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Modulating the endocannabinoid system in human health and disease: successes and failures

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Abstract

The discovery of the endocannabinoid system (ECS; comprising of G-protein coupled cannabinoid 1 and 2 receptors, their endogenous lipid ligands or endocannabinoids, and synthetic and metabolizing enzymes, triggered an avalanche of experimental studies that have implicated the ECS in a growing number of physiological/pathological functions. They also suggested that modulating ECS activity holds therapeutic promise for a broad range of diseases, including neurodegenerative, cardiovascular and inflammatory disorders, obesity/metabolic syndrome, cachexia, chemotherapy-induced nausea and vomiting, tissue injury and pain, among others. However, clinical trials with globally acting CB₁ antagonists in obesity/metabolic syndrome, and other studies with peripherally restricted CB_{1/2} agonists and inhibitors of the endocannabinoid metabolizing enzyme in pain introduced unexpected complexities, and suggested that better understanding of the pathophysiological role of the ECS is required in order to devise clinically successful treatment strategies, which will be critically reviewed in this brief synopsis.

Keywords

endocannabinoid system; disease; cannabinoids; human; clinical trials; therapeutic potential; pharmacology

Introduction

Although *Cannabis sativa* (marijuana plant) is one of the most ancient medicinal plants in the history of medicine[1], the clinical use of synthetic cannabinoids or medicinal plant extracts have been largely empirical and limited to a few specific indications related to pain, wasting disorders, and chemotherapy-induced nausea and vomiting, because of their socially undesirable psychoactive properties[2]. The discovery of endocannabinoids (ECs), which mimic some of the effects of synthetic cannabinoids *in vivo*, their G-protein coupled receptors (GPCR) as well as their synthetic and metabolizing enzymes, has prompted preclinical studies to explore the role of the ECS in health and disease[2–4]. These studies have been greatly facilitated by the introduction of mice deficient in cannabinoid receptors or the EC degrading enzymes, as well as selective cannabinoid receptor ligands and inhibitors of EC metabolism. The results of these studies have implicated the ECS in a variety of physiopathological processes, both in the peripheral and central nervous systems and in various peripheral organs[2]. They further suggested that modulating ECS activity may have therapeutic potential in almost all diseases affecting humans, including obesity/metabolic syndrome[5], diabetes and diabetic complications[6], neurodegenerative[7,8],

inflammatory[9], cardiovascular[10–12], liver[13,14], gastrointestinal[15], skin[16] diseases, pain[17,18], psychiatric disorders[19,20], cachexia[2], cancer[21,22], chemotherapy-induced nausea and vomiting[23], among many others[2]).

These investigations have also uncovered the remarkable complexity of the ECS, as exemplified by differences in the therapeutic profile of activating/inhibiting the same receptor in the CNS or in peripheral tissues, by the intriguing overlap between EC and eicosanoid signaling, or by the often opposite effects mediated by CB₁ and CB₂ receptors in disease models[2–4,6,24]. Similar complexities have emerged in clinical trials targeting the ECS. While globally acting (i.e. brain-penetrant) CB₁ antagonists/inverse agonists had therapeutic efficacy in obesity/metabolic syndrome, they elicited anxiety/depression in a small proportion of subjects, which has led to their withdrawal from the market worldwide and halted their further therapeutic development[5,25,26]. The first human trial with peripherally restricted mixed CB_{1/2} agonist(s) for pain has failed because of cardiovascular and metabolic side effects and hepatotoxicity[27,28]. Amplifying ECS tone by inhibiting EC metabolism was ineffective in alleviating osteoarthritic pain in human subjects[29,30]. Thus, we need to better understand the pathophysiological function of the ECS in humans, and have to refine the indications and design of clinical trials in order to successfully translate recent progress in cannabinoid biology into clinically effective treatment strategies.

In this brief synopsis we will discuss preclinical evidence implicating the ECS in human disease, and review treatment strategies that target the ECS for therapeutic gain in humans. Because of space limitations, we will often refer readers to recent overviews on specific subjects instead of original papers.

The endocannabinoid system (ECS)

Δ9-tetrahydrocannabinol (THC), the putative psychoactive ingredient of marijuana, as well as its endogenous counterparts anandamide (arachidonoyl ethanolamide) and 2-arachidonoylglycerol (2-AG) exert their primary effects through cannabinoid 1 and 2 (CB_{1/2}) receptors; 2-AG favors CB₂, while AEA binds with higher affinity to CB₁[2], but at higher concentrations may also modulate TRPV₁ and other receptors. Signaling by cannabinoid receptors is complex, as it may involve both G protein-dependent pathways, such as inhibition of adenylyl cyclase or modulation of ion channel function, and G protein-independent mechanisms, including activation of various MAPKs (p44/42MAPKs, p38, ERK and JNK) or ceramide signaling[2,31,32].

CB₁ receptors, the most abundant GPCR in the mammalian brain, mediate the socially undesirable psychoactive effects of Cannabis. Although their expression was initially considered to be restricted to the brain, more recent studies identified CB₁ receptors in virtually all peripheral tissues and cell types, albeit at much lower densities than in brain, and documented their important regulatory functions[2,3,5]. CB₂ receptors are largely restricted to immune and hematopoietic cells, although functionally relevant expression has been found in specific regions of the brain and in myocardium, gut, endothelial, vascular smooth muscle and Kupffer cells, exocrine and endocrine pancreas, bone, reproductive organs/cells, and in various tumors[4]. Both cannabinoid receptors may undergo rapid internalization and intracellular trafficking upon agonist exposure[33,34].

In the CNS, AEA and 2-AG are synthesized “on demand” and released to act as retrograde transmitters on CB₁ receptors[35–37]. They are not stored and are rapidly degraded after exerting a transient and localized effect[38]. The synthesis of ECs largely depends on the intracellular Ca²⁺-concentration. AEA is mainly formed via a two step-pathway, involving a Ca²⁺-dependent N-acyltransferase and N-acylphosphatidylethanolamine-hydrolyzing phospholipase D (NAPE-PLD), while diacylglycerol lipase and phospholipase Cβ are

mainly responsible for the biosynthesis of 2-AG[3,37]. The existence of additional, parallel biosynthetic pathways for AEA has also been proposed[39,40].

AEA and 2-AG are removed from the extracellular space by a process of cellular uptake and metabolism; however the putative transporter(s) involved have not yet been cloned, and are subjects of much recent controversy[41–43]. AEA is degraded primarily by fatty acid amide hydrolase (FAAH) and 2-AG is degraded by monoacylglycerol lipase (MAGL)[3,44], although additional enzymes have also been implicated in the degradation of both AEA and 2-AG[45,46]. Endocannabinoids may also be metabolized by cyclooxygenases, lipoxygenases and cytochrome P450, leading to the formation of bioactive metabolites which may activate CB receptor-independent mechanisms[24,47]. It is also important to note that FAAH and MAGL are also responsible for the degradation of numerous potentially bioactive lipids. Thus, the biological consequences of the inhibition of these enzymes are not necessarily due to enhanced EC levels. Some of the enzymes involved in EC synthesis/ degradation may exist in several forms and their activity may vary in different tissues or even in different regions of the same tissue[3,37,48–52].

In addition to AEA and 2-AG, several other EC-like molecules have been discovered, but their activities have not been studied in sufficient detail[53,54]. Interestingly, recent studies have identified novel peptide allosteric negative modulators of CB₁ receptors[55], the biological significance of which is yet to be determined. Additionally, the anti-inflammatory lipid lipoxin A4 may be an endogenous allosteric enhancer of CB₁ receptors[56]. A comprehensive overview of the ECS is beyond the scope of this chapter; instead, the reader is referred to several detailed reviews on this subject[3,24,37,57].

The endocannabinoid system in health and disease

Despite the ubiquitous expression of the various components of the ECS, their genetic ablation or pharmacological blockade in normal, healthy animals has minimal functional consequences, which suggests that the ECS has minimal or no tonic activity under normal physiological conditions[2,4]. On the other hand, an increase or decrease in ECS tone is associated with various pathological states, as a result of altered expression of CB receptors, endocannabinoid metabolizing enzymes and/or synthetic pathways, in a tissue-specific and time-dependent manner. Examples of selected pathologies in which dysregulation of the ECS was reported (in most cases upregulation of CB_{1/2} and/or increase in tissue levels of ECs) are shown in table 1, and have been summarized in more detail elsewhere[2–4,58,59]. In some cases, altered ECS activity is transient and forms part of the body's compensatory response to a particular insult, thus reducing symptoms and/or slowing progression of the disease (e.g. in neuropathic pain); in other cases, activation of the ECS may be pathogenic (e.g. in various forms of shock or diabetic complications) or may reflect a deficiency (e.g. in various tumors), the significance of which is yet to be determined[2].

From a therapeutic standpoint, identification of regional or tissue-specific changes in CB receptors is important, because of their possible selective targeting may mitigate unwanted side effects [59,60]. However, these changes can serve as a basis of successful drug development only as long as they are determined using appropriate tools (e.g. specific antibodies), the specificity of which needs to be carefully validated[4,61]. It is also very important to understand the underlying mechanisms of these alterations; for example, is the increase in the tissue level of an EC due to its increased biosynthesis or a decrease in its enzymatic degradation?

Cardiovascular consequences of targeting the ECS in health and disease

Since many promising drugs fail in clinical development because of cardiovascular side effects, it is important to briefly overview the cardiovascular consequences of modulating the ECS. ECs exert complex cardiovascular effects dominated by a decrease in blood pressure and myocardial contractility, mediated primarily by CB₁ receptors located in the myocardium, vasculature, and neurons in the central and autonomic nervous systems[2,62]. In cultured human coronary artery endothelial cells[63] and cardiomyocytes[64], CB₁ activation promotes stress signaling and cell death, and decreases contractility [10,12]. In contrast, activation of cardiovascular CB₂ receptors does not have adverse hemodynamic consequences[11]. CB₁, CB₂ or FAAH knockout mice have normal blood pressure, myocardial contractility and/or baroreflex sensitivity, indicating the minimal role of the ECS in normal cardiovascular regulation[2]. However, in several pathological conditions (e.g. shock, heart failure, cardiomyopathies, advanced liver cirrhosis) the ECS may become activated to promote hypotension/cardiodepression through cardiovascular CB₁ receptors[2,10]). CB₁ receptor signaling may also promote disease progression in preclinical models of heart failure[64–66] and atherosclerosis[67,68], and contributes to increased cardiovascular risk (e.g. plasma lipid alterations, abdominal obesity, hepatic steatosis, insulin and leptin resistance) in obesity/metabolic syndrome and diabetes, both in rodents and humans[5,69–71]. In contrast, CB₂ signaling in the heart and vasculature may activate cardioprotective mechanisms and limit inflammation[11].

Acute or chronic use of marijuana may decrease or increase heart rate and decrease blood pressure depending on the duration of the use, dose and route of administration[2,10]. Elevated resting heart rate is a known independent risk factor for cardiovascular disease in healthy men and women[72]. A recent controlled study at the National Institute on Drug Abuse evaluated the development of tolerance to the effects of oral synthetic THC in 13 healthy male daily cannabis smokers residing on a secure research unit over a period of 6 days[73]. Despite the development of tolerance to the subjective intoxicating effect of THC, no tolerance was observed to its hypotensive and tachycardic effects[73]. Another recent study of 72 young cannabis user men and 72 matched controls found increased heart rate variability in cannabis users[74]. Surinabant, a selective CB₁ antagonist, has recently been reported to inhibit THC-induced central nervous system and heart rate effects in humans, providing proof of principle that those effects were indeed mediated by CB₁ receptor activation[75]. At the 20th ICRS meeting in Sweden, AstraZeneca presented data from the first clinical studies with two novel, peripherally restricted, orally active mixed CB_{1/2} agonists (AZD1940 & AZD1704). The study was terminated due to adverse cardiovascular effects, weight gain and mild hepatotoxicity[27,28].

An increasing number of case reports associates marijuana smoking with precipitation of acute coronary syndrome (ACS)[76]. Alarmingly, this occurs mostly in young healthy subjects without any prior cardiovascular disease[77,78]. A retrospective study assessed the risk of ACS after exposure to marijuana smoke. It was found that the risk of myocardial infarction was highest during the first hour of exposure[79]. The effect of marijuana use on mortality following acute myocardial infarction was assessed in a prospective study involving 1913 adults hospitalized with myocardial infarction at 45 US hospitals between 1989 and 1994, with a median follow-up of 3.8 years. The results indicated that marijuana use may pose increased risk of infarction in susceptible individuals with coronary heart disease[80]. A more recent study evaluated the consequences of marijuana use and long-term mortality among survivors of acute myocardial infarction, and found that habitual marijuana use among patients presenting with acute MI was associated with an apparent increase in mortality rate (29% higher) over the following 18 years, though this did not reach statistical significance because of the limited sample size[81]. In the absence of large

scale, long term controlled studies with repeated measures of marijuana use, a firm conclusion on the long term impact of cannabis use on cardiovascular mortality cannot be drawn. Nevertheless, the above findings are of concern. Because THC is a relatively weak CB₁ agonist compared to many synthetic ligands, also activates cardioprotective CB₂ receptors and is a potent antioxidant, one may predict that the uncontrolled spread and use of mixtures of potent synthetic CB₁ agonists (spice, K2, etc.) used as recreational drugs, would lead to significantly greater cardiovascular morbidity. Indeed, in a recent case series in healthy children, myocardial infarction was precipitated by synthetic cannabinoid use[82], and another paper reported tachycardia, loss of consciousness and diffuse pain in two adolescents[83].

What is the situation regarding the ECS and cardiovascular pathology? As mentioned before, EC/CB₁ receptor signaling has been implicated as a pathogenic factor in rodent models of cardiovascular diseases, including atherosclerosis, shock and various forms of cardiomyopathy. However, ECs were also reported to exert protective effects, based mostly on ex vivo and indirect studies, via CB₂ and CB-receptor independent mechanisms. Clearly, selective CB₂ agonists exert beneficial effects in rodent models of myocardial infarction by limiting inflammatory cell infiltration (in cardiomyocytes the expression of CB₂ is very low, if any)[11]. To analyze the role of the ECS more directly, a recent study employed FAAH knockout mice with a 2.5–3-fold increase in myocardial AEA content. When such mice were used to induce various experimental models of cardiomyopathy, they displayed increased mortality, tissue injury and neutrophil infiltration in the heart, which could be partially rescued by CB₁ antagonists[66]. Consistently with this report, a recent study showed that FAAH deficiency enhanced intraplaque neutrophil recruitment in atherosclerotic mice and increased a proinflammatory immune response[84]. These findings indicate that the primary cardiovascular effects of elevated EC tone are deleterious and are mediated by CB₁ receptors.

In obese human subjects, increased plasma levels of AEA and 2-AG were strongly associated with coronary circulatory dysfunction, suggesting that plasma EC levels may be used as biomarkers of cardiovascular risk in obesity[85]. In another study, increased plasma AEA and 2-AG levels positively correlated with impaired coronary endothelial function in obese subjects[86]. In samples of epicardial fat from ischemic human hearts, upregulation of CB₁ was accompanied by downregulation of CB₂ and FAAH, compared to non-ischemic hearts[87]. CB₁ receptor density was significantly higher in atherosclerotic coronary artery sections from patients with unstable angina compared to those with stable angina[67]. G1359A polymorphism in the CB₁ receptor gene was also associated with coronary artery disease in the Chinese Han population, although the effect of this polymorphism on receptor function is unknown[88]. Both ECs were reported to inhibit human cardiac Kv4.3 channels at fairly low concentrations in ovary cells expressing Kv4.3 or in human cardiomyocytes in a receptor independent manner[89], a harbinger of pro-arrhythmic risk.

