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**Authors**

Makriyannis, A
Mechoulam, R
Piomelli, D

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# Therapeutic opportunities through modulation of the endocannabinoid system

Alexandros Makriyannis, Raphael Mechoulam, Daniele Piomelli *

*University of California, Department of Pharmacology, Irvine, CA 92697-4260, United States*

The discovery of the cannabinoid receptors and endocannabinoid ligands has generated a great deal of interest in identifying opportunities for the development of novel cannabinergic therapeutic drugs. Such an effort was first undertaken three decades ago by a number of pharmaceutical industries, but was rewarded with only modest success. However, the newly acquired knowledge on the physiological roles of the endocannabinoid system has significantly enhanced these prospects.

At the June 27, 2004 workshop "Future Directions in Cannabinoid Therapeutics II: From the Bench to the Clinic", sponsored by the University of California Center for Medicinal Cannabis Research, we on the Scientific Planning Committee were asked to identify the areas of research with the most immediate promise for the development of novel therapeutic agents. The Committee identified four broad areas involving modulation of the endocannabinoid system as particularly promising in this regard: agonists for central $CB_1$ cannabinoid receptors and peripheral $CB_2$ receptors, antagonists of $CB_1$ receptors, inhibitors of endocannabinoid deactivation, and endocannabinoid-like compounds that act through mechanisms distinct from $CB_1$ and $CB_2$ receptors activation. Below, we summarize the data presented at the Workshop and the consensus of its participants on the most exciting opportunities for drug discovery.

## 1. Cannabinoid agonists

Two endogenous agonists of cannabinoid receptors have been well characterized and are now widely used in research: anandamide (arachidonoylethanolamide), and 2-arachidonoylglycerol (2-AG). Both molecules derive chemically from the polyunsaturated fatty acid, arachidonic acid, which is used in nature as the starting material for other important signaling compounds, such as the eicosanoids. Additional endocannabinoid-related compounds present in the body include virodhamine, which may act as an endogenous antagonist of $CB_1$ receptors, and arachidonoylserine, which may engage an as-yet-uncharacterized cannabinoid-like receptor expressed in the vasculature.

As is well-known, the *Cannabis* plant contains more than 60 cannabinoids, which include $(-)$-$\Delta^9$-tetrahydrocannabinol ($\Delta^9$-THC), cannabigerol, cannabidiol, cannabinol, cannabichromene and cannabicyclol. Attention has been mostly focused on $\Delta^9$-THC, because of its multiple biological properties. Nevertheless, less studied compounds such as cannabidiol may also be important, although we do not yet know at which receptors they may act to achieve their effects. $\Delta^9$-THC is the only natural cannabinoid presently used in the clinic.

In addition to these plant-derived cannabinoids, an extensive set of synthetic cannabinergic agonists has been developed over the last 30 years. Products of these efforts include CP-55940 (Pfizer), created by opening one of the rings of the tricyclic $\Delta^9$-THC structure and introducing other small changes in its structure; HU-210 (Hebrew University), a very potent cannabinoid agonist resembling some $\Delta^9$-THC metabolites; and WIN55212-2 (Winthrop), which belongs to an altogether different class of chemicals, the aminoalkylindoles. Additionally, the metabolically stable synthetic analog of anandamide *R*-methanandamide (AM356) is routinely used as a pharmacological probe to circumvent the short half life of the natural substance.

Two important new additions to this armamentarium under discussion at the workshop include a peripherally acting cannabinoid agonist in preclinical development by Novartis for the treatment of neuropathic and inflammatory pain (structure as yet undisclosed), and

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* Corresponding author. Tel.: +1 9498246180; fax: +1 9498246305.
*E-mail address:* piomelli@uci.edu (D. Piomelli).

