Proc. Natl. Acad. Sci. USA
Vol. 96, pp. 5338–5339, May 1999

Commentary

High times for cannabis research

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Marijuana is by far the most widely used illicit drug in the Western world, with an estimated 20 million regular users in North America and Europe. Many thousands of patients with AIDS, multiple sclerosis, and other illnesses are also illegally self-medicating with cannabis in the belief that it provides them with a therapeutic benefit. The medical use of cannabis has been highlighted recently by the publication of the Institute of Medicine report *Marijuana and Medicine* (1).

The history of scientific research on cannabis in the 1990s is reminiscent of the development of research on morphine and related opiate drugs during the 1970s. In each case, what began as the study of a plant-derived psychoactive drug resulted in the discovery of a naturally occurring physiological control system in the mammalian brain. Thus, research on morphine led to the discovery of opiate receptors and the naturally occurring family of morphine-like peptides the endorphins. Research on the active principal of cannabis, Δ^9-tetrahydrocannabinol (THC) has lead to the discovery of cannabinoid receptors and more recently, the lipid derivatives anandamide and 2-arachidonyl glycerol, which are thought to represent the naturally occurring ligands for these receptors (for review, see ref. 2).

Two cannabinoid receptors have been described: the CB1 receptor, present both in the brain and in some peripheral organs, and the CB2 receptor, present only in the periphery on cells of the immune system (2). An obvious question is whether all of the effects of THC and other cannabinoids on the central nervous system are mediated by the CB1 receptor. It might be that some of the central effects of THC are mediated by actions at some other cannabinoid receptor whose identity has not yet been revealed. There have been two experimental approaches used to address this question: use of the powerful new drugs that act selectively as CB1 receptor antagonists and development of genetically modified strains of mice in which the expression of the CB1 receptor has been eliminated. The CB1 receptor knockout approach is the subject of reports by Zimmer *et al.* (3) and Steiner *et al.* (4) in this issue of the *Proceedings*, both emanating from the same laboratory at the National Institute of Mental Health (NIMH). Their findings need to be compared with those reported by Ledent *et al.* (5), who independently developed a CB1 knockout strain of mice. The CB1 receptor antagonists, epitomized by the compound SR1417161A (developed by the French company Sanofi), first became available in 1995 (6) and have been widely used in academic research studies in the past few years (2).

In an ideal world, all of these approaches would lead to the same conclusions, but as so often happens in research that addresses complex biological questions, this has not proved to be the case. As one of the most important potential indications for cannabis-based medicines is the control of pain, it is not surprising that this has been an important focus for many of the animal studies. A number of studies have used the CB1 antagonist drug SR141716A and reported that it completely blocked all of the pain-relieving effects of THC and related cannabinoids in various animal models of pain (2, 7–9). The CB1 antagonist drug given on its own, however, had no effect on the baseline sensitivity to pain stimuli in these animal studies. In agreement with these findings, Ledent *et al.* (5) found no change in pain thresholds in their $CB1^{-/-}$ mice using heat, mechanical pressure, or chemical irritants as pain stimuli. These animals also showed no analgesic response at all to THC in the one of the heat tests (hotplate) but retained a small but significant response to THC in the other heat test (tail immersion). The NIMH group (3) also found that the analgesic responses to THC in the hotplate test were abolished in the $CB1^{-/-}$ mice but, surprisingly, found that THC still gave a more or less normal analgesic response in the other heat test, the tail flick. On the other hand, they found that analgesic responses to a synthetic cannabinoid compound, HU210, were completely absent in the tail-flick test in the $CB1^{-/-}$ animals. Furthermore, Zimmer *et al.* (3) reported significant changes in baseline pain sensitivity in the $CB1^{-/-}$ mice when tested in the hotplate or formalin paw (chemical irritant) models but no changes in tail flick latency. It is possible that some of these differences among tests reflect the level in the central nervous system that is involved. The tail-flick and tail-immersion tests measure a spinal reflex, whereas both the hotplate and formalin tests measure behavioral responses that involve higher brain centers.

The European and NIMH groups (3, 5) were in agreement in finding that $CB1^{-/-}$ mice no longer exhibited some of the other characteristic responses to THC that are thought to be centrally mediated. These included THC-induced reduction in body temperature and spontaneous activity and THC-induced increases in immobility (catalepsy). Ledent *et al.* (5) also report an absence of the normal cardiovascular responses to THC (reduced blood pressure and heart rate) in $CB^{-/-}$ mice, although their resting heart rate and blood pressure remained normal.

