Chapter 6

The Endocannabinoid System and the Therapeutic Potential of Cannabinoids

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1. Introduction

Much has been written about the history of the medical uses of cannabis *(1)*. In the past two centuries, there have been numerous references to the use of cannabis extracts for a wide range of disorders *(2)*. In the early part of the 20th century, a standardized cannabis elixir was marketed in the United States. Following the introduction of synthetic drugs such as barbiturates and opioids into medicine, interest in cannabis elixir declined. The discovery of the primary active constitutent in marijuana, Δ^9-tetrahydrocannabinol (THC), in 1964 *(3)* rekindled interest in the area. However, the emphasis shifted to synthetic cannabinoids rather than the plant or plant extracts. For example, in the 1970s, clinical studies were conducted in an effort to determine the efficacy of THC as an analgesic *(4)*, antiemetic (5), antidepressant *(6,7)*, appetite stimulant *(7)*, and for treatment of glaucoma *(8)*. These efforts resulted in the approval of THC (dronabinol, Marinol™) for treatment of chemotherapy-induced nausea and vomiting in 1985 and for appetite stimulation in 1992.

There have been several attempts to develop THC derivatives for medical uses. Nabilone was found to have anxiolytic *(9)* and antiemetic properties *(10)* and is presently marketed as Cesamet™. Levonantradol was evaluated as an antiemetic *(11)* and analgesic *(12)* but was never approved for clinical use. Nabitan was studied clinically as an analgesic in cancer pain *(13)* but, like levonantradol, was never approved for use. However, the emphasis shifted back to cannabis in the early 1990s following the HIV epidemic. The lack of effective treatments for HIV led the advocacy community

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to demand more effective treatments and greater access to any material that might be beneficial for symptoms management. Hence, there has been increased attention to smoked marijuana not only for HIV patients, but also for a wide range of diseases. During this same period it became obvious that THC and marijuana were producing their effects through a newly discovered endocannabinoid system. The discovery of this biological system has provided opportunities for developing new medications that were not possible previously.

2. Endocannabinoid System

Although early structure–activity relationship *(14)* and initial receptor-binding studies *(15)* suggested the existence of cannabinoid receptors, it was not until the late 1980s that compelling evidence for a cannabinoid receptor emerged. Devane et al. *(16)* characterized a binding site that had all of the properties of a cannabinoid receptor. Shortly thereafter, the cannabinoid receptor was cloned, thereby verifying the existence of a specific target for cannabinoids *(17)*. Compton et al. *(18)* extended these characterizations by showing a strong correlation between binding affinity for this site and cannabinoid potency for a large number of cannabinoid analogs. This receptor is referred to as the CB_1 cannabinoid receptor. The cannabinoid receptor, while uniquely recognized by cannabinoids, is a member of a large family of receptors that are coupled to G proteins. CB_1 receptors are also found in brain and peripheral tissues that include sensory nerve fibers, the autonomic nervous system, testis, and immune cells *(19)*. Surprisingly, the CB_1 cannabinoid receptor was found to be present in very high quantities in the central nervous system, exceeding the levels of almost all neurotransmitter receptors. Although the CB_1 receptor is present throughout brain, the highest levels are found in brain structures associated with neurophysiological functions altered by cannabinoids *(20)*. The densest binding occurs in the basal ganglia (substantia nigra pars reticulata, globus pallidus, entropeduncular nucleus, and lateral caudate putamen) and the molecular layer of the cerebellum. Receptors in these regions are consistent with cannabinoid interference with movement. Intermediate levels of receptor binding are present in the CA pyramidal cell layers of the hippocampus, the dentate gryus, and layers I and VI of the cortex. The presence of CB_1 receptors in these regions is expected given the effects of cannabinoids on cognitive processes. The hippocampus stores memory and codes sensory information. The presence of cannabinoid receptors in regions associated with mediating brain reward (ventromedial striatum and nucleus accumbens) is consistent with the role that cannabinoids play in the neurobiology of reward *(21)*. Lower levels are found in the brainstem, hypothalamus, corpus callosum, and the deep cerebellum nuclei. At the cellular level, the CB_1 receptors are located predominantly on presynaptic terminals of γ-aminobutyric acid (GABA) and glutamate neurons. In the striatum they are present on glutamatergic terminals emanating from the cortex *(22)*, GABA interneurons *(23)*, and axon terminals of GABA-associated medium spiny neurons *(24)*. Cerebellar CB_1 receptors are present on excitatory terminals and GABA interneurons *(25)*.