BAY-387271 (Bayer), a centrally acting cannabinoid agonist in Phase II clinical studies for the treatment of stroke. The interest of the pharmaceutical industry in the application of cannabinoid agonists to the treatment of pain conditions is not recent. Indeed, most of the compounds now in experimental use derive from such an interest. Historically however cannabinoid agonist development has not proved clinically fruitful, largely because of the profound psychotropic side effects of centrally active cannabinoid agonists, hence the attention given to peripherally acting cannabinoids, which exhibit significant analgesic efficacy and low central activity in animal models.

Neuroprotection is a relatively new area for cannabinoid agonists, but one that appears to be already well advanced. Preclinical studies have made a convincing case for the efficacy of cannabinoid agents not only in experimental brain ischemia, but also in models of Parkinson's disease and other forms of degenerative brain disorders. The results of a Phase II clinical trial with BAY-387271 are awaited with great excitement.

Also highlighted during the conference were various derivatives of (−)cannabidiol. Particularly interesting in this regard was the compound (−)-7-hydroxy-4′-dimethylheptyl-cannabidiol (7-OH-DMH-CBD) a hydroxylated, dimethylheptylated cannabidiol, structurally related to HU-210. Like $\Delta^9$-THC, 7-OH-DMH-CBD is a potent inhibitor of electrically evoked contractions in the mouse vas deferens. However, 7-OH-DMH-CBD does not significantly bind to either $CB_1$ or $CB_2$ receptors and its inhibitory effects on muscle contractility are not blocked by $CB_1$ or $CB_2$ receptor antagonists, suggesting that the compound may target an as-yet-uncharacterized cannabinoid-like receptor. This hypothesis is reinforced by pharmacological experiments, which suggest that 7-OH-DMH-CBD displays anti-inflammatory and intestinal-relaxing properties, but does not exert overt psychoactive effects in mice. However, the nature of this hypothetical receptor and its relationship to other cannabinoid-like sites in the vasculature and in the brain hippocampus remains to be determined.

## 1.1. CB₂ agonists

Another way to reduce central side effects is to target peripheral $CB_2$ receptors, which are expressed in the brain only during inflammatory states and even then are limited to microglia. Selective $CB_2$ receptor agonists include the compounds AM1241 (University of Connecticut) and HU-308 (Hebrew University). Compounds of this type offer a great deal of promise in the treatment of pain and inflammation. Studies conducted on multiple animal models of pain have shown that the $CB_2$-selective agonist AM1241 has robust analgesic effects and is very potent in neuropathic pain models. These effects are maintained in $CB_1$-deficient, but not in $CB_2$-deficient mice.

## 1.2. Potential problems

Cannabinoid analogs are currently in development or being tested by Astra, Bayer, Endo, GSK, GW Pharmaceuticals, Indevus, Kadmus Pharmaceuticals, MAKScientific, and Pharmos. Some of the obvious issues in drug development are efficacy, side effects, and regulatory scheduling. With regard to efficacy, the primary clinical targets are pain, stroke, nausea, appetite, and cough. Potential side effects in addition to the well-known central psychotropic actions include cardiovascular and immune perturbations, as well as fertility problems. Finally, a major question from the perspective of drug industry is one of regulatory scheduling: that is, whether any drug acting either directly or indirectly through the endocannabinoid system will be subject to the restrictive scheduling of $\Delta^9$-THC, or whether scheduling will be determined by side effects, as is the case with most drugs.

## 2. CB₁ antagonists

A large number of pharmaceutical companies have started active $CB_1$ antagonist programs, mostly as a result of the clinical success of SR141716A (rimonabant), the first $CB_1$ antagonist to be developed. This molecule has successfully completed Phase III studies and is anticipated to become available within a year for the treatment of obesity and tobacco addiction. Rimonabant is an inverse $CB_1$ agonist with a $K_i$ of 11 nM at the $CB_1$ receptors and 1640 nM at $CB_2$. Additional agents currently in development include SLV-326 (Solvay) and LY320135 (Lilly). However, all of these compounds are inverse agonists. A series of neutral antagonists has been generated, but remains not as well characterized in the literature. Examples of this class are the compounds O-2654 (Organix) and AM5171 (University of Connecticut). As noted above, therapeutic areas for cannabinoid antagonists include obesity, drug addiction and perhaps CNS disorders.

