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CANNABINOID RECEPTORS AS THERAPEUTIC TARGETS

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■ **Abstract** CB1 and CB2 cannabinoid receptors are the primary targets of endogenous cannabinoids (endocannabinoids). These G protein–coupled receptors play an important role in many processes, including metabolic regulation, craving, pain, anxiety, bone growth, and immune function. Cannabinoid receptors can be engaged directly by agonists or antagonists, or indirectly by manipulating endocannabinoid metabolism. In the past several years, it has become apparent from preclinical studies that therapies either directly or indirectly influencing cannabinoid receptors might be clinically useful. This review considers the components of the endocannabinoid system and discusses some of the most promising endocannabinoid-based therapies.

INTRODUCTION

Cannabinoid receptors are part of the endocannabinoid system, which consists of cannabinoid receptors, endogenous cannabinoids (endocannabinoids), and the enzymes that synthesize and degrade endocannabinoids. Emerging evidence implicates endocannabinoids in a wide variety of physiological and pathophysiological processes. To date, most drugs used therapeutically that interact with the endocannabinoid system are derived from cannabis and produce their effects by activation of cannabinoid receptors. Regrettably, the psychoactivity of these compounds has prevented their widespread acceptance and application in Western medicine. In the past decade, the elucidation of the components of the endocannabinoid system and a better understanding of its role have broadened the therapeutic possibilities for its manipulation. For example, cannabinoid receptors can be directly manipulated by ligands that bind cannabinoid receptors or indirectly by drugs that modulate endocannabinoid levels. This review considers the components of the endocannabinoid system, their involvement in specific behaviors and diseases, and several promising clinical and preclinical studies. Because of space limitations not all potential therapies are considered. The interested reader is encouraged to consult recent reviews that either offer a different perspective or go into much greater depth on specific indications (1–9).

COMPONENTS OF THE ENDOCANNABINOID SIGNALING SYSTEM

The past 15 years have seen a tremendous advance in our understanding of the elements of the endocannabinoid system. During this period two cannabinoid receptors, CB1 and CB2, have been cloned; several endogenous cannabinoids have been identified; and the synthetic and degradative pathways for the endocannabinoids have been partially elucidated.

CANNABINOID RECEPTORS

The CB1 receptor was first cloned as an orphan receptor from a rat cDNA library based on its homology to the bovine substance K receptor (10). In the years preceding the cloning of CB1, a series of potent cannabinoid receptor agonists had been synthesized (11–13). By combining anatomical, molecular, and pharmacological approaches, the distribution and primary signaling mechanisms of the CB1 receptor were determined (14). Within a few years, a second cannabinoid receptor was found in a human promyelocytic cDNA library and designated the CB2 receptor on the basis of its homology to the CB1 receptor and similar ligand binding profile (15). In the CNS, CB1 receptors are most highly expressed on axons and nerve terminals, but ample functional evidence also supports their expression on somata (14). Their expression on glia is controversial and, if it occurs, is at a considerably lower density than on neurons (14). CB2 receptors are primarily found on immune cells. The highest levels of CB2 mRNA in peripheral blood cells are found in B lymphocytes > natural killer cells $\gg$ monocytes > neutrophils > T8 lymphocytes > T4 lymphocytes (16). Both CB1 and CB2 receptors belong to the superfamily of G protein–coupled receptors, coupling to inhibitory G proteins ($G_{i/o}$) (10, 15). As such, CB1 and CB2 receptors inhibit adenylyl cyclase and activate MAP kinase (14). In addition, CB1 receptors inhibit presynaptic N- and P/Q-type calcium channels and activate inwardly rectifying potassium channels (17, 18). Further signaling mechanisms involve focal adhesion kinase, phosphatidylinositol-3-kinase, sphingomyelinase, or nitric oxide synthase (19–22). Beyond CB1 and CB2, several intriguing studies support the existence of additional cannabinoid receptors (23–25). The cloning and pharmacological characterization of these receptors will likely blur the boundaries between classic cannabinoid agonists and other lipid mediators and will introduce a rich pharmacology of their own. However, the impact on these additional cannabinoid receptors must also be considered when endocannabinoid levels are manipulated.

ENDOGENOUS CANNABINOIDS (ENDOCANNABINOIDS)

The widespread distribution of cannabinoid receptors suggests the presence of an endogenous ligand, or endogenous cannabinoid (endocannabinoid). This hypothesis was validated by the identification of two families of endogenous cannabinoids

more than ten years ago. The prototypical endocannabinoid, anandamide, the amide of ethanolamine and arachidonic acid, was first isolated and identified as an endogenous cannabinoid by Mechoulam & Devane from porcine brain (26). A series of similar compounds, varying in the nature of the (unsaturated) fatty acid are also found in tissues. Some of these, for example homo-γ-linolenoylethanolamide and docosatetraenoylethanolamide, engage CB1 receptors (27). Others, such as palmitoylethanolamide (PEA) and oleoylathanolamide (OEA) do not, but have profound analgesic or anorexic effects, respectively (28, 29). The second endogenous cannabinoid, 2-arachidonyl glycerol (2-AG), was identified by the Sugiura and Mechoulam groups (30, 31), and its importance as an endogenous cannabinoid was established within a few years (32). In part because of its nonsignaling role as an intermediate in several lipid metabolic pathways, 2-AG is far more abundant than anandamide. Recently, amides of arachidonic acid such as N-arachidonyl dopamine, serine, and glycine have been described. Although some of these compounds have activity at cannabinoid receptors, they also affect many other targets and their pharmacology will not be considered here. Interested readers may refer to a recent review (33). Despite their superficial structural similarity, substantial differences between the anandamide and 2-AG families of endogenous cannabinoids must be noted and are an important source of therapeutic specificity. We review the differences in some detail, as they must be considered when assessing the possible therapeutic applications of drugs affecting various components of the endocannabinoid system.

The first difference is the route of synthesis. Anandamide and related acylamides are made following the hydrolysis of N-arachidonoyl (or another unsaturated fatty acid) phosphatidyl ethanolamine (NAPE) by a specific phospholipase D (34, 35). NAPE synthesis by an N-acyl transferase may be the rate-limiting step in anandamide formation (36) and is subject to regulation by calcium and cyclic AMP (37). In contrast, the major synthetic pathway of 2-AG formation is the hydrolysis of phosphatidylinositol by phospholipase C and diacylglycerol lipase (DGL) (38) with phosphatidylinositol hydrolysis by phospholipase A1 and lyso-phospholipase C being important in some tissues (39). As evidenced by their synthetic pathways, anandamide and 2-AG are "made on demand" rather than stored in vesicles, contrasting with classical neurotransmitters. The synthesis of anandamide and 2-AG is enhanced by neural activity, a feature that we address below.

Another important difference between the 2-AG and anandamide families is efficacy. Multiple studies with CB1 and CB2 receptors have found that anandamide is a low efficacy agonist, whereas 2-AG is highly efficacious (40–44). Interestingly, by a number of measures, the efficacy of anandamide at CB1 receptors is similar to that of Δ^9tetrahydrocannabinol (Δ^9THC), the major psychoactive component of cannabis. This leads to the intriguing possibility that the psychoactive effects of cannabis and Δ^9THC are due to a combination of mimicking anandamide's action at CB1 receptors while antagonizing 2-AG actions at these same receptors. This hypothesis is supported by the observation that a single very high dose (90 mg) of the CB1 antagonist rimonabant has limited efficacy in blocking the subjective

effects of cannabis (45). Even two weeks of rimonabant at 40 mg per day (twice the dose used in the phase III obesity trials, see below) produced similarly modest effects (46). Further support for this mixed agonist/antagonist mechanism of Δ^9THC psychoactivity comes from recent experiments in which we have found that Δ^9THC effectively antagonizes a form of endocannabinoid mediated short-term plasticity (see below) that is mediated by 2-AG (A. Straiker & K. Mackie, unpublished observations).

Endocannabinoid action appears to be terminated by a combination of uptake, possibly via a specific transporter (47), and hydrolysis. Although the transport process does not distinguish between the two endocannabinoid families, anandamide and 2-AG appear to be hydrolyzed by distinct enzymes in vivo. Anandamide and related ethanolamides are degraded by fatty acid amino hydrolase (FAAH). FAAH has been extensively studied by the Cravatt group and has several unique features that make it an attractive therapeutic target (48, 49). 2-AG is degraded by a monoacylglycerol lipase (MAG lipase) and possibly other lipases (50, 51). The characterization of MAG lipase is not as extensive as FAAH, and highly potent and selective MAG lipase inhibitors remain to be developed (52). Whether this is due to unfavorable intrinsic factors of the enzyme remains to be determined.