A second receptor subtype has been identified and is termed the CB_2 cannabinoid receptor *(26)*. The CB_2 receptor is present primarily in tissues that are associated

with immune function, including spleen, thymus, tonsils, bone marrow, pancreas, splenic macrophages/monocytes, mast cells, and peripheral blood leukocytes *(19)*. The messenger RNA for the CB_2 receptor varies considerably among various human blood cell populations, with B-lymphocytes > natural killer cells >> monocytes > polymorphonuclear neutrophils > T8-lymphocytes > T4-lymphocytes *(27)*. There is no evidence that this receptor subtype is associated with neuronal tissue. However, there is evidence that CB_2 receptors can be induced in microglia, a cell of macrophage lineage that is present in brain *(28)*. CB_1 and CB_2 receptors are activated by THC.

Several cannabinoid receptor signaling pathways have also been identified. Both cannabinoid receptor subtypes have the molecular signature of G protein-coupled receptors. Actually, evidence for a G protein-coupled cannabinoid receptor preceded the cloning of the CB_1 receptor *(29)*. There is strong evidence for CB_1 receptor coupling to multiple $G_{i/o}$ proteins *(30)*. The predominant effects of cannabinoids occur through inhibitory G protein function, including inhibition of adenylyl cylase, inhibition of calcium channels (N and Q types), as well as activation of inwardly rectifying potassium channels *(31,32)*. These actions are highly relevant to neurotransmitter release, as will be discussed later.

Although evidence of cannabinoid receptors and their signaling pathways was sufficient to establish biological relevance, identification of the natural ligands was essential for functional relevance. Three distinct arachidonoyl derivatives have been identified as natural ligands for the cannabinoid receptors. The amide anandamide *(33)*, the ester 2-arachidonoyl-glycerol *(34,35)*, and the 2-arachidonoyl glyceryl ether *(36)* have been identified thus far as endocannabinoids. These endogenous substances are considered endocannabinoids because they activate CB_1 cannabinoid receptors and produce effects that are consistent with CB_1 cannabinoid receptor activation. Moreover, the synthetic and degradative pathways for anandamide and 2-arachidonoylglycerol have been identified in relevant tissues.

There is substantial evidence that a calcium-dependent, energy-independent transacylase transfers arachidonic acid from the *sn*-1 position of phosphatidylcholine to the amino group in phosphatidylethanolamine to form *N*-arachidonoyl-phosphatidylethanolamine, with subsequent hydrolysis by a phospholipase D-type enzyme to form anandamide *(37)*. Inactivation of anandamide occurs primarily via fatty acid amide hydrolase, an enzyme that has been cloned (38). Blockade or deletion of this enzyme in mice greatly potentiates the actions of exogenously administered anandamide *(39)*. Diacylglycerol lipase synthesizes 2-arachidonoylglycerol *(40)*. This enzyme is required for axonal growth during development and for retrograde synaptic signaling at mature synapses. The inactivation of 2-arachidonoylglycerol occurs by a monoglyceride lipase *(41)*. Both of these synthetic and degradative 2-arachidonoylglycerol enzymes have been cloned.

The discovery that the endogenous cannabinoid system consists of two receptor subtypes, signaling pathways, endogenous ligands, and synthetic and metabolic pathways for these ligands provided unique opportunities to understand the mechanisms through which cannabinoids produce their effects. More importantly, the endogenous cannabinoid system provides a means for verifying whether cannabinoids are acting directly or indirectly to produce their wide range of pharmacological effects. At the

same time, the functional role of the endogenous cannabinoid system in normal physiological processes, as well as in disease states, is beginning to emerge. This chapter is confined to appetite, emesis, pain, and drug dependence.

