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Cannabinoids And The Cholinergic System

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Abstract: Δ^9-Tetrahydrocannabinol (THC) decreases EEG activation and causes slow waves in the cat. The EEG slow-wave activity is accompanied by a concomitant decrease in acetylcholine release from the neocortex. The findings suggest that THC depresses the brain stem activating system. Large doses of Δ^8- and Δ^9-THC increase brain acetylcholine levels in rodents such as the mouse and rat, but this effect is not seen with minimal doses of the cannabinoids which show behavioral effects. The most dramatic change produced by THC is that brain acetylcholine utilization is reduced primarily in the hippocampus.

It is still far from clear how cannabinoids produce their remarkable pharmacologic effects. The cannabinoids interact with many different neuronal and biochemical systems,[1,2] but which action is the most important in relation to therapeutic effects is still unknown. Marihuana and Δ^9-tetrahydrocannabinol (THC) have remarkable effects on human memory and cognition.[3–5] Recently, Ferraro[6] has reviewed the extensive literature on this subject. Although marihuana's acute effects on memory vary, he concluded that they are always detrimental to the marihuana user. Our animal neurochemical research suggests that these undesirable side effects of cannabis and probably other synthetic cannabinoids are related to a decrease in the release of neuronal acetylcholine (ACh) and its turnover in certain regions of the brain. This report summarizes these results from our laboratory. It should be pointed out that at least three other laboratories in the United States have been active in this field.[7–9] The results from all four laboratories differ in details, but all agree that cannabis preparations interact with the cholinergic system. Cannabinoids, especially the 7-OH derivatives, inhibit the twitch response of the guinea pig ileum to electric field stimulation,[10] a finding consistent with an ACh antirelease effect either indirectly or directly. There are a number of prominent side effects of cannabinoids in humans that also suggest an interaction with the cholinergic system, including dry mouth, tachycardia and bradycardia, drowsiness, sedation, and short-term memory loss.

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Methods

Mice, rats, and cats have been used in this research; details of the methods employed have been published elsewhere.[11–16] Briefly, a large variety of acetylcholine (ACh) assay techniques have been used including bioassays such as the frog rectus abdominus and the leech muscle, and chemical assays including the enzymatic choline kinase method, pyrolysis and chemical demethylation, gas chromatography-flame ion detection (GC-FID), gas chromatography-nitrogen detection (GC-ND), and gas chromatography-mass spectrometry (GC-MS). Current results, obtained using chemical demethylation and GC-ND or GC-MS, are fully consistent with the results obtained using bioassay and chemical assay techniques.

Results

Effects of Cannabinoids on Neocortical Acetylcholine Release in the Cat

Inasmuch as wakefulness involves a brain stem activating mechanism in the reticular formation and hypothalamus which has widespread neocortical and limbic system effects, it was postulated that THC would affect these systems. Both EEG and acetylcholine (ACh) release data were obtained to indicate that this indeed is the case.[13] It is known that the level of spontaneous release of ACh from the neocortex varies with the degree of anesthesia and level of brain stem transection. In order to assure a high level of spontaneous release, pretrigeminal brain stem transected preparations were used. Usually, the mean of three assays before and after drug administration was obtained. In our animals, the range in baseline release varied from a low of 8.0 to a high of 51.4 ng/cm^2/10 min, with a mean ± S.E. for 24 animals before drug of 22.9 ± 2.2 ng/cm^2/10 min 3 hours after halothane anesthesia and brain stem transection. The factors involved in the wide range observed in baseline release of ACh probably include residual anesthesia and postsurgical brain trauma.

THC in intravenous doses of 0.5 to 11.0 mg/kg produced high-voltage slow waves in the EEG of the neocortex of pretrigeminal midpontine transected cats. EEG high-voltage slow waves were more prominent in the frontal than the occipital cortical leads. The left frontal cortical lead which contained the bathing solution for collecting ACh showed EEG spikes and slow waves typical of the local effects of physostigmine. Small doses of THC tended to exaggerate these effects, but in larger doses they were reduced. The administration of (+)-amphetamine in intravenous doses of 2.5 mg/kg dramatically antagonized most of the effects induced by THC. Small doses of THC in the order of 0.5 mg/kg produced variable effects on ACh release. Some animals showed a definite increase in ACh release, while others showed a slight decrease. However, larger doses of THC produced consistent and progressively greater depression of ACh release. These effects are antagonized by (+)-amphetamine (Table I).

