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MARIJUANA

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Marijuana continues to garner considerable attention and is the subject of intense public debate and scientific scrutiny. It is unquestionably one of the most frequently used illicit drugs throughout the world. In Western countries, the pattern of use among age groups has not deviated significantly since the mid-1970s. The most prevalent use occurs in persons who are in their late teens and early twenties. Despite modest declines from the pinnacle of its use in the mid-1970s, there was an upsurge in use during the 1990s. Marijuana smoking is prevalent regardless of age, ethnicity, and sex. Epidemiologic data reveal that the rates of use during the year 2000 by United States students in grades 8, 10, and 12 were 17%, 32%, and 38%, respectively (1). There was a steady increase in daily marijuana use (defined as use on at least 20 occasions in the past 30 days) in all three age groups. For example, 2% of high school seniors used marijuana daily in 1991, with this figure rising to 6% in 1999. Two factors that undoubtedly contribute to the prevalence of marijuana use are the declining perception that marijuana produces harmful effects and the relative ease of acquiring marijuana.

Although marijuana has long been a subject of folklore medicine, interest as a potential therapeutic agent has intensified in recent times, likely as a result of numerous factors. The marijuana debate has increased, correlated with a period in our history marked by increased interest in nontraditional medications, increased awareness of several disease states not readily treated by current medications, and increased discourse about the public policy concerning recreational use of marijuana. Proponents cite a plethora of self-reports regarding the effectiveness of medical marijuana for a wide range of disorders, whereas opponents question its efficacy and point to potential deleterious effects of smoked marijuana. The scientific rationale for deciding the fate of marijuana as a therapeutic agent is often ignored.

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As the debate concerning potential therapeutic benefits and health consequences of acute and chronic exposure to marijuana continues, considerable new scientific evidence is emerging regarding the nature of cannabinoid effects both in vivo and in vitro and the endogenous system through which marijuana acts. The emphasis of this chapter is to summarize recent discoveries of the endogenous system as they relate to both putative adverse effects and therapeutic uses of marijuana and its psychoactive constituent, Δ^9 -tetrahydrocannabinol (THC). During the 1990s, numerous breakthroughs occurred that greatly increased our understanding of the cannabinoids. It is now evident that an endogenous cannabinoid system exists. A receptor has been characterized and cloned, second messenger systems identified, a putative endogenous ligand isolated and synthesized, and biochemical pathways for both synthesis and degradation of endogenous ligands described.

PHARMACOLOGY

Marijuana has prominent effects on the central nervous system as well as numerous peripheral effects in humans that are well recognized and were reviewed in the previous edition of this book. Acutely, cannabis produces an altered state of consciousness characterized by mild euphoria, relaxation, perceptual alterations including time distortion, and the enhancement of ordinary sensory experiences. Cognitive effects are also marked, such as impaired short-term memory. Motor skills and reaction time are also altered, so skilled activity of various kinds is frequently disrupted. Peripherally, marijuana produces prominent effects on the cardiovascular system characterized by tachycardia, and at high doses it can produce orthostatic hypotension. There are several other effects, such as antiemesis, analgesia, anticonvulsant action, and intraocular pressure lowering, discussed later. However, THC has provided most of the evidence for cannabinoid effects in laboratory animals. As for chronic marijuana use in humans, concerns arise because of potential long-term consequences on cognition and the development of tolerance and dependence. There has been considerable interest in cannabinoid effects on performance, cognition, and the development of dependence, discussed in the following sections.

Performance

Cannabinoids affect sensory, psychomotor, and cognitive function and the ability to perform certain tasks. There is little dispute that high doses of marijuana can disrupt performance when the task is difficult. As may be expected, the effects of marijuana on performance become more variable as the complexity of the task is simplified and the dose of marijuana is reduced. In a comprehensive review, Chait and Pierri concluded that marijuana, at doses that produce moderate levels of intoxication, can affect a wide range of learned and unlearned behaviors, including simple motor tasks, and more complex psychomotor and cognitive tasks (2). Cannabinoid-induced impairment of flying and driving has been documented in numerous studies, although interpretation of the results remains controversial. THC is frequently found in the blood of drivers involved in automobile accidents, and marijuana use has been associated with impaired field sobriety test performance.

One study compared the effects of marijuana on equilibrium and simulated driving (3). Marijuana smoking that produced a "high" also increased body sway and increased brake latency to a degree comparable to that found in persons with breath alcohol concentrations near 0.05%. Marijuana smoking also acutely produces decrements in smooth-pursuit eye tracking (4). Although robust acute effects of marijuana were found on subjective and physiologic measures, and on smooth-pursuit eye tracking performance, no effects were evident the day after administration, a finding indicating that the residual effects of smoking a single marijuana cigarette are minimal. Furthermore, perceptual motor speed and accuracy, two very important parameters of driving ability, seem to be impaired immediately after marijuana use (5).

Cognition

There is lack of consensus regarding the effects of Δ^9 -THC on memory and learning in that results are often inconsistent and test specific (2,6). Hall et al. concluded from clinical observations and cross-cultural studies that chronic marijuana use does not appear to produce severe gross impairment, but rather it may produce subtle cognitive deficits (7). The most frequently mentioned deficits were slower psychomotor performance, poorer perceptual motor coordination, and memory dysfunction. During the past few years, greater attention has been directed toward investigating specific cognitive deficits and relating these effects directly to marijuana use. Whereas THC appears to produce its greatest decrement in free recall or short-term memory, it has

been proposed that chronic marijuana use in adolescents may result in long-term memory impairment (6). There are also indications that persons with learning disabilities may be more susceptible to memory deficits (6). Almost all studies have shown that marijuana has no effect on retrieval of already-learned material. THC reliably alters the perception of time, with subjects overestimating elapsed time or experiencing an increase in the subjective rate of time (2). Evidence has emerged from several studies that chronic marijuana use after many years produces subtle cognitive changes, specifically with regard to attention, as well as organization and integration of complex information (8).

