1R01DA016768, grants from Spanish Ministry of Science and Technology (SAF2007-64062), and The European Commission (PHECOMP. LSH-F96-037669; NEWMOOD. LSHM-CT-2004-503474; GENADDICT. LSHM-CT-2004-05166).

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Addiction Biology, 13, 213–224
Addiction Biology

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