TABLE I

Effects of THC on Cat Brain Acetylcholine Release

No. of animals	Dose of THC (mg/kg)	Mean change in ACh release (%)
3	0.5	+19
2	1.0	− 9
2	1.5	− 7
1	3.5	−46
2	6.0	−55
1	11.0	−63

The mean ± S.E. acetylcholine concentration in rat brain for 12 control animals given no injection was 25.3 ± 0.8 nmole/Gm. Thirty minutes after Tween vehicle alone was given to 11 rats, brain ACh was 26.4 ± 1.3 nmole/Gm. This value was not significantly different from that achieved after no injection. After 1 μg acetylseco HC-3 (ASHC-3) was administered intraventricularly in 13 animals, brain ACh was significantly reduced to 20.3 ± 0.9 nmole/Gm ($P < 0.001$). When THC was given alone in doses of 3.2, 10, and 32 mg/kg intraperitoneally 30 minutes later, only the largest dose caused a significant increase in brain ACh above control ($P < 0.05$). Doses of THC of 5 mg/kg intravenously and 10 mg/kg intraperitoneally 1 hour later also did not significantly affect total brain ACh. On the other hand, in animals pretreated with ASHC-3, doses of 10 and 30 mg/kg THC intraperitoneally significantly elevated brain ACh to control levels ($P < 0.001$), indicating that ACh utilization was reduced.

Similar findings were observed in mouse brain in which 3.2 μg ASHC-3 was given intraventricularly. None of the control vehicles had any significant effect on mouse brain ACh 30 minutes later, whereas

TABLE II

Effects of THC Derivatives on Mouse Brain Acetylcholine Utilization

Treatment		Number	ACh (nmole/Gm)	
Subcutaneous†	Intraventricular	of animals.	Mean ± S.E.	P*
Vehicle**		8	18.6 ± 1.8	
Vehicle	NaBr	8	19.2 ± 2.0	NS
Vehicle	ASHC-3	31	9.5 ± 0.5	< 0.001
Δ^9-THC, 10 mg/kg	ASHC-3	8	11.5 ± 0.9	NS
Δ^9-THC, 32 mg/kg	ASHC-3	9	16.5 ± 1.3	< 0.001
Δ^9-THC, 56 mg/kg	ASHC-3	8	19.2 ± 2.0	< 0.001
Δ^8-THC, 32 mg/kg	ASHC-3	10	12.6 ± 1.7	< 0.05

* Group comparison Student's t-test.
** 4% Tween-20 in 0.9% saline.
† 3.2 mg, 30 minutes prior to sacrifice.

ASHC-3 reduced brain ACh levels significantly ($P < 0.001$). Both Δ^8- and Δ^9-THC significantly reduced mouse brain ACh utilization just as Δ^9-THC did in the rat (Table II).

Effects of THC on Regional Rat Brain Acetylcholine Levels and Utilization

Inasmuch as whole-brain acetylcholine (ACh) utilization was reduced by THC, it seemed important to localize this effect. Because the method involves the intraventricular injection of ASHC-3, brain areas close to the ventricles (hippocampus, hypothalamus, thalamus, and caudate) were studied. Male Holtzman rats (six per group) without intraventricular cannulation were given either THC (10 mg/kg, intraperitoneally) or vehicle and were sacrificed 1 hour later by microwave irradiation. Only slight, insignificant changes were found in the regional levels of ACh (Fig. 1). In view of the lack of any dramatic effects of THC on re-

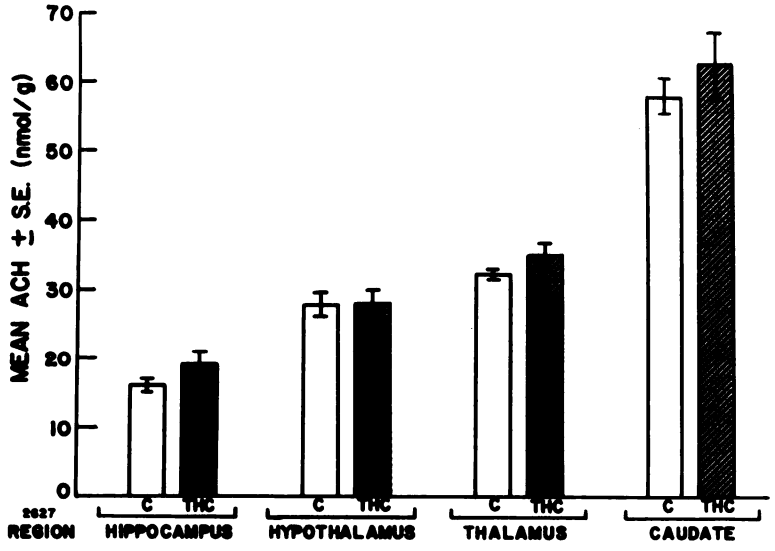

Fig. 1. Lack of effects of THC on regional brain acetylcholine levels in the rat brain.