These effects on memory have been supported by cannabinoid-induced deficits in several animal models. The naturally occurring cannabinoids as well as a wide range of synthetic compounds have been demonstrated to impair learning and memory in numerous laboratory animal memory tasks. Lichtman and Martin found that many synthetic cannabinoids impaired spatial memory in rats, as assessed by the eight-arm radial maze, and retarded completion time (9). Direct injection of cannabinoids into the hippocampus impaired memory, and this appeared specific to cognition because no other pharmacologic effects were produced (10).

Tolerance and Dependence

There is convincing evidence for the development of tolerance to THC in humans (11). Tolerance developed to a variety of THC's effects, after oral administration, including cannabinoid-induced decreases in cardiovascular and autonomic functions, increases in intraocular pressure, sleep disturbances, and mood changes (11). High doses of Δ^9 -THC were required for a sustained period of time to achieve behavioral tolerance. If the doses of Δ^9 -THC were sufficiently small and infrequent, little behavioral tolerance seemed to develop.

Although it is well known that cessation of chronic marijuana exposure does not result in severe withdrawal symptoms, numerous case reports attest to the development of dependence (12). Several early reports came from countries where potent marijuana was used for long periods of time. On deprivation of marijuana, users experienced auditory and visual hallucinations and irritability. The development of tolerance and dependence has been studied under rigorous and controlled conditions (12,13). In one study, high doses of marijuana extract or Δ^9 -THC were administered for up to 21 days, and the most prominent subjective symptoms were increased irritability and restlessness. Other symptoms included insomnia, anorexia, increased sweating, and mild nausea, although they were variable. Objective symptoms were increased body temperature, weight loss, and hand tremor. Readministration of a marijuana cigarette or oral Δ^9 -THC alleviated the objective and subjective effects, a finding suggesting the establishment of a withdrawal symptom. Similar findings were reported by Georgotas and

Zeidenberg in abstinent subjects who had smoked high quantities of marijuana on a long-term basis (13). One study (14) found that lower doses of THC (80 and 120 mg/day, orally, each for 4 days) initially produced ratings of "high," increased food intake over baseline by 35% to 45%, and decreased verbal interaction among participants (14). Tolerance developed to the subjective effects of THC but not to its effects on food intake or social behavior. Abstinence from THC produced anxious, depressed, and irritable symptoms, decreased the quantity and quality of sleep, and decreased food intake (14). A similar study conducted with marijuana cigarettes resulted in similar effects and led to the conclusion that abstinence symptoms may play a role in maintaining daily marijuana use, even at levels of use that do not produce tolerance (15).

Epidemiologic data support marijuana dependence as reviewed by Hall et al. (7). There are numerous cases in which persons seek treatment for dependence of which marijuana is the primary cause (16). These patients typically complained of being unable to stop or to decrease their marijuana use despite experiencing sleepiness, depression, inability to concentrate, and memorization difficulties that they directly attributed to marijuana exposure. Kandel and Davies reported similar problems in daily users of marijuana (17). Several groups of investigators have used DSM-III-R and DSM-IV criteria to diagnose marijuana dependence (18–20). With regard to prevalence of marijuana abuse and dependence, the strongest evidence was provided by the Epidemiological Catchment Area study involving 20,000 persons in five geographic areas of the United States (21). Approximately 4.4% of the population were diagnosed for marijuana abuse or dependence, and three-fifths of these met the criteria for dependence. After an extensive review of the literature, Hall et al. concluded that the risk of developing marijuana dependence was probably similar to that of alcohol, and daily use over a period of weeks to months results in the greatest risk of dependence development (7). Kandel and Davies estimated that the risk of dependence in near-daily marijuana users was one in three (17). Hall et al. estimated that the risk of developing dependence is 10% for those who ever used marijuana, with the risk rising to 20% to 30% for those using more than five times (7). Factors that have been associated with marijuana dependence include poor academic achievement, deviant behavior, rebelliousness, maladjustment, difficult parental relations, early initiation of drug use, and family history of drug use (7). The major complaints by marijuana-dependent persons are loss of control over drug use, cognitive and motivational impairments, lowered self-esteem, depression, and spousal discord. The risk of cannabis abuse and dependence was found to increase with the frequency of smoking occasions and slightly decreased with age (22). More severe comorbidity was associated with dependence compared with abuse, a finding suggesting that cannabis may be used to self-medicate major depression.

More recently, Budney et al. reported that most marijuana users seeking treatment for marijuana dependence had experienced symptoms consistent with either moderate or severe dependence (23). These investigators also reported that marijuana-dependent persons exhibit substantial problems (24). Comparison of marijuana- and cocaine-dependent patients revealed comparable substance-use histories and a range of impairments in both groups. However, the marijuana-dependent patients showed less severe dependence. The marijuana group was more ambivalent and less confident about stopping their marijuana use than the cocaine group was about stopping their cocaine use. The authors concluded that treatment-seeking, marijuana-dependent persons exhibit substantial problems and urged development of effective treatments for this population (24).

Some predisposing factors may contribute to marijuana dependence in some persons. Crowley et al. reported that juveniles diagnosed with both substance abuse and conduct disorders have serious problems related to cannabis, and most met standard adult criteria for cannabis dependence (25). Two-thirds of these cannabis-dependent patients reported withdrawal. The data indicate that for adolescents with conduct problems, cannabis use is not benign. Genetic risk factors may also contribute. Kendler et al. examined a large female twin population for lifetime cannabis use, heavy use, abuse, and dependence as defined by DSM-IV criteria (26). These investigators concluded that in women, genetic risk factors have a moderate impact on the probability of ever using cannabis and a strong impact on the liability to heavy use, abuse, and, probably, dependence. By contrast, the family and social environment substantially influences risk of ever using cannabis but plays little role in the probability of developing heavy cannabis use or abuse (26).