## 2.1. Obesity

The mechanism by which cannabinoid antagonists exert their anti-obesity effects is still not fully understood. Data on rimonabant presented at the workshop identified two possibilities. First, there is a loss of appetite. Mutant mice that are deficient in $CB_1$ receptors eat less than wild-type controls. Second, there is an increase in metabolic rate and a loss of fat mass. These effects may be linked, on the one hand, to the ability of rimonabant to affect corticotropin-releasing hormone (CRH), as suggested by the fact that $CB_1$ receptors colocalize with CRH receptors in the hypothalamus. This may be significant for explaining the drug's effects

on appetite drive, as it is known that CRH is anorexigenic. On the other hand, mice that lack $CB_1$ receptors display a hyperactivity of the hypothalamic-pituitary-adrenal axis, with increases in both ACTH and corticosterone. This phenotype may be important in regard to overall metabolic rate. Another possible mediator of the long-lasting effect on body weight reduction unrelated to altered food intake is the adipocyte, because $CB_1$ receptor activation causes lipogenesis, which is blocked by rimonabant.

## 2.2. Drug addiction

$CB_1$ cannabinoid receptors are present on the cell surface of neurons within the brain reward circuitry. Furthermore, endocannabinoids may be released from dopamine neurons in the ventral tegmental area (VTA), and from medium spiny neurons in the nucleus accumbens of the brain reward circuit. Additionally, endocannabinoids and $\Delta^9$-THC activate $CB_1$ receptors and by doing so regulate reward strength and drug craving. Though we do not know how this occurs, it is likely that these mechanisms extend to all drugs of abuse, because collectively these drugs show the propensity to increase VTA dopamine neuron activity, which might be coupled to augmented endocannabinoid production from the dopamine neurons themselves. Finally, cannabinoid receptor antagonists block the effects of endocannabinoids in these reward circuits.

Preclinical work shows that priming injections of cannabinoid agonists (WIN55212-2 and CP-55940) reinstate heroin-seeking behavior after a prolonged period of abstinence in rats trained to self-administer heroin. The cannabinoid antagonist rimonabant fully prevents heroin-induced reinstatement of heroin-seeking behavior. Additionally, rimonabant significantly attenuates cannabinoid-induced reinstatement of heroin-seeking behavior.

All these findings clearly support the hypothesis of a functional interaction between opioid and cannabinoid systems in the neurobiological mechanisms of relapse and might suggest a potential clinical use of cannabinoid antagonists for preventing relapse to heroin abuse. It has also been shown that cannabinoid antagonists can prevent drug reinstatement with cocaine, alcohol, and nicotine. Thus, it seems that the future of cannabinoid antagonists in substance abuse treatment is particularly promising, especially in the clinical setting, where polydrug abuse is exceedingly more common than isolated single-drug abuse.

## 2.3. Potential side effects

The available data suggest that $CB_1$ antagonism produces relatively mild side effects in people. Yet several potential risks were discussed and three in particular

received a great deal of attention. First, the possibility of neuropsychiatric sequelae, such as anhedonia and anxiety: preclinical studies have consistently shown such effects in animals, though they have not yet been observed in the clinic. Second, pain and hyperalgesia, because of the pervasive role played by the endocannabinoid system in the control of pain processing. Last, hypertension, as indicated by the contribution of the endocannabinoids to blood pressure regulation and the pressor effects of rimonabant in animal models of hypertension.