ENDOCANNABINOIDS AND NEURONAL PLASTICITY

The prominent presynaptic localization of CB1 receptors and their inhibition of calcium channels and activation of potassium channels suggest that they may modulate neurotransmission and affect neuronal excitability. Indeed, a large number of studies show that activation of CB1 receptors by both exogenous and endogenous cannabinoids suppresses neurotransmission (53). Furthermore, the enhancement of endocannabinoid synthesis during neural activity suggests that these ligands may inhibit neurotransmission. Indeed, endocannabinoid involvement in neuronal plasticity has now been shown to occur at many synapses. Space limitations preclude an extensive review of this topic; however, the generalities of this phenomenon are briefly summarized and the interested reader may consult detailed reviews (54, 55).

Endocannabinoid-mediated inhibition of neurotransmission comes in two forms, transient and long lasting. Transient, also termed DSI (depolarization-induced suppression of inhibition) or DSE (depolarization-induced suppression of excitation), relies on generation of endocannabinoids following increases in intracellular calcium—often from entry through calcium-permeant cell surface channels or release from intracellular stores, sometimes by activation of metabotropic receptors (56–60). DSI and DSE are of short duration, lasting tens of seconds, and localized; thus they may serve to rapidly modulate small ensembles of synapses (61). [However, activation of somatic CB1 receptors during DSE or DSI can suppress neurotransmitter release over a wide area by inhibiting spiking activity (62).] Long-lasting endocannabinoid-mediated inhibition of neurotransmission, one form of

long-term depression (LTD), is also ubiquitous (63–66). Here, endocannabinoids, often produced by group I metabotropic receptors during prolonged low-frequency stimulation, activate presynaptic CB1 receptors. These endocannabinoids set in motion a poorly understood process, culminating in long-lasting (at least tens of minutes) inhibition of neurotransmitter release. Endocannabinoid LTD (eLTD) only requires CB1 receptor activation for its initiation; once established eLTD is independent of CB1 receptor activation (63). eLTD is exquisitely sensitive to exogenous cannabinoids and cocaine, and thus its disruption may underlie some of the actions of these drugs (67, 68). The identity of the endocannabinoids involved in short- and long-term endocannabinoid plasticity remains to be identified with certainty. However, studies to date suggest 2-AG may underlie eLTD, whereas evidence supports a role for 2-AG and/or anandamide in DSE and DSI (69). Although the above discussion has been from the perspective on neuronal production of endocannabinoids, it is important to note that glial cells are prodigious producers of endocannabinoids (9).

A substantial literature describes a role for endocannabinoids in vascular regulation. Examples include vasodilation during sepsis (70) or cirrhosis (71) and a role in the regulation of cerebral blood flow (72, 73). In some cases CB1 receptors have been implicated, in others endocannabinoids appear to be interacting with novel receptors. Several recent reviews have considered this complicated topic (7, 25, 74). Possible cardiovascular actions of endocannabinoids and the implication of their antagonism need to be considered when using CB1 antagonists, as discussed below. For example, inhibition of FAAH and raising acylethanolamine levels can decrease blood pressure in hypertensive rats (75). However, to date, there is no suggestion of an increased incidence of hypertension in patients treated for up to a year with the CB1 antagonist, rimonabant (76).

Δ^9THC CANNABIS AND CANNABIS EXTRACTS

Synthetic Δ^9THC (dronabinol) is approved in the United States for treatment of nausea and vomiting associated with chemotherapy as well as an appetite stimulate in AIDS. The efficacy of Δ^9THC versus medical cannabis is the subject of a contentious debate, and a complete assessment of the topic is beyond the scope of this review (77). Nevertheless, two of the major issues to be considered are pharmacokinetic (e.g., oral versus inhaled) and the contribution of additional components of cannabis (e.g., cannabinol and cannabidiol) to therapeutic efficacy. Related to the medical cannabis question is the recent development of standardized cannabis extract, Sativex (78). Sativex is a standardized cannabis extract administered as a sublingual spray containing approximately equal quantities of Δ^9THC and cannabidiol, along with minor amounts of other cannabinoids. Limited clinical trials have been reported using this preparation in multiple sclerosis and neuropathic pain. These studies report modest improvement in spasticity and pain symptoms (79, 80).

CB1 RECEPTOR ANTAGONISTS

Of potential drugs affecting the endocannabinoid system, CB1 receptor antagonists have received the most attention and are the furthest along in clinical studies. The primary indication is for obesity, with secondary indications for disorders that have a prominent craving component. However, basic science and clinical studies suggest that CB1 antagonists have significant metabolic effects that extend beyond merely decreasing caloric intake. The rationale for using CB1 receptor antagonists as an anti-obesity drug is conceptually simple. It is widely appreciated that partaking of cannabis in its many preparations enhance appetite, and at least anecdotally, consumption of rich, nonnutritious foods (81). If this phenomenon is mediated by CB1 receptors, then a logical extension is that blocking these receptors might suppress appetite, leading to decreased food consumption and weight loss. As discussed below, CB1 receptor antagonists do decrease weight, but not for quite these reasons.

Rimonabant, also known as SR141716 or Acomplia®, was the first CB1 antagonist reported. It is a diarylpyrazole with nanomolar affinity for CB1 receptors and little affinity for the CB2 receptor (82). It shows inverse agonism both in heterologous expression systems and some in vivo preparations (83, 84). Many congeners of rimonabant have been synthesized and an SAR developed (14). One diarylpyrazole, AM251, deserves special mention owing to its high affinity and commercial availability. This compound has iodine substituted for the chlorine in the *para* position of the 5-phenol ring and has been frequently used for in vivo work. Although in most aspects it seems quite similar to rimonabant, differences have been described (24). Another chemical series that has given rise to relatively selective CB1 receptor antagonists are the substituted benzofurans, exemplified by the Lilly compound, LY320135 (85). However, this compound also is an inverse agonist, is less selective, and has poor oral bioavailability; so they have not been thoroughly studied (85). Given the intense commercial interest in CB1 receptor antagonists in the treatment of obesity and metabolic syndrome, additional CB1 antagonists with fundamentally different structures have been synthesized and characterized, and these are just now being reported in the scientific literature (85a).

CB1 Antagonists as Antiobesity Drugs

Low doses of CB1 receptor agonists enhance food consumption (86). Conversely, CB1 receptor antagonists decrease food consumption and body weight (1). The anorectic consequence of CB1 blockade is most prominent in lab animals with diet-induced obesity (87). CB1 receptor antagonists appear to have less effect on food consumption in mice fed a normal lab diet (88), but this finding is not universal (89). The anorexic effects of CB1 antagonists in rodents subside after only a few weeks. This is perhaps not surprising because feeding is such an important behavior for survival. Thus, after a month of CB1 antagonist treatment, there is no

difference in caloric intake between antagonist-treated and control animals (90). Importantly, for the therapeutic use of this class of drugs, the decreased weight in the antagonist-treated animals is maintained despite the now similar caloric intake (87). This seems to be a consequence of CB1-mediated effects in hepatocytes and adipocytes. In hepatocytes CB1 agonists enhance sterol response element-binding protein 1c (SREBP-1c) expression, which in turn increases acetyl-CoA carboxylase and fatty acid synthase expression (91). Furthermore, CB1 antagonists block the increase in fatty acid synthesis induced by a high-fat diet (91). Interestingly, CB1 agonists and antagonists have similar effects on SREBP-1c and FAS in the hypothalamus (91). This lends support to the concept that CB1 agonists regulate energy balance through shared central and peripheral mechanisms that are independent of classical CB1-mediated effects on neurotransmission. In adipocytes, CB1 activation appears to increase lipoprotein lipase activity (92). As for the effects described above, antagonism of this activation would increase lipolysis and favor a lean body phenotype. In summary, preclinical studies suggest that CB1 antagonists will have long-term efficacy for weight loss and improved lipid metabolism as a consequence of mechanisms that are primarily peripheral in origin. Thus, this class of drugs offers an exciting potential treatment for a disease that is accompanied by a significant public health cost. An interesting corollary of a major peripheral site of action for CB1 antagonists in obesity is that a CNS-impermeant CB1 antagonist might still be effective, while lessening the possibility of CNS-mediated adverse effects.