One of the difficulties in establishing the presence of cannabinoid dependence was the lack of a reliable animal model. Early attempts to demonstrate spontaneous withdrawal after cessation of chronic marijuana or THC treatment resulted in equivocal findings. However, the development of a specific cannabinoid antagonist, SR 141617A, made it possible to precipitate withdrawal in rats (27,28), mice (29), and dogs (30) treated chronically with THC. The physical withdrawal syndrome for cannabinoids and opioids in rodents shares many of the same characteristics. It is also clear that, in humans, THC is an essential reinforcing component in marijuana (31). Contrary to most drugs abused by humans, it has been difficult to train animals to self-administer cannabinoids. Although the physical characteristics of cannabinoids probably contribute to this difficulty, the general opinion persists that cannabinoids lack rewarding effects and therefore are devoid of dependence liability. However, Martelotta et al. demonstrated that the synthetic cannabinoid agonist WIN 55,212-2 was intravenously self-administered by mice in a concentration-dependent manner according to an inverted U-shaped curve (32).

Therefore, it appears that WIN 55,212-2 elicits both rewarding and aversive effects, depending on the concentration used. It may well be that these dual properties have hindered the development of a THC model of self-administration. Nevertheless, these studies clearly demonstrate that cannabinoid self-administration is not confined to humans.

ENDOGENOUS CANNABINOID SYSTEM

Cannabinoid Receptors

It is now widely recognized that most of the neurobehavioral and peripheral actions of marijuana and THC result from activation of selective receptors, two of which, named CB₁ and CB₂, have been cloned and characterized (33,34). The development of transgenic mice lacking the genes encoding for either of these two receptors, the CB₁ and CB₂-receptor knockout mice (35–37), have provided conclusive evidence that the effects of THC on motor behavior, body temperature, cardiovascular function, and nociception, on the one hand, and on some immunologic responses, on the other hand, are mediated by CB₁ and CB₂ receptors, respectively.

CB₁ receptors are widely distributed throughout mammalian tissues and have been found not only in the central and peripheral nervous systems, but also in both male and female reproductive organs, immune cells, the gastrointestinal tract, the liver, and the heart (38). In the central nervous system, CB₁ receptors are most abundant in the hippocampus (i.e., the dentate gyrus and CA pyramidal cells), the basal ganglia (namely, the substantia nigra pars reticulata, globus pallidus, caudate putamen), and the cerebellum and the olfactory bulb (39), findings in agreement with the inhibitory actions of THC on memory and cognitive functions, spontaneous activity, locomotion, motor coordination, and posture. Lower density of CB₁ receptors is present in discrete nuclei of other brain regions such as the hypothalamus, brainstem, thalamus, and limbic forebrain, thus possibly accounting for THC activity on body temperature, appetite, supraspinal mechanisms of pain perception, sensory perception, and mood or reward. CB₁ receptors are associated with nerve fibers and axon terminals, but not in the neuronal soma. This pattern is consistent with the presynaptic inhibitory effects of cannabinoids on neurotransmitter release in the brain (see ref. 40 for review). CB₁expressing cells in mouse forebrain can be divided into distinct neuronal subpopulations. Most of the cells that highly express CB₁ are GABAergic neurons belonging mainly to the cholecystokinin-positive type of interneurons (basket cells). In the hippocampus, amygdala, and entorhinal cortex area, CB₁ mRNA is present at low but significant levels in many non-GABAergic cells that can be considered as projecting principal neurons. These data are in good agreement with the observation that cannabinoids act on principal glutamatergic circuits as well as modulate local GABAergic inhibitory circuits by inhibiting glutamate and GABA release. Virtually all striatal projection neurons contain CB₁ mRNA, which is also expressed in putative GABAergic interneurons that enable functional interactions between the direct and indirect striatal output pathways (41). CB₁ mRNA is found in striatonigral neurons that contain dynorphin and substance P and striatopallidal neurons that contain enkephalin, whereas local circuit neurons in striatum that contain somatostatin or acetylcholine do not synthesize CB₁ mRNA.

The presence of CB_1 receptors in sensory (42) and autonomic peripheral fibers (43,44) has been reported. CB₁ receptors seem to be mostly restricted to spinal interneurons, rather than at the axonal level (45), thus possibly accounting for spinal mechanisms of pain control ascribed to psychotropic cannabinoids. However, indirect evidence also exists for the presence of CB₁ receptors in peripheral sensory afferents (46), a finding thus supporting the concept that cannabinoids may also exert analgesia at the peripheral level. The presence of CB₁ receptors in parasympathetic and sympathetic fibers, on the other hand, may be at the basis of the vascular and smooth muscle-relaxing activity of THC through inhibition of norepinephrine and acetylcholine release, respectively (43,44). There is no evidence for the presence of CB₂ receptors in the central nervous system, except for their expression in microglia. Clearly, given that CB₂ receptors seem to be mostly confined to cells of the immune system (34), it would not be surprising to find these proteins only in those central nervous system cells deputed to immune defense, such as the microglia and resident mast cells. This finding may explain some of the neuroprotective activities of cannabinoids in vivo (see later).