Thus it is clear that activation of CB₁ receptors by synthetic ligands or ECs is associated with adverse cardiovascular consequences, which must be very carefully weighed during preclinical/clinical development of new drugs targeting the ECS.

Activation of CB_{1/2} receptors: THC, synthetic agonists and cannabinoid extracts

THC (Dronabinol; Marinol) and its synthetic analogue Nabilone (Cesamet) have been approved by the FDA for treatment of chemotherapy-induced nausea and vomiting and for stimulating appetite in wasting disorders (e.g. AIDS, tumor cachexia, etc). Sativex, an oromucosal spray containing THC and the non-psychoactive plant cannabinoid, cannabidiol,

has recently been approved in Canada, the UK and several other European countries for the symptomatic relief of neuropathic pain and spasticity associated with multiple sclerosis, and as adjunctive analgesic treatment for adults with advanced cancer. However, the therapeutic utility of THC and its synthetic analogs are limited by their unwanted psychotropic effects mediated by central CB₁ receptors. Here, we will summarize only the clinically most relevant indications.

Earlier preclinical studies suggested that ECs or plant-derived cannabinoids exert neuroprotective effects in the CNS by: 1) modulating excitability and calcium homeostasis via effects on various ion channels (Ca²⁺, Na⁺, K⁺), intracellular Ca²⁺ stores and gap junctions and *N*-methyl d-aspartate (NMDA) receptors; 2) attenuating excitatory glutamatergic transmissions and modulating synaptic plasticity via presynaptic CB₁ receptors; 3) inducing CB₁ receptor-mediated hypothermia; 4) exerting antioxidant effects; 5) modulating immune responses and the release of pro-inflammatory mediators by CB₁, CB₂, and non CB₁/CB₂ receptors on microglia, astrocytes, macrophages, neutrophils, lymphocytes and neurons[2]. Numerous recent studies have suggested that many of the previously described protective effects of synthetic CB₁ ligands were in fact attributable to centrally-mediated hypothermia and/or receptor-independent antioxidant/anti-inflammatory effects of the compounds, and ECs through the activation of CB₁ receptors may also promote tissue injury and neurodegeneration (for example in stroke and other forms of I/R injury)[6,90–92].

Historical documents reveal that one of the earliest uses of cannabis was to treat pain [93]. Studies in modern times initially focused on CB₁ receptors and demonstrated beneficial effects of cannabinoids in rodent models of acute and chronic pain. The results suggested that the observed antinociceptive effects have complex mechanisms involving actions in the CNS, spinal cord, and peripheral sensory nerves[2,94]. Recent evidence also implicates CB₂ receptors in the antihyperalgesic activity of cannabinoids[95,96], however the exact mechanisms and cellular targets are elusive because of the lack of reliable antibodies for CB₂[4].

In humans, the analgesic activity of THC and other cannabinoids is less clear-cut, as cannabinoids are relatively weak analgesics compared to opiates, even when they do show efficacy[2]. The clinical data on THC, CBD and their combinations have been comprehensively reviewed elsewhere[97,98]. The primary focus of these studies has been the safety/efficacy and symptom relief (e.g. bladder incontinence, limb spasticity, pain and sleep quality) in multiple sclerosis (MS) or other pain-related conditions. Three studies demonstrated that cannabis extract in MS patients improved urinary incontinence[98]. A number of controlled and blinded trials evaluating the efficacy of oral or sublingual cannabis/Sativex on spasticity in MS found that at doses that lack overt psychoactivity, these drugs show no or minimal efficacy, as assessed by the objective outcomes using the Ashworth Scale. However, the treatment consistently improved subjective, patient-assessed endpoints (spasms, pain, spasticity, sleep quality). Follow-up studies using a patient assessed Numeric Rating Scale for spasticity showed significant benefits of Sativex compared to placebo[98]. One could argue that some of the benefits observed could be due mood improvement (patients feel subjective improvement), but since only some of the symptoms were improved (spasticity, pain and sleep quality), this may not be the case. In patients treated with THC for one year, improvements using the Ashworth Scale were reported[98]. Zhornitsky and Potvin meta-analyzed the data of 33 studies with cannabidiol alone or in various combinations with THC, the rationale for combining THC and CBD being to attenuate the psychoactive effects of THC by CBD, based on empirical evidence obtained in some studies. Among these studies, 16 had been conducted in healthy subjects and 17 in clinical populations, including 4 in MS, 3 in neuropathic and cancer pain, 4 in schizophrenia

and bipolar mania, 2 in social anxiety disorder, and one each in cancer-related anorexia, Huntington's disease, insomnia, and epilepsy [97]. The authors concluded that depending on the study and on the THC/CBD ratio, CBD may prolong/intensify or inhibit THC-induced effects. In some of these studies THC or CBD+THC was more effective in reducing pain, but in others CBD alone also exerted (or completely lacked) analgesic properties. Notably, several of these studies used multiple pain assessment scores, and the treatments were effective when evaluated by some, but not by other scales[97]. In one of the studies in which oral administration of CBD+THC in MS was not effective in improving symptoms, immunological analysis surprisingly revealed a certain pro-inflammatory effect of the drug[97]. The authors also concluded that preliminary clinical evidence suggests that high-dose oral CBD may have therapeutic benefits in social anxiety disorder, insomnia and epilepsy, but may also cause mental sedation[97].

Taken together, the above mentioned studies in MS show consistent improvements in subjective rather than quantitative symptomatic outcome measures (including pain), which supports the beneficial effects of cannabinoid based medicines in neuropathic pain associated with MS. The relatively poor efficacy observed in some clinical studies may be attributable to pharmacokinetic problems such as first pass effects via liver and slow absorption via the oral route of administration, which may also limit the success of self-titration[98]. In most of these studies, formulations containing THC frequently caused generally mild to moderate side effects. However, with individual dose-titration, which can be better achieved by using the oromucosal Sativex spray, side effects can be further attenuated. Initial dose-titration may also help in select responders and excluding non-responders early. Future clinical studies should explore how cannabinoid-based medicines affect MS progression. In light of the preclinical data, the combination of THC with CBD appears to be the most promising, given the neuroprotective effects of CBD observed in numerous preclinical studies[99].

There is considerable interest in developing THC-based medicines for other forms of pain, such as pain associated with cancer or diabetic neuropathy. However, under these conditions we should also carefully weigh the potential effect of the treatment on cancer and/or diabetes progression. Regarding cancer, although numerous studies suggest that THC may slow down the growth/progression of certain types of cancers in preclinical models, others suggest that THC may in fact promote cancer growth, and cannabinoid receptor deletion or inhibition is beneficial[2,4,22]. In addition, results of a clinical study evaluating the association between ECS activity and survival and pain in pancreatic cancer indicate that although patients with high CB₁ receptor expression in enlarged nerves in pancreatic ductal adenocarcinoma had a lower combined pain score (intensity, frequency, duration), they had significantly shorter survival[100]. For CBD, the evidence more clearly suggests potential benefits in multiple preclinical tumor models[99]. In the case of diabetes and diabetic complications, there is strong evidence (both preclinical and clinical) that CB₁ activation promotes primary diabetes and also contributes to all diabetic complications (including neuropathy), and CB₁ antagonists can prevent or reverse these changes as well as insulin resistance[6,69,101].

Interestingly, analysis of cross-sectional data from the National Health and Nutrition Examination Survey (NHANES III, 1988–1994) indicated that marijuana use was independently associated with a lower prevalence of diabetes mellitus [102], and glucose tolerance and insulin sensitivity were found unchanged in chronic marijuana smokers [103]. In view of the demonstrated ability of acute marijuana smoking to induce insulin resistance[104], these findings may reflect desensitization of peripheral CB₁ receptors in chronic users. Further clinical studies are needed to analyze the differential mechanisms involved in the acute and chronic effects of marijuana use on glycemic control.

Nevertheless, in light of the overwhelming preclinical and clinical evidence suggesting that CB₁ receptor activation contributes to diabetes development and its complications (cardiovascular, neuropathy, retinopathy, and nephropathy)[6], and a recent study by Centers for Disease Control and Prevention (CDC) associating cases of acute kidney injury with synthetic cannabinoid use [105], the use of THC would be risky from a clinical point of view in patients with established diabetes. Diabetic patients also have impaired immune functions and wound healing, which could be adversely affected by immunosuppressive/immunomodulatory drugs such as THC. In contrast, CBD demonstrated beneficial effects due to its anti-inflammatory and antioxidant properties both in preclinical models of primary diabetes and in models of all major diabetic complications, which is encouraging for its potential testing in diabetic patients[6].

As mentioned above, THC and its synthetic analogue Nabilone are used to treat chemotherapy-induced nausea and vomiting and to stimulate appetite in cachexia associated with AIDS or terminal tumors[2]. In case of AIDS, recent controlled studies in non-human primates showed unexpectedly that chronic THC administration prior to and during simian immunodeficiency virus infection ameliorates disease progression, attenuates viral load and tissue inflammation, significantly reducing morbidity and mortality of virus-infected macaques[106], which is very encouraging.

There is considerable preclinical and clinical evidence that the combination of THC with opioids or non-steroidal anti-inflammatory drugs may enhance their efficacy in pain and also limit their side effects,[2,95,96]. Recently, it has become clear that cannabinoid analgesia is predominantly mediated via peripheral CB₁ receptors in nociceptors[107], providing the rationale for selectively targeting peripheral CB₁ receptors by peripherally restricted (brain impermeable) agonists, thereby eliminating the undesirable CNS consequences of CB₁ stimulation[71]. Astra Zeneca developed 2 novel peripherally restricted, orally bioavailable CB_{1/2} agonists (AZD1940 & AZD1704). Despite their mixed agonist activity at CB₁ and CB₂ receptors, analgesic efficacy in rodent models was mainly driven by CB₁ receptors, validated through the use of CB₁ selective antagonist and knockout mice[27]. The clinical efficacy of AZD1940 as a pain reliever was tested in two single-dose, phase II studies (human capsaicin and 3rd molar extraction models) and in a multiple ascending doses (MAD) study performed in subjects with chronic low-back pain. The 2 single-dose, phase II studies showed no efficacy at the primary endpoints (pain intensity and heat pain threshold for capsaicin study)[28]. In the multiple ascending dose study where AZD1940 was administered for 12 days, repeated dosing led to slow compound accumulation, significant weight gain and elevation of hepatic transaminases. AZD1704 also induced profound hypotensive effects[28]. Thus, the analgesic efficacy of peripherally restricted CB₁ agonists remains to be established in humans. Whereas their cardiovascular and metabolic side effects confirm the role of CB₁ receptors in these functions in humans, they further limit their usefulness as therapeutic agents. Whereas the above studies of Astra Zeneca with novel, peripherally restricted, orally bioavailable CB_{1/2} agonists did not indicate CB₂ involvement in preclinical models of analgesia, other studies suggest that CB₂ activation may attenuate certain types of pain[95,96]. CB₂-selective peripherally restricted agonists (instead of mixed CB_{1/2} agonists) may offer better optimization of dosing in humans, as metabolic and cardiovascular side effects are less likely to occur.

Inhibition of the CB₁ receptors: global and peripherally restricted CB₁ antagonists

Recent preclinical studies provided compelling evidence that ECs modulate food intake, energy balance, glucose and lipid metabolism through CB₁ receptors expressed in the brain and various peripheral tissues, such as fat, liver, and skeletal muscle[5,70,108,109].

Treatment with brain-penetrant CB₁ receptor antagonists/inverse agonists resulted in improvements of multiple cardiovascular risk factors both in preclinical studies and in clinical trials in obese/overweight subjects[110–116]. Parallel preclinical studies clearly demonstrated that reduced food intake was not the primary mechanism responsible for the weight reducing effect of CB₁ antagonists, and suggested that peripheral energy metabolism might be directly under EC control[5]. These studies demonstrated that ECs promote lipogenesis in adipose tissue and liver, but inhibit fatty acid oxidation and mitochondrial biogenesis, while CB₁ antagonists exert opposite effects[5]. Meanwhile, clinical trials revealed that a small but statistically significant fraction of subjects treated with the CB₁ inverse agonist rimonabant exhibited anxiety, depression and/or suicidal ideations, eventually leading to withdrawal of rimonabant from the market in over 50 countries and discontinuation of the therapeutic development of this class of compounds[117].

By that time, several lines of evidence strongly suggested that selective inhibition of peripheral CB₁ receptors may preserve much of the metabolic benefit of global CB₁ blockade while minimizing side effects due to blockade of CB₁ receptors in the CNS[5]. A proof of principle study by Tam et al.[118] demonstrated that chronic treatment of DIO mice with AM6545, the first high affinity, selective, peripherally restricted neutral CB₁ antagonist, improved glucose tolerance, insulin sensitivity, plasma lipid profile, and also reversed fatty liver, but was less effective than its parent compound rimonabant in reducing body weight, as it did not affect caloric intake. This study also provided evidence for the importance of CB₁ receptors in hepatocytes in the development of diet-induced insulin resistance. A subsequent study provided additional mechanistic insight by demonstrating that CB₁-mediated hepatic insulin resistance involves ER stress-dependent impairment of insulin signaling as well as reduced insulin clearance[119]. In a follow-up study a highly potent, selective, and brain impermeable CB₁ receptor inverse agonist, JD5037, was even more effective in improving metabolic parameters in mouse models of obesity, and it not only improved cardiometabolic risk but had antiobesity and hypophagic effects by reversing leptin resistance[101]. This compound is currently undergoing toxicology screening as a prelude to its clinical testing.

As discussed above, we have learned important lessons from the first clinical trials aiming to attenuate pain with the peripherally restricted mixed CB_{1/2} agonists, which were terminated because of the excessive weight gain, hepatotoxicity, and cardiovascular adverse effects. Interestingly, this side effect profile strongly supports the rationale for the development and therapeutic use of peripherally restricted CB₁ antagonists in humans[27,28].

Activation of CB₂ receptors by selective agonists

Overwhelming evidence for the therapeutic potential of EC/CB₂ receptor signaling in some of the major pathologies affecting humans have been reviewed recently[4]. An important consideration for the therapeutic development of selective CB₂ receptor agonists is the absence of psychoactive effects, coupled with anti-inflammatory and tissue protective activity of these ligands in numerous preclinical disease models[4].

CB₂ receptors are predominantly expressed in peripheral blood immune cells where the level of their expression is strongly modulated by pro-inflammatory and other stimuli, largely depending on the experimental conditions[120]. Initial studies focusing on the immunomodulatory effects of THC and other cannabinoid ligands *in vivo* in rodents and *in vitro* in human immune cell cultures demonstrated immunosuppressive effects in T and B lymphocytes, NK cells and macrophages, and most likely involved both CB₁ and CB₂ receptors as well as CB receptor-independent mechanisms[9,120,121]. ECs were also found to modulate T and B cell proliferation and apoptosis, immune cell activation and

inflammatory cytokine production, chemotaxis and inflammatory cell migration, and macrophage-mediated killing of sensitized cells[9,120,122]. These generally inhibitory effects were ligand- and cell type-dependent and were also influenced by the experimental conditions used[9,120,123,124]. A complicating factor is the agonist-induced rapid internalization and trafficking of CB₂ receptors in vitro, which can confound the interpretation of results[33,34]. The effects of ECs or synthetic analogs on microglia activation/migration also appear to be largely experimental condition-dependent[123].

