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Review

Molecular and cellular basis of cannabinoid and opioid interactions

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Abstract

Cannabinoids and opioids have been shown to possess several similar pharmacological effects, including analgesia and stimulation of brain circuitry that are believed to underlie drug addiction and reward. In recent years, these phenomena have supported the possible existence of functional links in the mechanisms of action of both types of drugs. The present review addresses the recent advances in the study of biochemical and molecular mechanisms underlying opioid and cannabinoid interaction. Several hypothesis have been formulated to explain this cross-modulation including the release of opioid peptides by cannabinoids or endocannabinoids by opioids and interaction at the level of receptor and/or their signal transduction mechanisms. Moreover it is important to consider that the nature of cannabinoid and opioid interaction might differ in the brain circuits mediating reward and in those mediating other pharmacological properties, such as antinociception. While in vitro studies point to the presence of interaction at various steps along the signal transduction pathway, studies in intact animals are frequently contradictory pending on the used species and the adopted protocol. The presence of reciprocal alteration in receptor density and efficiency as well as the modification in opioid/cannabinoid endogenous systems often do not reflect the behavioral results. Further studies are needed since a better knowledge of the opioid–cannabinoid interaction may lead to exciting therapeutic possibilities.

Keywords: Cannabinoids; Opioids; Signalling; Endogenous systems

Contents

1. Introduction

Cannabinoids and opioids are two separate groups of psychoactive drugs that share a similar pharmacological profile: both induce analgesia, catalepsy, hypothermia, motor depression, hypotension, immunosuppression, sedation and reward effects (Manzanares et al., 1999; Massi et al., 2001; Varvel et al., 2004). In the cell, they activate different receptors (mu, delta and kappa opioid, and CB1, CB2 cannabinoid receptors), which are coupled to Gi/Go GTP-binding proteins that inhibit adenylyl cyclase activity, block voltage-dependent calcium channels, activate potassium channels and stimulate the MAP kinase cascade (for review see Childers, 1991; Childers et al., 1992; Howlett, 1995). Cannabinoid and opioid receptors are mainly located

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presynaptically where their activation causes inhibition of the release of different neurotransmitters (Mansour et al., 1995; Schlicker and Kathmann, 2001).

Anatomical studies have reported a similar distribution of CB1 cannabinoid and mu-opioid receptors in the dorsal horn of the spinal cord (Welch and Stevens 1992; Hohmann et al., 1999; Salio et al., 2001) and in several structures within the central nervous system (CNS). Brain areas such as the caudate putamen, dorsal hippocampus, and substantia nigra are rich in both cannabinoid and opioid receptors (Mansour et al., 1988; Herkenham et al., 1991; Mailleux and Vanderhaeghen, 1992; Rodriguez et al., 2001), and the co-localization of both types of receptors is possible. Other brain structures, such as the periaqueductal gray (PAG), raphe nuclei, central medial thalamic nuclei and the medial basal hypothalamus contain more moderate levels of cannabinoid and opioid binding sites but play an important role in antinociception (Lichtman et al., 1996) and in neuroendocrine effects (Fernandez-Ruiz et al., 1997; Romero et al., 1998a).

In vitro and in vivo assays highlight the similarities between the effects of opioids and cannabinoids, raising the possibility of interactions between them. These drugs seem to interact in their analgesic effects, as demonstrated by the ability of opioid and cannabinoid antagonists to reverse cannabinoid/opioid-induced analgesia (Welch, 1993; Reche et al., 1996a,b; Cichewicz et al., 1999). The co-administration of opioid and cannabinoid receptor agonists enhanced the antinociceptive effect compared with either drug alone in different models of acute pain (Cichewicz et al., 1999; Smith et al., 1998; Welch and Eads, 1999; Cichewicz and McCarthy, 2003). Synergism also occurs at subeffective or submaximal doses of cannabinoids and opioids and these effects were blocked by cannabinoid receptor and opioid receptor antagonists (Reche et al., 1996a; Smith et al., 1998; Cichewicz, 2004), suggesting that low doses of delta-9-tetrahydrocannabinol (THC) in conjunction with low doses of morphine could be an alternative regimen to reduce the need to escalate opioid doses, while increasing the opioid's potency.