Clinical studies with CB1 antagonists are encouraging. A large (1507 enrolled, 920 completed) clinical study investigating CB1 blockade in obesity (RIO-Europe) has recently been published (76). The primary findings were that rimonabant (Acomplia) at a daily dose of 20 mg per day together with a mildly hypocaloric diet led to sustained weight loss of an average of 7 kg versus a weight loss of 2 kg in the placebo group treated with diet alone. More impressive than the weight loss were the improvements in the rimonabant-treated group in lipid profiles, central obesity, insulin resistance, and the incidence of metabolic syndrome. Because of the central role of CB1 receptors in several pathways potentially involved in anxiety, memory extinction, etc., there was theoretical concern that chronic CB1 blockade might be accompanied by significant psychiatric issues. This fear was not borne out with similar rates of depression in the 20 mg rimonabant and placebo groups. Overall, modestly higher rates of gastrointestinal symptoms, dizziness, and arthralgias were seen in the 20 mg rimonabant group. The results of two other large trials (RIO-North America and RIO-Lipids) have been reported at scientific meetings, with similar results (93, 94). Overall the current data are very encouraging that CB1 antagonists will be clinically useful drugs in treating the metabolic consequences of obesity. However, as with any new drug, the role they will assume in clinical therapeutics will not become apparent until their safety and efficacy have been established in a much larger and more heterogeneous patient population.

CB1 Antagonists and Craving

Another area of excitement for the CB1 antagonists is in the treatment of drug abuse. A link between the endocannabinoids and drugs of abuse has been long suspected based on animal studies. Because this topic has been reviewed in depth by several authors, only the highlights are considered here (95–97). From a large number of laboratory experiments, there appears to be a reciprocal relationship between the motivational or rewarding aspects of endogenous opioids and the endocannabinoids (98). Specifically, CB1 antagonists attenuate the rewarding properties of opioids, and the rewarding properties of opioids are absent in CB1 knockout mice (99, 100). Furthermore, in heroin-treated rats, CB1 receptor agonists enhance reinstatement, and CB1 receptor antagonists decrease reinstatement (101, 102). Interestingly, this effect of CB1 receptor antagonists was most marked with less favorable response ratios, that is, when more effort is required to receive a heroin injection. Conversely, the rewarding effects of Δ^9THC are attenuated by opioid receptor blockade and knockout of mu opioid receptors (103, 104). However, opiate receptors do not appear to be involved in the subjective effects of Δ^9THC, as an opioid receptor antagonist does not block these effects (105). The situation for other drugs of abuse has parallels, but a unifying theme remains to be identified (or, more likely doesn't exist, emphasizing the pleomorphic mechanisms underlying drug abuse). For example, CB1 receptors seem involved in some responses to nicotine—CB1 antagonists block nicotine-induced conditioned place preference (CPP) and nicotine-induced dopamine release in the nucleus accumbens (106–108). [Interestingly, CB1 antagonists do not decrease accumbens dopamine release in response to opiates (109).] These effects suggest that CB1 receptor blockade may decrease the strength of specific environmental cues associated with receiving nicotine. Another potentially important role for the endocannabinoid system is in the reinforcing effects of alcohol. CB1 receptor activation enhances alcohol consumption while blocking these receptors decreases consumption and deletion of the receptor reduces alcohol-induced CPP (110–112).

Clinical trials with rimonabant will determine if the preclinical studies discussed are relevant to drug abuse in humans. The usefulness of CB1 antagonism in smoking cessation has been investigated in the STRATUS-US trial (94). This was a short (10 week) clinical trial enrolling 787 moderate cigarette smokers. Those receiving rimonabant at 20 mg per day were almost twice (36% versus 20%) as likely to have sustained abstinence during the last four weeks of the trial compared to the placebo group. The rimonabant group also lost approximately half a pound during treatment, whereas the placebo group gained almost two and half pounds. The disparity in weight change was more marked in obese patients and is particularly interesting as most smoking cessation therapies led to weight gain after cessation. There were no reported differences in side effects between the two groups in this short trial.

Another potential application of CB1 blockade for which there is strong preclinical evidence is in treating alcohol abuse (96, 113). A clinical trial examining

the efficacy of rimonabant in alcoholism sponsored by the National Institute on Alcohol Abuse and Alcoholism (NIAAA) is now underway. Although financial considerations dampen enthusiasm for conducting clinical trials on the effectiveness of CB1 antagonists in heroin or cocaine abuse prior to regulatory approval, it is likely that they will be tested for efficacy in other "craving" disorders if CB1 antagonists are approved for clinical use.

One factor to be considered in the application of CB1 blockade for the treatment of craving-based disorders is whether it will retain its effectiveness during prolonged treatment. Although the long-term (2 year) effectiveness of rimonabant in obesity and metabolic syndrome seems established, this appears to be based on suppression of metabolic pathways involved in lipogenesis, whereas its attenuation of food consumption (presumably a behavioral effect that may share similarities to the craving aspects of drug abuse) is transient. Thus, it remains to be determined if CB1 antagonism will be an effective long-term strategy for the treatment of craving disorders.

CB2 RECEPTOR AGONISTS

The physiological role of CB2 receptors remains to be fully defined. However, several intriguing preclinical studies suggest that agonists at this receptor may be clinically useful. Multiple animal studies suggest that chronic pain may be one such indication. Still more preliminary studies also suggest a role of CB2 receptors in the maintenance of bone density and the progression of atherosclerotic lesions. A particularly attractive feature of selective CB2 agonists, such as AM1241, HU308, and JWH133, as therapeutics is that they are devoid of known psychoactivity.

Cannabis and its extracts have long been used to treat painful conditions. The possible mechanisms involved have been the subject of many investigations. Although CB1 activation may be analgesic (114), several studies clearly show that CB2 agonists are also effective in chronic pain models. Specifically, CB2 agonists are analgesic in neuropathic pain models, peripheral inflammatory models, and some sensitization models (115–121). The anatomical site of CB2 analgesic action is currently being studied, with two discrete loci emerging. The first potential site of action is the skin. Here it has been demonstrated that in thermal hypersensitivity models, CB2 agonists enhance beta-endorphin release from keratinocytes (117). As expected from a mechanism involving opiates, analgesia produced by CB2 agonists in this model is naloxone sensitive. In addition, CB2 agonists are analgesic in neuropathic pain models (116, 122). Interestingly, the neuropathic pain models of spinal nerve root ligation or sciatic nerve ligature are accompanied by robust upregulation of CB2 mRNA expression in spinal microglia as they become activated in the days following the lesion (123). It is conceivable that the activated microglia participate in the allodynia and/or hyperalgesia that are a hallmark of these neuropathic models, and stimulation of microglia CB2 receptors attenuates this microglia-neuron communication. In contrast, a chronic inflammatory pain

model, injection of Freund's adjuvant, did not increase spinal CB2 mRNA (123), despite CB2 agonists being effective analgesics in inflammatory models. At the present time, a synthesis of these studies suggests that in neuropathic pain models following nerve injury, CB2 agonists may be analgesic by their effects on spinal microglia, whereas in the peripheral models CB2 agonists might be acting by decreasing beta-endorphin release in the dermis. Ongoing experiments will sort out these and additional details in the coming years. PEA is another lipid mediator that should be considered in the context of the peripheral actions of CB2 receptors. Acting locally, PEA is an effective analgesic in inflammatory pain models, and CB2 antagonists block this analgesia (28). However, PEA does not bind to cannabinoid receptors. A potential resolution to this paradox is that PEA activates a receptor on cells resident in skin that in turn release endocannabinoids activating CB2 receptors.

The involvement of CB2 receptors in bone growth was initially unexpected. It derives from the observation that CB2 knockout mice have a markedly decreased bone mass compared to their littermates expressing the receptor (124). That these mouse results might be relevant to humans comes from the observation that a particular (silent) single nucleotide polymorphism in CB2 correlates strongly with osteoporosis in a cohort of women (124). The therapeutic possibilities of these results are emphasized by the observation that a CB2 agonist (HU308) decreases bone loss following ovariectomy in mice (124). The combination of these results and those on the efficacy of CB2 agonists in chronic pain is provocative, and may lead to the development of an analgesic drug useful for several chronic pain states that does not have the detrimental effects on bone density and (presumably) bone healing that are associated with the nonsteroidal anti-inflammatory drugs. However, a role for CB1 and CB2 receptors in stimulating osteoclast activity has also been reported (125). The discrepancy between these two studies may be a function of experimental model, choice of agonists and antagonists, or the knockout models employed.