An important recent development has been the identification of low levels of CB₂ receptor expression in tissues previously thought to be devoid of these receptors. These include specific regions of the brain[125–127], spinal cord and dorsal root ganglia[17,95,128], neurons in the myenteric and submucosal plexus of the enteric nervous system[129–131], in myocardium or cardiomyocytes[64,65,132], human vascular smooth muscle and endothelium[25,133–135], activated hepatic stellate cells[136,137], Kupffer cells[138], in reproductive organs/cells[139,140], colonic epithelial cells[141], bone[142–144], mouse and human exocrine and endocrine pancreas[145–148], and in various human tumors[149]. Further studies are needed to fully explore the function of CB₂ receptors at these sites.

More importantly, disease-induced changes – usually increases - in CB₂ receptor expression have been reported (Table 1), and synthetic CB₂ receptor agonists exerted protective effects in a variety of preclinical disease models and pathological conditions[4], ranging from cardiovascular disorders[11], various forms of ischemic-reperfusion injury[90], gastrointestinal and liver inflammation[13,150,151], autoimmune and neurodegenerative disorders[7,152–154], kidney[4] and bone disorders[143,144], cancer[149,155–157], and pain[17,95].

As for the therapeutic potential of CB₂ agonists, it is important to point out that while under conditions of a sterile inflammatory response CB₂ agonists may limit injury, in pathogen-induced inflammation the immunosuppressive effects of the CB₂ receptor activation may enhance or even inflict tissue damage, and may also lead to accelerated cancer growth in certain types of tumors, as reviewed recently[4]. In order to successfully target CB₂ in selected human diseases it is imperative to identify the exact cellular location and disease-induced, time-dependent changes in the expression of CB₂ receptors. This will necessitate the development of improved research tools, such as more reliable and specific antibodies. This is particularly important, because in many injury models CB₂ agonists appear to be most effective when given before the initiation of the insult, and may lose their efficacy or even promote inflammation when given at later time points[4]. Thus, a better understanding of the underlying pathology and its effects on CB₂ expression is required for the development of meaningful therapeutic approaches. Before going to clinical development for a particular indication, it is also important to confirm previous preclinical findings with novel and more selective CB₂ agonists, since currently available ligands may not be entirely specific. Better knowledge of the pharmacokinetics and metabolism of ligands is also essential, particularly given the bell-shaped dose-response often seen with recently available CB₂ agonists in various disease models[4]. The reason for the latter may be that, when used at higher doses, currently used CB₂ agonists may also activate CB₁ receptors, particularly when the relative expression of CB₁ over CB₂ is high. Our understanding of the complexities of CB₂ receptor signaling is still limited, and one must also consider important interspecies differences in CB₂ receptor signaling and in the pharmacology of CB₂ ligands[158].

Problems with the use of peripherally restricted CB_{1/2} agonists for pain relief due to cardiovascular and metabolic side effects have been discussed above. A plausible alternative could be the testing of peripherally restricted selective CB₂ agonists for analgesia in

humans, as such compounds would be expected to be devoid of cardiometabolic liabilities. However, the preclinical data with AZD1940 & AZD1704 indicate that the analgesic efficacy of this class of compounds was mainly driven by the CB₁ receptor[27] which, if confirmed in humans, would limit the promise of this approach. Nevertheless, the therapeutic development of selective CB₂ receptor ligands (agonists or inverse agonists/antagonists depending on the pathology and its stage) is still a promising strategy for a number of disease conditions, provided the issues discussed above are successfully resolved[4].

Inhibition of EC metabolism, cellular uptake or biosyntheses

The hypothesis behind the therapeutic inhibition of EC degradation was that increasing EC tissue levels would be less likely to cause psychoactive effects than would the use of synthetic CB₁ ligands (endocannabinoids are biosynthesized and degraded in a site and time-dependent manner), while the beneficial effects of CB_{1/2} activation, such as analgesia, would be maintained[159]. In support of this, FAAH knockout mice or mice treated with a FAAH inhibitor have elevated AEA levels in the brain and other tissues, are supersensitive to exogenous AEA and exhibit CB₁ receptor-mediated hypoalgesia[160,161] and reduced anxiety, but do not display catalepsy, a marker for psychoactivity in humans[162]. The antinociceptive effect of FAAH inhibitors, likely mediated through increases in AEA and PEA levels which activate CB_{1/2}, PPAR α , and/or TRPV1 [163], was investigated in acute and chronic rodent models of pain[164]. Most of the initial results were based on using URB597, which irreversibly inhibits FAAH both in the CNS and a periphery[164]. Recent studies with a peripherally restricted FAAH inhibitor, URB937, showed efficacy in neuropathic and inflammatory pain[165], confirming that the analgesic effects of AEA are initiated at the peripheral sites[107]. However, similar to direct acting peripheral CB_{1/2} agonists, URB597 has both hypotensive[166] and diabetogenic effects[167] mediated by CB₁ receptors, and FAAH knockout mice are also prone to diet-induced obesity and diabetes[168]. The diabetogenic effect of URB597 has been attributed to blocking FAAH in the liver, and the novel FAAH inhibitor AM3506, which does not block FAAH in the liver due to its rapid uptake and metabolism by hepatocytes, was found to be devoid of glycemic side effects in rodents[167]. FAAH antagonism may also promote fat accumulation and insulin resistance through centrally mediated hypothyroidism[169].

The analgesic effects of FAAH inhibition in preclinical models prompted the development of PF-04457845, an irreversible FAAH inhibitor with excellent analgesic efficacy in animal models[29,170], which was selected for clinical development. In a randomised, placebo-controlled, phase II clinical trial PF-04457845 was recently evaluated in patients with osteoarthritic pain of the knee[30]. The results clearly demonstrated that PF-04457845 inhibited FAAH activity in white blood cells and raised the concentrations of various fatty acid amides 3.5–10 fold, which persisted for up to 2 weeks after discontinuation of the drug, and did not affect cognitive functions in test subjects. However, the study failed to show any analgesic efficacy of PF-04457845, while the NSAID naproxen, used as positive control, was effective[30]. These results were also highlighted and discussed in a recent editorial[171].

A promising alternative indication for the therapeutic use of FAAH antagonists is post-traumatic stress syndrome (PTSD). The FAAH inhibitor AM3506 was recently found effective in increasing fear extinction in a CB₁ receptor-dependent manner in a mouse model of PTSD, and human carriers of a low-expressing FAAH variant displayed quicker habituation of amygdala reactivity to threat, as detected by brain imaging[172].

The main rationale for the development of MAGL inhibitors, which metabolize 2-AG, is similar to the rationale for FAAH inhibitors. Numerous recent studies demonstrated that MAGL inhibition or genetic deletion exerts antiemetic[173], antineoplastic[174], and anxiolytic and antinociceptive effects in rodents[175], protects against brain injury[176,177], acute liver injury/inflammation[138] and colitis either via enhancing CB_{1/2} signaling or by attenuating eicosanoid synthesis in specific tissues, such as the brain and the liver[178], or by the combination of both. In case of cancer, MAGL inhibition modulates fatty acid release for the synthesis of protumorigenic signaling lipids[174]), as reviewed recently[179,180].

While the above preclinical findings are indeed exciting, they also highlight important limitations. 1) Raising the tissue levels of ECs may promote the formation of cyclooxygenase-, lipoxygenase- and cytochrome P450-derived pro-inflammatory metabolites[47,181]. 2) Some of the prostaglandins which were attenuated by MAGL inhibitors have well documented tissue protective functions. 3) While the dual effect of MAGL inhibition on attenuating eicosanoid and enhancing EC signaling can be beneficial in certain tissues (e.g. brain and liver) where MAGL links the EC and eicosanoid systems through hydrolysis of 2-AG, in other tissues it can promote inflammation and injury (e.g. in the myocardium) through the non-CB mechanisms described above (the cardiotoxicity of COX-2 inhibitors is well documented in humans). 4) Chronic MAGL inhibition leads to functional antagonism of the ECS[175]. 5) As previously discussed, very strong preclinical and clinical evidence suggests that in cardiovascular disease and diabetes/diabetic complications endocannabinoids through CB₁ and most likely through the first two mechanisms described above promote cardiovascular injury. 6) There is growing evidence that ECs exert proinflammatory effects in various disease models through both CB₁-dependent and -independent mechanisms[6]. This is supported by a recent study demonstrating that inhibition of EC synthesis is anti-inflammatory in macrophages[182]; 7) Various isoforms of metabolizing enzymes (e.g. FAAH) may have distinct functions[52], and the functional properties of rodent and human FAAH may also be different[183]. 8) Most of the benefits observed with inhibitors of FAAH or MAGL were reported in acute models; the safety of chronic inhibition of these enzymes has not yet been determined, particularly in pathological situations. 9) The use of irreversible inhibitors of FAAH and MAGL could be a disadvantage for accurate dose titration and would make it difficult to treat toxicity[164].

Conclusions and future directions

Recent clinical studies provided evidence that cannabinoid based medicines with controlled doses of plant derived cannabinoids can provide symptomatic relief in a subset of patients suffering from pain and spasticity associated with MS and certain other types of pain, and there is hope based on preclinical studies that these medications would also positively modulate disease progression. Synthetic cannabinoids are also useful in subset of patients with wasting disorders and chemotherapy-induced nausea and vomiting. There are numerous promising new targets (plant-derived cannabinoids, peripherally restricted CB₁ antagonists, selective CB₂ agonists, inhibitors of endocannabinoid metabolism/transport) “in waiting” which have been reviewed here. However, it is clear that for the successful translation of preclinical findings to clinical practice, better understanding of the pathological role of the ECS in various diseases, of potential side effects of targeting this system, and of endocannabinoid pharmacology is required, coupled with the development of improved research tools to dissect these processes (see also Figure 1 and Table 2).

Future studies should focus on rigorous evaluation of the CB receptor dependent/ independent, and hypothermia-independent effects of THC in preclinical models (e.g. in

tissue injury, cancer, inflammation, etc.) using global and tissue/cell specific knockout mice and to identify potential novel targets/mechanisms of action of THC and other plant derived cannabinoid, coupled with identification of non-psychoactive constituents in cannabis extracts with potential therapeutic effects. Novel highly selective, orally available non-toxic cannabinoid ligands should be developed and evaluated in preclinical disease models. Large animal studies (e.g. canine, pig, primate) should confirm the efficacy of cannabinoid ligands obtained in rodent disease models before initiating human trials. Development of specific novel antibodies for CB_{1/2} receptors and endocannabinoid metabolic enzymes (FAAH, MAGL, DAGL α/β) validated by using positive and negative controls is essential to accurately assess the time-dependent changes in CB_{1/2} receptors and metabolic enzyme expressions in diseased animal and human tissues, in order to understand the human relevance of these changes. Our limited knowledge should be expanded in understanding the CB_{1/2} receptor trafficking, signaling and their interspecies differences. Development of reliable radio-ligands suitable for human imaging studies and research could contribute to our better understanding the role of ECS in human health and disease.

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List of abbreviations

| | |
|--------------------------------|--|
| 2-AG | 2-arachidonoylglycerol |
| AEA | anandamide or arachidonoyl ethanolamide |
| CB_{1/2} | cannabinoid receptor 1 or 2 |
| CBD | cannabidiol |
| EC(s) | endocannabinoid(s) |
| ECS | the endocannabinoid system |
| FAAH | fatty acid amide hydrolase |
| GPCR | G-protein coupled receptor |
| MAGL | monoacylglycerol lipase |
| MAPKs | mitogen-activated protein kinases |
| NAPE-PLD | N-acyltransferase and N-acylphosphatidylethanolamine-hydrolyzing phospholipase D |
| PPARα | peroxisome proliferator-activated receptor α |
| THC | Δ 9-tetrahydrocannabinol |
| TRPV₁ | transient receptor potential cation channel subfamily V member 1 |

References

1. Hanus LO. Pharmacological and therapeutic secrets of plant and brain (endo)cannabinoids. *Med Res Rev.* 2009; 29:213–71. [PubMed: 18777572]
2. Pacher P, Batkai S, Kunos G. The endocannabinoid system as an emerging target of pharmacotherapy. *Pharmacol Rev.* 2006; 58:389–462. [PubMed: 16968947]
3. Di Marzo V. Targeting the endocannabinoid system: to enhance or reduce? *Nat Rev Drug Discov.* 2008; 7:438–55. [PubMed: 18446159]

4. Pacher P, Mechoulam R. Is lipid signaling through cannabinoid 2 receptors part of a protective system? *Prog Lipid Res.* 2011; 50:193–211. [PubMed: 21295074]
5. Kunos G, Tam J. The case for peripheral CB(1) receptor blockade in the treatment of visceral obesity and its cardiometabolic complications. *British journal of pharmacology.* 2011; 163:1423–31. [PubMed: 21434882]
6. Horvath B, Mukhopadhyay P, Hasko G, Pacher P. The endocannabinoid system and plant-derived cannabinoids in diabetes and diabetic complications. *The American journal of pathology.* 2012; 180:432–42. [PubMed: 22155112]
7. Centonze D, Finazzi-Agro A, Bernardi G, Maccarrone M. The endocannabinoid system in targeting inflammatory neurodegenerative diseases. *Trends Pharmacol Sci.* 2007; 28:180–7. [PubMed: 17350694]
8. Skaper SD, Di Marzo V. Endocannabinoids in nervous system health and disease: the big picture in a nutshell. *Philosophical transactions of the Royal Society of London Series B, Biological sciences.* 2012; 367:3193–200.
9. Klein TW. Cannabinoid-based drugs as anti-inflammatory therapeutics. *Nat Rev Immunol.* 2005; 5:400–11. [PubMed: 15864274]
10. Pacher P, Mukhopadhyay P, Mohanraj R, Godlewski G, Batkai S, Kunos G. Modulation of the endocannabinoid system in cardiovascular disease: therapeutic potential and limitations. *Hypertension.* 2008; 52:601–7. [PubMed: 18779440]
11. Steffens S, Pacher P. Targeting cannabinoid receptor CB(2) in cardiovascular disorders: promises and controversies. *British journal of pharmacology.* 2012; 167:313–23. [PubMed: 22612332]
12. Montecucco F, Di Marzo V. At the heart of the matter: the endocannabinoid system in cardiovascular function and dysfunction. *Trends in pharmacological sciences.* 2012; 33:331–40. [PubMed: 22503477]
13. Lotersztajn S, et al. CB2 receptors as new therapeutic targets for liver diseases. *Br J Pharmacol.* 2008; 153:286–9. [PubMed: 17952109]
14. Tam J, Liu J, Mukhopadhyay B, Cinar R, Godlewski G, Kunos G. Endocannabinoids in liver disease. *Hepatology.* 2011; 53:346–55. [PubMed: 21254182]
15. Izzo AA, Camilleri M. Emerging role of cannabinoids in gastrointestinal and liver diseases: basic and clinical aspects. *Gut.* 2008; 57:1140–55. [PubMed: 18397936]
16. Biro T, Toth BI, Hasko G, Paus R, Pacher P. The endocannabinoid system of the skin in health and disease: novel perspectives and therapeutic opportunities. *Trends Pharmacol Sci.* 2009; 30:411–20. [PubMed: 19608284]
17. Guindon J, Hohmann AG. Cannabinoid CB2 receptors: a therapeutic target for the treatment of inflammatory and neuropathic pain. *Br J Pharmacol.* 2008; 153:319–34. [PubMed: 17994113]
18. Guindon J, Hohmann AG. The endocannabinoid system and pain. *CNS Neurol Disord Drug Targets.* 2009; 8:403–21. [PubMed: 19839937]
19. Mechoulam R, Parker LA. The Endocannabinoid System and the Brain. *Annual review of psychology.* 2012
20. Hillard CJ, Weinlander KM, Stuhr KL. Contributions of endocannabinoid signaling to psychiatric disorders in humans: genetic and biochemical evidence. *Neuroscience.* 2012; 204:207–29. [PubMed: 22123166]
21. Guindon J, Hohmann AG. The endocannabinoid system and cancer: therapeutic implication. *British journal of pharmacology.* 2011; 163:1447–63. [PubMed: 21410463]
22. Velasco G, Sanchez C, Guzman M. Towards the use of cannabinoids as antitumour agents. *Nature reviews Cancer.* 2012; 12:436–44.
23. Parker LA, Rock EM, Limebeer CL. Regulation of nausea and vomiting by cannabinoids. *British journal of pharmacology.* 2011; 163:1411–22. [PubMed: 21175589]
24. Piscitelli F, Di Marzo V. “Redundancy” of endocannabinoid inactivation: new challenges and opportunities for pain control. *ACS chemical neuroscience.* 2012; 3:356–63. [PubMed: 22860203]
25. Pacher P, Steffens S. The emerging role of the endocannabinoid system in cardiovascular disease. *Semin Immunopathol.* 2009; 31:63–77. [PubMed: 19357846]