Transduction Mechanisms

Studies have revealed that activation of the α subunits of G_i/ Go proteins, with subsequent inhibition of adenylate cyclase through both CB₁ and CB₂ receptors (47), blockade of voltage-activated calcium (Ca²⁺) channels of the N- and P/Qtype through CB₁ receptors (48), and activation of inwardly rectifying potassium channels through CB₁ receptors (49), may not be the sole intracellular signaling messages delivered by psychoactive cannabinoids. There is now evidence for the coupling of CB₁, but not CB₂ receptors, to G_s proteins, with consequent activation of adenylyl cyclase. It is not clear yet whether this effect may explain the biphasic nature of cannabinoid effects on behavior in several tests. Another typical consequence of CB₁-receptor activation is the modulation of nitric oxide (NO) release. In neurons, THC and synthetic and endogenous cannabinoids can either stimulate (50) or inhibit (51) NO formation. The former effect results in inhibition of dopamine release from invertebrate ganglia, whereas the inhibition of NO release in granule cerebellar cells seems to result from inhibition of voltage-activated Ca²⁺ channels. In any case, modulation of NO levels may result in changes in cyclic guanosine monophosphate intracellular concentrations. Finally, protein phosphorylation catalyzed by mitogen-activated protein kinase is coupled to both CB₁- and CB₂-receptor stimulation (52). This intracellular effect, together with inhibition of the cyclic adenosine monophosphate (cAMP)-dependent protein kinase A, is at the basis of cannabinoid action on the expression of several genes such as krox-24 in HL60 cells (52) or the prolactin receptor and the high-affinity receptors trk for the nerve growth factor in human breast cancer cells (53). Mitogen-activated protein kinase activation by cannabinoids may occur independently from inhibition of protein kinase A (52), or it may result in part from inhibition of cAMP formation (53). Likewise, CB₁-induced activation of focal adhesion kinase in hippocampal slices, an effect suggested to lead to modulation by cannabinoids of synaptic plasticity and learning, results from inhibition of adenylate cyclase and protein kinase A.

Endogenous Ligands (Endocannabinoids)

Since the mid-1990s, several fatty acid derivatives have been isolated from mammalian tissues and have been shown to mimic the pharmacologic properties of THC. Not all these substances, however, can displace high-affinity cannabinoid ligands from selective binding sites in membrane preparations containing the CB₁ or the CB₂ receptor. Anandamide (arachidonoylethanolamide, AEA), the amide of arachidonic acid with ethanolamine, was the first of such compounds to be isolated (54). The other prominent endogenous ligand is a glycerol ester, 2-arachidonoyl glycerol (2-AG) (55). These compounds share the ability to bind to and to activate CB₁ and (particularly in the case of 2-AG) CB₂ receptors. Therefore, they induce a series of pharmacologic effects in vitro and in vivo that are, to some extent, similar to those exerted by THC. Other fatty acid derivatives, such as palmitoylethanolamide and oleamide, do not have high affinity for either CB₁ or CB₂ receptors, and yet they exhibit pharmacologic actions that in some cases resemble those of THC (56). The molecular mode of action of these latter compounds is still a subject for investigation.

Several structure-activity relationship studies have been carried out on AEA and have revealed that this compound does share with THC some of the structural prerequisites necessary for interaction with the CB₁ receptor. This relationship can be best appreciated with a successful conformational model (57), in which AEA may assume a low-energy conformation resulting in the superimposition of its *n*-pentyl chain, carbonyl amide group and ethanolamine hydroxyl group, respectively, with the *n*-pentyl chain, the phenolic hydroxyl group and the C-9 hydroxyl group in 9-*nor*-9β-OH-hexahydrocannabinol, a potent THC analogue. Structure-activity relationship studies for the interaction with CB₂ receptors have not been performed yet, the sole exception being the article by Sugiura et al. (58) on the structural moieties necessary to 2-AG analogues to induce Ca²⁺ tran-

sients in HL60 cells through these receptors. Interestingly, in this study, AEA was shown to be a very weak and partial agonist at CB_2 receptors. Whatever its role as an endogenous agonist at CB_2 receptors, AEA, and much more so its metabolically stable analogues (R)-methanandamide and 2'-fluoro-2-methyl-arachidonoyl-ethanolamide, act as relatively potent (K_i between 12 and 100 nM) and selective CB_1 -receptor agonists, and thus can be considered useful pharmacologic tools for studies on the bioactivity of endocannabinoids.

AEA shows, in some cases, effects qualitatively and quantitatively different from those of classic cannabinoids, possibly in part because of the rapid metabolism of this compound both in vitro and in vivo (59), and because AEA is a partial agonist in some functional assays of CB₁ activity (60). In the brain, AEA was shown to exert inhibitory actions on learning and memory (61) and to modulate the extrapyramidal control of motor behavior (62). These effects probably result from the capability of AEA to induce, by activation of CB₁ receptors, modulation of neurotransmitter (e.g., glutamate, GABA, dopamine) release, action or reuptake through intracellular signaling events similar to those described earlier for THC (40). This neuromodulatory action may also underlie AEA regulation of hormone release at the level of the hypothalamus-pituitary-adrenal axis, as well as the antinociceptive effects of the compound through both spinal and supraspinal mechanisms (63).

Endocannabinoid levels in tissues and cells can be modulated through the regulation of either their biosynthesis or inactivation. It is commonly accepted that the AEA and 2-AG are not stored as such in cells, but rather are synthesized and are directly released by cells "on demand," after Ca²⁺ influx into the cell (such as that occurring in neurons on depolarization or in mast cells after IgE-mediated activation) and the hydrolysis of phospholipid precursors (40). For, example, AEA is produced in neurons and leukocytes together with other N-acyl-ethanolamines (NAEs) from the hydrolysis of the corresponding N-acyl-phosphatidyl-ethanolamines (NAPEs) (64). This reaction is catalyzed by a Ca²⁺-independent phospholipase D, whereas a Ca²⁺-dependent trans-acylase catalyzes the formation of NAPEs form phosphatidylethanolamine and the fatty acids on the sn-1 position of phosphoglycerides. Several mechanisms for the inactivation of endocannabinoids have been identified in neuronal and blood cells. A membrane-bound intracellular hydrolase catalyzes AEA hydrolysis after its diffusion into neuronal cells and leukocytes (64). A mechanism for the facilitated diffusion of AEA into cells according to its concentration gradient across the plasma membrane has been partially characterized as a saturable, temperature-sensitive, selective and sodium-independent "carrier" (64). This "carrier," probably a protein, may be used for both the reuptake by and the release from cells of AEA. The major enzyme responsible for AEA hydrolysis is the fatty acid amide hydrolase (FAAH), cloned so far in four different mammalian species (65).

