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Hearing on Medical Marijuana
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Introduction

The resin secreted by the flowers and leaves of marijuana (*Cannabis sativa*) contains a family of chemically related molecules that are unique to this plant (1). The most-studied and best-understood member of this family is Δ^9-tetrahydrocannabinol (Δ^9-THC). Δ^9-THC has a complex set of pharmacological properties that stem from its ability to bind to selective receptor proteins found on the surface of neurons and other cells throughout the human body. These proteins are called *cannabinoid receptors*. Another quantitatively relevant chemical constituent of marijuana is cannabidiol (CBD). CBD is pharmacologically active, but its actions are different from those of Δ^9-THC and are not mediated by cannabinoid receptors. The receptor responsible for the effects of CBD has not been identified. There are many other chemical constituents in marijuana, but they are present in the plant at very low levels, relative to Δ^9-THC or CBD, and their contribution to the overall effects of the drug are unknown (1).

The binding of Δ^9-THC to cannabinoid receptors in the brain causes the characteristic mental state that accompanies marijuana intoxication (the 'high'). Experienced users describe this condition as a combination of enhanced sociability, quickened mental associations, increased appetite for sweet and fatty foods, alterations in the perception of time and space, and heightened sensitivity to certain sensory stimuli (e.g., sounds or colors) (2). These subjective feelings are the reason people like marijuana, but occur with, and are outlasted by, impairments in cognition, judgment and motor coordination. When Δ^9-THC reaches high levels in blood, some users even experience unpleasant sensations such as panic, paranoid thoughts and hallucinations (2). In mice and rats, moderate doses of pure Δ^9-THC produce a standard set of measurable behavioral and physiological responses that include lowered motor activity and body temperature along with reduced pain sensation and increased feeding (3). The concomitant administration of drugs that block one specific subtype of cannabinoid receptors,

called CB_1, blunts both the effects of Δ^9-THC in animals (3) and those of smoked marijuana in human volunteers (4).

As one would expect from its intoxicating effects, marijuana is primarily consumed for recreational purposes (http://www.nida.gov), but its medical use is also expanding. In fact, since its partial legalization in the US, medical marijuana has been gaining popularity at an increasing speed. In this testimony, I will briefly describe the properties of cannabinoid receptors and outline potential therapeutic applications of marijuana and other agents that activate those receptors. I will turn then to consider the endogenous neurotransmitter system that normally engages cannabinoid receptors, and discuss how our growing knowledge of such system might lead, first, to a better understanding of the value and risk associated with the medicinal use of marijuana and, second, to the discovery of safer and more effective medicines for pain, anxiety and other disease conditions.

Cannabinoid receptors

The existence of cannabinoid receptors was postulated more than thirty years ago, when it was discovered that man-made chemicals created to replicate the effects of Δ^9-THC were able to bind a specific site in brain membranes and, by doing so, cause biochemical responses inside brain cells (14). The subsequent mapping of cannabinoid-binding sites in the rat brain (15) and the molecular cloning of the first cannabinoid receptor gene, now called CB_1 (16), established the presence of unique cell-surface receptors that recognize Δ^9-THC and its synthetic mimics. A second such receptor, CB_2, was subsequently identified (17).

CB_1 receptors are highly concentrated in regions of the human brain that are implicated in the physiological and psychological effects of marijuana. For example, substantial numbers of receptors are found in structures involved in cognitive functions and in the processing of pleasurable stimuli (18). CB_1 is also present in many cell types outside the brain, including pain-sensing neurons, innate-immune cells (e.g. macrophages), adipocytes, hepatocytes and

skeletal muscle cells. This broad distribution reflects the importance of endogenous cannabinoid (endocannabinoid) substances in the peripheral control of energy balance, pain and inflammation (19, 20), among other functions. I will come back to these substances later in my testimony. In addition to CB_1, the brain contains a relatively small number of CB_2 receptors (21). However, this receptor subtype is found at much higher levels in cells of the immune system – such as B-lymphocytes, macrophages and microglia – as well as in bone cells (osteoclasts and osteoblasts) (21). Its contributions to endocannabinoid signaling in the brain appear to be important, but are still subject of unsettled debate (22).