26. Di Marzo V. Play an ADAGIO with a STRADIVARIUS: the right patient for CB1 receptor antagonists? *Nat Clin Pract Cardiovasc Med.* 2008; 5:610–2. [PubMed: 18695695]
27. Groblewski, T., et al. PRE-CLINICAL PHARMACOLOGICAL PROPERTIES OF NOVEL PERIPHERALLY-ACTING CB1-CB2 AGONISTS. Proceedings of 20th Annual Symposium of the International Cannabinoid Research Society; Lund, Sweden. 2010.
28. Groblewski, T., et al. PERIPHERALLY-ACTING CB1-CB2 AGONISTS FOR PAIN: DO THEY STILL HOLD PROMISE?. Proceedings of the 20th Annual Symposium of the International Cannabinoid Research Society; Lund, Sweden. 2010.
29. Li GL, Winter H, Arends R, Jay GW, Le V, Young T, Huggins JP. Assessment of the pharmacology and tolerability of PF-04457845, an irreversible inhibitor of fatty acid amide hydrolase-1, in healthy subjects. *British journal of clinical pharmacology.* 2012; 73:706–16. [PubMed: 22044402]
30. Huggins JP, Smart TS, Langman S, Taylor L, Young T. An efficient randomised, placebo-controlled clinical trial with the irreversible fatty acid amide hydrolase-1 inhibitor PF-04457845, which modulates endocannabinoids but fails to induce effective analgesia in patients with pain due to osteoarthritis of the knee. *Pain.* 2012; 153:1837–46. [PubMed: 22727500]
31. Howlett AC, et al. International Union of Pharmacology. XXVII. Classification of cannabinoid receptors. *Pharmacol Rev.* 2002; 54:161–202. [PubMed: 12037135]
32. Pertwee RG, et al. International Union of Basic and Clinical Pharmacology. LXXIX. Cannabinoid Receptors and Their Ligands: Beyond CB1 and CB2. *Pharmacol Rev.* 2010; 62:588–631. [PubMed: 21079038]
33. Atwood BK, Wager-Miller J, Haskins C, Straiker A, Mackie K. Functional Selectivity in CB2 Cannabinoid Receptor Signaling and Regulation: Implications for the Therapeutic Potential of CB2 Ligands. *Mol Pharmacol.* 2012; 81:250–63. [PubMed: 22064678]
34. Kleyer J, Nicolussi S, Taylor P, Simonelli D, Furger E, Anderle P, Gertsch J. Cannabinoid receptor trafficking in peripheral cells is dynamically regulated by a binary biochemical switch. *Biochemical pharmacology.* 2012; 83:1393–412. [PubMed: 22387618]
35. Devane WA, et al. Isolation and structure of a brain constituent that binds to the cannabinoid receptor. *Science.* 1992; 258:1946–9. [PubMed: 1470919]
36. Mechoulam R, et al. Identification of an endogenous 2-monoglyceride, present in canine gut, that binds to cannabinoid receptors. *Biochem Pharmacol.* 1995; 50:83–90. [PubMed: 7605349]
37. Wang J, Ueda N. Biology of endocannabinoid synthesis system. *Prostaglandins Other Lipid Mediat.* 2009; 89:112–9. [PubMed: 19126434]
38. Di Marzo V, Bifulco M, De Petrocellis L. The endocannabinoid system and its therapeutic exploitation. *Nat Rev Drug Discov.* 2004; 3:771–84. [PubMed: 15340387]
39. Liu J, et al. Multiple pathways involved in the biosynthesis of anandamide. *Neuropharmacology.* 2008; 54:1–7. [PubMed: 17631919]
40. Simon GM, Cravatt BF. Anandamide Biosynthesis Catalyzed by the Phosphodiesterase GDE1 and Detection of Glycerophospho-N-acyl Ethanolamine Precursors in Mouse Brain. *J Biol Chem.* 2008; 283:9341–9. [PubMed: 18227059]
41. Fowler CJ. Anandamide uptake explained? *Trends in pharmacological sciences.* 2012; 33:181–5. [PubMed: 22297258]
42. Piomelli D. Transport of endocannabinoids across the plasma membrane and within the cell. *FEBS Lett.* 2013
43. Kaczocha M, Vivieca S, Sun J, Glaser ST, Deutsch DG. Fatty acid-binding proteins transport N-acylethanolamines to nuclear receptors and are targets of endocannabinoid transport inhibitors. *The Journal of biological chemistry.* 2012; 287:3415–24. [PubMed: 22170058]
44. Cravatt BF, Lichtman AH. Fatty acid amide hydrolase: an emerging therapeutic target in the endocannabinoid system. *Curr Opin Chem Biol.* 2003; 7:469–75. [PubMed: 12941421]
45. Ueda N, Tsuboi K, Uyama T. N-acylethanolamine metabolism with special reference to N-acylethanolamine-hydrolyzing acid amidase (NAAA). *Progress in lipid research.* 2010; 49:299–315. [PubMed: 20152858]
46. Ueda N, Tsuboi K, Uyama T, Ohnishi T. Biosynthesis and degradation of the endocannabinoid 2-arachidonoylglycerol. *Biofactors.* 2011; 37:1–7. [PubMed: 21328621]

47. Rouzer CA, Marnett LJ. Endocannabinoid oxygenation by cyclooxygenases, lipoxygenases, and cytochromes P450: cross-talk between the eicosanoid and endocannabinoid signaling pathways. *Chemical reviews*. 2011; 111:5899–921. [PubMed: 21923193]
48. Cravatt BF, Lichtman AH. The enzymatic inactivation of the fatty acid amide class of signaling lipids. *Chem Phys Lipids*. 2002; 121:135–48. [PubMed: 12505696]
49. McKinney MK, Cravatt BF. Structure and function of fatty acid amide hydrolase. *Annu Rev Biochem*. 2005; 74:411–32. [PubMed: 15952893]
50. Fezza F, De Simone C, Amadio D, Maccarrone M. Fatty acid amide hydrolase: a gate-keeper of the endocannabinoid system. *Subcell Biochem*. 2008; 49:101–32. [PubMed: 18751909]
51. Palkovits M, Harvey-White J, Liu J, Kovacs ZS, Bobest M, Lovas G, Bago AG, Kunos G. Regional distribution and effects of postmortal delay on endocannabinoid content of the human brain. *Neuroscience*. 2008; 152:1032–9. [PubMed: 18343585]
52. Fu J, et al. A catalytically silent FAAH-1 variant drives anandamide transport in neurons. *Nature neuroscience*. 2012; 15:64–9.
53. Di Marzo V, De Petrocellis L. Endocannabinoids as regulators of transient receptor potential (TRP) channels: A further opportunity to develop new endocannabinoid-based therapeutic drugs. *Curr Med Chem*. 2010; 17:1430–49. [PubMed: 20166923]
54. Hanus LO, Mechoulam R. Novel natural and synthetic ligands of the endocannabinoid system. *Curr Med Chem*. 2010; 17:1341–59. [PubMed: 20166928]
55. Bauer M, et al. Identification and quantification of a new family of peptide endocannabinoids (Pepcans) showing negative allosteric modulation at CB1 receptors. *The Journal of biological chemistry*. 2012; 287:36944–67. [PubMed: 22952224]
56. Pamplona FA, et al. Anti-inflammatory lipoxin A4 is an endogenous allosteric enhancer of CB1 cannabinoid receptor. *Proceedings of the National Academy of Sciences of the United States of America*. 2012; 109:21134–9. [PubMed: 23150578]
57. Ueda N. Metabolism of endocannabinoids: old and new pathways. *FEBS Lett*. 2013
58. Di Marzo V. Endocannabinoids: synthesis and degradation. *Rev Physiol Biochem Pharmacol*. 2008; 160:1–24. [PubMed: 18481028]
59. Miller LK, Devi LA. The highs and lows of cannabinoid receptor expression in disease: mechanisms and their therapeutic implications. *Pharmacological reviews*. 2011; 63:461–70. [PubMed: 21752875]
60. Pertwee RG. Targeting the endocannabinoid system with cannabinoid receptor agonists: pharmacological strategies and therapeutic possibilities. *Philosophical transactions of the Royal Society of London. Series B, Biological sciences*. 2012; 367:3353–63.
61. Atwood BK, Mackie K. CB2: a cannabinoid receptor with an identity crisis. *Br J Pharmacol*. 2010; 160:467–79. [PubMed: 20590558]
62. Pacher P, Batkai S, Kunos G. Cardiovascular pharmacology of cannabinoids. *Handb Exp Pharmacol*. 2005:599–625. [PubMed: 16596789]
63. Rajesh M, Mukhopadhyay P, Hasko G, Liaudet L, Mackie K, Pacher P. Cannabinoid-1 receptor activation induces reactive oxygen species-dependent and -independent mitogen-activated protein kinase activation and cell death in human coronary artery endothelial cells. *British journal of pharmacology*. 2010; 160:688–700. [PubMed: 20590572]
64. Mukhopadhyay P, et al. CB1 cannabinoid receptors promote oxidative stress and cell death in murine models of doxorubicin-induced cardiomyopathy and in human cardiomyocytes. *Cardiovasc Res*. 2010; 85:773–84. [PubMed: 19942623]
65. Mukhopadhyay P, et al. Pharmacological inhibition of CB1 cannabinoid receptor protects against doxorubicin-induced cardiotoxicity. *J Am Coll Cardiol*. 2007; 50:528–36. [PubMed: 17678736]
66. Mukhopadhyay P, et al. Fatty acid amide hydrolase is a key regulator of endocannabinoid-induced myocardial tissue injury. *Free Radic Biol Med*. 2011
67. Sugamura K, et al. Activated endocannabinoid system in coronary artery disease and antiinflammatory effects of cannabinoid 1 receptor blockade on macrophages. *Circulation*. 2009; 119:28–36. [PubMed: 19103987]

68. Dol-Gleizes F, et al. Rimonabant, a selective cannabinoid CB1 receptor antagonist, inhibits atherosclerosis in LDL receptor-deficient mice. *Arterioscler Thromb Vasc Biol.* 2009; 29:12–8. [PubMed: 18845788]
69. Kunos G, Osei-Hyiaman D, Liu J, Godlewski G, Batkai S. Endocannabinoids and the control of energy homeostasis. *J Biol Chem.* 2008; 283:33021–5. [PubMed: 18694938]
70. Di Marzo V. The endocannabinoid system in obesity and type 2 diabetes. *Diabetologia.* 2008; 51:1356–67. [PubMed: 18563385]
71. Kunos G, Osei-Hyiaman D, Batkai S, Sharkey KA, Makriyannis A. Should peripheral CB(1) cannabinoid receptors be selectively targeted for therapeutic gain? *Trends Pharmacol Sci.* 2009; 30:1–7. [PubMed: 19042036]
72. Cooney MT, Vartiainen E, Laatikainen T, Juolevi A, Dudina A, Graham IM. Elevated resting heart rate is an independent risk factor for cardiovascular disease in healthy men and women. *American heart journal.* 2010; 159:612–619. e3. [PubMed: 20362720]
73. Gorelick DA, et al. Tolerance to Effects of High-Dose Oral {Delta}9- Tetrahydrocannabinol and Plasma Cannabinoid Concentrations in Male Daily Cannabis Smokers. *Journal of analytical toxicology.* 2012
74. Schmid K, Schonlebe J, Drexler H, Mueck-Weymann M. The effects of cannabis on heart rate variability and well-being in young men. *Pharmacopsychiatry.* 2010; 43:147–50. [PubMed: 20191442]
75. Klumbers LE, et al. Surinabant, a selective CB(1) antagonist, inhibits THC-induced central nervous system and heart rate effects in humans. *British journal of clinical pharmacology.* 2012
76. Singla S, Sachdeva R, Mehta JL. Cannabinoids and atherosclerotic coronary heart disease. *Clinical cardiology.* 2012; 35:329–35. [PubMed: 22278660]
77. Pratap B, Korniyenko A. Toxic effects of marijuana on the cardiovascular system. *Cardiovascular toxicology.* 2012; 12:143–8. [PubMed: 22194141]
78. Leblanc A, et al. Cannabis and myocardial infarction without angiographic stenosis in young patient: guilty or not guilty? A case report. *Annales de cardiologie et d'angiologie.* 2011; 60:154–8.
79. Mittleman MA, Lewis RA, Maclure M, Sherwood JB, Muller JE. Triggering myocardial infarction by marijuana. *Circulation.* 2001; 103:2805–9. [PubMed: 11401936]
80. Mukamal KJ, Maclure M, Muller JE, Mittleman MA. An exploratory prospective study of marijuana use and mortality following acute myocardial infarction. *American heart journal.* 2008; 155:465–70. [PubMed: 18294478]
81. Frost L, Mostofsky E, Rosenbloom JI, Mukamal KJ, Mittleman MA. Marijuana use and long-term mortality among survivors of acute myocardial infarction. *American heart journal.* 2013; 165:170–5. [PubMed: 23351819]
82. Mir A, Obafemi A, Young A, Kane C. Myocardial infarction associated with use of the synthetic cannabinoid K2. *Pediatrics.* 2011; 128:e1622–7. [PubMed: 22065271]
83. Heath TS, Burroughs Z, Thompson AJ, Tecklenburg FW. Acute intoxication caused by a synthetic cannabinoid in two adolescents. *The journal of pediatric pharmacology and therapeutics: JPPT: the official journal of PPAG.* 2012; 17:177–81.
84. Lenglet S, et al. Fatty Acid Amide Hydrolase Deficiency Enhances Intraplaque Neutrophil Recruitment in Atherosclerotic Mice. *Arteriosclerosis, thrombosis, and vascular biology.* 2012
85. Quercioli A, et al. Elevated endocannabinoid plasma levels are associated with coronary circulatory dysfunction in obesity. *Eur Heart J.* 2011; 32:1369–78. [PubMed: 21303779]
86. Quercioli A, et al. Coronary vasomotor control in obesity and morbid obesity: contrasting flow responses with endocannabinoids, leptin, and inflammation. *JACC Cardiovascular imaging.* 2012; 5:805–15. [PubMed: 22897994]
87. Cappellano G, et al. Different Expression and Function of the Endocannabinoid System in Human Epicardial Adipose Tissue in Relation to Heart Disease. *The Canadian journal of cardiology.* 2012
88. Liu R, Zhang Y. G1359A polymorphism in the cannabinoid receptor-1 gene is associated with coronary artery disease in the Chinese Han population. *Clinical laboratory.* 2011; 57:689–93. [PubMed: 22029183]