A common feature of opioids and cannabinoids is their long-term activity. Continuous use of these drugs leads to tolerance and addiction. Chronic exposure to opioid agonists induced tolerance to the antinociceptive effect of THC (Bloom and Dewey, 1978; Hine, 1985; Smith et al., 1994; Thorat and Bhargava, 1994). Similarly, chronic THC induced tolerance to the antinociceptive effect of opioids (Smith et al., 1994; Welch, 1997). However, some other studies did not detect cross-tolerance (Mao et al., 2000), and Rubino et al. (1997) even found a potentiation of the antinociceptive effects of THC in rats made tolerant to morphine.

Cross-dependence between opioid and cannabinoid compounds has also been reported: cannabinoids replaced morphine and suppressed opioid withdrawal signs (Bhargava, 1976, 1978; Hine et al., 1975; Vela et al., 1995; Yamaguchi et al., 2001; Del Arco et al., 2002); the opioid antagonist naloxone precipitated abstinence symptoms in THC-tolerant rats (Hirschhorn and Rosecrans, 1974; Kaymakcalan et al., 1977) and the cannabinoid antagonist SR141716A precipitated abstinence in morphine-dependent rats (Navarro et al., 1998). Chronic blockade of CB1 receptor signaling by administering SR141716A during the development of opioid dependence reduced withdrawal symptoms (Rubino et al., 2000; Mas-Nieto et al., 2001).

A growing body of literature attests to the interaction between opioids and cannabinoids with respect to reward processes. The most direct experimental method of assessing reinforcing properties of drugs is the self-administration paradigm. Thus, interactions between cannabinoids and the opioid system have been found in drug self-administration studies. For example, SR141716A reduced self-administration of heroin in rats or mice (Chaperon et al., 1998; Braida et al., 2001; Mas-Nieto et al., 2001; Navarro et al., 2001; De Vries et al., 2003); in turn, the opioid antagonist naloxone or naltrexone reduced self-administration of THC in squirrel monkeys (Tanda et al., 2000; Justinova et al., 2003, 2004) or self-administration of the CB1 agonist CP-55,940 in rats (Braida et al., 2001). Moreover, the cannabinoid antagonists were able to prevent heroin-seeking behavior after a long period of extinction (Fattore et al., 2003; Caille and Parsons, 2003; Solinas et al., 2003) in either a fixed-ratio schedule of reinforcement, or a progressive-ratio schedule. Cross-sensitization between opioids and cannabinoids has also been reported (Lamarque et al., 2001; Cadoni et al., 2001; Pontieri et al., 2001a,b).

Finally, the interaction between these drugs has been further confirmed in knock-out mice although the results are often contradictory. The absence of the CB1 cannabinoid receptor did not modify the antinociceptive effects induced by different opioid agonists in the hot plate and tail-immersion tests. In contrast, the stress-induced opioid mediated responses were inhibited in CB1 mutants. These results indicated that the CB1 receptors were not involved in the antinociceptive responses to exogenous opioids, but that a physiological interactions between the opioid and cannabinoid systems was necessary to allow the development of opioid-mediated responses to stress (Valverde et al., 2000a). While most of the acute effects of opiates were unaffected in CB1 knock-out mice, opioid dependence and reward properties were reduced, suggesting a reduction in morphine's reinforcing activity (Ledent et al., 1999, Martin et al., 2000; Cossu et al., 2001). On the other hand, cannabinoid addiction was reduced in mice lacking opioid receptors (Valverde et al., 2000b; Ghozland et al., 2002; Castane et al., 2003), indicating that the opioid systems were involved in cannabinoid dependence. However, Rice et al. (2002) reported that CB1 knock-out mice readily acquired a conditioned place preference with morphine.