In the brain, CB_1 receptors are primarily found at synapses – the structures that connect neurons to one another. Two important consequences of CB_1 receptor activation are the suppression of neuronal excitability (the neurons respond less to stimuli) and the reduction of neurotransmitter release (the neurons secrete smaller quantities of neurotransmitters) (23). Persistent occupation of CB_1 receptors by Δ^9-THC starts a molecular process, called *tolerance*, that progressively lowers the effects of the drug (24). In animals, tolerance to Δ^9-THC is associated with, and presumably caused by, a partially reversible decrease in the number of CB_1 receptors, combined with impaired ability of the receptors to elicit biochemical signals in cells (24, 25). Likewise, in humans, positron emission tomography (PET) imaging studies have demonstrated that chronic marijuana use dampens CB_1 levels in cortical structures and that abstinence reverses this effect (26).

Despite lingering popular notions to the contrary, long-term marijuana exposure can lead to physical dependence (27, 6). Epidemiological surveys indicate that 8 to 9% of adults and 17% of teenagers who try marijuana become addicted (28), and that the number of Americans who are now dependent on the drug – approximately 2.7 million according to recent estimates (6) – is close to that of people suffering from schizophrenia (≈2.2 million).

Current medical applications of marijuana and other cannabinoid agents

In States where it is legal to do so, physicians recommend medical marijuana primarily to relieve chronic pain resistant to standard analgesics, control muscle spasms caused by multiple sclerosis, improve appetite and alleviate nausea, prevent epileptic seizures, and reduce symptoms of inflammatory bowel disease (5; 6). Some of these indications are rooted in the history of Western medicine – *Cannabis* was listed in European and US pharmacopeias of the 19th and early 20th century as an analgesic, anticonvulsant and hypnotic (7) – and are at least partially backed by preclinical and clinical evidence. This is the case for chronic neuropathic pain (8-11), spasticity in multiple sclerosis (13) and, to a lesser extent, inflammatory bowel disease (12). However, for most other claimed indications sound data are not yet available. Research is needed to fill these gaps in knowledge.

To harness the potential therapeutic benefits of marijuana, a variety of man-made ('synthetic') chemicals that bind to and activate cannabinoid receptors have been produced (3). Some of these agents are highly potent in experimental animals. But, while desirable in a research setting, potency can be detrimental in the clinic because it can translate into unacceptable levels of mind-altering activity. Because of this looming side effect, the three currently licensed drugs that target cannabinoid receptors do not stray much from plant-derived Δ^9-THC. A synthetic version of the compound is marketed under the international non-proprietary name of *dronabinol* and the trade name of *Marinol®,* and is employed clinically to increase appetite and decrease nausea in people with acquired immunodeficiency syndrome (AIDS) or undergoing cancer chemotherapy, and to alleviate chronic pain. (Note that synthetic and pure plant-derived Δ^9-THC are pharmacologically identical.) A close chemical analog of Δ^9-THC, *nabilone* (*Cesamet®*), is prescribed for similar indications.

Both dronabinol and nabilone are given by oral administration and have a slow onset of action, reducing their attractiveness to recreational marijuana users who often (albeit not always) seek the quicker 'buzz' given by the smoked drug (29). In addition to these synthetic compounds, a standardized *Cannabis sativa* extract called *nabiximols* (*Sativex*®) has been approved in various parts of the world, but not yet in the United States, for the symptomatic relief of pain and muscle spasticity in multiple sclerosis and as an adjunctive analgesic in cancer patients (3). Nabiximols is administered as an oromucosal spray, a formulation strategy that improves the bioavailability of its active constituents. A meta-analysis of 666 patients concluded that nabiximols reduces spasticity and is well tolerated (30).

Along with Δ^9-THC, nabiximols contains approximately equal amounts of CBD, the other main compound present in the *Cannabis* resin. CBD does not activate cannabinoid receptors, but displays nonetheless an important pharmacological profile that may include antiepileptic (34) and antipsychotic (31-33) activities. Current theories attribute these effects to interactions with a diverse array of molecular targets, a non-exhaustive list of which comprises the serotonin 5-HT_1 receptor, the transient receptor potential vanilloid type-1 channel, the α3 and α1 glycine receptors, the orphan G-protein-coupled receptor GPR55, and the equilibrative nucleoside transporter (34). This plethora of mechanisms might reflect a true polypharmaceutical action of CBD or, more likely, the fact that we are still missing something fundamental about the cellular events triggered by this molecule. The reader is referred to recent reviews with focus on schizophrenia (33), epilepsy (34), neuropathic pain (35) and stroke (36).