89. Amoros I, Barana A, Caballero R, Gomez R, Osuna L, Lillo MP, Tamargo J, Delpon E. Endocannabinoids and cannabinoid analogues block human cardiac Kv4.3 channels in a receptor-independent manner. *J Mol Cell Cardiol.* 48:201–10. [PubMed: 19616555]
90. Pacher P, Hasko G. Endocannabinoids and cannabinoid receptors in ischaemia-reperfusion injury and preconditioning. *Br J Pharmacol.* 2008; 153:252–62. [PubMed: 18026124]
91. Bisogno T, Di Marzo V. Cannabinoid Receptors and Endocannabinoids: Role in Neuroinflammatory and Neurodegenerative Disorders. *CNS Neurol Disord Drug Targets.* 2010
92. Fowler CJ, Rojo ML, Rodriguez-Gaztelumendi A. Modulation of the endocannabinoid system: neuroprotection or neurotoxicity? *Exp Neurol.* 2010; 224:37–47. [PubMed: 20353772]
93. Mechoulam R, Hanus L. A historical overview of chemical research on cannabinoids. *Chem Phys Lipids.* 2000; 108:1–13. [PubMed: 11106779]
94. Hohmann AG, Suplita RL 2nd. Endocannabinoid mechanisms of pain modulation. *AAPS J.* 2006; 8:E693–708. [PubMed: 17233533]
95. Anand P, Whiteside G, Fowler CJ, Hohmann AG. Targeting CB2 receptors and the endocannabinoid system for the treatment of pain. *Brain Res Rev.* 2009; 60:255–66. [PubMed: 19150370]
96. Rahn EJ, Hohmann AG. Cannabinoids as pharmacotherapies for neuropathic pain: from the bench to the bedside. *Neurotherapeutics.* 2009; 6:713–37. [PubMed: 19789075]
97. Zhornitsky S, Potvin S. Cannabidiol in humans—the quest for therapeutic targets. *Pharmaceuticals.* 2012; 5:529–552.
98. Baker AL, Thornton LK, Hides L, Dunlop A. Treatment of cannabis use among people with psychotic disorders: a critical review of randomised controlled trials. *Current pharmaceutical design.* 2012; 18:4923–37. [PubMed: 22716135]
99. Izzo AA, Borrelli F, Capasso R, Di Marzo V, Mechoulam R. Non-psychotropic plant cannabinoids: new therapeutic opportunities from an ancient herb. *Trends Pharmacol Sci.* 2009; 30:515–27. [PubMed: 19729208]
100. Michalski CW, et al. Cannabinoids in pancreatic cancer: correlation with survival and pain. *Int J Cancer.* 2008; 122:742–50. [PubMed: 17943729]
101. Tam J, et al. Peripheral cannabinoid-1 receptor inverse agonism reduces obesity by reversing leptin resistance. *Cell metabolism.* 2012; 16:167–79. [PubMed: 22841573]
102. Rajavashisth TB, Shaheen M, Norris KC, Pan D, Sinha SK, Ortega J, Friedman TC. Decreased prevalence of diabetes in marijuana users: cross-sectional data from the National Health and Nutrition Examination Survey (NHANES) III. *BMJ open.* 2012; 2:e000494.
103. Muniyappa R, et al. Metabolic effects of chronic cannabis smoking. *Diabetes Care.* 2013; 36:1–8. [PubMed: 23390628]
104. Hollister LE, Reaven GM. Delta-9-tetrahydrocannabinol and glucose tolerance. *Clin Pharmacol Ther.* 1974; 16:297–302. [PubMed: 4851289]
105. Acute kidney injury associated with synthetic cannabinoid use -multiple States, 2012. *MMWR Morbidity and mortality weekly report.* 2013; 62:93–8. [PubMed: 23407124]
106. Molina PE, et al. Cannabinoid administration attenuates the progression of simian immunodeficiency virus. *AIDS research and human retroviruses.* 2011; 27:585–92. [PubMed: 20874519]
107. Agarwal N, et al. Cannabinoids mediate analgesia largely via peripheral type 1 cannabinoid receptors in nociceptors. *Nat Neurosci.* 2007; 10:870–9. [PubMed: 17558404]
108. Osei-Hyiaman D, et al. Endocannabinoid activation at hepatic CB1 receptors stimulates fatty acid synthesis and contributes to diet-induced obesity. *J Clin Invest.* 2005; 115:1298–305. [PubMed: 15864349]
109. Pagotto U, Marsicano G, Cota D, Lutz B, Pasquali R. The emerging role of the endocannabinoid system in endocrine regulation and energy balance. *Endocr Rev.* 2006; 27:73–100. [PubMed: 16306385]
110. Despres JP, Golay A, Sjostrom L. Effects of rimonabant on metabolic risk factors in overweight patients with dyslipidemia. *N Engl J Med.* 2005; 353:2121–34. [PubMed: 16291982]

111. Van Gaal LF, Rissanen AM, Scheen AJ, Ziegler O, Rossner S. Effects of the cannabinoid-1 receptor blocker rimonabant on weight reduction and cardiovascular risk factors in overweight patients: 1-year experience from the RIO-Europe study. *Lancet*. 2005; 365:1389–97. [PubMed: 15836887]
112. Scheen AJ, Van Gaal LG, Despres JP, Pi-Sunyer X, Golay A, Hanotin C. Rimonabant improves cardiometabolic risk profile in obese or overweight subjects: overview of RIO studies. *Rev Med Suisse*. 2006; 2:1916–23. [PubMed: 16972542]
113. Despres JP, Ross R, Boka G, Almeras N, Lemieux I. Effect of rimonabant on the high-triglyceride/low-HDL-cholesterol dyslipidemia, intraabdominal adiposity, and liver fat: the ADAGIO-Lipids trial. *Arterioscler Thromb Vasc Biol*. 2009; 29:416–23. [PubMed: 19112166]
114. Nissen SE, et al. Effect of rimonabant on progression of atherosclerosis in patients with abdominal obesity and coronary artery disease: the STRADIVARIUS randomized controlled trial. *Jama*. 2008; 299:1547–60. [PubMed: 18387931]
115. Rosenstock J, Hollander P, Chevalier S, Iranmanesh A. SERENADE: the Study Evaluating Rimonabant Efficacy in Drug-naïve Diabetic Patients: effects of monotherapy with rimonabant, the first selective CB1 receptor antagonist, on glycemic control, body weight, and lipid profile in drug-naïve type 2 diabetes. *Diabetes Care*. 2008; 31:2169–76. [PubMed: 18678611]
116. Hollander PA, Amod A, Litwak LE, Chaudhari U. Effect of rimonabant on glycemic control in insulin-treated type 2 diabetes: the ARPEGGIO trial. *Diabetes Care*. 33:605–7. [PubMed: 20009090]
117. Di Marzo V, Despres JP. CB1 antagonists for obesity--what lessons have we learned from rimonabant? *Nat Rev Endocrinol*. 2009; 5:633–8. [PubMed: 19844251]
118. Tam J, et al. Peripheral CB1 cannabinoid receptor blockade improves cardiometabolic risk in mouse models of obesity. *The Journal of clinical investigation*. 2010; 120:2953–66. [PubMed: 20664173]
119. Liu J, et al. Hepatic cannabinoid receptor-1 mediates diet-induced insulin resistance via inhibition of insulin signaling and clearance in mice. *Gastroenterology*. 2012; 142:1218–1228. [PubMed: 22307032]
120. Klein TW, Newton C, Larsen K, Lu L, Perkins I, Nong L, Friedman H. The cannabinoid system and immune modulation. *J Leukoc Biol*. 2003; 74:486–96. [PubMed: 12960289]
121. Cabral GA, Staab A. Effects on the immune system. *Handb Exp Pharmacol*. 2005:385–423. [PubMed: 16596782]
122. Cencioni MT, Chiurchiu V, Catanzaro G, Borsellino G, Bernardi G, Battistini L, Maccarrone M. Anandamide suppresses proliferation and cytokine release from primary human T-lymphocytes mainly via CB2 receptors. *PLoS One*. 5:e8688. [PubMed: 20098669]
123. Miller AM, Stella N. CB2 receptor-mediated migration of immune cells: it can go either way. *Br J Pharmacol*. 2008; 153:299–308. [PubMed: 17982478]
124. Buckley NE. The peripheral cannabinoid receptor knockout mice: an update. *Br J Pharmacol*. 2008; 153:309–18. [PubMed: 17965741]
125. Van Sickle MD, et al. Identification and functional characterization of brainstem cannabinoid CB2 receptors. *Science*. 2005; 310:329–32. [PubMed: 16224028]
126. Onaivi ES. Neuropsychobiological evidence for the functional presence and expression of cannabinoid CB2 receptors in the brain. *Neuropsychobiology*. 2006; 54:231–46. [PubMed: 17356307]
127. Viscomi MT, Oddi S, Latini L, Pasquariello N, Florenzano F, Bernardi G, Molinari M, Maccarrone M. Selective CB2 receptor agonism protects central neurons from remote axotomy-induced apoptosis through the PI3K/Akt pathway. *J Neurosci*. 2009; 29:4564–70. [PubMed: 19357281]
128. Beltramo M. Cannabinoid type 2 receptor as a target for chronic -pain. *Mini Rev Med Chem*. 2009; 9:11–25. [PubMed: 19149657]
129. Wright KL, Duncan M, Sharkey KA. Cannabinoid CB2 receptors in the gastrointestinal tract: a regulatory system in states of inflammation. *Br J Pharmacol*. 2008; 153:263–70. [PubMed: 17906675]

130. Marquez L, Suarez J, Iglesias M, Bermudez-Silva FJ, Rodriguez de Fonseca F, Andreu M. Ulcerative colitis induces changes on the expression of the endocannabinoid system in the human colonic tissue. *PLoS One*. 2009; 4:e6893. [PubMed: 19730730]
131. Duncan M, et al. Cannabinoid CB2 receptors in the enteric nervous system modulate gastrointestinal contractility in lipopolysaccharide-treated rats. *Am J Physiol Gastrointest Liver Physiol*. 2008; 295:G78–G87. [PubMed: 18483180]
132. Bouchard JF, Lepicier P, Lamontagne D. Contribution of endocannabinoids in the endothelial protection afforded by ischemic preconditioning in the isolated rat heart. *Life Sci*. 2003; 72:1859–70. [PubMed: 12586223]
133. Rajesh M, Mukhopadhyay P, Hasko G, Huffman JW, Mackie K, Pacher P. CB2 cannabinoid receptor agonists attenuate TNF-alpha-induced human vascular smooth muscle cell proliferation and migration. *Br J Pharmacol*. 2008; 153:347–57. [PubMed: 17994109]
134. Rajesh M, et al. CB2-receptor stimulation attenuates TNF-alpha-induced human endothelial cell activation, transendothelial migration of monocytes, and monocyte-endothelial adhesion. *Am J Physiol Heart Circ Physiol*. 2007; 293:H2210–8. [PubMed: 17660390]
135. Rajesh M, et al. Cannabinoid-2 receptor agonist HU-308 protects against hepatic ischemia/reperfusion injury by attenuating oxidative stress, inflammatory response, and apoptosis. *J Leukoc Biol*. 2007; 82:1382–9. [PubMed: 17652447]
136. Julien B, et al. Antifibrogenic role of the cannabinoid receptor CB2 in the liver. *Gastroenterology*. 2005; 128:742–55. [PubMed: 15765409]
137. Mallat A, Lotersztajn S. Endocannabinoids and liver disease. I. Endocannabinoids and their receptors in the liver. *Am J Physiol Gastrointest Liver Physiol*. 2008; 294:G9–G12. [PubMed: 17975129]
138. Cao Z, et al. Monoacylglycerol Lipase Controls Endocannabinoid and Eicosanoid Signaling and Hepatic Injury in Mice. *Gastroenterology*. 2013 in press.
139. Wang H, Dey SK, Maccarrone M. Jekyll and hyde: two faces of cannabinoid signaling in male and female fertility. *Endocr Rev*. 2006; 27:427–48. [PubMed: 16682502]
140. Maccarrone M. Endocannabinoids: friends and foes of reproduction. *Prog Lipid Res*. 2009; 48:344–54. [PubMed: 19602425]
141. Rousseaux C, et al. *Lactobacillus acidophilus* modulates intestinal pain and induces opioid and cannabinoid receptors. *Nat Med*. 2007; 13:35–7. [PubMed: 17159985]
142. Bab I, Ofek O, Tam J, Rehnelt J, Zimmer A. Endocannabinoids and the regulation of bone metabolism. *J Neuroendocrinol*. 2008; 20(Suppl 1):69–74. [PubMed: 18426503]
143. Bab I, Zimmer A. Cannabinoid receptors and the regulation of bone mass. *Br J Pharmacol*. 2008; 153:182–8. [PubMed: 18071301]
144. Bab I, Zimmer A, Melamed E. Cannabinoids and the skeleton: from marijuana to reversal of bone loss. *Ann Med*. 2009; 41:560–7. [PubMed: 19634029]
145. Michalski CW, et al. Cannabinoids ameliorate pain and reduce disease pathology in cerulein-induced acute pancreatitis. *Gastroenterology*. 2007; 132:1968–78. [PubMed: 17484889]
146. Michalski CW, et al. Cannabinoids reduce markers of inflammation and fibrosis in pancreatic stellate cells. *PLoS One*. 2008; 3:e1701. [PubMed: 18301776]
147. Bermudez-Silva FJ, et al. Presence of functional cannabinoid receptors in human endocrine pancreas. *Diabetologia*. 2008; 51:476–87. [PubMed: 18092149]
148. Petrella C, et al. Cannabinoid agonist WIN55,212 in vitro inhibits interleukin-6 (IL-6) and monocyte chemo-attractant protein-1 (MCP-1) release by rat pancreatic acini and in vivo induces dual effects on the course of acute pancreatitis. *Neurogastroenterol Motil*. 2010
149. Guzman M. Cannabinoids: potential anticancer agents. *Nat Rev Cancer*. 2003; 3:745–55. [PubMed: 14570037]
150. Izzo AA, Camilleri M. Cannabinoids in intestinal inflammation and cancer. *Pharmacol Res*. 2009; 60:117–25. [PubMed: 19442536]
151. Izzo AA, Sharkey KA. Cannabinoids and the gut: new developments and emerging concepts. *Pharmacol Ther*. 2010; 126:21–38. [PubMed: 20117132]