CB2 ligands may have therapeutic utility in other chronic inflammatory diseases. An intriguing study that we briefly consider is a very recent report that low-dose Δ^9THC treatment in mice slows the progression of atherosclerotic lesions in ApoE −/− mice fed an atherogenic diet (126). This effect of Δ^9THC was blocked by the CB2 antagonist, SR144258, suggesting CB2 involvement (126). These results are congruent with the concept that Δ^9THC has anti-inflammatory properties (127–130). It will be interesting to see if selective CB2 agonists are similarly effective. Although these results need to be confirmed and extended, they are quite exciting and may lead to an entirely new application for CB2 agonists. CB2 signaling may also be involved in the neurodegeneration associated with plaque development in Alzheimer's disease (130a) and the inflammatory response accompanying retroviral encephalitis (130b), such as that occurring with HIV.

At their current stage of development, the CB2 ligands are at an exciting point. However, several fundamental questions remain. Perhaps the most basic is a better understanding of the physiological role(s) of the CB2 receptor in immune

responses. Also needed is a thorough assessment of the pharmacological properties, for example, intrinsic efficacy at the relevant signaling pathways, of the current generation of CB2 agonists and antagonists. Another is the consequence of long-term CB2 activation. CB2 receptors rapidly desensitize, at least when expressed in heterologous expression systems (131). Whether CB2 agonists will maintain their efficacy in treating neuropathic pain over a period of months remains to be determined. Finally, CB2 receptors in different species vary considerably in their distal carboxy termini (132). As this region is important in many aspects of GPCR signaling, it will be necessary to determine that the regulation and function of CB2 signaling important for its therapeutic actions are maintained across species.

FATTY ACID AMINO HYDROLASE (FAAH)

As mentioned above, FAAH seems to be the major degradative enzyme for anandamide and related amides in vivo (49). In contrast to the findings of in vitro studies, FAAH does not appear to metabolize 2-AG to a significant extent in vivo (133). Thus, drugs that selectively inhibit FAAH would increase N-acylethanolamine levels without affecting those of 2-AG. FAAH's active site is distinct from other mammalian hydrolases favoring the development of selective FAAH inhibitors. Indeed, in addition to the less specific trifluoromethyl ketone inhibitors, at least two families of FAAH inhibitors have been developed, the alpha-ketoheterocycles and the carbamates (134–136). Clues that these inhibitors might be therapeutically useful can be inferred from a number of studies that have either looked at anandamide levels following a behavioral manipulation or investigated consequences of FAAH inhibition. For example, midbrain anandamide levels are increased following injection of Freund's adjuvant (137) and treatment with the CB1 antagonist, SR141716, typically increases pain behaviors (138). Taken together, these results suggest that endocannabinoid release is enhanced following nociceptive stimulation and that inhibiting the degradation of these endocannabinoids (here, presumably an N-acylethanolamine) might be therapeutically beneficial. Results from both FAAH knockout mice and specific FAAH inhibitors support this notion. Genetic deletion of FAAH greatly increases brain levels of anandamide and other acylethanolamides, but doesn't change levels of 2-AG. FAAH knockout mice have elevated pain thresholds in several analgesic tests (139). Furthermore, the analgesia produced by selective carbamate-based FAAH inhibitors (URB532, URB597), as well as the reversible alpha-ketoheterocyclic FAAH inhibitors (OL92, OL135), is blocked by CB1 antagonists (140, 141). The carbamate FAAH inhibitors are also efficacious in two anxiolysis models (140).

Importantly for their therapeutic application, selective FAAH inhibitors do not produce the catalepsy, hypothermia, or hyperphagia that are seen with direct CB1 receptor agonists (140, 141). Taken together, these observations emphasize that with FAAH inhibitors, it is feasible to produce local increases in endocannabinoids associated with behaviorally meaningful effects and that these inhibitors activate

cannabinoid signaling in a much more selective way than is possible with CB1 agonists.

ENDOCANNABINOID TRANSPORT INHIBITORS

It is commonly assumed—although not rigorously proven—that endocannabinoid action is terminated, in part, by their uptake into cells. This putative mechanism appears similar for anandamide and 2-AG, with some mild differences in the effect of the degraded endocannabinoid on the process (142). However, whether endocannabinoid transport occurs through a specific transporter or by nonspecific means has been the subject of some debate (47, 143). The initial pharmacological probe used to study the putative endocannabinoid membrane transporter (EMT), AM404, inhibited uptake, but it also interacted with CB1 receptors and activated VR1 channels at higher concentrations. In addition, it is a substrate for FAAH. These multiple actions often made it difficult to interpret studies done with AM404, particularly in vivo studies. This led to a hypothesis that endocannabinoid passage across membranes was passive and carrier independent, solely as a consequence of their metabolism by FAAH (or MAG lipase). Thus, the reported inhibition of transport by AM404 was merely inhibition of anandamide degradation, accumulation of nondegraded endocannabinoid, and loss of the concentration gradient driving passive diffusion (144). However, the development of more potent and specific EMT inhibitors that have less activity toward FAAH, VR1, and CB1 clearly shows that it is possible to dissociate inhibition of FAAH (which would decrease endocannabinoid transport because of intracellular accumulation of endocannabinoid) from authentic transmembrane endocannabinoid transport (145–148). These compounds, typified by UCM707 and AM1172, will be useful in determining the therapeutic utility of inhibiting endocannabinoid uptake. For example, UCM707 augments the hypokinetic and analgesic effects of a subtherapeutic dose of systemically administered anandamide (149). Although pointing toward a transport process, it needs to be recognized that these inhibitor studies do not differentiate between the possibilities that the EMT is a transmembrane transporter, a carrier protein, or another entity. Implicit in the preceding discussion is that the primary role of the EMT is to terminate the action of endocannabinoids. However, strong evidence also suggests that the EMT may also play a role in the release of endocannabinoids (64). Whether this role offers therapeutic possibilities remains to be determined.

PERSPECTIVES FOR THE FUTURE

These are exciting times for drugs targeting cannabinoid receptors and the endocannabinoid system. A rich variety of drugs are being developed and novel indications elucidated. It is likely that CB1 antagonists will first receive regulatory

approval for metabolic indications, followed by smoking cessation, if the current trend of promising clinical studies continues. The cannabis extract Sativex has received approval in Canada, and regulatory approval is pending in Europe. However, it remains to be seen how widely this preparation will be accepted. Based on preclinical studies and their lack of psychoactivity, CB2 agonists have a strong potential for treatment of pain and some promise in osteoporosis and cardiovascular disease. Inhibitors of the enzymes that lead to the synthesis of endocannabinoids have not received much attention from a therapeutic perspective, in part because there is no strong evidence that an excess of endocannabinoid is detrimental. With their high specificity, FAAH inhibitors remain an exciting potential therapeutic, possibly for pain or anxiety, although clinical studies have yet to be performed. Although no specific and potent MAG lipase inhibitors have been reported, such compounds will certainly be useful for research purposes and may be clinically valuable. Now that the preponderance of evidence supports the existence of an endocannabinoid membrane transporter and selective drugs have been reported, preclinical studies should be forthcoming to identify possible therapeutic targets. The past fifteen years have seen a burgeoning in our understanding of the endocannabinoid system, and it is likely that the next five years will see much of this knowledge translated into useful therapeutic agents.