In closing this section, it is important to point out that the effects of all cannabinoid agents are strongly dose-dependent (67). For example, marijuana can cause euphoria and relaxation or, conversely, dysphoria and panic, depending on the dosage and level of user's experience with the drug (6). This 'inverted-U' dose-response curve might reflect the engagement of non-cannabinoid receptors or, more likely, a process of differential recruitment-desensitization of CB_1 receptors in areas of the brain that regulate mental function in opposing ways.

Potential medical applications of cannabinoid agents

In addition to the uses outlined above, dronabinol (oral synthetic Δ^9-THC), nabilone (oral Δ^9-THC analogue) and nabiximols (*Cannabis* extract spray) have been investigated, with some initial promising results, in three psychiatric pathologies that currently lack good therapeutic options: cannabis-use disorder, post-traumatic stress disorder and Tourette's syndrome.

Cannabis use disorder (CUD)

According to the fifth edition of the *Diagnostic and Statistical Manual* (DSM-5), treatment for CUD should ideally address both marijuana dependence and withdrawal. No such treatment exists, however (37). Based on the relative success of agonist replacement therapy in other types of addiction (tobacco, opiates), it stands to reason that cannabinoid receptor agonists might be beneficial in CUD. This expectation was confirmed by a controlled human laboratory study, which showed that nabilone prolongs abstinence and decreases marijuana self-administration in relapsing subjects (38). The same study also reported that nabilone ameliorates all primary symptoms of marijuana withdrawal: it lowers irritability scores, improves sleep quality and normalizes food intake and sociability (38). The usefulness of agonist replacement therapy in the treatment of marijuana withdrawal is also supported by human laboratory and clinical studies with dronabinol and nabiximols (27, 39, 40). Unlike nabilone, however, dronabinol and nabiximols do not significantly alleviate marijuana dependence (40, 41).

Post-traumatic stress disorder (PTSD)

It is estimated that 6.4% to 7.8% of the population in the United States suffers from PTSD (52) and that the prevalence of this disabling anxiety disorder rises up to 23.6-30.5% in combat veterans (53). Antidepressant drugs that inhibit serotonin and norepinephrine uptake have been approved by the FDA for the treatment of PTSD, but there is still a strong need for better

therapies (54). Many afflicted war veterans report using marijuana as self-medication (55, 56) and there are in fact several clues that cannabinoid receptor activation might help alleviate the symptoms of PTSD. In an open-label trial of 47 persons diagnosed with the disease, nabilone produced a significant reduction in the number and intensity of nightmares experienced by the subjects (57). These results are in line with those of a double-blind placebo-controlled trial in 20 subjects suffering from anxiety, which reported marked effects of low-dose nabilone relative to placebo (58). An earlier human laboratory study had also suggested anxiolytic properties for low-dose nabilone (59). These findings can only be viewed as preliminary, but do encourage additional research.

Tourette's syndrome

Antipsychotic drugs reduce motor and phonic tics in many Tourette's patients, but also cause intolerable side effects, while α_2-adrenergic agonists, although widely used, lack consistent support for their efficacy (42). Two double-blind placebo-controlled trials suggest that oral Δ^9-THC might offer an alternative to those drugs (43, 44). Both trials reported positive effects of the cannabinoid agent, which were accompanied by mild and transient adverse events. Nevertheless, the total number of patients (28) and the effect size were small in those studies (42). Research on this topic has recently slowed down, but a few case studies (45, 46) and many basic science findings offer good reasons to continue. CB_1 receptors are expressed at high levels in dopamine-sensitive cortico-striatal networks of the human brain (47), which have been implicated in Tourette's pathology (48). Activation of CB_1 receptors localized to these networks functionally counters the motor stimulation caused by dopamine D_2 agonists (49, 50). These data, along with anecdotal reports of marijuana self-medication by people with Tourette's (51), provide a plausible rationale to reexamine the usefulness of cannabinoid agonists in this disorder.