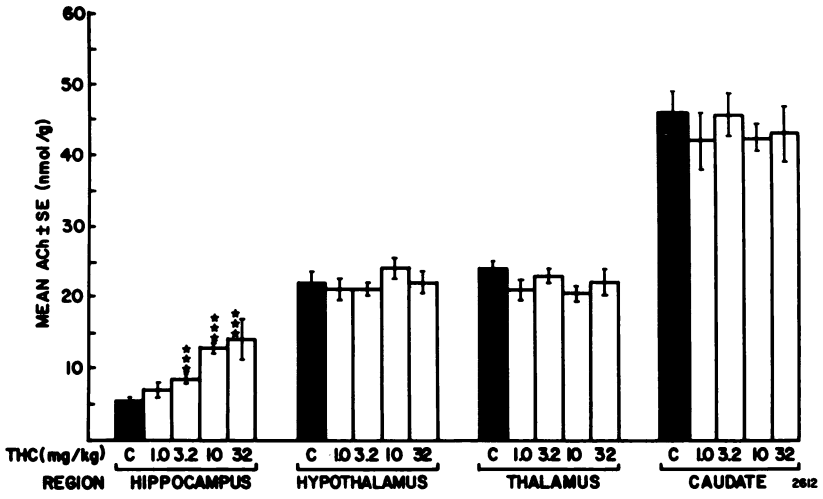

*Fig. 2. Effects of THC on regional brain acetylcholine utilization in the rat brain. ***P < 0.01 group comparison Student's t-test.*

gional brain ACh levels, its effects on regional brain ACh utilization were examined. Male Holtzman rats (six per group) were given intraperitoneally 1.0 to 32 mg/kg THC suspended in 4% Tween 20–0.9% NaCl and sacrificed 1 hour later by focused microwave irradiation. The procedure differed from that when using normal animals in that polyethylene cannulas (PE10) were implanted under light ether anesthesia 48 hours prior to drug administration. Each rat was implanted with an intraventricular cannula 4.5 mm below the cranial surface. Two days later, on the experimental day, 30 minutes before sacrifice, ASHC-3 (3.2 μg) was injected into the left lateral ventricle through the cannula. Acetylcholine levels in the hypothalamus, thalamus, hippocampus, and the caudate nucleus were then assayed (Fig. 2). No significant alteration of ACh utilization was found in the hypothalamus, thalamus, or caudate nucleus. However, the previously depleted hippocampal levels of ACh increased in a dose-effect manner, ranging from 123 (1.0 mg/kg) to 243 per cent (32 mg/kg).

Discussion

Among the many neurochemical actions of the cannabinoids is their effect on the release and turnover of acetylcholine. Whether any of the potential therapeutic effects of cannabinoids are related to a cholinergic mechanism is certainly questionable. However, some of the potential undesirable side effects of cannabinoids are almost certainly related to a decrease in ACh release and turnover.

Large doses of 10 mg/kg or more of THC elevate rat brain ACh and reduce its turnover. Inasmuch as doses of 3.2 mg/kg THC already depress acquisition of rat shuttle box behavior, the failure of this dose to affect total rat brain ACh indicates a dissociation of total brain ACh and THC behavioral effects. Regional brain ACh studies indicate that THC, in doses of 3.2 mg/kg intraperitoneally in rats, produces localized hippocampal ACh effects.

Our finding of a decreased rat brain hippocampal ACh turnover by THC suggests that at least this neurochemical effect may be related to an unwanted side effect of cannabinoid administration. It would be of great interest to examine other synthetic cannabinoids such as nabilone and levonantradol for similar effects. If these agents also reduce short-term memory, they may be of especial interest therapeutically to anesthesiologists. If indeed these new can-

nabinoids do affect short-term memory, it would be of value to study their effects on brain ACh release, content, utilization, and turnover.