Evidence of functional interactions between cannabinoid and opioid systems has recently been provided for feeding behavior too (CB1 agonists stimulated and cannabinoid

antagonists reduced food intake whereas naloxone blocked this effect Arnone et al., 1997; Kirkham and Williams, 2001; Williams and Kirkham, 2002) and for anxiolytic activity, where the effects of a low dose of THC were blocked by the mu-opioid antagonist beta-funaltrexamine and the delta-opioid naltrindole, but not by the kappa-opioid antagonist binaltorphimine (Berrendero and Maldonado, 2002). Moreover, Marin et al. (2003) have shown that in rats the anxiogenic-like effect of CP-55,940 in the plus-maze was antagonized by the kappa opioid receptor antagonist nor-binaltorphimine, but not by either a mu-(cyprodime) or a delta-(naltrindole) receptor antagonist, suggesting that the k receptor system participated in the anxiogenic-like effect of CP-55,940.

The present review addresses recent advances related to the biochemical and molecular mechanisms underlying opioid and cannabinoid behavioral interactions. Several authors have suggested that both drugs share links in their molecular mechanisms of action, although this is still debated. It is important to consider that the nature of cannabinoid and opioid interactions might differ in the brain circuits mediating reward and in those mediating other pharmacological properties, such as antinociception. Several hypotheses have been formulated to explain the interaction between cannabinoid and opioid systems, including interaction at the level of the receptor and signal transduction system and the release of opioid peptides by cannabinoids or endocannabinoids by opioids.

1.1. In vitro studies

Cell lines provide a suitable experimental model to study the cellular mechanism of cannabinoid/opioid interactions. The N18TG2 cell line expresses both delta-opioid and CB1-cannabinoid receptors (Devane et al., 1986; Law et al., 1982; Abood et al., 1997). In these cells, opioids and cannabinoids both inhibited cAMP production (Devane et al., 1986; Law et al., 1982) through a pertussis toxin (PTX)-sensitive G-protein (Devane et al., 1986; Law et al., 1982; Howlett, 1995), and long-term exposure to either opioid or cannabinoid agonists induced desensitization to the ability of each drug to inhibit cAMP production (Dill and Howlett, 1988; Law et al., 1982).

Since opioid and cannabinoid agonists bind to different receptors, the two signaling pathways may first converge at the level of the G-protein. The activation of G-proteins by various agonists can be conveniently studied by measuring [^{35}S]GTPγS binding to cell membranes. Shapira et al. (1998) reported that when the opioid agonist etorphine and the cannabinoid agonist desacetyl-levonantradol (DALN) were applied together in N18TG2 membranes, the stimulation of [^{35}S]GTPγS binding was similar to the arithmetic sum of the two separate effects. This additivity persisted even after partial ablation of the G-protein reservoir with a low concentration of pertussis toxin, indicating that opioid and cannabinoid receptors activated different pools of G proteins in these cells. The two pools may involve either different subtypes of PTX-sensitive G-proteins, or different pools of the same protein which are separately compartmentalized within the cell. These experimental results suggest that opioid and cannabinoid receptors in N18TG2 cells were coupled to their own G-proteins, and the two signaling pathways converged only at the level of adenylyl cyclase.

Chronic treatment of N18TG2 cells with either opioid or cannabinoid agonists induced desensitization to the respective drug while revealing asymmetric cross-tolerance between the two drugs. Chronic exposure to DALN induced homologous desensitization and did not reduce the effect of etorphine on [^{35}S]GTPγS binding, whereas long-term exposure to etorphine caused partial desensitization to the cannabinoid's effect on G-protein. Di Toro et al. (1998) described a similar finding in NG108,15, another cell line that expresses both the CB1 and delta-opioid receptor. Prolonged exposure of NG108,15 cells to THC led to delta-opioid receptor down-regulation, with significant attenuation of the ability of enkephalin to inhibit forskolin-stimulated cAMP production. SR141716A blocked the effect of THC on delta-opioid receptor desensitization and down-regulation, indicating that they involve activation of the cannabinoid receptor.