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LITERATURE CITED

1. Black SC. 2004. Cannabinoid receptor antagonists and obesity. *Curr. Opin. Investig. Drugs* 5:389–94
2. Di Carlo G, Izzo AA. 2003. Cannabinoids for gastrointestinal diseases: potential therapeutic applications. *Expert Opin. Investig. Drugs* 12:39–49
3. Di Marzo V, Bifulco M, De Petrocellis L. 2004. The endocannabinoid system and its therapeutic exploitation. *Nat. Rev. Drug Discov.* 3:771–84
4. Drysdale AJ, Platt B. 2003. Cannabinoids: mechanisms and therapeutic applications in the CNS. *Curr. Med. Chem.* 10:2719–32
5. Jarvinen T, Pate DW, Laine K. 2002. Cannabinoids in the treatment of glaucoma. *Pharmacol. Ther.* 95:203–20
6. Kirkham TC, Williams CM. 2004. Endocannabinoid receptor antagonists: potential for obesity treatment. *Treat. Endocrinol.* 3:345–60
7. Mendizabal VE, Adler-Graschinsky E. 2003. Cannabinoid system as a potential target for drug development in the treatment of cardiovascular disease. *Curr. Vasc. Pharmacol.* 1:301–13
8. Russo EB. 2004. Clinical endocannabinoid deficiency (CECD): can this concept explain therapeutic benefits of cannabis in migraine, fibromyalgia, irritable bowel syndrome and other

treatment-resistant conditions? *Neuro. Endocrinol. Lett.* 25:31–39
9. Walter L, Stella N. 2004. Cannabinoids and neuroinflammation. *Br. J. Pharmacol.* 141:775–85
10. Matsuda LA, Lolait SJ, Brownstein MJ, Young AC, Bonner TI. 1990. Structure of a cannabinoid receptor and functional expression of the cloned cDNA. *Nature* 346:561–64
11. Compton DR, Gold LH, Ward SJ, Balster RL, Martin BR. 1992. Aminoalkylindole analogs: cannabimimetic activity of a class of compounds structurally distinct from delta 9-tetrahydrocannabinol. *J. Pharmacol. Exp. Ther.* 263:1118–26
12. Little PJ, Compton DR, Johnson MR, Melvin LS, Martin BR. 1988. Pharmacology and stereoselectivity of structurally novel cannabinoids in mice. *J. Pharmacol. Exp. Ther.* 247:1046–51
13. Melvin LS, Milne GM, Johnson MR, Subramaniam B, Wilken GH, Howlett AC. 1993. Structure-activity relationships for cannabinoid receptor-binding and analgesic activity: studies of bicyclic cannabinoid analogs. *Mol. Pharmacol.* 44:1008–15
14. Howlett AC, Barth F, Bonner TI, Cabral G, Casellas P, et al. 2002. International Union of Pharmacology. XXVII. Classification of cannabinoid receptors. *Pharmacol. Rev.* 54:161–202
15. Munro S, Thomas KL, Abu-Shaar M. 1993. Molecular characterization of a peripheral receptor for cannabinoids. *Nature* 365:61–65
16. Bouaboula M, Rinaldi M, Carayon P, Carillon C, Delpech B, et al. 1993. Cannabinoid-receptor expression in human leukocytes. *Eur. J. Biochem.* 214: 173–80
17. Mackie K, Lai Y, Westenbroek R, Mitchell R. 1995. Cannabinoids activate an inwardly rectifying potassium conductance and inhibit Q-type calcium currents in AtT20 cells transfected with rat brain cannabinoid receptor. *J. Neurosci.* 15:6552–61
18. Twitchell W, Brown S, Mackie K. 1997. Cannabinoids inhibit N- and P/Q-type calcium channels in cultured rat hippocampal neurons. *J. Neurophysiol.* 78: 43–50
19. Derkinderen P, Toutant M, Burgaya F, Le Bert M, Siciliano JC, et al. 1996. Regulation of a neuronal form of focal adhesion kinase by anandamide. *Science* 273:1719–22
20. Gomez Del Pulgar T, De Ceballos ML, Guzman M, Velasco G. 2002. Cannabinoids protect astrocytes from ceramide-induced apoptosis through the phosphatidylinositol 3-kinase/protein kinase B pathway. *J. Biol. Chem.* 277: 36527–33
21. Sanchez C, Galve-Roperh I, Rueda D, Guzman M. 1998. Involvement of sphingomyelin hydrolysis and the mitogen-activated protein kinase cascade in the delta9-tetrahydrocannabinol-induced stimulation of glucose metabolism in primary astrocytes. *Mol. Pharmacol.* 54: 834–43
22. Waksman Y, Olson JM, Carlisle SJ, Cabral GA. 1999. The central cannabinoid receptor (CB1) mediates inhibition of nitric oxide production by rat microglial cells. *J. Pharmacol. Exp. Ther.* 288:1357–66
23. Di Marzo V, Griffin G, De Petrocellis L, Brandi I, Bisogno T, et al. 2002. A structure/activity relationship study on arvanil, an endocannabinoid and vanilloid hybrid. *J. Pharmacol. Exp. Ther.* 300: 984–91
24. Hajos N, Freund TF. 2002. Pharmacological separation of cannabinoid sensitive receptors on hippocampal excitatory and inhibitory fibers. *Neuropharmacology* 43:503–10
25. Randall MD, Kendall DA, O'Sullivan S. 2004. The complexities of the cardiovascular actions of cannabinoids. *Br. J. Pharmacol.* 142:20–26

26. Devane WA, Hanus L, Breuer A, Pertwee RG, Stevenson LA, et al. 1992. Isolation and structure of a brain constituent that binds to the cannabinoid receptor. *Science* 258:1946–49
27. Felder CC, Briley EM, Axelrod J, Simpson JT, Mackie K, Devane WA. 1993. Anandamide, an endogenous cannabimimetic eicosanoid, binds to the cloned human cannabinoid receptor and stimulates receptor-mediated signal transduction. *Proc. Natl. Acad. Sci. USA* 90:7656–60
28. Calignano A, La Rana G, Piomelli D. 2001. Antinociceptive activity of the endogenous fatty acid amide, palmitylethanolamide. *Eur. J. Pharmacol.* 419: 191–98
29. Fu J, Gaetani S, Oveisi F, Lo Verme J, Serrano A, et al. 2003. Oleylethanolamide regulates feeding and body weight through activation of the nuclear receptor PPAR-alpha. *Nature* 425:90–93
30. Mechoulam R, Ben-Shabat S, Hanus L, Ligumsky M, Kaminski NE, et al. 1995. Identification of an endogenous 2-monoglyceride, present in canine gut, that binds to cannabinoid receptors. *Biochem. Pharmacol.* 50:83–90
31. Sugiura T, Kondo S, Sukagawa A, Nakane S, Shinoda A, et al. 1995. 2-Arachidonoylglycerol: a possible endogenous cannabinoid receptor ligand in brain. *Biochem. Biophys. Res. Commun.* 215:89–97
32. Stella N, Schweitzer P, Piomelli D. 1997. A second endogenous cannabinoid that modulates long-term potentiation. *Nature* 388:773–78
33. Bradshaw HB, Walker JM. 2005. The expanding field of cannabimimetic and related lipid mediators. *Br. J. Pharmacol.* 144:459–65
34. Di Marzo V, Fontana A, Cadas H, Schinelli S, Cimino G, et al. 1994. Formation and inactivation of endogenous cannabinoid anandamide in central neurons. *Nature* 372:686–91
35. Okamoto Y, Morishita J, Tsuboi K, Tonai T, Ueda N. 2004. Molecular characterization of a phospholipase D generating anandamide and its congeners. *J. Biol. Chem.* 279:5298–305
36. Cadas H, di Tomaso E, Piomelli D. 1997. Occurrence and biosynthesis of endogenous cannabinoid precursor, N-arachidonoyl phosphatidylethanolamine, in rat brain. *J. Neurosci.* 17:1226–42
37. Cadas H, Gaillet S, Beltramo M, Venance L, Piomelli D. 1996. Biosynthesis of an endogenous cannabinoid precursor in neurons and its control by calcium and cAMP. *J. Neurosci.* 16:3934–42
38. Bisogno T, Howell F, Williams G, Minassi A, Cascio MG, et al. 2003. Cloning of the first sn1-DAG lipases points to the spatial and temporal regulation of endocannabinoid signaling in the brain. *J. Cell Biol.* 163:463–68
39. Piomelli D. 2003. The molecular logic of endocannabinoid signalling. *Nat. Rev. Neurosci.* 4:873–84
40. Breivogel CS, Childers SR. 2000. Cannabinoid agonist signal transduction in rat brain: comparison of cannabinoid agonists in receptor binding, G-protein activation, and adenylyl cyclase inhibition. *J. Pharmacol. Exp. Ther.* 295:328–36
41. Breivogel CS, Selley DE, Childers SR. 1998. Cannabinoid receptor agonist efficacy for stimulating [35S]GTPgammaS binding to rat cerebellar membranes correlates with agonist-induced decreases in GDP affinity. *J. Biol. Chem.* 273:16865–73
42. Gonsiorek W, Lunn C, Fan X, Narula S, Lundell D, Hipkin RW. 2000. Endocannabinoid 2-arachidonyl glycerol is a full agonist through human type 2 cannabinoid receptor: antagonism by anandamide. *Mol. Pharmacol.* 57:1045–50
43. Luk T, Jin W, Zvonok A, Lu D, Lin XZ, et al. 2004. Identification of a potent and