## 3. Endocannabinoid deactivation inhibitors

The endocannabinoid signaling system differs from classical neurotransmitter systems, picking up where classical neurotransmitters leave off. That is, the activation of receptors ($D_2$, glutamate, NMDA) initiates a series of chemical events that leads to the release of endocannabinoids from the postsynaptic spine – the final step of which is the enzymatic production and subsequent release of anandamide and/or 2-AG. Once released, the endocannabinoids are then directed to the presynaptic cell and the $CB_1$ receptor responds by inhibiting further release of that cell's neurotransmitters. The termination of this cascade is accomplished via a transporter that internalizes the endocannabinoids, after which intracellular enzymes such as fatty-acid amide hydrolase (FAAH) break them down.

There is a general consensus that endocannabinoids are transported into cells via a facilitated diffusion mechanism. This process may differ both kinetically and pharmacologically from cell to cell. In brain neurons, endocannabinoid transport is blocked by certain agents, which include the compounds AM404, OMDM-8 and AM1172 (University of Connecticut/University of California). However, the pharmacological properties of these drugs in vivo are only partially understood.

Once inside cells, endocannabinoids are hydrolyzed by three principal enzyme systems. FAAH is a key enzyme of anandamide deactivation in the brain. Potent and selective FAAH inhibitors have been developed and shown to exert profound antianxiety and antihypertensive effects in animals. The latter effects were discussed at length at the workshop, highlighting the important role of anandamide in two important examples of vascular allostasis – shock and hypertension. In addition to FAAH, another amide hydrolase has been recently characterized, which may participate in the degradation of anandamide and other fatty-acid ethanolamides such as oleoylethanolamine (OEA). This amidase prefers acid pH values and has a different tissue distribution than FAAH, being notably high in lung, spleen and inflammatory cells. Inhibitors of this enzyme are being developed. Finally, 2-AG is hydrolyzed by an enzymatic system separate from FAAH, which probably involves

a monoacylglycerol lipase recently cloned from the rat brain. Inhibitors of this enzyme are currently under development.

### 3.1. Direct vs. indirect agonists

What are the therapeutic advantages and drawbacks of using a direct agonist vs. an indirect agonist? Several parallels can be drawn to the well-known SSRIs (selective serotonin reuptake inhibitors), which have shown such powerful and useful therapeutic applications in effecting indirect agonism of the serotonergic system. Indeed, there is ample evidence that pharmacological profiles for the indirectly-acting agonists can generally be attributed to enhanced selectivity based on more localized action. A prime reason for favoring the indirect agonism approach is the possibility of obtaining new drugs devoid of the psychoactive effects and perceived abuse potential of directly acting $CB_1$ agonists. If we accept the postulate of on-demand modulation of endocannabinoid signaling as contributing to some disease states, we are likely to witness the development of more specific medications acting indirectly such as inhibitors of cannabinoid uptake or breakdown.

### 3.2. Endocannabinoid-like compounds

An avenue through which endocannabinoid-like fatty-acid ethanolamides have been shown to accomplish their effects is through binding to intracellular receptors like the ligand-activated transcription factor peroxisome proliferators-activate receptor-$\alpha$ (PPAR-$\alpha$). OEA exerts profound satiety-inducing, weight-reducing and anti-inflammatory effects in rodents, which are absent in mice deficient in PPAR-$\alpha$ and are closely mimicked by synthetic PPAR-$\alpha$ agonists. Furthermore, OEA was found to bind to and activate PPAR-$\alpha$ with high affinity ($K_D$ of 37 nM) in vitro. These findings suggest that OEA is an endogenous PPAR-$\alpha$ agonist involved in the regulation of energy balance. An analogous role has been recently suggested for palmitoylethanolamide, the antinflammatory properties of which have been known for decades and are now been exploited in veterinary medicine.

### 4. Conclusions

The discovery of the endocannabinoid signaling system — with its network of ligands, membrane receptors, and regulatory proteins — has revealed a score of potential new therapeutic targets in the fields of obesity, drug abuse, pain, stroke and hypertension. Most of these targets must still be fully validated through pharmacology and genetics, and better ligands for these targets are still needed. Yet, it is clear that endocannabinoid modulation has become one of the exciting new areas of current drug discovery, and that efforts to fill these gaps are well worthwhile.