Endogenous cannabinoids

Δ^9-THC usurps the place normally held by two marijuana-like molecules produced by the body. These substances, called endocannabinoids, are generated 'on demand', activate cannabinoid receptors near their site of production, and are then rapidly degraded. There are two known endocannabinoids, anandamide and 2-arachidonoylglycerol (2-AG), each serving distinct biological functions. Researchers have developed pharmacological agents that selectively stop the degradation of either substance and, by doing so, enhance its actions. These agents can be used to unmask the functions served by each endocannabinoid in physiology and pathology, as well as starting points for novel classes of therapeutic drugs.

Anandamide is broken down inside cells by the protein fatty acid amide hydrolase (FAAH) (83). Pharmacological blockade of FAAH activity causes anandamide to accumulate, and consequently heightens anandamide-mediated signaling at cannabinoid receptors (60). FAAH inhibition may be valuable therapeutically because many beneficial consequences of CB_1 receptor activation (e.g., relief of pain and anxiety, mood elevation) might be achieved with equal efficacy and fewer undesired effects, compared to marijuana or Δ^9-THC, by protecting endogenously formed anandamide from degradation. For example, the FAAH inhibitor URB597 decreases isolation-induced ultrasonic vocalizations in rat pups, and increases the time spent in the open arms of an elevated zero maze, two results that are suggestive of an anti-anxiety action (60). Moreover, URB597 enhances active stress-coping behaviors in animals, which points to potential antidepressant properties (61). On the other hand, the peripherally restricted FAAH inhibitor URB937, which increases anandamide levels only outside the brain and spinal cord, is profoundly analgesic in animal models of acute and chronic pain (20). Several FAAH inhibitors have successfully passed safety testing in animals and humans, and are now undergoing clinical studies for various indications.

The degradation of 2-AG is mediated by the enzyme monoacylglycerol lipase (MGL) (92). Similarly to FAAH inhibitors, agents that block MGL stop the degradation of 2-AG, causing this substance to accumulate and persistently activate cannabinoid receptors (81, 91, 92). MGL inhibitors have demonstrated a wide array of pharmacological activities in animal models of pain, anxiety and Alzheimer's disease, among others (81, 94). MGL inhibitors are currently undergoing preclinical development.

Physiological roles of the endocannabinoid system

Animal experiments have implicated the endocannabinoid system in a broad variety of physiological processes. Strong evidence points to crucial roles for this system in the following functions:

- **Pain.** The endocannabinoids work in concert with the opioid system – the target of powerful painkillers such as morphine and oxycodone – to control the flow of pain-related information from peripheral tissues to the spinal cord and the brain. This provides a biological rationale for the use of marijuana in the treatment of chronic pain – which also has gained initial support, as we have seen before, from preclinical and clinical studies (8-10).
- **Response to stress.** Stress stimulates the endocannabinoid system in the brain. Endocannabinoids, in turn, strengthen the ability of the organism to cope with stress. Stress is an important causative factor in anxiety and depression, and animal experiments suggest that endocannabinoid signaling protects against these two affective disorders. This may provide a rationale for the use of marijuana in the treatment of PTSD, which has a strong anxiety component, but clinical evidence is scant and further research is needed in this area. One important point that needs clinical data pertains to the dose levels at which marijuana might reduce anxiety *vs* those at which its ability to induce tolerance (i.e. loss of cannabinoid receptors) might cause opposite effects.