152. Fernandez-Ruiz J, Romero J, Velasco G, Tolon RM, Ramos JA, Guzman M. Cannabinoid CB2 receptor: a new target for controlling neural cell survival? *Trends Pharmacol Sci.* 2007; 28:39–45. [PubMed: 17141334]
153. Cabral GA, Raborn ES, Griffin L, Dennis J, Marciano-Cabral F. CB2 receptors in the brain: role in central immune function. *Br J Pharmacol.* 2008; 153:240–51. [PubMed: 18037916]
154. Fernandez-Ruiz J. The endocannabinoid system as a target for the treatment of motor dysfunction. *Br J Pharmacol.* 2009; 156:1029–40. [PubMed: 19220290]
155. Pisanti S, Bifulco M. Endocannabinoid system modulation in cancer biology and therapy. *Pharmacol Res.* 2009; 60:107–16. [PubMed: 19559362]
156. Stella N. Cannabinoid and cannabinoid-like receptors in microglia, astrocytes, and astrocytomas. *Glia.* 2010; 58:1017–30. [PubMed: 20468046]
157. Fowler CJ, Gustafsson SB, Chung SC, Persson E, Jacobsson SO, Bergh A. Targeting the endocannabinoid system for the treatment of cancer—a practical view. *Curr Top Med Chem.* 2010; 10:814–27. [PubMed: 20370711]
158. Ndong C, O'Donnell D, Ahmad S, Groblewski T. Cloning and pharmacological characterization of the dog cannabinoid CB(2)receptor. *European journal of pharmacology.* 2011; 669:24–31. [PubMed: 21871882]
159. Makriyannis A, Mechoulam R, Piomelli D. Therapeutic opportunities through modulation of the endocannabinoid system. *Neuropharmacology.* 2005; 48:1068–71. [PubMed: 15885714]
160. Cravatt BF, Demarest K, Patricelli MP, Bracey MH, Giang DK, Martin BR, Lichtman AH. Supersensitivity to anandamide and enhanced endogenous cannabinoid signaling in mice lacking fatty acid amide hydrolase. *Proc Natl Acad Sci U S A.* 2001; 98:9371–6. [PubMed: 11470906]
161. Lichtman AH, Shelton CC, Advani T, Cravatt BF. Mice lacking fatty acid amide hydrolase exhibit a cannabinoid receptor-mediated phenotypic hypoalgesia. *Pain.* 2004; 109:319–27. [PubMed: 15157693]
162. Kathuria S, et al. Modulation of anxiety through blockade of anandamide hydrolysis. *Nat Med.* 2003; 9:76–81. [PubMed: 12461523]
163. Starowicz K, Makuch W, Osikowicz M, Piscitelli F, Petrosino S, Di Marzo V, Przewlocka B. Spinal anandamide produces analgesia in neuropathic rats: possible CB(1)-and TRPV1-mediated mechanisms. *Neuropharmacology.* 2012; 62:1746–55. [PubMed: 22178705]
164. Roques BP, Fournie-Zaluski MC, Wurm M. Inhibiting the breakdown of endogenous opioids and cannabinoids to alleviate pain. *Nature reviews Drug discovery.* 2012; 11:292–310.
165. Clapper JR, et al. Anandamide suppresses pain initiation through a peripheral endocannabinoid mechanism. *Nature neuroscience.* 2010; 13:1265–70.
166. Batkai S, et al. Endocannabinoids acting at cannabinoid-1 receptors regulate cardiovascular function in hypertension. *Circulation.* 2004; 110:1996–2002. [PubMed: 15451779]
167. Godlewski G, et al. Inhibitor of fatty acid amide hydrolase normalizes cardiovascular function in hypertension without adverse metabolic effects. *Chemistry & biology.* 2010; 17:1256–66. [PubMed: 21095576]
168. Tourino C, Oveisi F, Lockney J, Piomelli D, Maldonado R. FAAH deficiency promotes energy storage and enhances the motivation for food. *Int J Obes (Lond).* 34:557–68. [PubMed: 20029375]
169. Brown WH, et al. Fatty acid amide hydrolase ablation promotes ectopic lipid storage and insulin resistance due to centrally mediated hypothroidism. *Proceedings of the National Academy of Sciences of the United States of America.* 2012; 109:14966–71. [PubMed: 22912404]
170. Ahn K, et al. Mechanistic and pharmacological characterization of PF-04457845: a highly potent and selective fatty acid amide hydrolase inhibitor that reduces inflammatory and noninflammatory pain. *The Journal of pharmacology and experimental therapeutics.* 2011; 338:114–24. [PubMed: 21505060]
171. Di Marzo V. Inhibitors of endocannabinoid breakdown for pain: not so FA(AH)cile, after all. *Pain.* 2012; 153:1785–6. [PubMed: 22785079]
172. Gunduz-Cinar O, et al. Convergent translational evidence of a role for anandamide in amygdala-mediated fear extinction, threat processing and stress-reactivity. *Molecular psychiatry.* 2012

173. Sticht MA, Long JZ, Rock EM, Limebeer CL, Mechoulam R, Cravatt BF, Parker LA. Inhibition of monoacylglycerol lipase attenuates vomiting in *Suncus murinus* and 2-arachidonoyl glycerol attenuates nausea in rats. *British journal of pharmacology*. 2012; 165:2425–35. [PubMed: 21470205]
174. Nomura DK, Long JZ, Niessen S, Hoover HS, Ng SW, Cravatt BF. Monoacylglycerol lipase regulates a fatty acid network that promotes cancer pathogenesis. *Cell*. 2010; 140:49–61. [PubMed: 20079333]
175. Schlosburg JE, et al. Chronic monoacylglycerol lipase blockade causes functional antagonism of the endocannabinoid system. *Nat Neurosci*.
176. Piro JR, et al. A dysregulated endocannabinoid-eicosanoid network supports pathogenesis in a mouse model of Alzheimer's disease. *Cell reports*. 2012; 1:617–23. [PubMed: 22813736]
177. Carloni S, et al. Pretreatment with the monoacylglycerol lipase inhibitor URB602 protects from the long-term consequences of neonatal hypoxic-ischemic brain injury in rats. *Pediatric research*. 2012; 72:400–6. [PubMed: 22821058]
178. Nomura DK, et al. Endocannabinoid hydrolysis generates brain prostaglandins that promote neuroinflammation. *Science*. 2011; 334:809–13. [PubMed: 22021672]
179. Mulvihill MM, Nomura DK. Therapeutic potential of monoacylglycerol lipase inhibitors. *Life sciences*. 2012
180. Fowler CJ. Monoacylglycerol lipase -a target for drug development? *British journal of pharmacology*. 2012; 166:1568–85. [PubMed: 22428756]
181. Gatta L, Piscitelli F, Giordano C, Boccella S, Lichtman A, Maione S, Di Marzo V. Discovery of prostamide F2alpha and its role in inflammatory pain and dorsal horn nociceptive neuron hyperexcitability. *PLoS One*. 2012; 7:e31111. [PubMed: 22363560]
182. Hsu KL, Tsuboi K, Adibekian A, Pugh H, Masuda K, Cravatt BF. DAGLbeta inhibition perturbs a lipid network involved in macrophage inflammatory responses. *Nature chemical biology*. 2012; 8:999–1007.
183. Di Venere A, et al. Rat and human fatty acid amide hydrolases: overt similarities and hidden differences. *Biochimica et biophysica acta*. 2012; 1821:1425–33. [PubMed: 22877990]
184. Di Filippo C, Rossi F, Rossi S, D'Amico M. Cannabinoid CB2 receptor activation reduces mouse myocardial ischemia-reperfusion injury: involvement of cytokine/chemokines and PMN. *J Leukoc Biol*. 2004; 75:453–9. [PubMed: 14657208]
185. Montecucco F, Lenglet S, Braunersreuther V, Burger F, Pelli G, Bertolotto M, Mach F, Steffens S. CB(2) cannabinoid receptor activation is cardioprotective in a mouse model of ischemia/reperfusion. *J Mol Cell Cardiol*. 2009; 46:612–20. [PubMed: 19162037]
186. Defer N, et al. The cannabinoid receptor type 2 promotes cardiac myocyte and fibroblast survival and protects against ischemia/reperfusion-induced cardiomyopathy. *FASEB J*. 2009; 23:2120–30. [PubMed: 19246487]
187. Lamontagne D, Lepicier P, Lagneux C, Bouchard JF. The endogenous cardiac cannabinoid system: a new protective mechanism against myocardial ischemia. *Arch Mal Coeur Vaiss*. 2006; 99:242–6. [PubMed: 16618028]
188. Weis F, Beiras-Fernandez A, Sodian R, Kaczmarek I, Reichart B, Beiras A, Schelling G, Kreth S. Substantially altered expression pattern of cannabinoid receptor 2 and activated endocannabinoid system in patients with severe heart failure. *J Mol Cell Cardiol*. 2010; 48:1187–93. [PubMed: 19931541]
189. Batkai S, et al. Endocannabinoids acting at vascular CB1 receptors mediate the vasodilated state in advanced liver cirrhosis. *Nat Med*. 2001; 7:827–32. [PubMed: 11433348]
190. Moezi L, Gaskari SA, Lee SS. Endocannabinoids and liver disease. V. endocannabinoids as mediators of vascular and cardiac abnormalities in cirrhosis. *Am J Physiol Gastrointest Liver Physiol*. 2008; 295:G649–53. [PubMed: 18703639]
191. Batkai S, Mukhopadhyay P, Harvey-White J, Kechrid R, Pacher P, Kunos G. Endocannabinoids acting at CB1 receptors mediate the cardiac contractile dysfunction in vivo in cirrhotic rats. *Am J Physiol Heart Circ Physiol*. 2007; 293:H1689–95. [PubMed: 17557913]
192. Batkai S, Pacher P. Endocannabinoids and cardiac contractile function: pathophysiological implications. *Pharmacol Res*. 2009; 60:99–106. [PubMed: 19569260]

193. Montecucco F, et al. Regulation and possible role of endocannabinoids and related mediators in hypercholesterolemic mice with atherosclerosis. *Atherosclerosis*. 2009; 205:433–41. [PubMed: 19187936]
194. Mach F, Steffens S. The role of the endocannabinoid system in atherosclerosis. *J Neuroendocrinol*. 2008; 20(Suppl 1):53–7. [PubMed: 18426500]
195. Steffens S, et al. Low dose oral cannabinoid therapy reduces progression of atherosclerosis in mice. *Nature*. 2005; 434:782–6. [PubMed: 15815632]
196. Montecucco F, Burger F, Mach F, Steffens S. CB2 cannabinoid receptor agonist JWH-015 modulates human monocyte migration through defined intracellular signaling pathways. *Am J Physiol Heart Circ Physiol*. 2008; 294:H1145–55. [PubMed: 18178718]
197. Pacher P, Ungvari Z. Pleiotropic effects of the CB2 cannabinoid receptor activation on human monocyte migration: implications for atherosclerosis and inflammatory diseases. *Am J Physiol Heart Circ Physiol*. 2008; 294:H1133–4. [PubMed: 18203843]
198. Montecucco F, et al. The activation of the cannabinoid receptor type 2 reduces neutrophilic protease-mediated vulnerability in atherosclerotic plaques. *European heart journal*. 2012; 33:846–56. [PubMed: 22112961]
199. Naccarato M, Pizzuti D, Petrosino S, Simonetto M, Ferigo L, Grandi FC, Pizzolato G, Di Marzo V. Possible Anandamide and Palmitoylethanolamide involvement in human stroke. *Lipids Health Dis*. 9:47. [PubMed: 20470384]
200. Hillard CJ. Role of cannabinoids and endocannabinoids in cerebral ischemia. *Curr Pharm Des*. 2008; 14:2347–61. [PubMed: 18781985]
201. Muthian S, Rademacher DJ, Roelke CT, Gross GJ, Hillard CJ. Anandamide content is increased and CB1 cannabinoid receptor blockade is protective during transient, focal cerebral ischemia. *Neuroscience*. 2004; 129:743–50. [PubMed: 15541895]
202. Zhang M, Adler MW, Abood ME, Ganea D, Jallo J, Tuma RF. CB2 receptor activation attenuates microcirculatory dysfunction during cerebral ischemic/reperfusion injury. *Microvasc Res*. 2009; 78:86–94. [PubMed: 19332079]
203. Zhang M, Martin BR, Adler MW, Razdan RK, Jallo JI, Tuma RF. Cannabinoid CB(2) receptor activation decreases cerebral infarction in a mouse focal ischemia/reperfusion model. *J Cereb Blood Flow Metab*. 2007; 27:1387–96. [PubMed: 17245417]
204. Zhang M, Martin BR, Adler MW, Razdan RK, Ganea D, Tuma RF. Modulation of the balance between cannabinoid CB(1) and CB(2) receptor activation during cerebral ischemic/reperfusion injury. *Neuroscience*. 2008; 152:753–60. [PubMed: 18304750]
205. Baty DE, et al. Cannabinoid CB2 receptor activation attenuates motor and autonomic function deficits in a mouse model of spinal cord injury. *Clin Neurosurg*. 2008; 55:172–7. [PubMed: 19248685]
206. Murikinati S, et al. Activation of cannabinoid 2 receptors protects against cerebral ischemia by inhibiting neutrophil recruitment. *FASEB J*. 2010; 24:788–98. [PubMed: 19884325]
207. Kohro S, Imaizumi H, Yamakage M, Masuda Y, Namiki A, Asai Y. Reductions in levels of bacterial superantigens/cannabinoids by plasma exchange in a patient with severe toxic shock syndrome. *Anaesth Intensive Care*. 2004; 32:588–91. [PubMed: 15675223]
208. Kohro S, Imaizumi H, Yamakage M, Masuda Y, Namiki A, Asai Y, Maruyama I. Anandamide absorption by direct hemoperfusion with polymixin B-immobilized fiber improves the prognosis and organ failure assessment score in patients with sepsis. *J Anesth*. 2006; 20:11–6. [PubMed: 16421670]
209. Csoka B, et al. CB2 cannabinoid receptors contribute to bacterial invasion and mortality in polymicrobial sepsis. *PLoS One*. 2009; 4:e6409. [PubMed: 19641602]
210. Tschop J, et al. The cannabinoid receptor 2 is critical for the host response to sepsis. *J Immunol*. 2009; 183:499–505. [PubMed: 19525393]
211. Kurabayashi M, Takeyoshi I, Yoshinari D, Matsumoto K, Maruyama I, Morishita Y. 2-Arachidonoylglycerol increases in ischemia-reperfusion injury of the rat liver. *J Invest Surg*. 2005; 18:25–31. [PubMed: 15804949]
212. Batkai S, et al. Cannabinoid-2 receptor mediates protection against hepatic ischemia/reperfusion injury. *Faseb J*. 2007; 21:1788–800. [PubMed: 17327359]