Because the biosynthetic precursors for AEA and 2-AG, by being products of membrane phospholipid remodeling, are likely to occur in most animal tissues, the two endocannabinoids are probably to be found, at least in minute amounts, as ubiquitous metabolites. However, for these compounds to work as endogenous agonists of CB₁ and CB₂ receptors, their tissue concentrations need to be increased up to at least 50 to 100 nM after cell stimulation (e.g., neuronal depolarization, immune challenge) and subsequent activation of the proteins involved in their biosynthesis and release. Furthermore, the inactivation of endocannabinoids may be subject to regulation. In agreement with possible regulation of endocannabinoid levels under physiologic and pathologic conditions, the amounts of AEA or 2-AG have been found to vary during brain development, to be higher in some of the brain regions with the highest density of CB1 receptors, such as the basal ganglia and the hippocampus (66), to decrease and increase in the striatum and limbic forebrain, respectively, of rats after chronic treatment with THC (67), to be inversely correlated with spontaneous activity in the globus pallidus of reserpine-treated rats (68), to vary during pregnancy in mouse uterus, levels of these agents being maximal when the uterus is least receptive to embryo implantation (69), and to be enhanced during septic or hemorrhagic shock in rat macrophages and platelets (70,71). Indeed, several possible regulative mechanisms have been reported for both the biosynthesis and inactivation of AEA and 2-AG in isolated, intact cells.

PHYSIOLOGIC ROLE OF ENDOGENOUS SYSTEM

The finding of variations in AEA and 2-AG levels during physiologic or pathologic conditions, together with observations of their pharmacological activity *in vivo* and *in vitro*, provide useful information on the possible biological role played by these compounds. Additionally, *in vivo* pharmacologic studies carried out by administering selective cannabinoid receptor antagonists may reveal a possible endocannabinoid-induced "tone" of CB₁- and CB₂-receptor activation during certain conditions, although the capability of the antagonists so far developed to behave as inverse agonists as well (72) should always be taken into account.

Pain

Extensive studies (see refs. 63 and 73 for review) have been carried out demonstrating the involvement of endocannabinoids in the control of nociception and, in particular, chronic and inflammatory pain. Electrical stimulation of the periaqueductal gray was shown to induce CB₁-mediated

analgesia while leading to the release of AEA in microdialysates from this region of the brainstem (74). Moreover, the injection of formalin into the paw induced a nociceptive response concomitantly to the release of AEA from the periaqueductal gray and thereby established an correlation between supraspinal nociception and endocannabinoid release. In fact, an earlier investigation had suggested that an endocannabinoid tone may down-modulate pain perception through CB₁ receptors in another region of the brainstem, the rostral ventromedial medulla, through the same circuit previously shown to contribute to the pain-suppressing effects of morphine (75). However, other studies have shown that blockade of the action or expression of spinal CB₁ receptors by SR141716A or a CB₁-receptor antisense oligonucleotide, respectively, leads to hyperalgesia (76), a finding thus suggesting the existence of an endocannabinoid tone down-modulating nociceptive response also at the spinal level. The same group gained evidence for the presence of CB₁ receptors in peripheral sensory afferents in the skin, and for their involvement in the control of inflammatory pain (77). It may well be that an endocannabinoid and CB₁/CB₂ receptors mediate tone controlling pain at the peripheral level, because local administration of the antagonist for each receptor subtype leads to hyperalgesia and exogenous AEA blocks the painful response of mice to formalin injection. Several studies, taken together with that by Meng et al. (75) and Walker et al. (74), suggest that if endocannabinoids tonically modulate inflammatory pain perception, they may do so at sites different from those of inflammation.

There has been considerable interest in determining what role, if any, opioids play in cannabinoid-induced antinociception. In one study, marijuana produced significant dosedependent antinociception (increased finger withdrawal latency) and behavioral effects. Naltrexone did not significantly influence marijuana dose-effect curves, a finding suggesting no role of endogenous opiates in marijuana-induced antinociception under these conditions (78). Conversely, it has been shown that cannabinoids stimulate release of endogenous opioids that contribute to cannabinoid antinociception (79). Meng et al. showed that the rostral ventromedial medulla that contributes to the pain-suppressing effects of morphine is also required for the analgesic effects of cannabinoids (75). Although cannabinoids produce analgesia by modulating rostral ventromedial medulla neuronal activity in a manner similar to morphine, their actions are not identical. They also show that endogenous cannabinoids tonically regulate pain thresholds in part through the modulation of rostral ventromedial medulla neuronal activity. These authors concluded that analgesia produced by cannabinoids and opioids involves similar brainstem circuitry and that cannabinoids are indeed centrally acting analgesics with a new mechanism of action.

Memory

The effects of cannabinoids on memory in rats are also blocked by a specific cannabinoid antagonist, SR 141716A, a finding providing strong evidence that these effects are mediated through cannabinoid receptors in the brain (9). Mallet and Beninger used a two-component instrumental discrimination task, consisting of a conditional discrimination, and a non-match-to-position to assess recent or working memory (61). These investigators found that both THC and anandamide impaired performance, an effect that could be attenuated with the administration of the CB₁-receptor antagonist SR141716A. These results suggest that anandamide-induced memory disruption is mediated by CB receptors. Studies have shown that THC produces memory deficits similar to those produced by neurochemical lesions of the hippocampus. A possible role for cannabinoid receptors and endogenous cannabinoids may be to regulate the storage and retrieval of information (80).

As discussed previously, cannabinoid receptors are highly expressed in the hippocampus, a brain region that is believed to play an important role in certain forms of learning and memory. The notion that endocannabinoids are involved in the control of learning and memory processes at the level of the hippocampus is supported by several different types of observations. First, both AEA and 2-AG inhibit hippocampal long-term potentiation (81) and modulate the release of glutamate or acetylcholine from hippocampal slices (40). Second, AEA modulates both short-term and longterm memory (61). Third, SR141716A enhances long-term potentiation, a finding thus suggesting a CB₁-receptor tone in the control of this process. Fourth, CB₁-receptor knockout mice exhibit enhancement of memory as well as of longterm potentiation (82). Finally, CB₁ receptors, AEA, and FAAH are found in high levels in the hippocampus of humans, rats, and mice (66). These findings suggest that constitutive activation of CB1 receptors in this brain region leads to inhibition of learning and memory processes.