highly efficacious, yet slowly desensitizing CB1 cannabinoid receptor agonist. *Br. J. Pharmacol.* 142:495–500
44. Mackie K, Devane WA, Hille B. 1993. Anandamide, an endogenous cannabinoid, inhibits calcium currents as a partial agonist in N18 neuroblastoma cells. *Mol. Pharmacol.* 44:498–503
45. Huestis MA, Gorelick DA, Heishman SJ, Preston KL, Nelson RA, et al. 2001. Blockade of effects of smoked marijuana by the CB1-selective cannabinoid receptor antagonist SR141716. *Arch. Gen. Psychiatry* 58:322–28
46. Huestis MA, Preston KL, Heishman SJ, Boyd ST, Mondick J, et al. 2004. Antagonism of inhalded cannabis by single and multiple doses of SR141716 (rimonabant) in male subjects with a history of cannabis use. *2004 Symp. Cannabinoids*, p. 86 , Paestum, Italy
47. McFarland MJ, Barker EL. 2004. Anandamide transport. *Pharmacol. Ther.* 104: 117–35
48. Cravatt BF, Lichtman AH. 2003. Fatty acid amide hydrolase: an emerging therapeutic target in the endocannabinoid system. *Curr. Opin. Chem. Biol.* 7:469–75
49. McKinney MK, Cravatt BF. 2005. Structure and function of fatty acid amide hydrolase. *Annu. Rev. Biochem.* 74:411–32
50. Dinh TP, Carpenter D, Leslie FM, Freund TF, Katona I, et al. 2002. Brain monoglyceride lipase participating in endocannabinoid inactivation. *Proc. Natl. Acad. Sci. USA* 99:10819–24
51. Dinh TP, Kathuria S, Piomelli D. 2004. RNA interference suggests a primary role for monoacylglycerol lipase in the degradation of the endocannabinoid 2-arachidonoylglycerol. *Mol. Pharmacol.* 66:1260–64
52. Ghafouri N, Tiger G, Razdan RK, Mahadevan A, Pertwee RG, et al. 2004. Inhibition of monoacylglycerol lipase and fatty acid amide hydrolase by analogues of 2-arachidonoylglycerol. *Br. J. Pharmacol.* 143:774–84
53. Freund TF, Katona I, Piomelli D. 2003. Role of endogenous cannabinoids in synaptic signaling. *Physiol. Rev.* 83: 1017–66
54. Alger BE. 2002. Retrograde signaling in the regulation of synaptic transmission: focus on endocannabinoids. *Prog. Neurobiol.* 68:247–86
55. Gerdeman GL, Lovinger DM. 2003. Emerging roles for endocannabinoids in long-term synaptic plasticity. *Br. J. Pharmacol.* 140:781–89
56. Kim J, Isokawa M, Ledent C, Alger BE. 2002. Activation of muscarinic acetylcholine receptors enhances the release of endogenous cannabinoids in the hippocampus. *J. Neurosci.* 22:10182–91
57. Kreitzer AC, Regehr WG. 2001. Retrograde inhibition of presynaptic calcium influx by endogenous cannabinoids at excitatory synapses onto Purkinje cells. *Neuron* 29:717–27
58. Varma N, Carlson GC, Ledent C, Alger BE. 2001. Metabotropic glutamate receptors drive the endocannabinoid system in hippocampus. *J. Neurosci.* 21: RC188
59. Wilson RI, Kunos G, Nicoll RA. 2001. Presynaptic specificity of endocannabinoid signaling in the hippocampus. *Neuron* 31:453–62
60. Wilson RI, Nicoll RA. 2001. Endogenous cannabinoids mediate retrograde signalling at hippocampal synapses. *Nature* 410:588–92
61. Carlson G, Wang Y, Alger BE. 2002. Endocannabinoids facilitate the induction of LTP in the hippocampus. *Nat. Neurosci.* 5:723–24
62. Kreitzer AC, Carter AG, Regehr WG. 2002. Inhibition of interneuron firing extends the spread of endocannabinoid signaling in the cerebellum. *Neuron* 34: 787–96
63. Chevaleyre V, Castillo PE. 2003. Heterosynaptic LTD of hippocampal

GABAergic synapses: a novel role of endocannabinoids in regulating excitability. *Neuron* 38:461–72

64. Gerdeman GL, Ronesi J, Lovinger DM. 2002. Postsynaptic endocannabinoid release is critical to long-term depression in the striatum. *Nat. Neurosci.* 5:446–51
65. Marsicano G, Wotjak CT, Azad SC, Bisogno T, Rammes G, et al. 2002. The endogenous cannabinoid system controls extinction of aversive memories. *Nature* 418:530–34
66. Robbe D, Kopf M, Remaury A, Bockaert J, Manzoni OJ. 2002. Endogenous cannabinoids mediate long-term synaptic depression in the nucleus accumbens. *Proc. Natl. Acad. Sci. USA* 99:8384–88
67. Fourgeaud L, Mato S, Bouchet D, Hemar A, Worley PF, Manzoni OJ. 2004. A single in vivo exposure to cocaine abolishes endocannabinoid-mediated long-term depression in the nucleus accumbens. *J. Neurosci.* 24:6939–45
68. Mato S, Chevaleyre V, Robbe D, Pazos A, Castillo PE, Manzoni OJ. 2004. A single in-vivo exposure to delta 9THC blocks endocannabinoid-mediated synaptic plasticity. *Nat. Neurosci.* 7:585–86
69. Chevaleyre V, Castillo PE. 2004. Endocannabinoid-mediated metaplasticity in the hippocampus. *Neuron* 43: 871–81
70. Wagner JA, Varga K, Kunos G. 1998. Cardiovascular actions of cannabinoids and their generation during shock. *J. Mol. Med.* 76:824–36
71. Batkai S, Jarai Z, Wagner JA, Goparaju SK, Varga K, et al. 2001. Endocannabinoids acting at vascular CB1 receptors mediate the vasodilated state in advanced liver cirrhosis. *Nat. Med.* 7:827–32
72. Bloom AS, Tershner S, Fuller SA, Stein EA. 1997. Cannabinoid-induced alterations in regional cerebral blood flow in the rat. *Pharmacol. Biochem. Behav.* 57:625–31
73. Stein EA, Fuller SA, Edgemond WS, Campbell WB. 1998. Selective effects of the endogenous cannabinoid arachidonylethanolamide (anandamide) on regional cerebral blood flow in the rat. *Neuropsychopharmacology* 19:481–91
74. Kunos G, Batkai S, Offertaler L, Mo F, Liu J, et al. 2002. The quest for a vascular endothelial cannabinoid receptor. *Chem. Phys. Lipids* 121:45–56
75. Batkai S, Pacher P, Osei-Hyiaman D, Radaeva S, Liu J, et al. 2004. Endocannabinoids acting at cannabinoid-1 receptors regulate cardiovascular function in hypertension. *Circulation* 110:1996–2002
76. Van Gaal LF, Rissanen AM, Scheen AJ, Ziegler O, Rossner S. 2005. 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* 365:1389–97
77. Iversen L. 2003. Cannabis and the brain. *Brain* 126:1252–70
78. Smith PF. 2004. GW-1000. GW Pharmaceuticals. *Curr. Opin. Investig. Drugs* 5:748–54
79. Berman JS, Symonds C, Birch R. 2004. Efficacy of two cannabis based medicinal extracts for relief of central neuropathic pain from brachial plexus avulsion: results of a randomised controlled trial. *Pain* 112:299–306
80. Wade DT, Makela P, Robson P, House H, Bateman C. 2004. Do cannabis-based medicinal extracts have general or specific effects on symptoms in multiple sclerosis? A double-blind, randomized, placebo-controlled study on 160 patients. *Mult. Scler.* 10:434–41
81. Foltin RW, Fischman MW, Byrne MF. 1988. Effects of smoked marijuana on food intake and body weight of humans living in a residential laboratory. *Appetite* 11:1–14
82. Rinaldi-Carmona M, Barth F, Heaulme M, Shire D, Calandra B, et al. 1994.