3. Appetite

The desire to consume food represents one of the fundamental physiological processes essential for survival. It is therefore not surprising that appetite is regulated by a highly complex integration of hormonal and neuronal systems to maintain homeostasis. Disruptions of these homeostatic mechanisms can result in either food deprivation or excess eating. Appetite is also easily disrupted in many disease states, such as cancer and HIV infection.

There is ample evidence that the endogenous cannabinoid system plays a role in appetite homeostasis. Although both marijuana and THC have been shown to stimulate appetite, direct evidence for the involvement of cannabinoid receptors was provided by a study in which CB_1 receptor knockout mice ate less than wild-type mice following food restriction *(42)*. The selective antagonist, rimonabant (SR 141716), provided additional support for CB_1 receptor involvement in that this compound reduced food intake in wild-type but not CB_1 knockout mice *(42)*. There are several lines of evidence indicating that the brain is a prominent site for cannabinoid regulation of appetite. For example, the hypothalamus contains both CB_1 receptors and the endocannabinoids anandamide and 2-arachidonoylglycerol. Direct injections of anandamide into the hypothalamus of rats induced hyperphagia, an effect that was blocked by the CB_1 receptor antagonist rimonabant *(43)*. In addition, there is evidence of an interrelationship between the endocannabinoids and leptin, a key anorexigenic agent that is secreted by adipose tissue and acts within the hypothalamus at the arcuate nucleus to suppress appetite-stimulating peptides and stimulate the activity of appetite-reducing peptides. Di Marzo et al. *(42)* demonstrated that acute treatment with leptin reduces the levels of anandamide and 2-arachidonoyl glycerol in the hypothalamus of normal rats. On the other hand, these endocannabinoids were elevated in obese leptin-deficient *ob/ob* and obese leptin-receptor-deficient *db/db* mice.

A second central component of cannabinoid-mediated food intake likely involves reward pathways and the hedonic aspect of eating. Higgs et al. *(44)* recently demonstrated that both THC and anandamide increased sucrose intake in rats, whereas rimonabant decreased it. Fasting increases levels of anadamide and 2-arachidonoylglycerol in the nucleus accumbens, a brain structure crucial for reward *(45)*. Levels of endocannabinoids were not changed in satiated rats. In diet-induced obese rats there was a significant decrease in CB_1 receptor density in hippocampus, cortex, nucleus accumbens, and entopeduncular nucleus, but not in hypothalamus *(46)*. Collectively, these data strongly implicate a central mechanism for endocannabinoid influence on diet.

There are also several suggestions that endocannabinoids act peripherally to regulate metabolism. Cota et al. *(47)* found CB_1 receptors in adipocytes, thereby raising the possibility of a direct peripheral lipogenic mechanism. Furthermore, rimonabant stimulated Acrp30 (adiponectin) messenger RNA expression in adipose tissue and reduced hyperinsulinemia in obese (*fa/fa*) rats *(48)*. At present, there is no evidence

that CB_1 receptor agonists produce opposing effects. Nevertheless, these findings suggest that the endocannabinoid system may have a direct effect on energy balance and lipid metabolism.

Based on the above findings, it seems logical that the endocannabinoid system could be manipulated for the purpose of treating either weight loss or obesity *(49)*. Indeed, one of the most consistent effects of smoking marijuana is an increase in appetite. A recent study compared marijuana smoking with oral THC, and both treatments increased food intake *(50)*. However, the results in patient populations have been less definitive. Beal et al. *(51)* examined the effects of THC on appetite and weight in patients with AIDS-related anorexia. They reported modest improvement in appetite and mood along with stabilization in weight. Several early investigations showed that THC increased appetite in cancer patients *(52,53)*. More recently, Jatoi et al. *(54)* compared megestrol acetate with THC for palliating cancer-associated anorexia. They found that megestrol acetate provided superior anorexia palliation among advance cancer patients. On the other hand, Nelson et al. *(55)* evaluated the effects of THC on appetite in advanced cancer patients suffering from anorexia. Most patients completed the 28-day study and experienced improved appetite. With regard to the CB_1 receptor antagonist rimonabant, it has been shown to be effective in reducing food intake in both laboratory animals (described earlier) and in promoting weight loss in humans during recent phase III clinical trials.