There is evidence that memory deficits induced by cannabinoids may be mediated through cholinergic and dopaminergic systems (83). The systemic administration of THC reduced hippocampal extracellular acetylcholine concentrations while impairing working memory in rats. Both effects were blocked by the CB₁ cannabinoid and D2 dopamine receptor antagonists and potentiated by the D2 dopamine receptor agonist quinpirole. The inhibition of hippocampal extracellular acetylcholine concentration and working memory produced by the combination of (-)- quinpirole and THC was suppressed by either CB₁ cannabinoid and D2 dopamine receptor antagonists. These researchers concluded that cannabinoid impairment of working memory and inhibition of hippocampal extracellular acetylcholine concentration are mediated by the concomitant activation of D2 and CB₁ receptors.

Movement

Central cannabinoid receptors are densely located in the output nuclei of the basal ganglia (globus pallidus, substantia nigra pars reticulata), a finding suggesting their involvement in the regulation of motor activity. However, different approaches have not managed to give a precise role of endocannabinoids in the inhibition of spontaneous activity and induction of catalepsy in rodents, typical of all CB₁-receptor agonists (84). In fact, although CB₁-receptor knockout mice seem to have different baseline locomotor activity than wildtype mice, it is not clear whether deletion of the CB₁-receptor gene in these transgenic animals leads to hypermotility (35) or hypomotility (36). However, an endogenous cannabinoid tone negatively controlling spontaneous activity and motor behavior is supported by the finding that AEA, but not 2-AG, is released in microdialysates from the dorsal striatum of freely moving rats (85), and the levels of AEA are also very high in the substantia nigra and external layer of the globus pallidus (68).

In this latter brain region, endocannabinoid levels are inversely correlated with spontaneous activity in the reserpine-treated rat, an animal model of Parkinson disease, in which dopamine and other catecholamines in the striatum are depleted (68). AEA levels in the striatum of normal rats are increased by selective stimulation of D2 dopamine receptors by quinpirole, whereas the CB₁ antagonist SR141716A strongly enhances quinpirole-induced movement in both normal and reserpine-treated rats (68,85). These data suggest that the endocannabinoid system may act as a brake on dopaminergic stimulation of movement in the basal ganglia, and an exaggerated endocannabinoid tone in this region may produce (or at least contribute to) parkinsonian symptoms in rats (68). Further evidence for such suggestions has been provided by the finding that tolerance to the motor inhibitory actions of THC in rats chronically treated with the cannabinoid is accompanied not only by down-regulation of cannabinoid receptors in the striatum, but also by a significant decrease of endocannabinoid levels in this brain area (67).

Craving, Appetite Stimulation, and Reward

The finding of CB₁ receptors in the arcuate nucleus and the medial preoptic area of the hypothalamus, the presence of endocannabinoids and their biosynthetic precursors in the hypothalamus and pituitary, and the effect of endocannabinoids on body temperature, food intake, and pituitary hormone release suggest a role for endocannabinoids in the control of hypothalamic functions, and in particular on appetite and hormone release. Indeed, the CB₁-receptor–selective antagonist, SR141716A, inhibits palatable food intake in rodents (86). It has not been established whether

this effect results from the inverse agonist properties of SR141716A (72) or from its blockade of a food-intake stimulatory tone by endocannabinoids. Another brain region possibly involved in the control of appetite and craving is the limbic forebrain and, more particularly, the nucleus accumbens. In this brain area, cannabinoids, by enhancing the release of dopamine from dopaminergic terminals originating in the ventral tegmental area, may exert reinforcing actions on the effects of other drugs of abuse or, under more physiologic conditions, may participate in the regulation of feelings of craving and reward (87). Furthermore, it was found that chronic treatment of rats with THC causes an almost fourfold increase of AEA levels (and no down-regulation of cannabinoid receptors) in the limbic forebrain (67). It is possible that dopamine released in the nucleus accumbens on chronic treatment with THC triggers AEA formation, as previously shown for the dorsal striatum (85). Conversely, dopamine may be released in this region after the activation of CB₁ receptors by AEA. Indeed, studies carried out with CB₁-receptor knockout mice showed reduced opioid dependence (35), as well as lack of morphine-induced dopamine release in the nucleus accumbens of these transgenic animals (88). Thus, contrary to the basal ganglia, endocannabinoids released in the nucleus accumbens may act to enhance the action or release of dopamine, thereby participating in reward, craving, and pleasure or in the reinforcement of drug of abuse effects. There are indications that withdrawal from chronic cannabinoid administration is associated with reduced dopaminergic transmission in the limbic system, similar to that observed with other addictive drugs, a finding consistent with a role in drug craving and relapse into drug addiction or in the reinforcing effects of drugs of abuse.

Neuroprotection

The possibility that endocannabinoids may play a role in diminishing cellular or neuronal damage is of particular relevance to neurodegenerative disorders. The suggestion that endocannabinoids may have a neuroprotective function during cell injury stems from the finding that a similar role was proposed also for other ethanolamide of fatty acids (89), as well as for both psychoactive and nonpsychoactive cannabinoids. This hypothesis is supported by the finding that stimuli leading to high intracellular Ca²⁺ concentrations (e.g., glutamate-induced excitotoxicity) and noxious agents such as ethanol and sodium azide lead to increased synthesis of AEA and related compounds in neuronal cells. Cannabinoid receptors do not appear to be involved in this elevation of Ca²⁺. In fact AEA, via CB₁ receptors, produces the opposite effect. It inhibits Ca²⁺ influx into neurons through voltage-gated Ca²⁺ channels and counteracts membrane permeability to Ca²⁺ through N-methyl-D-aspartate receptor-coupled channels. Therefore, endocannabinoids should be able to inhibit glutamate-induced excitotoxicity (or other pathologic conditions arising from high intracellular Ca²⁺ concentrations) by acting at CB₁ receptors, particularly because they do not share the antioxidant effects of some synthetic cannabinoids. In conclusion, further studies are necessary to assess whether and through what mechanisms AEA and 2-AG prevent neuronal damage.