213. Ishii Y, Sakamoto T, Ito R, Yanaga K. F2-isoprostanes and 2-arachidonylglycerol as biomarkers of lipid peroxidation in pigs with hepatic ischemia/reperfusion injury. *J Surg Res.* 2010; 161:139–45. [PubMed: 19439322]
214. Mendez-Sanchez N, et al. Endocannabinoid receptor CB2 in nonalcoholic fatty liver disease. *Liver Int.* 2007; 27:215–9. [PubMed: 17311616]
215. Deveaux V, et al. Cannabinoid CB2 receptor potentiates obesity-associated inflammation, insulin resistance and hepatic steatosis. *PLoS One.* 2009; 4:e5844. [PubMed: 19513120]
216. Agudo J, Martin M, Roca C, Molas M, Bura AS, Zimmer A, Bosch F, Maldonado R. Deficiency of CB2 cannabinoid receptor in mice improves insulin sensitivity but increases food intake and obesity with age. *Diabetologia.* 2010; 53:2629–40. [PubMed: 20835701]
217. Rajesh M, et al. Cannabinoid 1 receptor promotes cardiac dysfunction, oxidative stress, inflammation, and fibrosis in diabetic cardiomyopathy. *Diabetes.* 2012; 61:716–27. [PubMed: 22315315]
218. Cote M, Matias I, Lemieux I, Petrosino S, Almeras N, Despres JP, Di Marzo V. Circulating endocannabinoid levels, abdominal adiposity and related cardiometabolic risk factors in obese men. *Int J Obes (Lond).* 2007; 31:692–9. [PubMed: 17224929]
219. Barutta F, et al. Protective role of cannabinoid receptor type 2 in a mouse model of diabetic nephropathy. *Diabetes.* 2011; 60:2386–96. [PubMed: 21810593]
220. Barutta F, et al. Cannabinoid receptor 1 blockade ameliorates albuminuria in experimental diabetic nephropathy. *Diabetes.* 59:1046–54. [PubMed: 20068137]
221. Annuzzi G, et al. Differential alterations of the concentrations of endocannabinoids and related lipids in the subcutaneous adipose tissue of obese diabetic patients. *Lipids Health Dis.* 9:43. [PubMed: 20426869]
222. Siegmund SV, Schwabe RF. Endocannabinoids and liver disease. II. Endocannabinoids in the pathogenesis and treatment of liver fibrosis. *Am J Physiol Gastrointest Liver Physiol.* 2008; 294:G357–62. [PubMed: 18006606]
223. Munoz-Luque J, et al. Regression of fibrosis after chronic stimulation of cannabinoid CB2 receptor in cirrhotic rats. *J Pharmacol Exp Ther.* 2008; 324:475–83. [PubMed: 18029545]
224. Zyromski NJ, Mathur A, Pitt HA, Wade TE, Wang S, Swartz-Basile DA, Prather AD, Lillemoe KD. Cannabinoid receptor-1 blockade attenuates acute pancreatitis in obesity by an adiponectin mediated mechanism. *J Gastrointest Surg.* 2009; 13:831–8. [PubMed: 19225848]
225. Borrelli F, Izzo AA. Role of acylethanolamides in the gastrointestinal tract with special reference to food intake and energy balance. *Best Pract Res Clin Endocrinol Metab.* 2009; 23:33–49. [PubMed: 19285259]
226. Wright K, Rooney N, Feeney M, Tate J, Robertson D, Welham M, Ward S. Differential expression of cannabinoid receptors in the human colon: cannabinoids promote epithelial wound healing. *Gastroenterology.* 2005; 129:437–53. [PubMed: 16083701]
227. Kimball ES, Schneider CR, Wallace NH, Hornby PJ. Agonists of cannabinoid receptor 1 and 2 inhibit experimental colitis induced by oil of mustard and by dextran sulfate sodium. *Am J Physiol Gastrointest Liver Physiol.* 2006; 291:G364–71. [PubMed: 16574988]
228. Storr MA, Keenan CM, Zhang H, Patel KD, Makriyannis A, Sharkey KA. Activation of the cannabinoid 2 receptor (CB2) protects against experimental colitis. *Inflamm Bowel Dis.* 2009; 15:1678–85. [PubMed: 19408320]
229. Storr MA, et al. Targeting endocannabinoid degradation protects against experimental colitis in mice: involvement of CB1 and CB2 receptors. *J Mol Med.* 2008; 86:925–36. [PubMed: 18493729]
230. Mukhopadhyay P, et al. Cannabinoid-2 receptor limits inflammation, oxidative/nitrosative stress, and cell death in nephropathy. *Free Radic Biol Med.* 2010; 48:457–67. [PubMed: 19969072]
231. Mukhopadhyay P, et al. CB1 cannabinoid receptors promote oxidative/nitrosative stress, inflammation and cell death in a murine nephropathy model. *Br J Pharmacol.* 2010; 160:657–68. [PubMed: 20590569]
232. Horvath B, Mukhopadhyay P, Kechrid M, Patel V, Tanchian G, Wink DA, Gertsch J, Pacher P. beta-Caryophyllene ameliorates cisplatin-induced nephrotoxicity in a cannabinoid 2 receptor-dependent manner. *Free radical biology & medicine.* 2012; 52:1325–33. [PubMed: 22326488]

233. Lim JC, Lim SK, Han HJ, Park SH. Cannabinoid receptor 1 mediates palmitic acid-induced apoptosis via endoplasmic reticulum stress in human renal proximal tubular cells. *J Cell Physiol.*
234. Benito C, Nunez E, Tolon RM, Carrier EJ, Rabano A, Hillard CJ, Romero J. Cannabinoid CB2 receptors and fatty acid amide hydrolase are selectively overexpressed in neuritic plaque-associated glia in Alzheimer's disease brains. *J Neurosci.* 2003; 23:11136–41. [PubMed: 14657172]
235. Benito C, Romero JP, Tolon RM, Clemente D, Docagne F, Hillard CJ, Guaza C, Romero J. Cannabinoid CB1 and CB2 receptors and fatty acid amide hydrolase are specific markers of plaque cell subtypes in human multiple sclerosis. *J Neurosci.* 2007; 27:2396–402. [PubMed: 17329437]
236. Ramirez BG, Blazquez C, Gomez del Pulgar T, Guzman M, de Ceballos ML. Prevention of Alzheimer's disease pathology by cannabinoids: neuroprotection mediated by blockade of microglial activation. *J Neurosci.* 2005; 25:1904–13. [PubMed: 15728830]
237. Maresz K, Carrier EJ, Ponomarev ED, Hillard CJ, Dittel BN. Modulation of the cannabinoid CB2 receptor in microglial cells in response to inflammatory stimuli. *J Neurochem.* 2005; 95:437–45. [PubMed: 16086683]
238. Maresz K, et al. Direct suppression of CNS autoimmune inflammation via the cannabinoid receptor CB1 on neurons and CB2 on autoreactive T cells. *Nat Med.* 2007; 13:492–7. [PubMed: 17401376]
239. Kim K, Moore DH, Makriyannis A, Abood ME. AM1241, a cannabinoid CB2 receptor selective compound, delays disease progression in a mouse model of amyotrophic lateral sclerosis. *Eur J Pharmacol.* 2006; 542:100–5. [PubMed: 16781706]
240. Shoemaker JL, Seely KA, Reed RL, Crow JP, Prather PL. The CB2 cannabinoid agonist AM-1241 prolongs survival in a transgenic mouse model of amyotrophic lateral sclerosis when initiated at symptom onset. *J Neurochem.* 2007; 101:87–98. [PubMed: 17241118]
241. Price DA, et al. WIN55,212-2, a cannabinoid receptor agonist, protects against nigrostriatal cell loss in the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine mouse model of Parkinson's disease. *Eur J Neurosci.* 2009; 29:2177–86. [PubMed: 19490092]
242. Benito C, Tolon RM, Pazos MR, Nunez E, Castillo AI, Romero J. Cannabinoid CB2 receptors in human brain inflammation. *Br J Pharmacol.* 2008; 153:277–85. [PubMed: 17934510]
243. Palazuelos J, et al. Microglial CB2 cannabinoid receptors are neuroprotective in Huntington's disease excitotoxicity. *Brain.* 2009; 132:3152–64. [PubMed: 19805493]
244. Palazuelos J, et al. The CB(2) cannabinoid receptor controls myeloid progenitor trafficking: involvement in the pathogenesis of an animal model of multiple sclerosis. *J Biol Chem.* 2008; 283:13320–9. [PubMed: 18334483]
245. Sagredo O, et al. Cannabinoid CB2 receptor agonists protect the striatum against malonate toxicity: relevance for Huntington's disease. *Glia.* 2009; 57:1154–67. [PubMed: 19115380]
246. Tolon RM, Nunez E, Pazos MR, Benito C, Castillo AI, Martinez-Orgado JA, Romero J. The activation of cannabinoid CB2 receptors stimulates in situ and in vitro beta-amyloid removal by human macrophages. *Brain Res.* 2009; 1283:148–54. [PubMed: 19505450]
247. De March Z, et al. Cortical expression of brain derived neurotrophic factor and type-1 cannabinoid receptor after striatal excitotoxic lesions. *Neuroscience.* 2008; 152:734–40. [PubMed: 18313855]
248. Mestre L, Docagne F, Correa F, Loria F, Hernangomez M, Borrell J, Guaza C. A cannabinoid agonist interferes with the progression of a chronic model of multiple sclerosis by downregulating adhesion molecules. *Mol Cell Neurosci.* 2009; 40:258–66. [PubMed: 19059482]
249. Loria F, et al. An endocannabinoid tone limits excitotoxicity in vitro and in a model of multiple sclerosis. *Neurobiol Dis.* 2010; 37:166–76. [PubMed: 19815071]
250. Pertwee RG. The therapeutic potential of drugs that target cannabinoid receptors or modulate the tissue levels or actions of endocannabinoids. *AAPS J.* 2005; 7:E625–54. [PubMed: 16353941]
251. Calignano A, La Rana G, Giuffrida A, Piomelli D. Control of pain initiation by endogenous cannabinoids. *Nature.* 1998; 394:277–81. [PubMed: 9685157]
252. Hanus L, et al. HU-308: a specific agonist for CB(2), a peripheral cannabinoid receptor. *Proc Natl Acad Sci U S A.* 1999; 96:14228–33. [PubMed: 10588688]

253. Malan TP Jr, Ibrahim MM, Deng H, Liu Q, Mata HP, Vanderah T, Porreca F, Makriyannis A. CB2 cannabinoid receptor-mediated peripheral antinociception. *Pain*. 2001; 93:239–45. [PubMed: 11514083]
254. Clayton N, Marshall FH, Bountra C, O’Shaughnessy CT. CB1 and CB2 cannabinoid receptors are implicated in inflammatory pain. *Pain*. 2002; 96:253–60. [PubMed: 11972997]
255. Ibrahim MM, et al. Activation of CB2 cannabinoid receptors by AM1241 inhibits experimental neuropathic pain: pain inhibition by receptors not present in the CNS. *Proc Natl Acad Sci U S A*. 2003; 100:10529–33. [PubMed: 12917492]
256. Ibrahim MM, et al. CB2 cannabinoid receptor activation produces antinociception by stimulating peripheral release of endogenous opioids. *Proc Natl Acad Sci U S A*. 2005; 102:3093–8. [PubMed: 15705714]
257. Ibrahim MM, et al. CB2 cannabinoid receptor mediation of antinociception. *Pain*. 2006; 122:36–42. [PubMed: 16563625]
258. Nackley AG, Makriyannis A, Hohmann AG. Selective activation of cannabinoid CB(2) receptors suppresses spinal fos protein expression and pain behavior in a rat model of inflammation. *Neuroscience*. 2003; 119:747–57. [PubMed: 12809695]
259. Nackley AG, Suplita RL 2nd, Hohmann AG. A peripheral cannabinoid mechanism suppresses spinal fos protein expression and pain behavior in a rat model of inflammation. *Neuroscience*. 2003; 117:659–70. [PubMed: 12617970]
260. Nackley AG, Zvonok AM, Makriyannis A, Hohmann AG. Activation of cannabinoid CB2 receptors suppresses C-fiber responses and windup in spinal wide dynamic range neurons in the absence and presence of inflammation. *J Neurophysiol*. 2004; 92:3562–74. [PubMed: 15317842]
261. Quartilho A, Mata HP, Ibrahim MM, Vanderah TW, Porreca F, Makriyannis A, Malan TP Jr. Inhibition of inflammatory hyperalgesia by activation of peripheral CB2 cannabinoid receptors. *Anesthesiology*. 2003; 99:955–60. [PubMed: 14508331]
262. Elmes SJ, Jhaveri MD, Smart D, Kendall DA, Chapman V. Cannabinoid CB2 receptor activation inhibits mechanically evoked responses of wide dynamic range dorsal horn neurons in naive rats and in rat models of inflammatory and neuropathic pain. *Eur J Neurosci*. 2004; 20:2311–20. [PubMed: 15525273]
263. Hohmann AG, Farthing JN, Zvonok AM, Makriyannis A. Selective activation of cannabinoid CB2 receptors suppresses hyperalgesia evoked by intradermal capsaicin. *J Pharmacol Exp Ther*. 2004; 308:446–53. [PubMed: 14610224]
264. Scott DA, Wright CE, Angus JA. Evidence that CB-1 and CB-2 cannabinoid receptors mediate antinociception in neuropathic pain in the rat. *Pain*. 2004; 109:124–31. [PubMed: 15082134]
265. Whiteside GT, Lee GP, Valenzano KJ. The role of the cannabinoid CB2 receptor in pain transmission and therapeutic potential of small molecule CB2 receptor agonists. *Curr Med Chem*. 2007; 14:917–36. [PubMed: 17430144]
266. Rahn EJ, Zvonok AM, Thakur GA, Khanolkar AD, Makriyannis A, Hohmann AG. Selective activation of cannabinoid CB2 receptors suppresses neuropathic nociception induced by treatment with the chemotherapeutic agent paclitaxel in rats. *J Pharmacol Exp Ther*. 2008; 327:584–91. [PubMed: 18664590]
267. Muller-Vahl KR, Emrich HM. Cannabis and schizophrenia: towards a cannabinoid hypothesis of schizophrenia. *Expert Rev Neurother*. 2008; 8:1037–48. [PubMed: 18590475]
268. Andreasson S, Allebeck P, Engstrom A, Rydberg U. Cannabis and schizophrenia. A longitudinal study of Swedish conscripts. *Lancet*. 1987; 2:1483–6. [PubMed: 2892048]
269. De Marchi N, De Petrocellis L, Orlando P, Daniele F, Fezza F, Di Marzo V. Endocannabinoid signalling in the blood of patients with schizophrenia. *Lipids Health Dis*. 2003; 2:5. [PubMed: 12969514]
270. Ishiguro H, et al. Brain cannabinoid CB2 receptor in schizophrenia. *Biol Psychiatry*. 2010; 67:974–82. [PubMed: 19931854]
271. Khan A, Kendall DA, Fone KCF. The effects of the cannabinoid CB2 receptor antagonist, AM630, on isolation rearing – induced behavioural deficits in rats. *Schizophrenia Research*. 2010; 117:391–392.