To better clarify whether different subtypes of opioid and cannabinoid receptors, when co-expressed in the same cell, share a common reservoir, or use different pools of G protein, Shapira and coworkers (2000) used the [^{35}S]GTPγS binding method to measure the activation of G proteins by opioid and cannabinoid agonists in N18TG2 neuroblastoma cells, that endogenously co-express opioid and cannabinoid receptors, and in transiently transfected COS-7 cells. Their findings indicated a fundamental difference between transfected and native receptors with regard to their coupling to GTP-binding proteins. In COS-7 cells, the stimulation of [^{35}S]GTPγS binding through the combined presence of cannabinoid and opioid agonists was similar to the effect of either agonist alone, suggesting that the transfected opioid and cannabinoid receptors share the same pool of G proteins. In contrast, in the neuroblastoma cells, natively expressing opioid and cannabinoid receptors, the combined effect of the opioid and cannabinoid agonists was additive, suggesting that the two receptors activate different pools of G proteins. More recently, the Sarne group (Shapira et al., 2003), using HEK-293 and COS-7 cells co-transfected with delta-opioid and CB1 cannabinoid receptors, examined the effect of prolonged exposure to either etorphine or a cannabinoid (DALN) agonist on DOR and CB1 density and on the ability of the agonist to activate G proteins and inhibit cAMP production. In HEK-293 cells, etorphine induced both homologous and heterologous desensitization, while DALN induced only the former. This asymmetric cross-desensitization coincided with asymmetric cross-down-regulation: etorphine down-regulated the binding of the cannabinoid while DALN failed to reduce the binding of

the opioid. In contrast, COS-7 cells presented a two-way cross-desensitization between cannabinoid and opioid agonists, and DALN down-regulated the opioid binding in these cells. Thus a complete correlation was observed between down-regulation and reduction in cell responsiveness. The differences between the two cell lines-both quantitative and qualitative-may reflect their different enzymatic profiles.

In summary, these studies suggest that cross-tolerance in these cell lines takes place at the cellular level and can be detected at various steps along the signal transduction pathway.

Finally, Massi et al. (2003), using cultured splenocytes, found cross-desensitization in the inhibitory effects on cAMP production after chronic exposure to opiates and cannabinoids, strengthening the hypothesis that at the immune level too cAMP might be part of the intracellular pathway shared by opiates and cannabinoids.

1.2. Interactions between opioids and cannabinoids in intact animals

This section will outline the most important advances that have been made in clarifying the molecular mechanism underlying the pharmacological interactions between cannabinoids and opioids in intact animals.

1.2.1. Receptor and transduction systems

Mainly during chronic treatment these compounds might interact at the level of receptor or signal transduction mechanisms. Several studies have investigated whether the development of morphine tolerance in animals resulted in changes in cannabinoid receptor density or expression that might explain the presence or absence of behavioral cross-tolerance. For example, Thorat and Bhargava (1994) found that in morphine-dependent mice given THC there was a significant reduction in the analgesic effect of the cannabinoid as compared to placebo controls, suggesting the presence of cross-tolerance. Despite the existence of behavioral cross-tolerance these authors did not find changes in CB1 receptor binding. In contrast, Rubino et al. (1997) showed hypersensitivity to THC's analgesic effect in morphine-tolerant animals, that seemed to be due to the enhanced density of cannabinoid receptors and expression observed in their brains. Other studies, without examining any behavioral correlates, have found that chronic morphine exposure produced divergent and region-dependent effects on CB1 receptor binding and mRNA levels. Depending on the animals used-mice or rats-and the protocol of administration, some authors reported no significant changes in CB1 receptors in mice chronically treated with morphine (Thorat and Bhargava, 1994; Romero et al., 1998b); others, in cannabinoid binding studies in morphine-tolerant rats, found either a decrease in the cerebellum and hippocampus (Viganò et al., 2003), or an increase in the caudate putamen and limbic structures (Gonzalez et al., 2002). In addition, Gonzalez et al. (2003) examined the alteration in cannabinoid receptors in several brain regions of rats during morphine abstinence syndrome. They reported a decrease in CB1 receptor binding in the mid-brain and cerebral cortex, areas implicated in drug dependence. These discordant findings presumably reflect the different species (rats or mice), strains and protocols used (mild or strong) to induce morphine tolerance/dependence, since these might influence the cannabinoid system differently.

Only a few studies have examined changes in opioid receptor density in animals tolerant to cannabinoids. Here again, some authors reported no alteration in μ-opioid receptor binding, apart from the presence of behavioral cross-tolerance (Thorat and Bhargava, 1994), while others demonstrated that repeated administration of cannabinoid agonists increased mu-opioid receptor density in several brain areas, the degree of magnitude and time-related effect being dependent upon the brain region examined (Corchero et al., 2004; Parolaro, 2003, personal communication).