MEDICAL MARIJUANA

Scientific Justification

The nonmedical use of marijuana has a very long history, primarily for its mind-altering effects and the sense of wellbeing that it can provide. Therefore, the potential use of marijuana for diseases of the brain is a logical extension of the popularity of the use of the material in producing moodaltering effects. The initial therapeutic uses proposed for marijuana included the treatment of mental disorders and pain. As more information about the pharmacologic effects of the plant material emerged, other potential therapeutic uses became apparent. Since the 1970s, investigators have proposed many different therapeutic uses for marijuana including, but not limited to, nausea and vomiting induced by cancer chemotherapeutic agents, the wasting syndrome accompanying AIDS, mental illness, convulsions, glaucoma, cognition disorders, muscle spasticity, and neuropathic pain (90).

The consequences of the social use of marijuana, both real and as often exaggerated by opponents and proponents, have caused increased anxiety on both sides of the controversy over the medical use of marijuana. Strong proponents of the use of smoked marijuana for the treatment of various syndromes and diseases argue that smoking marijuana has produced beneficial effects in at least one disease state that could not be achieved by the oral administration THC or by any other treatment modality. Opponents are concerned with the deleterious effects of the smoked marijuana, especially the prolonged use of this plant material. The issue is further complicated by the fact that many strong proponents of the use of marijuana in medicine also advocate for its legal recreational use. Conversely, those who are opposed to its use, especially by adolescents and young adults who may be especially vulnerable to problems of abuse, effects on energy, memory, and acquisition of interpersonal skills, have not always considered the possible benefits with the same degree of objectivity as would be afforded other potential therapeutic agents. One of the major problems contributing to this dilemma is the lack of well-controlled studies attesting to the efficacy and the safety of marijuana in humans. Such studies require a reasonable hypothesis to be tested and an appropriate investigation under conditions that completely eliminate the possibility of subjectivity in the measurements. This controversy is not likely to be resolved until such studies are forthcoming.

The need for an approved medical use of marijuana itself

is questioned by some investigators because of the availability of marijuana's active constituent. THC was approved for the treatment of the nausea and vomiting associated with cancer chemotherapy in the 1980s and for the treatment of the wasting syndrome in AIDS patients in the early 1990s. It has been moved from Schedule II to Schedule III. Marijuana proponents counter that the therapeutic benefits derived from smoked marijuana are the result of many chemicals in the plant, not solely THC. There are, however, toxic pulmonary consequences to marijuana smoking. Further, the smoking route of administration used for marijuana has advantages over the oral route used for the administration of THC. The onset of action is faster while at the same time allowing the smoker to titrate blood levels better. The heightened interest in medical marijuana has not yet been translated into a satisfactory resolution of the differences. There is no doubt of the severity of the conditions and diseases for which marijuana or THC has been proposed. The availability of alternative delivery systems (e.g., aerosol) and alternative synthetics that have the desired therapeutic effect with minimal intoxicating effects are needed to resolve the controversy. Even then, however, it is likely that some will argue that it is the intoxicating effect combined with the other effects that makes marijuana particularly useful.

Medication with Plant Material

Active Constituents

Hundreds of compounds have been isolated and identified from the marijuana plant (91). Most have been shown to have minimal pharmacologic activity, and most exist in the plant in very small quantities. The major active constituent in marijuana has been shown to be THC. The pharmacologic profile of THC is essentially the same as that of smoked marijuana, and the evidence is now overwhelming that the predominant effects of marijuana on the brain result from this compound. Other cannabinoids such as Δ^8 -THC, cannabidiol, and cannabinol have been studied and have been shown to have interesting pharmacologic profiles. The Δ^8 -THC isomer produces many of the same effects as the Δ^9 - isomer (THC), but it is generally less potent, and the quantity of Δ^8 -THC in the plant material usually is less than that of THC. Cannabidiol and cannabinol are of interest because they exist in reasonable quantities in the plant and produce interesting pharmacologic effects in some systems but also are considerably devoid of activity on the central nervous system, especially in relation to mental health, memory, and cognition. The lack of effects on the central nervous system is an advantage in the potential use of one of these agents or an analogue to treat a disease or a condition with a locus of action outside the brain. The search for other cannabinoids that could have therapeutic potential has shifted almost totally to the synthetic chemistry process.

THC Content

The identification of THC as the active agent in marijuana stimulated a concentrated effort to quantitate the amount of this material in various samples of the cannabis plant. The initially reported concentrations of THC in confiscated marijuana were approximately 2% but have increased to more than 4% during the past few years (92). It was found that by altering the soil conditions and the environment, the concentration could be increased several fold. As one would expect, the pharmacologic effects of smoking marijuana are directly related to the concentration of THC. Advances in biogenetic engineering as applied to agriculture suggest that manipulations could be made to increase the concentration of THC even higher. Concentrations of more than 20% have been reported in some marijuana grown under artificial conditions in the Netherlands. How available increased-potency marijuana is in the United states remains unclear.

Consistency

As described earlier, the concentration of the active constituent in marijuana can vary over a large range. This variation clearly complicates the delivery of a consistent dose of medication. It would not be practical to quantitate the concentration of the active ingredient in each cigarette before its consumption. Most of the proposed indications for the medical use of marijuana require chronic administration, which magnifies the problem of inconsistent dosing. Administration of any drug through smoking presents an additional problem when a standard procedure does not exist for preparing cigarettes with a constant quantity of plant material in each cigarette. Further, the variability from individual to individual in the size and the rate of puffing produces another variable for the consumption of drugs by this route of administration. Even in the same patient, the volume of smoke inhaled can often differ from time to time.