- **Feeding and energy balance.** Endocannabinoids both inside and outside the brain exert a tight regulatory control over food intake and body weight (70). These substances are viewed as 'regulators of thrift' that stimulate hunger and help maintain body fat. Consistent with this view, drugs that block endocannabinoid signaling at CB_1 receptors curb the appetite for food and lower body weight in animals and humans (72,73). Future clinical studies on marijuana should monitor these important parameters, which can have a strong negative impact on general health.
- **Cognition and memory.** The endocannabinoid system regulates in subtle and complex ways the brain's ability to process information and store it as new memories. Activation of cannabinoid receptors by Δ^9-THC disrupts normal cognition in a dose-dependent manner, and cognitive impairment is a common effect of marijuana use. This unwanted effect should be carefully evaluated in future studies of medicinal marijuana. In particular, more research needs to be done on the impact of the drug on automobile driving. Recent work suggests that marijuana-impaired drivers drive more slowly, pass other drivers less frequently, and maintain a greater following distance behind other cars than do alcohol-impaired or non-impaired drivers. Nevertheless, caution is warranted especially in light of the difficulty in assessing the level of impairment caused by marijuana, relative to the impairment caused by alcohol.
- **Control of natural rewards.** Adaptive pleasurable stimuli (e.g., sweet foods) engage brain neurotransmitters, such as dopamine, which are exquisitely sensitive to control by the endocannabinoid system. The tight connection between endocannabinoids and reward pathways in the brain likely underpins the addictive properties of marijuana, but it might also be leveraged to combat addiction. A case in point is provided by a genetic study that reported a statistically significant interaction between marijuana use and a single nucleotide polymorphism of the human FAAH gene. The study found that, among subjects who tried marijuana, those carrying a genetic variation of FAAH (C385A) that

causes reduced enzyme expression and activity were significantly less likely to become dependent on the drug. Because individuals who carry the C385A FAAH mutant are expected to have higher-than-normal levels of anandamide in the brain, it is reasonable to speculate that enhanced anandamide-mediated signaling may reduce the susceptibility to develop cannabis-use disorder. A corollary of this hypothesis is that FAAH inhibitors might be useful in the treatment of this condition (100).

The endocannabinoid system in adolescence

Adolescents are especially vulnerable to the impact of marijuana exposure. Brain networks controlling human cognition and affect are still actively developing during the teenage years (101,102). The plasticity of these structures – that is, their ability to change in response to demands of the environment – makes them particularly sensitive to the chronic effects of marijuana (103,104). Indeed, there is an overall consensus across epidemiological surveys that adolescence-onset use of the drug is associated with impairments in cognition and affective functioning that continue into adulthood even after use has stopped (105). For example, a prospective study of 1,037 individuals followed from birth to age 38 years found a significant association between prolonged marijuana exposure in adolescence and cognitive decline later on in life (106). Increased risk of developing neuropsychiatric disorders – including addiction, depression and schizophrenia – has also been convincingly documented (107,108). In agreement with these epidemiological findings, a relatively small but growing set of animal studies indicate that adolescent exposure to Δ^9-THC, causes long-term impairments in sociality and memory, increased reward seeking, and dysregulated affect (for review, see 104).

The endocannabinoid system is a plausible target for the persistent effects of marijuana on the adolescent brain. Experiments in rodents suggest that this signaling complex contributes to the control of neuronal migration, axonal guidance and synaptogenesis during prenatal and postnatal brain development [105-107]. Moreover, in adolescent and adult life, the

endocannabinoid system constitutes the molecular scaffold for a retrograde signaling mechanism that regulates crucial forms of short-term and long-term synaptic plasticity throughout the brain [108,109]. Along with other neurotransmitter systems, endocannabinoid signaling undergoes profound modifications during adolescence. Animal studies have shown that production of anandamide and expression of CB_1 receptors reach peak levels around mid-adolescence and decrease in adulthood [110,111]. This transient upward regulation may reflect the important role played by endocannabinoid signals in two defining features of adolescent behavior – the heightened sensitivity to natural and drug rewards, and the increased propensity to seek novelty and take risks [112]. Thus, age-dependent plasticity in the ECB system likely puts adolescents at particular risk to disruption/alteration of this system by Δ^9-THC. In this context, it is imperative for research to address the persistent effects of adolescent-onset exposure to medicinal cannabis.

Conclusions

The last two decades of research on endocannabinoids and their receptors have greatly expanded our understanding of these unconventional signaling molecules and the roles they play in mental health. Information from the synapse to the patient has illuminated how Δ^9-THC and other exogenous cannabinoids hijack the endocannabinoid signaling system, leading to serious side effects, but at the same time providing promising opportunities for therapeutic intervention. While important questions remain, it is nevertheless clear that the medical potential of marijuana, along with its risks, can no longer be ignored.

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