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Discussion of the Paper

Dr. Dewey: As Dr. Domino indicated, my colleagues, Frank Vocci, Hem Tripathi and I have been working on this problem as well. We have studied the effects of 9 different cannabinoids on acetylcholine turnover in mice. The animals were sacrificed 20 minutes after intraperitoneal administration of the compounds. We found that 30 mg/kg of Δ^9-THC (a dose which causes a decrease in locomotor activity) decreases acetylcholine turnover, as measured by the radioenzymatic method of McCamman and Goldberg. 11-Hydroxy-Δ^9-THC is more active than the parent compound, and is also more active in altering the behavior of mice. The effect of Δ^8-THC (30 mg/kg) is not significantly different from control but is significantly different from the 3 mg/kg dose. In general we observed effects on acetylcholine turnover similar to effects previously reported for other neurotransmitter systems; i.e. low doses stimulate while high doses depress the noradrenergic and dopaminergic system. In slight contrast to Dr. Domino's results, cannabinol (10 and 30 mg/kg) depressed acetylcholine synthesis. Cannabidiol was completely without effect. β-HHC (synthesized by May and Wilson) at 30 mg/kg significantly decreased acetylcholine synthesis. The α-isomer, which has neither analgetic nor significant psychotomimetic effects, reduced acetylcholine synthesis only at a dose of 100 mg/kg. "Abnormal cannabidiol" (synthesized by Razdan of SISA) was inactive even at doses up to 100 mg/kg. 1-Methoxy-Δ^8-THC, like "abnormal cannabidiol" had minimal effect on the central nervous system below doses of 100 mg/kg, at which dose it also decreased the synthesis of acetylcholine. Thus we concur with the hypothesis that, in mice, cannabinoids lead to a decrease in acetylcholine in the hippocampus which may be related to psychotomimetic activity; however in 5 other brain areas there were no other significant effects in mice with any of these compounds.

Dr. Cash: Is it possible to measure acetylcholine release by monitoring spontaneous miniature end-plate potentials in peripheral muscle?

Dr. Domino: Yes. It is simple for the neuromuscular junction which is a nicotinic cholinergic effect. However, patients taking marijuana or THC show mostly a muscarinic cholinergic response. The guinea pig ileum has been used, but poor aqueous solubility has prevented extensive study of cannabinoids in this system. The most effective cholinergic anti-release cannabinoids are the more water soluble OH-derivatives.

Dr. Perez-Reyes: The first effect of marijuana in human beings is cerebral activation. Euphoria, increase in thought processes, and increase of imagery and sensory perception; all of these point to activation. After this activation, the effect becomes sedative, with the subject seeming to be exhausted.

Dr. Domino: We have preliminary evidence for a facilitating effect on cortical acetylcholine release with very low doses of THC. The number of experiments is small, but the data are consistent with the clinical observations.

Dr. Braude: Do you see the same effects after chronic and acute administration? Do you find comparable effects with phencyclidine?

Dr. Domino: No, just the opposite. Phencyclidine is amphetamine-like in facilitating the turnover of acetylcholine. Unfortunately, we don't have any data following chronic administration of THC, although I would expect tolerance development.

Dr. Clark: In humans we found (Clark, W.C., Goetz, R. R., McCarthy, R. H., Bemporad, B. and Zeidenberg, P. In G. G. Nahas and W. D. M. Paton (Eds.) *Marihuana: Biological Effects*. Oxford: Pergamon Press,

1979) only a slight effect on short-term memory; what was most disturbed was recall. Information got in, and was stored, but the subjects could not recall it unless we gave them a hint. The difficulty with retrieval of the information appeared to occur at an early stage of the memory process. The subjects failed to encode it in a systematic manner into a memory trace. In going from animal to human work we do not want to look at the short-term memory side as much as at the recall from longer term memory, but I do not know how you do that in animals.

Dr. Domino: That is precisely what happens as we get older—we have trouble with recall from memory. Thus I have been interested in marijuana and other cannabinoids as models of memory deficits in the geriatric patient.

Dr. Mechoulam: With cannabidiol, Costa showed that there is decrease in acetylcholine turnover. At my suggestion, he then compared two optical isomers, only one of which is psychotropically active, as well as a much more potent dimethylheptyl analog. The psychotropically inactive isomer caused essentially no change in acetylcholine turnover. The psychotropically active compound caused a large increase in acetylcholine turnover; so I think there is some correlation between cannabinoid psychic effects and acetylcholine turnover. (Revuelta *et al., Brain Research* **195**, 445, 1980).

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