Unwanted Side Products

The administration of a drug by smoking plant material causes other problems because many substances are being taken in along with the active ingredient. Each of these substances has its pharmacologic and toxicologic effects. Further, these other substances may either potentiate or interfere with the effects of the active ingredient. It is abundantly clear from the vast literature on the smoking of other products, predominantly tobacco, that numerous compounds are produced in the burning process. These pyrolysis products also have their own pharmacologic and toxicologic profile, and as with the other ingredients in the plant material, these pyrolysis products have the potential to alter the effects of the active ingredient. The major problem is the inability to control the exposure of the patient carefully to

a consistent and correct dose of the active ingredient and to establish a treatment regimen that is devoid of interference from other chemicals in the preparation or made during the administration process.

Optimal Delivery

One of the major concerns with the potential use of marijuana, and, for that matter with any of the cannabinoids, is the observation that these agents produce a multiplicity of effects, and they all seem to occur at similar doses. One possible way to overcome this problem is to develop a delivery mechanism that limits the distribution of the drug to the desired site of action. This is particularly difficult when the site of action is in the brain, as it is with the cannabinoids and their potential usefulness in treating symptoms of mental illness. One potential therapeutic use of the cannabinoids is in the treatment of glaucoma. Local administration directly into the eye is the preferred mechanism of drug delivery. One of the problems of using cannabinoids in this fashion is that they are insoluble and must be administered in a vehicle, which may have deleterious effects when it is placed in the eye. An optimal delivery for this indication would be to have a water-soluble cannabinoid (93) with good efficacy in lowering intraocular pressure that can be applied directly to the eye and not be irritating. The direct application of such a drug would provide the intended therapeutic effect and would not produce the undesirable side effects that would be observed if the drug were absorbed into the general circulation.

The reemergence on the debate of the use of marijuana for medicinal purposes has also been the impetus for developing an acceptable delivery form of aerosolized cannabinoids. A nebulizer was used to generate an aerosol with sufficiently small particle size such that exposure to rodents produced pharmacologic effects, These results demonstrate that the development of an aerosolized form of cannabinoids for human medicinal use is feasible (94).

Future Developments

Selective Receptor Agonists and Selective Pharmacologic Profiles

Because the cannabinoids have multiple effects on so many different body functions at approximately the same dose, considerable effort continues to be directed toward identifying the portion of the cannabinoid molecule that is most responsible for each unique pharmacologic effect. The identification of multiple cannabinoid receptors and the observation that certain cannabinoids have selectivity for one type of receptor over the other are encouraging (95,96). Further research into different receptor types and the identification of specific endogenous ligands for each of these receptor types will provide guidance for the medicinal chemist syn-

thesizing new and, one hopes, more selective cannabinoids. The hypothesis that each receptor subtype has its own specific ligand, as in the case of AEA preference for the CB₁ receptor, is a reasonable approach. These investigations will be guided by the continued progress in the efforts of researchers to identify and understand the cellular and molecular effects of the cannabinoids. Mechanisms may be found that will help to provide selectivity resulting from the effects of the cannabinoids on an intracellular site of action. The more detail we know about the genetic influences and the structural makeup of the receptors and other cellular elements, the better we will be able to design cannabinoids with selective activity.

Another approach to identifying cannabinoids with more selectivity of action is to investigate the interactions of exogenously administered substances with AEA and other endogenous cannabinoids. If one were to hypothesize that an altered tonic activity of the endogenous cannabinoid system is at the basis of some neurologic disorders, there are again two approaches that could be taken in the search for more selective agents. One would be to alter either the synthesis or degradation of the endocannabinoids, and the other is to modulate their actions. An example of the latter approach would be to compete with or block the receptor or to interfere with the signaling events through which the endogenous compound produces its effect. The advantage of these approaches would be that drugs interfering with endocannabinoid metabolism or action would exhibit higher effects in those tissues where the levels and activity of endocannabinoids are pathologically altered.

Selective Transport Blockers and Enzyme Inhibitors

Several blockers of AEA-facilitated transport have been developed so far, but only two were shown to enhance the actions of AEA in vivo or in vitro. The most widely used one, AM404, potentiates AEA analgesic effects in the hot plate test and inhibition of adenylate cyclase (97), although it also activates vanilloid receptors. Inhibitors of FAAH have also been developed (98), a potent and selective one of which, palmitylsulfonylfluoride (AM347), acts as a covalent inhibitor (99). The most potent irreversible FAAH inhibitor developed so far is methylarachidonoylfluorophosphonate (100), which unfortunately also binds to CB₁ receptors. Future studies will have to establish whether defective biosynthesis or exaggerated metabolism of endocannabinoids contributes to pathologic conditions, and, therefore, whether therapeutically useful drugs can be developed using these or more selective inhibitors of AEA degradation. These compounds are likely to act most efficaciously only at the site where AEA and 2-AG levels are pathologically altered, and, also for this reason, they will be devoid of undesired psychotropic side effects.

Receptor Antagonists

Ideally, the development of an antagonist to any new drug would be an asset to provide protection against an accidental overdose or to reverse the effects in a hypersensitive individual. Clearly, an antagonist is a very useful tool for studies directed toward elucidation of the pharmacologic profile of the agonist. The development of an antagonist as a new therapeutic agent requires the same demonstration of efficacy and safety needed for a cannabinoid agonist. As discussed earlier, a cannabinoid agonist may have potential therapeutic uses for disorders that have characteristics opposite to the pharmacologic effects of THC. Conversely, cannabinoid antagonists may have potential in treating memory impairment, obesity, and perhaps certain psychiatric disorders associated with marijuana use. The basis of the therapeutic usefulness of a cannabinoid antagonists rests on the premise that the endogenous cannabinoid system is under tonic control, and the antagonist can either block the actions of the endogenous ligand or exert inverse agonist effects by interactions with the cannabinoid receptor.

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