4. *Emesis*

Although emesis has a dramatic impact on appetite, the mechanisms underlying emesis trials and nausea/vomiting are quite distinct. In contrast to the predominant role of the hypothalamus in appetite, the postrema-nucleus tractus solatarius in the brainstem plays an essential role in emesis. Additionally, the dopaminergic, cholinergic, and serotonergic systems in the gastrointestinal tract can contribute to emesis. Several animal studies indicate a direct role for endocannabinoid modulation of emesis. Darmani et al. *(56)* showed that CB_1 receptor agonists reduced cisplatin-induced emesis in the least shrew, whereas the antagonist rimonabant produced the opposite effects. Similar findings were reported with cannabinoid agonists that attenuated lithium-induced vomiting in the musk shrew *(57,58)*. In addition, combinations of inactive doses of THC and ondansetron were effective in blocking vomiting in the musk shrew *(58)*. The musk shrew has also been used to study conditioned retching, an animal model of anticipatory nausea and vomiting. THC completely suppressed conditioned retching in this model *(59)*. In addition, cannabinoid agonists suppressed lithium-induced conditioned rejection, a model of nausea in rats *(60)*. Opioids are known to be powerful emetogenic agents. Activation of the cannabinoid system was also effective in blocking opioid-induced vomiting in ferrets *(61)*. CB_1 cannabinoid receptors were strongly implicated in that rimonabant blocked the action of cannabinoid agonists in this model. Importantly, Darmani et al. *(62)* found prominent CB_1 receptor binding in the nucleus tractus solartius of the shrew. The exact nature of the role played by endocannabinoids is unclear at this time. A metabolically stable analog of anandamide blocked vomiting, whereas another endocannabinoid, 2-arachidonoylglycerol, was emetogenic *(62)*.

As for clinical evidence, anecdotal reports of patients smoking marijuana to control chemotherapy-induced nausea and vomiting provided the initial clues. These reports led to clinical studies with THC in which it was found to be useful in patients whose chemotherapy-induced nausea and vomiting were refractory to other standard antiemetics available at that time *(63)*. Plasse et al. *(53)* reported that combinations of THC and prochlorperazine resulted in enhancement of efficacy as measured by duration of episodes of nausea and vomiting and by severity of nausea. In addition, the incidence of psychotropic effects from THC appeared to be decreased by concomitant administration of prochlorperazine. The combination was significantly more effective than was either single agent in controlling chemotherapy-induced nausea and vomiting *(64)*. Nabilone, a synthetic derivative of THC, was also reported to be an effective oral antiemetic drug for moderately toxic chemotherapy *(65)*. Cannabinoids have also been found to be effective in treating nausea and vomiting in children undergoing chemotherapy *(66,67)*. As for the current status of antiemetics, serotonergic anatagonists such as ondansetron have become the standards for managing emesis. These agents have proven to be effective in preventing chemotherapy-induced nausea and vomiting in most patients. However, delayed nausea and vomiting are less well controlled. Therefore, the search for more effective agents continues. Combination therapy with ondansetron and THC has not been fully explored. In addition, there is a need for a higher-efficacy CB_1 receptor agonist with fewer side effects.