Finally, Sandra Welch's group (Cichewicz and Welch, 2003) reported that chronic treatment with a low-dose combination of THC and morphine avoided the development of morphine tolerance while maintaining a high antinociceptive effect. Down-regulation of all three types of opioid receptor proteins was not seen in the mid-brain of combination-treated mice, thus demonstrating the correlation between the absence of tolerance to morphine and the prevention of changes in opioid receptor protein levels in neurons involved in pain transmission (Cichewicz et al., 2001).

To conclude, although differences in species, strains and methods used to induce morphine/cannabinoid tolerance and dependence might account for some of the differences in results, it seems likely that the effect of opioid activation on cannabinoid receptors and vice versa is hard to define because of several region-dependent links in the mechanisms of action of both compounds.

A second interesting hypothesis is the existence of interactions at the post-receptor level. This would be supported by the fact that the receptors for both compounds act through similar intracellular signaling mechanisms. Hence, cross-tolerance or mutual potentiation might be possible through different degrees of efficiency of agonist-induced receptor activation, causing alterations in signal transduction.

Like for receptor proteins, results reported by several groups show some differences. Viganò et al. (2003) found that prolonged exposure to morphine in rats significantly lowered cannabinoid-induced [^{35}S]GTPγS binding in the limbic area, while Gonzalez et al. (2003) showed an increase of cannabinoid-stimulated [^{35}S]GTPγS binding in the cortex and a decrease in the brainstem of morphine-dependent rats. Romero et al. (1998b) noted that morphine-dependent mice had higher agonist-stimulated [^{35}S]GTPγS binding only in the substantia nigra and central gray substance.

In any case, the alterations in receptor efficiency appear to be anatomically discrete and relatively small, their

possible functional significance depending on the brain region where they occur. None of these changes were seen in the brain of rats given acute morphine. When the alterations were found in areas closely related to the mechanisms of drug addiction (e.g. limbic structures, nucleus accumbens) it is tempting to speculate that pharmacological manipulation of the cannabinoid system might offer a new tool to reduce morphine tolerance and dependence.

1.2.2. Endogenous systems

Recent papers pointed to the reciprocal importance of endogenous opioid and cannabinoid systems in either acute or chronic effects of the two drugs. It was clearly demonstrated that the endogenous opioid system was involved in cannabinoid actions either in pharmacological studies showing that several opioid antagonists could block the cannabinoid responses or in knock-out mice. However, some contradictory results have been reported with these two experimental approaches.

A plausible explanation of how endogenous opioids are involved in the effects of cannabinoids is that these drugs may increase the synthesis or release of endogenous opioids. Thus, acute administration of THC and other exogenous cannabinoid agonists, but not anandamide, raised extracellular levels of endogenous dynorphin in the spinal cord (Houser et al., 2000; Pugh et al., 1997; Mason et al., 1999; Welch and Eads, 1999), a structure vital for the transmission and control of the nociceptive message, and of endogenous enkephalin in the nucleus accumbens, a limbic area involved in the control of reward and emotional responses (Valverde et al., 2001).

A correlation between antinociception and increased dynorphin levels suggested that these endogenous opioids interact with the kappa opioid receptors to mediate the antinociceptive effect of THC and its enhancement by kappa opioids (Welch and Eads, 1999; Mason et al., 1999). In addition, the discovery of a two-way cross-tolerance between THC and CP-55,940 to kappa agonists in the tail-flick test (Smith et al., 1994) confirmed that cannabinoids interact with kappa opioids to influence antinociception. However, the endogenous cannabinoid anandamide produced antinociception through a mechanism different from that of THC; anandamide did not increase dynorphin A nor was its antinociceptive effect sensitive to nor-binaltorphimine (nor-BNI), though the drug's rapid breakdown by fatty acid amide hydrolase (Deutsch et al., 2001) may have prevented it from producing this effect.