SR141716A, a potent and selective antagonist of the brain cannabinoid receptor. *FEBS Lett.* 350:240–44

83. Bouaboula M, Perrachon S, Milligan L, Canat X, Rinaldi-Carmona M, et al. 1997. A selective inverse agonist for central cannabinoid receptor inhibits mitogen-activated protein kinase activation stimulated by insulin or insulin-like growth factor 1. Evidence for a new model of receptor/ligand interactions. *J. Biol. Chem.* 272:22330–39
84. Pan X, Ikeda SR, Lewis DL. 1998. SR 141716A acts as an inverse agonist to increase neuronal voltage-dependent Ca2+ currents by reversal of tonic CB1 cannabinoid receptor activity. *Mol. Pharmacol.* 54:1064–72
85. Felder CC, Joyce KE, Briley EM, Glass M, Mackie KP, et al. 1998. LY320135, a novel cannabinoid CB1 receptor antagonist, unmasks coupling of the CB1 receptor to stimulation of cAMP accumulation. *J. Pharmacol. Exp. Ther.* 284: 291–97

85a. Smith RA, Fathi A. 2005. Recent advances in the research and development of CB_1 antagonists. *IDrugs* 8:53–66

86. Williams CM, Rogers PJ, Kirkham TC. 1998. Hyperphagia in pre-fed rats following oral delta9-THC. *Physiol. Behav.* 65:343–46
87. Ravinet Trillou C, Arnone M, Delgorge C, Gonalons N, Keane P, et al. 2003. Anti-obesity effect of SR141716, a CB1 receptor antagonist, in diet-induced obese mice. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 284:R345–53
88. Di Marzo V, Goparaju SK, Wang L, Liu J, Batkai S, et al. 2001. Leptin-regulated endocannabinoids are involved in maintaining food intake. *Nature* 410:822–25
89. Verty AN, McGregor IS, Mallet PE. 2004. Consumption of high carbohydrate, high fat, and normal chow is equally suppressed by a cannabinoid receptor antagonist in non-deprived rats. *Neurosci. Lett.* 354:217–20
90. Colombo G, Agabio R, Diaz G, Lobina C, Reali R, Gessa GL. 1998. Appetite suppression and weight loss after the cannabinoid antagonist SR 141716. *Life Sci.* 63:PL113–17
91. Osei-Hyiaman D, Depetrillo M, Pacher P, Liu J, Radaeva S, et al. 2005. Endocannabinoid action at hepatic CB1 receptors regulates fatty acid synthesis: role in diet-induced obesity. *J. Clin. Invest.* 115:1298–305
92. Cota D, Marsicano G, Tschop M, Grubler Y, Flachskamm C, et al. 2003. The endogenous cannabinoid system affects energy balance via central orexigenic drive and peripheral lipogenesis. *J. Clin. Invest.* 112:423–31
93. Boyd ST, Fremming BA. 2005. Rimonabant—a selective CB1 antagonist. *Ann. Pharmacother.* 39:684–90
94. Cleland JG, Ghosh J, Freemantle N, Kaye GC, Nasir M, et al. 2004. Clinical trials update and cumulative meta-analyses from the American College of Cardiology: WATCH, SCD-HeFT, DINAMIT, CASINO, INSPIRE, STRATUS-US, RIO-lipids and cardiac resynchronisation therapy in heart failure. *Eur. J. Heart Fail.* 6:501–8
95. Fattore L, Cossu G, Spano MS, Deiana S, Fadda P, et al. 2004. Cannabinoids and reward: interactions with the opioid system. *Crit. Rev. Neurobiol.* 16:147–58
96. Le Foll B, Goldberg SR. 2005. Cannabinoid CB1 receptor antagonists as promising new medications for drug dependence. *J. Pharmacol. Exp. Ther.* 312:875–83
97. Lupica CR, Riegel AC, Hoffman AF. 2004. Marijuana and cannabinoid regulation of brain reward circuits. *Br. J. Pharmacol.* 143:227–34
98. Corchero J, Manzanares J, Fuentes JA. 2004. Cannabinoid/opioid crosstalk in the central nervous system. *Crit. Rev. Neurobiol.* 16:159–72
99. Chaperon F, Soubrie P, Puech AJ, Thiebot MH. 1998. Involvement of central

cannabinoid (CB1) receptors in the establishment of place conditioning in rats. *Psychopharmacology* 135:324–32

100. Cossu G, Ledent C, Fattore L, Imperato A, Bohme GA, et al. 2001. Cannabinoid CB1 receptor knockout mice fail to self-administer morphine but not other drugs of abuse. *Behav. Brain Res.* 118:61–65
101. De Vries TJ, Homberg JR, Binnekade R, Raaso H, Schoffelmeer AN. 2003. Cannabinoid modulation of the reinforcing and motivational properties of heroin and heroin-associated cues in rats. *Psychopharmacology* 168:164–69
102. Solinas M, Panlilio LV, Antoniou K, Pappas LA, Goldberg SR. 2003. The cannabinoid CB1 antagonist N-piperidinyl-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methylpyrazole-3-carboxamide(SR-141716A) differentially alters the reinforcing effects of heroin under continuous reinforcement, fixed ratio, and progressive ratio schedules of drug self-administration in rats. *J. Pharmacol. Exp. Ther.* 306:93–102
103. Chen JP, Paredes W, Li J, Smith D, Lowinson J, Gardner EL. 1990. Delta 9-tetrahydrocannabinol produces naloxone-blockable enhancement of presynaptic basal dopamine efflux in nucleus accumbens of conscious, freely-moving rats as measured by intracerebral microdialysis. *Psychopharmacology* 102:156–62
104. Ghozland S, Matthes HW, Simonin F, Filliol D, Kieffer BL, Maldonado R. 2002. Motivational effects of cannabinoids are mediated by mu-opioid and kappa-opioid receptors. *J. Neurosci.* 22: 1146–54
105. Wachtel SR, de Wit H. 2000. Naltrexone does not block the subjective effects of oral delta(9)-tetrahydrocannabinol in humans. *Drug Alcohol Depend.* 59:251–60
106. Castane A, Valjent E, Ledent C, Parmentier M, Maldonado R, Valverde O. 2002. Lack of CB1 cannabinoid receptors modifies nicotine behavioural responses, but not nicotine abstinence. *Neuropharmacology* 43:857–67
107. Cohen C, Perrault G, Voltz C, Steinberg R, Soubrie P. 2002. SR141716, a central cannabinoid (CB(1)) receptor antagonist, blocks the motivational and dopamine-releasing effects of nicotine in rats. *Behav. Pharmacol.* 13:451–63
108. Valjent E, Mitchell JM, Besson MJ, Caboche J, Maldonado R. 2002. Behavioural and biochemical evidence for interactions between delta 9-tetrahydrocannabinol and nicotine. *Br. J. Pharmacol.* 135:564–78
109. Tanda G, Pontieri FE, Di Chiara G. 1997. Cannabinoid and heroin activation of mesolimbic dopamine transmission by a common mu1 opioid receptor mechanism. *Science* 276:2048–50
110. Gallate JE, Mallet PE, McGregor IS. 2004. Combined low dose treatment with opioid and cannabinoid receptor antagonists synergistically reduces the motivation to consume alcohol in rats. *Psychopharmacology* 173:210–16
111. Gallate JE, Saharov T, Mallet PE, McGregor IS. 1999. Increased motivation for beer in rats following administration of a cannabinoid CB1 receptor agonist. *Eur. J. Pharmacol.* 370:233–40
112. Mechoulam R, Parker L. 2003. Cannabis and alcohol—a close friendship. *Trends Pharmacol. Sci.* 24:266–68
113. Basavarajappa BS, Hungund BL. 2002. Neuromodulatory role of the endocannabinoid signaling system in alcoholism: an overview. *Prostaglandins Leukot. Essent. Fatty Acids* 66:287–99
114. Walker JM, Huang SM. 2002. Cannabinoid analgesia. *Pharmacol. Ther.* 95: 127–35
115. Hohmann AG, Farthing JN, Zvonok AM, Makriyannis A. 2004. Selective activation of cannabinoid CB2 receptors suppresses hyperalgesia evoked by intradermal capsaicin. *J. Pharmacol. Exp. Ther.* 308:446–53