5. Pain

Animal studies have firmly established cannabinoid-induced analgesia in a wide array of acute and chronic pain models *(68)*. Most of this evidence is based on CB_1 receptor agonists such as THC and related synthetic derivatives. It has been firmly established that these effects are being mediated through the endocannabinoid system. First, there is an excellent correlation between cannabinoid analgesics and their affinity for the CB_1 receptor *(69)*. Second, the CB_1 receptor antagonist rimonabant is effective in blocking the analgesic effects of cannabinoid agonists *(70,71)*. As expected, the endogenous ligands anandamide and 2-arachidonoylglycerol exhibit analgesic properties when administered to laboratory animals *(34,72)*. Mice with genetic deletion of fatty acid amidohydrolase, the enzyme that hydrolyzes anandamide, exhibit enhanced analgesic activity with exogenously administered anandamide *(39)*. More importantly, these animals have elevated endogenous anandamide levels as well as an increased pain threshold, evidence that supports a physiological role for endocannabinoids in pain perception. Additional evidence for endocannabinoid pain modulation includes cannabinoid suppression of spinal and thalamic nociceptive neurons, identification of spinal, supraspinal, and peripheral sites of action, as well as evidence that endocannabinoids are released upon electrical stimulation of the periaqueductal gray and following inflammation in the periphery *(73,74)*.

Although nociceptive events will stimulate the release of endocannabinoids, the exact nature of their actions on pain neurotransmission remains to be fully established. CB_1 receptors are located predominantly on presynaptic terminals, and their activation results in the inhibition of the neurotransmitter released at this site. Hohman et al.

examined the distribution of CB_1 receptors in rat dorsal root ganglion and found them present in only a subset of neurons containing substance P and calcitonin gene-related peptide *(75)*. There is evidence for localization of CB_1 receptors on neurons containing endogenous opioids. Welch and Stevens *(76)* demonstrated that cannabinoid agonists potentiated morphine analgesia in laboratory animals. This laboratory later demonstrated that THC, but not anandamide, stimulates the release of dynorphin A *(77)*. While there is an abundance of data illustrating interactions between the opioid and cannabinoid systems, the exact nature of these interactions remains to be elucidated.

Although there is strong evidence that the endocannabinoid system regulates pain pathways, the effectiveness of CB_1 agonists as analgesics has been equivocal. Despite intense efforts to develop cannabinoid analgesics, there has been little success in devising a CB_1 receptor agonist that is devoid of behavioral effects. For example, Noyes et al. *(78)* found that oral THC was as efficacious as codeine in producing analgesia in a patient population, but its behavioral side effects precluded the use of higher doses. As for synthetic cannabinoid derivatives that might be useful as analgesics, nabitan is one such analog that was evaluated in at least two studies. Jochimsen et al. *(79)* failed to observe pain relief in cancer patients, and there was some evidence for increased pain sensitivity. On the other hand, another research group *(13)* reported analgesia comparable to that of codeine in cancer patients. Levonantradol, another cannabinoid derivative, elicited some benefit for postoperative surgical pain but only at doses that produced significant behavioral disturbances *(80)*. Several recent clinical studies have found THC to lack sufficient efficacy in postoperative pain *(81)*, neuropathic pain *(82)*, and refractory neuropathic pain *(83)*. On the other hand, THC was found to exert some benefit in treating intractable neuropathic pain in two adolescents *(84)*. A review of clinical studies regarding cannabinoid agonist treatment of cancer pain led the author to conclude that the present studies do not justify the use of cannabinoid agonists for pain management *(85)*.

The evidence suggests that the CB_1 receptor agonists that have been developed thus far are unlikely to be highly efficacious in controlling high-intensity pain. However, the possibility remains that they might be useful in more moderate pain, particularly in case in which some of the typical cannabinoid side effects (sedation, dizziness, etc.) might be more tolerated. Theoretically, CB_1 receptor agonists should be effective as adjuvants to other analgesics. Numerous preclinical studies have shown that THC will enhance opioid analgesia. However, in a recent study in human experimental pain models, THC offered relatively small additive analgesic effects when combined with morphine *(86)*. It remains to be determined whether similar results would occur in pain patients.