The groups differed, however, in their findings on the effects of the $CB^{-/-}$ knockout on baseline motility. Ledent *et al.* (5) found that the knockout mice exhibited higher levels of spontaneous running activity, even when placed in fear-inducing novel environments (open field, elevated plus maze). This finding is consistent with the observation that THC and other cannabinoids cause reductions in spontaneous activity and at least one report that the CB1 antagonist SR141716 caused an increase in spontaneous activity in mice (7). Zimmer *et al.* (3), paradoxically, found that their $CB1^{-/-}$ mice displayed reduced activity in the open-field test and an increased tendency to immobility in a test of catalepsy. The accompanying paper by Steiner *et al.* (4) provides a rationale to explain this apparent anomaly, by providing evidence of alterations in the expression of neurotransmitter and neuropeptide genes in neurons in the motor control centers of the mouse brain, the basal ganglia. They found increased levels of mRNA for the γ-aminobutyric acid biosynthetic enzyme, glutamate decarboxylase, and the neuropeptides substance P, dynorphin and enkephalin, in output neurons of the mouse striatum, especially in those regions that were normally enriched in CB1 receptors. The authors point out that such alterations in the

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Table 1. Comparison of CB1 receptor knockout reports and literature on effects of CB1 antagonist SR141716A

Response	Source		
	Zimmer *et al.* (3)	Ledent *et al.* (5)	SR141716A
Spontaneous activity	Reduced	Increased	Increased
Pain sensitivity			
Hotplate test	Reduced	Unchanged	Unchanged
Tail flick test	Unchanged	Unchanged	Unchanged
Chemical irritants	Reduced	Unchanged	Unchanged
THC			
Tail flick/or immersion	±Normal (tail flick)	Much reduced (tail immersion test)	Abolished
Hot plate test	Abolished	Abolished	Abolished
Hypothermia	Abolished	Abolished	Abolished
THC-induced hypomotility and catalepsy	Abolished	Abolished	Abolished
Remaining effects	High dose-induced diarrhea, postural changes	Weak analgesic response (tail immersion)	None

basal ganglia might account for the alterations in spontaneous activity observed in the $CB1^{-/-}$ animals; their results, however, remain at variance with those of the apparently similar strain of animals tested by Ledent *et al.* (5). A summary of the findings with $CB1^{-/-}$ mice and the CB1 antagonist drug SR141716A is given in Table 1.

Zimmer *et al.* (3) also describe some unusual effects of high doses of THC (50–100 mg/kg) in the $CB1^{-/-}$ mice. These effects, including strong diarrhea and abnormal posture, head movements, and grooming, are not seen in normal wild-type mice in response to THC. The doses of THC needed to elicit these effects, however, were very high, and it is hard to know how to interpret these observations. A dose of THC of 0.2 mg/kg is strongly intoxicant in man.

There is increasing evidence that the cannabinoid and opiate systems represent parallel but overlapping physiological control mechanisms, particularly in their involvement in the control of pain sensitivity (10). Ledent *et al.* (5) pursued this relationship in their studies of the $CB1^{-/-}$ mice. They found that the knockout mice showed normal analgesic responses to morphine (tail-flick and hotplate tests). However, the $CB1^{-/-}$ mice seemed to find morphine less rewarding; they proved less likely to self-administer morphine by intravenous injection. When morphine-dependent animals were challenged with the opiate antagonist drug naloxone, the behavioral signs of opiate withdrawal were less severe in the $CB1^{-/-}$ animals, suggesting again a possible involvement of cannabinoid mechanisms in the euphoriant effects of opiates and in the development of opiate dependence. The $CB1^{-/-}$ mice did not show any tendency to self-administer the synthetic cannabinoid WIN55,212-2 and displayed no withdrawal signs when, after repeated treatment with THC, they were challenged with the antagonist SR141716A, suggesting that the CB1 receptor does mediate the rewarding properties of cannabis and is involved in the development of dependence.

Overall, these new findings provide many valuable new insights into cannabinoid mechanisms in the brain, despite some disagreement between the NIMH and European reports. There is general agreement that the CB1 receptor plays a key role in mediating many, if not all, of the important central nervous system effects of THC and related cannabinoids. It seems likely also that the endogenous cannabinoid system may play a role in the modulation of pain sensitivity and the control of tonic activity in the output motor systems of the brain, although it remains unclear to what extent the cannabinoid mechanisms are activated under normal resting conditions. Like the opiate mechanisms in brain, it is possible that they are only called into play in response to some perturbation in the animal's environment or circumstances. No doubt further research in this now active area will provide answers to some of the remaining questions.

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