Cannabinoid-opioid interactions also persist after chronic drug administration. Corchero et al. reported that chronic treatment with THC increased prodynorphin and proenkephalin gene expression in the rat spinal cord (Corchero et al., 1997a), and propiomelanocortin gene expression (its post-translation product is beta-endorphin) in the arcuate nucleus of the hypothalamus (Corchero et al., 1997b). Manzanares et al. (1998) subsequently showed an increase in proenkephalin mRNA in the ventro-medial nucleus of the hypothalamus and in the PAG matter of rats treated chronically with THC and metanandamide. All these brain areas are closely related to spinal and supraspinal circuits regulating nociceptive pathways, suggesting that supraspinal mu and spinal kappa receptors are involved differently in the interaction between cannabinoid and opioid systems to regulate nociception (Reche et al., 1998).

THC induced analgesia is reduced in prodynorphin−/− mice (Zimmer et al., 2001). However, recent reports using wild-type and prodynorphin−/− mice found that WIN55212-2 and THC were equipotent, suggesting an endogenous opioid-independent mechanism of cannabinoid antinociception in the spinal cord (Gardell et al., 2002). In contrast, mice lacking mu, kappa, and delta opioid receptor genes developed the same degree of tolerance as the wild type to THC after chronic administration, showing that the suppression of opioid receptors has no important consequences on the development of cannabinoid tolerance (Zimmer et al., 2001; Ghozland et al., 2002; Maldonado and Valverde, 2003). In partial disagreement with this conclusion, Castane et al. (2003) showed that in knock-out mice with double deletion of mu and delta receptors, tolerance to the THC hypothermic effect developed more slowly, but the development of tolerance to antinociceptive and hypolocomotor effects was not affected.

The role of the endogenous opioid system in cannabinoid dependence has also been investigated. Several studies have looked at THC withdrawal in mice whose opioid system has been genetically altered. The somatic expression of cannabinoid withdrawal was dramatically attenuated in pre-proenkephalin knock-out mice compared to wild-type mice (Valverde et al., 2000b) and in mu opioid receptor-deficient mice (Lichtman et al., 2001), indicating the potential importance of the endogenous opioid system. In contrast, the global withdrawal score in THC-dependent mice challenged with SR141716A was not affected by deletion of mu, kappa and delta receptors (Ghozland et al., 2002). Strain differences in the two studies might have contributed to the differences in cannabinoid withdrawal in mu opioid-deficient mice. Whereas Lichtman et al. (2001) used mice with a C57Bl/6 background, Maldonado's group used 1:1 hybrids from the 129/SV and C57Bl/6 strain (Ghozland et al., 2002).

Finally, Castane et al. (2003) reported that in multiple mice deficient in both mu and delta opioid receptors, the withdrawal score was significantly lower, suggesting that a cooperative action of both receptors–mu and delta–was required for the expression of THC dependence. Taken together these data indicate that opioid systems have a role in cannabinoid dependence.

It has also been suggested that cannabinoid-induced up-regulation of opioid gene expression might be relevant for cannabinoid reward. Prolonged administration of different cannabinoid receptor agonists to rats increased proenkephalin gene expression in the caudate-putamen, nucleus

accumbens, paraventricular and ventromedial hypothalamic nuclei and medial mamillary nucleus (Manzanares et al., 1998). In view of the fact that these modifications were in areas involved with the complex circuits mediating drug dependence, this paper concluded that an interaction between cannabinoid and enkephalinergic systems may be part of a molecular integrative response to behavioral and neurochemical alterations that may underlie cannabinoid rewarding properties.

A variety of animal paradigms have traditionally been employed to directly and indirectly investigate the rewarding/reinforcing effects of drugs and their molecular mechanisms, most notably drug self-administration, conditioned place preference (CPP), and intracranial self-stimulation techniques.

Self-administration studies and CPP experiments suggested the endogenous opioid systems are involved in the motivational effects of cannabinoids. For example, the self-administration and CPP produced by CP-55,940 in rats were blocked by naloxone (Braida et al., 2001) and the opioid antagonist naltrexone reduced the reinforcing effects of THC in squirrel monkeys (Justinova et al., 2004). In addition, absence of mu-opioid receptors abolished THC place preference and deletion of kappa-opioid receptors ablatet THC place aversion and furthermore unmasked THC place preference suggesting that an opposing activity of mu and kappa-opioid receptors in modulating reward pathways forms the basis for the dual euphoric-dysphoric activity of THC (Ghozland et al., 2002). In double-deleted MOR/DOR knock-out mice the place preference to THC was reduced (Castane et al., 2003). In line with these results, the ability of THC to lower the threshold for intra-cranial self-stimulation (ICSS) was blocked by naloxone (Gardner et al., 1988; Gardner and Lowinson, 1991) providing more support for the theory that functional mu opioid mechanisms were necessary for the expression of cannabinoid-induced reward.