There are several possible explanations for the discrepancy between the analgesic effects of CB_1 receptor agonists in laboratory animals and humans. Certainly, higher doses can be administered to laboratory animals, and hence greater analgesic effects achieved, than in humans. Pharmacokinetics may also play a very important part. The studies that have been carried out thus far have relied on oral administration of THC, a route that does not allow for easy optimization of treatment. Efforts are underway to develop alternative formulations of THC to allow for other routes of administration.

Rectal suppositories of THC hemisuccinate have been found to be effective in treating spasticity and pain *(87)*. A water-soluble analog of THC has been developed that may be appropriate for intravenous use *(88)*. There have been recent studies demonstrating that topical administration of cannabinoids produce analgesic effects *(89)*. Moreover, topical administration produced a synergistic interaction with spinally administered cannabinoids. A separate group of investigators reported an analgesic interaction between topical opioids and cannabinoids administered either topically or spinally *(90)*. These observations reinforce the notion that treatment regimens of opioid and cannabinoids combinations have yet to be optimized clinically. Unfortunately, a topical preparation of THC or related cannabinoid is not yet available for clinical use. Another attractive approach is the inhalation route. An inhalation formulation of THC was developed years ago, but unfortunately it produced bronchial irritation *(91)*. The recent develop of a THC aerosol delivered through a metered-dose inhaler holds promise *(92)*.

The discussion so far has been devoted to nonselective CB_1 and CB_2 agonists, such as THC, because most of the analgesic literature has been generated with these compounds. The discovery of the CB_2 receptor in nonneuronal tissues such as immune cells attracted interest in its potential modulation of immune function. However, there are now numerous reports that CB_2 selective agonists have analgesic properties. One such CB_2 selective agonist is AM 1241, which was shown to be highly active in a thermal pain model in rats *(93)*. It was also shown to suppress capsaicin-induced hyperalgesia *(94)*. HU 308 is another CB_2 selective agonist that has been reported to produce analgesic effects in rodents *(95)*. The advantage of these compounds is that they are devoid of the behavioral effects produced by CB_1 selective agonists. At present there are no reports of clinical efficacy of CB_2 selective agonists.

6. Drug Dependence

Marijuana dependence has long been a controversial issue, in part as a result of the lack of understanding of drug dependence. It is clear that a major physical withdrawal syndrome does not occur upon abrupt cessation of marijuana use. Certainly, dependence on many substances occurs without a prominent physical aspect of the syndrome. What is clear is that continual use of marijuana can lead to dependence as defined by the *Diagnostic and Statistical Manual of Mental Disorders*, 4th ed. criteria, or essentially the inability to the user to exert control over their use. In actual fact, an abrupt cannabinoid withdrawal syndrome was described in humans following discontinuation of a rather rigorous treatment regimen of THC *(96,97)*. Studies in more recent times have used treatment regimens that more closely reflect typical marijuana use patterns and have also demonstrated an abstinence symptom that included subjective effects of anxiety, irritability, and stomach pain, as well as decreases in food intake, following abrupt withdrawal from continued administration of either oral THC *(98)* or marijuana smoke inhalation *(99)*. There have been several efforts to devise strategies for treating marijuana dependence. Haney et al. *(100)* found that bupropion worsened mood during marijuana withdrawal. The antidepressant nefazodone provided partial relief *(101)*. They also demonstrated that oral THC decreased marijuana craving and withdrawal signs during abstinence *(102)*.

Demonstrating a well-defined abstinence withdrawal syndrome following prolonged cannabinoid administration in laboratory animals also presented challenges. Several unconditional behavioral effects, including hyperirritability, tremors, and anorexia, were reported to occur during THC abstinence *(103)*, while other studies failed to observe abrupt withdrawal effects following chronic THC administration in dogs *(104)* or rats *(105,106)*. Abrupt withdrawal from chronic THC has been reported in rhesus monkeys *(107)*. The fact that readministration of THC reversed the withdrawal effects suggested that the animals were cannabinoid-dependent. The development of rimonabant *(70)*, a selective CB_1 receptor cannabinoid antagonist, represented the first opportunity to determine whether a physical withdrawal syndrome could be precipitated with an antagonist challenge. Antagonist-precipitated withdrawal is much easier and more reliable to quantitate than withdrawal following abrupt cessation of the dependence-producing drug. Indeed, a robust withdrawal syndrome was observed in THC-treated rats that were challenged with rimonabant *(108,109)*. Subsequent studies verified precipitated withdrawal in both mice *(110)* and dogs *(111)*. Another contribution of rimonabant was that it enabled investigators to carefully document the symptoms of withdrawal as well as the time course, both of which are critical for assessing abrupt withdrawal. Subsequently, Aceto et al. *(112)* were able to document abrupt withdrawal following cessation of infusion with the synthetic CB_1 receptor agonist WIN 55,212.