116. Ibrahim MM, Deng H, Zvonok A, Cockayne DA, Kwan J, et al. 2003. Activation of CB2 cannabinoid receptors by AM1241 inhibits experimental neuropathic pain: pain inhibition by receptors not present in the CNS. *Proc. Natl. Acad. Sci. USA* 100:10529–33
117. Ibrahim MM, Porreca F, Lai J, Albrecht PJ, Rice FL, et al. 2005. CB2 cannabinoid receptor activation produces antinociception by stimulating peripheral release of endogenous opioids. *Proc. Natl. Acad. Sci. USA* 102:3093–98
118. Malan TP Jr, Ibrahim MM, Deng H, Liu Q, Mata HP, et al. 2001. CB2 cannabinoid receptor-mediated peripheral antinociception. *Pain* 93:239–45
119. Nackley AG, Makriyannis A, Hohmann AG. 2003. Selective activation of cannabinoid CB(2) receptors suppresses spinal fos protein expression and pain behavior in a rat model of inflammation. *Neuroscience* 119:747–57
120. Nackley AG, Zvonok AM, Makriyannis A, Hohmann AG. 2004. 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.* 92:3562–74
121. Quartilho A, Mata HP, Ibrahim MM, Vanderah TW, Porreca F, et al. 2003. Inhibition of inflammatory hyperalgesia by activation of peripheral CB2 cannabinoid receptors. *Anesthesiology* 99:955–60
122. Elmes SJ, Jhaveri MD, Smart D, Kendall DA, Chapman V. 2004. 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.* 20:2311–20
123. Zhang J, Hoffert C, Vu HK, Groblewski T, Ahmad S, O'Donnell D. 2003. Induction of CB2 receptor expression in the rat spinal cord of neuropathic but not inflammatory chronic pain models. *Eur. J. Neurosci.* 17:2750–54
124. Bab I. 2005. The skeleton: stone bones or stoned heads? In *Cannabinoids as Therapeutics*, ed. R. Mechoulam, pp. 201–6. Basel, Switzerland: Birkhauser Verlag AG
125. Idris AI, van't Hof RJ, Greig IR, Ridge SA, Baker D, et al. 2005. Regulation of bone mass, bone loss and osteoclast activity by cannabinoid receptors. *Nat. Med.* 11:774–79
126. Steffens S, Veillard NR, Arnaud C, Pelli G, Burger F, et al. 2005. Low dose oral cannabinoid therapy reduces progression of atherosclerosis in mice. *Nature* 434:782–86
127. Coffey RG, Snella E, Johnson K, Pross S. 1996. Inhibition of macrophage nitric oxide production by tetrahydrocannabinol in vivo and in vitro. *Int. J. Immunopharmacol.* 18:749–52
128. Lyman WD, Sonett JR, Brosnan CF, Elkin R, Bornstein MB. 1989. Delta 9-tetrahydrocannabinol: a novel treatment for experimental autoimmune encephalomyelitis. *J. Neuroimmunol.* 23:73–81
129. Srivastava MD, Srivastava BI, Brouhard B. 1998. Delta9 tetrahydrocannabinol and cannabidiol alter cytokine production by human immune cells. *Immunopharmacology* 40:179–85
130. Zurier RB. 2003. Prospects for cannabinoids as anti-inflammatory agents. *J. Cell Biochem.* 88:462–66
130a. Benito C, Nunez, E, Tolon, RM, Carrier EJ, Rabano A, et al. 2003. Cannabinoid CB2 receptors and fatty acid amide hydrolase are selectively overexpressed in neuritic plaque-associated glia in Alzheimer's disease brains. *J. Neurosci.* 23:11136–41
130b. Benito C, Kim WK, Chavarria I, Hillard CJ, Mackie K, et al. 2005. A glial endogenous cannabinoid system is upregulated in the brains of macques with

simian immunodeficiency virus-induced encephalitis. *J. Neurosci.* 25:2530–36

131. Bouaboula M, Dussossoy D, Casellas P. 1999. Regulation of peripheral cannabinoid receptor CB2 phosphorylation by the inverse agonist SR 144528. Implications for receptor biological responses. *J. Biol. Chem.* 274:20397–405
132. Brown SM, Wager-Miller J, Mackie K. 2002. Cloning and molecular characterization of the rat CB2 cannabinoid receptor. *Biochim. Biophys. Acta* 1576:255–64
133. Lichtman AH, Hawkins EG, Griffin G, Cravatt BF. 2002. Pharmacological activity of fatty acid amides is regulated, but not mediated, by fatty acid amide hydrolase in vivo. *J. Pharmacol. Exp. Ther.* 302:73–79
134. Boger DL, Miyauchi H, Du W, Hardouin C, Fecik RA, et al. 2005. Discovery of a potent, selective, and efficacious class of reversible alpha-ketoheterocycle inhibitors of fatty acid amide hydrolase effective as analgesics. *J. Med. Chem.* 48: 1849–56
135. Mor M, Rivara S, Lodola A, Plazzi PV, Tarzia G, et al. 2004. Cyclohexylcarbamic acid 3′- or 4′-substituted biphenyl-3-yl esters as fatty acid amide hydrolase inhibitors: synthesis, quantitative structure-activity relationships, and molecular modeling studies. *J. Med. Chem.* 47:4998–5008
136. Tarzia G, Duranti A, Tontini A, Piersanti G, Mor M, et al. 2003. Design, synthesis, and structure-activity relationships of alkylcarbamic acid aryl esters, a new class of fatty acid amide hydrolase inhibitors. *J. Med. Chem.* 46:2352–60
137. Walker JM, Huang SM, Strangman NM, Tsou K, Sanudo-Pena MC. 1999. Pain modulation by release of the endogenous cannabinoid anandamide. *Proc. Natl. Acad. Sci. USA* 96:12198–203
138. Richardson JD, Aanonsen L, Hargreaves KM. 1997. SR 141716A, a cannabinoid receptor antagonist, produces hyperalgesia in untreated mice. *Eur. J. Pharmacol.* 319:R3–4
139. Lichtman AH, Shelton CC, Advani T, Cravatt BF. 2004. Mice lacking fatty acid amide hydrolase exhibit a cannabinoid receptor-mediated phenotypic hypoalgesia. *Pain* 109:319–27
140. Kathuria S, Gaetani S, Fegley D, Valino F, Duranti A, et al. 2003. Modulation of anxiety through blockade of anandamide hydrolysis. *Nat. Med.* 9:76–81
141. Lichtman AH, Leung D, Shelton CC, Saghatelian A, Hardouin C, et al. 2004. Reversible inhibitors of fatty acid amide hydrolase that promote analgesia: evidence for an unprecedented combination of potency and selectivity. *J. Pharmacol. Exp. Ther.* 311:441–48
142. Beltramo M, Piomelli D. 2000. Carrier-mediated transport and enzymatic hydrolysis of the endogenous cannabinoid 2-arachidonylglycerol. *Neuroreport* 11: 1231–35
143. Hillard CJ, Jarrahian A. 2003. Cellular accumulation of anandamide: consensus and controversy. *Br. J. Pharmacol.* 140: 802–8
144. Glaser ST, Abumrad NA, Fatade F, Kaczocha M, Studholme KM, Deutsch DG. 2003. Evidence against the presence of an anandamide transporter. *Proc. Natl. Acad. Sci. USA* 100:4269–74
145. de Lago E, Fernandez-Ruiz J, Ortega-Gutierrez S, Viso A, Lopez-Rodriguez ML, Ramos JA. 2002. UCM707, a potent and selective inhibitor of endocannabinoid uptake, potentiates hypokinetic and antinociceptive effects of anandamide. *Eur. J. Pharmacol.* 449:99–103
146. Fegley D, Kathuria S, Mercier R, Li C, Goutopoulos A, et al. 2004. Anandamide transport is independent of fatty-acid amide hydrolase activity and is blocked by the hydrolysis-resistant inhibitor AM1172. *Proc. Natl. Acad. Sci. USA* 101:8756–61
147. Lopez-Rodriguez ML, Viso A, Ortega-Gutierrez S, Fowler CJ, Tiger G, et al.

2003. Design, synthesis, and biological evaluation of new inhibitors of the endocannabinoid uptake: comparison with effects on fatty acid amidohydrolase. *J. Med. Chem.* 46:1512–22

148. Ortega-Gutierrez S, Hawkins EG, Viso A, Lopez-Rodriguez ML, Cravatt BF. 2004. Comparison of anandamide transport in FAAH wild-type and knockout neurons: evidence for contributions by both FAAH and the CB1 receptor to anandamide uptake. *Biochemistry* 43:8184–90
149. Lopez-Rodriguez ML, Viso A, Ortega-Gutierrez S, Fowler CJ, Tiger G, et al. 2003. Design, synthesis and biological evaluation of new endocannabinoid transporter inhibitors. *Eur. J. Med. Chem.* 38:403–12

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