Several studies have investigated whether opioid addiction involves functional changes in the endocannabinoid system. As briefly described in the introduction, CB1 receptors may be necessary for the expression of several effects of opiates, so CB1 antagonists may offer a novel approach for treating opiate addiction. Besides the pharmacological findings, further evidence of a role for CB1 receptors comes from studies with mutant cannabinoid CB1 receptor knock-out mice. Though most of the acute effects of opiates were unaffected, these mice were unable to learn to self-administer morphine and the severity of the withdrawal syndrome was strongly reduced, suggesting a reduction in morphine's reinforcing property (Ledent et al., 1999; Cossu et al., 2001). Similarly, morphine-induced place preference was abolished in CB1(−/−) mice, though a place preference could still be established with cocaine (Martin et al., 2000).

A contradiction emerges when one considers that the severity of opioid withdrawal is reduced by cannabinoid agonists or by deletion of the CB1 receptor. The apparent paradox can be reconciled if one takes into account that cannabinoid agonists are typically given only during naloxone challenge, while there is no CB1 signal in the mutant during the entire development of dependence.

Finally, some studies used a protocol that induces morphine tolerance or dependence to see whether there are functional changes in the endocannabinoid levels in term of concentration of anandamide (AEA) and 2-arachidoylglycerol (2-AG), the two major endogenous ligands of cannabinoid receptors. Chronic morphine exposure markedly lowered 2-AG contents without changing AEA levels in several brain regions (striatum, cortex, hippocampus, limbic area and hypothalamus), showing that the treatment has different effects on the regulatory mechanisms controlling AEA and 2-AG homeostasis in the brain (Viganò et al., 2003). Gonzalez et al. (2003), using a well-established method to induce physical dependence to morphine, reported that besides the alterations in CB1 receptor levels and efficiency, there were no changes in the contents of AEA in the brain regions considered. To conclude, rewarding effects of drugs have been associated with effects on forebrain mechanisms associated with natural reward, particularly dopamine release in the nucleus accumbens. The well-known enhancement of presynaptic dopamine efflux after acute THC in the nucleus accumbens and the medial prefrontal cortex (Chen et al., 1990a,b, 1991, 1993; Navarro et al., 1993; Tanda et al., 1997) appeared to involve endogenous opioids since naloxone fully blocked this effect (Chen et al., 1990b). Tanda et al. (1997) showed that the opioid antagonist naloxone, infused into the ventral tegmentum, prevented the effects of cannabinoids and heroin on dopamine transmission. Thus, THC and heroin exerted similar effects on mesolimbic dopamine transmission through a common mu-opioid receptor mechanism in the ventral mesencephalic tegmentum.

One possible mechanism might be that cannabinoid receptor activation changes the levels of endogenous peptides in mesolimbic areas that, in turn, influence dopaminergic activity. The CB1 stimulation may enhance mesolimbic dopamine by increasing endogenous activity at mu-opioid receptors, while simultaneously exerting an opposite aversive influence by increasing endogenous activity at kappa-opioid receptors (Ghozland et al., 2002). These competing mechanisms could be regulated differently by a host of factors, helping to explain many of the discrepancies in the animal literature.

2. Conclusion

Besides the recognized similarities between the effects of cannabinoids and opioids, progress towards understanding the molecular basis for these similarities and the degree to which the endogenous opioid and endocannabinoid systems

interact still has far to go. Further advances may lead to exciting therapeutic possibilities. For example, low doses of a cannabinoid agonist may prove useful for patients being treated with morphine for pain. The cannabinoids may help relieve the effects of opiate withdrawal and, finally, manipulation of the endogenous cannabinoid system may aid in efforts to develop new therapeutic protocols.

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