Although it was important to demonstrate that abrupt and precipitated withdrawal can be documented, most dependence-producing agents will also be self-administered by laboratory animals. Unfortunately, THC is not readily self-administered by animals. There was an early report that rats would self-administer THC *(113)*. However, it has not been an easy task to get rats to self-administer cannabinoids *(114)*. It has now been shown that THC can be reliably self-administered in squirrel monkeys *(115,116)*.

There is now increasing knowledge that the endocannabinoid system participates in dependence on drugs other than THC. There has always been considerable interest in the interactions of cannabinoids and opioids as it relates to dependence. Naloxone has been reported to precipitate withdrawal effects in rats treated chronically with THC *(117,118)*. Conversely, naloxone was ineffective in precipitating withdrawal in THC-dependent monkeys *(107)*, pigeons *(104)*, or mice *(119)*. It has long been known that THC produces a moderate attenuation of naloxone-precipitated withdrawal in morphine-dependent mice *(120,121)* and rats *(122,123)*. The endogenous cannabinoids anandamide *(124)* and 2-arachidonoylglycerol *(125)* have both been reported to decrease naloxone-induced morphine withdrawal.

Actually, the availability of mice lacking either μ-opioid or CB_1 receptors has greatly advanced our understanding of the interrelationship between the opioid and endocannabinoid systems. CB_1 receptor knockout mice exhibited substantial decreases in both morphine self-administration and naloxone-precipitated morphine withdrawal *(126)*. In addition, rimonabant reduced the rewarding responses of morphine in the conditioned place preference paradigm *(127)*. Co-administration of rimonabant and morphine led to decreases in naloxone-precipitated wet dog shakes and jumping but had no effects on other indices of opioid withdrawal, including paw tremors, ptosis, sniffing, and body tremors *(127)*. Repeated administration of rimonabant in rats

implanted with morphine pellets reduced some, but not all, naloxone precipitated withdrawal effects *(128)*.

The converse also appears to be true, in that opioid receptors may play a modulatory role on cannabinoid dependence. Rimonabant-precipitated THC withdrawal symptoms were significantly diminished in pre-proenkephalin-deficient mice compared to the wild-type mice *(129)*. Similarly, mice lacking the μ-opioid receptor exhibited significant attenuation of rimonabant-precipitated withdrawal signs compared with the wild-type controls. These findings implicate a role for opioid system in the modulation of cannabinoid dependence.

The finding that modulation of the endocannabinoid system is capable of influencing opioid dependence—and vice versa—raises the possibility that the CB_1 receptor antagonist might influence opioid dependence. Indeed, Navarro et al. *(130)* found that rimonabant was capable of blocking heroin self-administration in rats. Several other laboratories evaluated CB_1 receptor agonists and antagonists for their ability to influence reinstatement of heroin self-administration *(131,132)*. They found that several CB_1 receptor agonists restored heroin-seeking behavior, whereas rimonabant prevented reinstatement.

The question arises as to whether the endocannabinoid system is involved in dependence to drugs other than opioids. De Vries et al. *(133)* reported that the potent CB_1 receptor agonist HU210 provoked relapse to cocaine seeking after prolonged withdrawal periods. In addition, rimonabant attenuated relapse induced by re-exposure to cocaine-associated cues or cocaine itself, but not relapse induced by exposure to stress. On the other hand, another laboratory reported that a CB_1 receptor agonist attenuated the effects of cocaine on brain self-stimulation thresholds, whereas rimonabant did not alter cocaine's effects *(134)*. These findings suggest that the endocannabinoid system plays a greater role in relapse to cocaine use than in maintaining cocaine self-administration.