272. Grinspoon L, Bakalar JB. Marihuana as medicine. A plea for reconsideration. *JAMA*. 1995; 273:1875–6. [PubMed: 7776506]
273. Grinspoon L, Bakalar JB, Zimmer L, Morgan JP. Marijuana addiction. *Science*. 1997; 277:749. author reply 750–2. [PubMed: 9273692]
274. Onaivi ES, et al. Functional expression of brain neuronal CB2 cannabinoid receptors are involved in the effects of drugs of abuse and in depression. *Ann N Y Acad Sci*. 2008; 1139:434–49. [PubMed: 18991891]
275. Garcia-Gutierrez MS, Perez-Ortiz JM, Gutierrez-Adan A, Manzanares J. Depression-resistant endophenotype in mice overexpressing cannabinoid CB(2) receptors. *Br J Pharmacol*. 2010; 160:1773–84. [PubMed: 20649579]
276. Hu B, Doods H, Treede RD, Ceci A. Depression-like behaviour in rats with mononeuropathy is reduced by the CB2-selective agonist GW405833. *Pain*. 2009; 143:206–12. [PubMed: 19345493]
277. Garci AGMA, Manzanares J. Overexpression of CB2 cannabinoid receptors decreased vulnerability to anxiety and impaired anxiolytic action of alprazolam in mice. *J Psychopharmacol*. 2010
278. Richardson D, et al. Characterisation of the cannabinoid receptor system in synovial tissue and fluid in patients with osteoarthritis and rheumatoid arthritis. *Arthritis Res Ther*. 2008; 10:R43. [PubMed: 18416822]
279. Blazquez C, Carracedo A, Barrado L, Real PJ, Fernandez-Luna JL, Velasco G, Malumbres M, Guzman M. Cannabinoid receptors as novel targets for the treatment of melanoma. *FASEB J*. 2006; 20:2633–5. [PubMed: 17065222]
280. Zheng D, Bode AM, Zhao Q, Cho YY, Zhu F, Ma WY, Dong Z. The cannabinoid receptors are required for ultraviolet-induced inflammation and skin cancer development. *Cancer Res*. 2008; 68:3992–8. [PubMed: 18483286]
281. McKallip RJ, Lombard C, Fisher M, Martin BR, Ryu S, Grant S, Nagarkatti PS, Nagarkatti M. Targeting CB2 cannabinoid receptors as a novel therapy to treat malignant lymphoblastic disease. *Blood*. 2002; 100:627–34. [PubMed: 12091357]
282. Guida M, et al. The levels of the endocannabinoid receptor CB2 and its ligand 2-arachidonoylglycerol are elevated in endometrial carcinoma. *Endocrinology*. 2010; 151:921–8. [PubMed: 20133454]

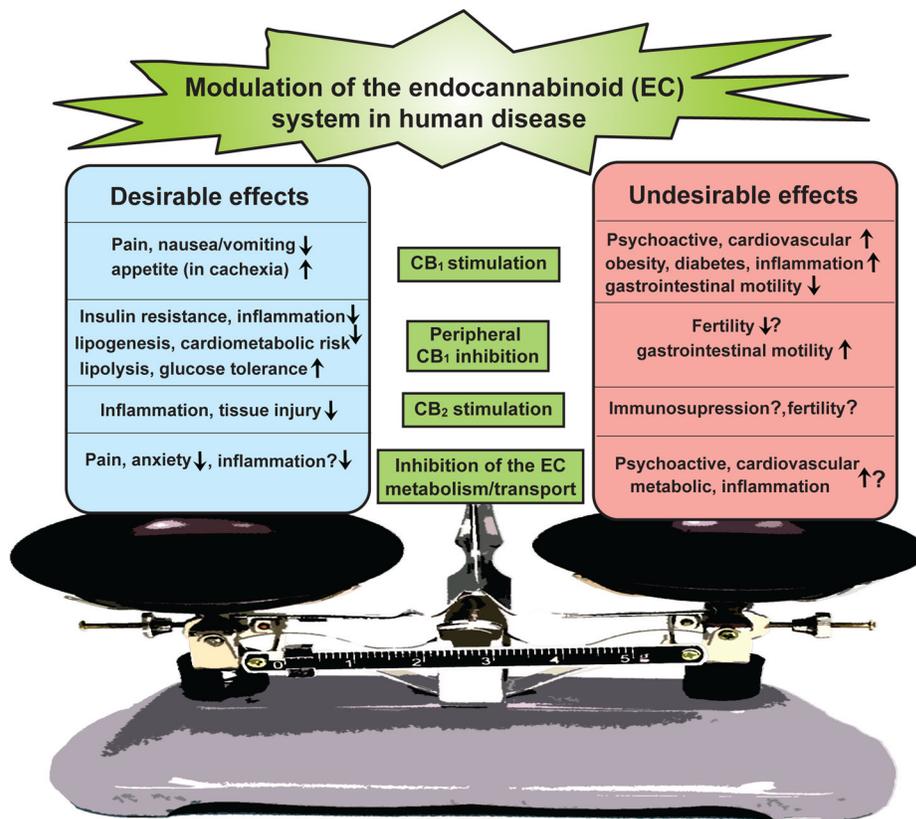


Figure 1.
Cannabinoid therapeutics: finding the right balance

Table 1

Examples of the dysregulation of the ECS in disease

| Disease, sample (R: rodent; P: pig; C: canine; H: human) | Expression/changes in CB _{1/2} | Changes in endocannabinoid levels | Proposed role of CB receptors in disease | Reference |
|--|--|--|--|-----------------------------|
| Myocardial infarction ischemia/reperfusion injury (R, P, H) | Myocardium. In human epicardial adipose tissues of ischemic hearts upregulation of CB ₁ and PKA, accompanied by CB ₂ and FAAH downregulation, increased iNOS/eNOS ration and reduced cell survival signaling | Increase in circulating immune cells or in serum of obese patients with adverse cardiovascular events. Elevated endocannabinoid plasma levels are strongly associated with coronary dysfunction in obese human subjects. | CB ₂ : decrease in leukocyte infiltration and enhancement of pro-survival pathways; CB ₁ : contribution to cardiovascular dysfunction, cell death/dysfunction in human endothelial cells and cardiomyocytes; central hypothermia (the latter is only in rodents and can be protective) | [11,12,76,85–87,90,184–187] |
| Heart failure, cardiomyopathies (R, H) | Myocardium, cardiomyocytes, endothelial cells | Myocardium, cardiomyocytes, circulating immune cells and platelets | CB ₂ : attenuation of inflammation/injury; CB ₁ : promotion of cardiac dysfunction and cell death in cardiomyocytes and endothelial cells | [64,65,186,188–192] |
| Atherosclerosis, restenosis (R, H) | Infiltrating and other immune cells, vascular smooth muscle and endothelium | Serum, atherosclerotic plaques | CB ₂ : context dependent attenuation or promotion of vascular inflammation (monocyte chemotaxis, infiltration and activation) and factors of plaque stability; attenuation of vascular smooth muscle proliferation; CB ₁ : increase of vascular inflammation and/or plaque vulnerability | [67,84,133,134,193–198] |
| Stroke, spinal cord injury (R, H) | Brain, microglia, infiltrating immune cells, endothelium | Serum, brain | CB ₂ : attenuation of inflammation (endothelial activation, leukocyte infiltration), and tissue injury; attenuation of motor and autonomic deficits in a mouse model of spinal cord injury; | [90,199–206] |

| Disease, sample (R: rodent; P: pig; C: canine; H: human) | Expression/changes in CB _{1/2} | Changes in endocannabinoid levels | Proposed role of CB receptors in disease | Reference |
|---|--|---|--|--------------------------|
| | | | CB ₁ : promotes hypothermia-dependent protection, but if hypothermia is compensated ineffective or enhances injury | |
| Cirrhotic cardiomyopathy (R, H) | N.D. | Myocardium, circulating immune cells and platelets | CB ₂ : attenuation of hypotension by decreasing liver inflammation; CB ₁ : contribution to cardiovascular dysfunction | [189–192] |
| Septic shock by live bacteria (R, H) | N.D. | Serum | CB ₂ : decrease or increase in inflammation and tissue injury most likely by affecting bacterial load; CB ₁ : contribution to cardiovascular collapse | [10,207–210] |
| Hepatic ischemia-reperfusion injury (R, P, H) | Inflammatory immune cells, activated endothelium | Liver, serum, hepatocytes, Kupffer and endothelial cells | CB ₂ : attenuation of inflammation (endothelial activation, leukocyte chemotaxis, infiltration and activation), oxidative stress, and tissue injury; CB ₁ : promotion of liver injury | [135,138,211–213] |
| Obesity, nonalcoholic fatty liver disease, diabetic complications (R, H) | Hepatocytes, inflammatory cells, adipocytes, certain neurons, sites of diabetic complications (kidneys, retina and myocardium) | Liver, adipose tissue, brain, skeletal muscle, diabetic kidneys, hearts, retinas, serum | CB ₂ : Enhancement of high fat diet-induced steatosis and inflammation or attenuation of obesity associated one with age; CB ₁ : increase in fat storage, decrease in metabolism, promotion of insulin and leptin resistance and inflammation in adipose tissue and in the liver | [5,6,70,101,108,214–221] |
| Liver fibrosis, cirrhosis, alcohol-induced liver injury (R, H) | Activated Stellate cells, inflammatory cells, hepatocytes, Kupffer cells | Liver, serum, inflammatory cells | CB ₂ : Attenuation of fibrosis and injury/inflammation; CB ₁ : increase in fibrosis/injury | [14,136,137,191,222,223] |
| Pancreatitis (R, H) | Pancreas | Inflamed pancreas | CB ₂ : Attenuation of inflammation; CB ₁ : context dependent effect | [145,146,148,224] |

| Disease, sample (R: rodent; P: pig; C: canine; H: human) | Expression/changes in CB _{1/2} | Changes in endocannabinoid levels | Proposed role of CB receptors in disease | Reference |
|--|---|--|---|---------------------------------|
| Inflammatory bowel disease, colitis, diverticulitis (R, H) | Epithelial cells, infiltrating inflammatory cells, enteric nerves | Inflamed gut | Attenuation of inflammation and visceral sensitivity | [130,151,225–229] |
| Nephropathy (R, H) | Kidney, human proximal tubular cells, podocytes | Kidney | CB ₂ : attenuation of inflammation (chemokine signaling and chemotaxis, inflammatory cell infiltration and endothelial activation) and oxidative stress; CB ₁ : promotion of inflammation/injury | [105,219,220,230–233] |
| Neurodegenerative/neuroinflammatory disorders (multiple sclerosis, Alzheimer's, Parkinson's and Huntington's disease, spinal cord injury) (R, H) | Microglia, inflammatory cells, brain lesions, neurons? | Brain, spinal fluid | CB ₂ : attenuation of inflammation (microglia activation, secondary immune cell infiltration), facilitation of neurogenesis; CB ₁ : attenuation of excitotoxicity, hypothermia; context dependent effect on injury/inflammation | [27,91,92,152,205,234–250] |
| Pain (R) | Inflammatory cells, certain neurons | Site of induced chronic inflammatory pain | CB ₂ : attenuation of inflammatory pain via unknown mechanism(s); CB ₁ : attenuation of various forms of pain by inhibiting neurotransmission | [17,95,96,251–266] |
| Psychiatric disorders (anxiety and depression, schizophrenia) (R, H) | Glial, inflammatory cells, neurons? | Blood, cerebrospinal fluid, brain (increased in schizophrenia, but decreased in brain in depression) | CB ₂ : largely unexplored, In rodent models of depression/anxiety it may modulate CNS inflammation and either attenuate or promote anxiety like behavior; CB ₁ : context dependent effect on anxiety, improved sleep | [19,267–277] |
| Rheumatoid arthritis (H) | N.D. | Synovial fluid, synovia | CB ₂ : attenuation of the autoimmune inflammatory response; CB ₁ : attenuation of pain | [278] |
| Cancer (R, H) | In various tumors or cancer cells | Various tumors | CB _{1/2} : context dependent attenuation or promotion of tumor growth (apoptosis, | [279–282] [2,22,149,155,157] |

| Disease, sample (R: rodent; P: pig; C: canine; H: human) | Expression/changes in CB _{1/2} | Changes in endocannabinoid levels | Proposed role of CB receptors in disease | Reference |
|--|---|-----------------------------------|--|-----------|
| | | | angiogenesis, proliferation, etc.) | |

Table 2

Potential approaches/directions for future success

| Therapeutic approach (<i>target</i>) | Possible directions/ approaches for success | Possibly therapeutic indications in humans (realistic) | Potential/expected adverse effects |
|--|--|--|--|
| THC based medicines, cannabinoid based extracts (CB_1, CB_2 and unrelated antioxidant anti-inflammatory mechanisms) | <ul style="list-style-type: none"> • Optimization of route of administration, dosing and indication • Better selection criteria for trials, identification of potential positive responders by initial titration • Placebo controlled trials to establish short and long term efficacy in given indications • Long term controlled studies to determine possible disease modifying effects (e.g. in multiple sclerosis) and adverse consequences (e.g. immune and/or cardiovascular effects, etc.) • Combination approaches in pain to achieve better efficacy and fewer side effects (e.g. with opioids, non-steroid anti-inflammatory drugs, etc.) • Optimization of the extract composition for improved benefit/risk profile | <ul style="list-style-type: none"> • Symptomatic relief in certain forms of pain and spasticity (as in neurodegenerative disorders such as multiple sclerosis) • Stimulation of appetite in patients with wasting disorders • Attenuation of chemotherapy-induced nausea and vomiting • Topical administration in certain skin disorders? • Non-psychoactive constituents of marijuana, such as cannabidiol or their analogs, may have therapeutic utility in certain forms of acute tissue injury, inflammatory disorders, diabetes and diabetic complications | <ul style="list-style-type: none"> • In case of THC- containing formulations, effects related to CB_1 stimulation at higher doses (e.g. psychoactive, cardiovascular, metabolic side effects) and potential modulation of immune responses |
| Peripherally restricted CB_1 agonists (<i>peripheral CB_1</i>) | <ul style="list-style-type: none"> • Evaluation of the feasibility of the topical/local use of peripherally restricted CB_1 agonists in certain forms of pain and skin conditions (e.g. pruritus) | <ul style="list-style-type: none"> • Topical/local use in certain forms of pain and skin conditions/diseases? (the systematic administration/use is not likely because of the established adverse cardiovascular and metabolic consequences of this approach) | <ul style="list-style-type: none"> • Cardiovascular • Metabolic • Kidney • Gastrointestinal (decreased motility) • Pro-inflammatory? |

| Therapeutic approach (<i>target</i>) | Possible directions/ approaches for success | Possibly therapeutic indications in humans (realistic) | Potential/expected adverse effects |
|---|--|--|---|
| Peripherally restricted or global CB₂ agonists (<i>peripheral CB₂</i>) | <ul style="list-style-type: none"> • Reevaluation of human indications based on previous failures of trials with mixed peripherally restricted CB_{1/2} agonists • Search for new indications • More preclinical and clinical research to understand the significance of tissue and time specific changes in CB₂ receptor expression in pathological conditions • Development of novel, specific and orally available ligands for proof of the principle studies; evaluation of toxicology and pharmacokinetics | <ul style="list-style-type: none"> • Various forms of acute tissue injuries associated with inflammation (stroke, myocardial infarction, traumatic injury, organ transplantation, etc.) • Various forms of inflammatory diseases if the antiinflammatory effects are confirmed in humans | <ul style="list-style-type: none"> • Most likely related to effects on immune and hematopoietic system • Effects on fertility? |
| Peripherally restricted CB₁ antagonists, inverse agonists (<i>peripheral CB₁</i>) | <ul style="list-style-type: none"> • Development and testing of new ligands, toxicology and safety studies in rodents, large animals, and humans • Proof of the principle studies in large animals and humans | <ul style="list-style-type: none"> • Diabetes and diabetic complications, • Cardiometabolic syndrome • Kidney disease? | <ul style="list-style-type: none"> • Gastrointestinal (increased motility) • Effects on fertility? |
| Inhibition of EC metabolism, cellular uptake or biosynthesis (CB_{1/2}, TRPV₁ and nuclear receptors, prostaglandin and leukotriene signaling) | <ul style="list-style-type: none"> • Preclinical research to identify the putative endocannabinoid transporter(s), and to better understand the tissue, time, and disease specific metabolism of endocannabinoids to various other bioactive mediators (e.g. prostaglandins, leukotriens, etc.) • Reevaluation of human indications based on previous | <ul style="list-style-type: none"> • Pain? • Certain disorders associated with anxiety? • Certain forms of acute tissue injury? | <ul style="list-style-type: none"> • Similar, but acutely less pronounced than with CB₁ agonists. However, long term use may be associated with adverse effects similar to COX2 inhibitors (e.g. cardiovascular). • Pro-inflammatory effects in certain cases? |

| Therapeutic approach (<i>target</i>) | Possible directions/ approaches for success | Possibly therapeutic indications in humans (realistic) | Potential/expected adverse effects |
|--|--|--|------------------------------------|
| | failures of trials with FAAH inhibitors in pain <ul style="list-style-type: none">• Search for new indications, better and more selective ligands | | |