Another drug that is frequently used in conjunction with marijuana is alcohol. There are several indications that the endocannabinoid system may influence alcohol intake. It has been shown that rimonabant will decrease alcohol self-administration in laboratory animals *(135)* and that alcohol preference is reduced by rimonabant *(136)*. Also, alcohol withdrawal symptoms are absent in CB_1 receptor knockout mice, which provides further support for a role of the endocannabinoid system in alcohol dependence. Rimonabant has also been evaluated for its potential effects on the motivational effects of nicotine in the rat *(137)*. Rimonabant decreased nicotine self-administration but did not substitute for nicotine nor antagonize the nicotine cue in a nicotine-discrimination procedure. It also blocked nicotine-induced dopamine release in the shell of the nucleus accumbens and the bed nucleus of the stria terminalis *(137)*. Dopamine release induced by ethanol in the nucleus accumbens was also reduced by rimonabant.

The fact that the endocannabinoid system is an active participant in the dependence on a wide range of drugs argues that it may play a fundamental role in the perturbation of reward pathways that underlie drug dependence. These results suggest that activation of the endogenous cannabinoid system may participate in the motivational and dopamine-releasing effects of nicotine and ethanol as well as possibly other

drugs of abuse. Thus, CB_1 receptor antagonists may be effective in treating drug dependence induced by opioids, psychomotor stimulants, nicotine, and ethanol, in addition to marijuana.

7. Summary

Because the endocannabinoid system represents an important target for addressing symptoms arising from numerous disease states, the ability to manipulate this system becomes of paramount importance. At present, the only means of activating the endocannabinoid system is with CB_1 and CB_2 receptor agonists. The disadvantage of CB_1 receptor agonists is that they have a broad pharmacological spectrum of action that limits their clinical utility. Attempts to develop CB_1 receptor agonists that have improved the therapeutic-to-adverse effect ratio have met with limited success. However, the new evidence that is emerging regarding the multiple signaling pathways activated by the CB_1 receptor provides encouragement that development of agonists with improved pharmacological profile is possible. Moreover, structure–activity relationship studies continually provide new chemical templates for agents that activate the CB_1 receptor. In the near term, the most likely success will come from new formulations of current CB_1 receptor agonists that are already approved for clinical use.

As for selective CB_2 receptor agonists, there is intense interest in these compounds as potential therapeutic agents because they will be devoid of the behavioral effects that currently plague the CB_1 receptor agonists. The fact that selective CB_2 receptor agonists have been found to be effective in some animal models of pain provides an exciting possibility for development of new analgesics.

Efforts are also underway to develop inhibitors of the enzymes that degrade anandamide. Indeed, deletion of this enzyme in mice through genetic engineering resulted in elevated anandamide levels and increased resistance to pain *(39)*. Highly potent inhibitors of this enzyme have also been synthesized *(138)*. By elevating anandamide levels, these inhibitors represent an entirely new strategy for activating the endocannabinoid system. Elevation of 2-arachidonoylglycerol levels could occur through the blockade of monoglyceride lipase, the enzyme that metabolizes this endocannabinoid *(41)*. There are at present no selective inhibitors of this enzyme.

It is also abundantly clear that attenuating the endocannabinoid system has important therapeutic uses. The CB_1 receptor antagonist rimonabant has been shown to be effective in both animal models and clinical trials for treatment of decreased appetite and increased weight loss. Moreover, it has been shown to alter alcohol, cocaine, heroin, and nicotine dependence. Another potential means of attenuating the endocannabinoid system is through inhibition of the synthesis of anandamide and 2-arachidonolyglycerol. Although these enzymes have been identified, there are at present no inhibitors shown to have potential as therapeutic agents in, for example, obesity or drug dependence.

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