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Chronic Marijuana Smoke Exposure in the Rhesus Monkey IV: Neurochemical Effects and Comparison to Acute and Chronic Exposure to Delta-9-Tetrahydrocannabinol (THC) in Rats

SYED F. ALI,[1] GLENN D. NEWPORT, ANDREW C. SCALLET, MERLE G. PAULE, JOHN R. BAILEY AND WILLIAM SLIKKER, JR.

Division of Reproductive and Developmental Toxicology
National Center for Toxicological Research, Jefferson, AR 72079

ALI, S. F., G. D. NEWPORT, A. C. SCALLET, M. G. PAULE, J. R. BAILEY AND W. SLIKKER, JR. *Chronic marijuana smoke exposure in the rhesus monkey IV: Neurochemical effects and comparison to acute and chronic exposure to delta-9-tetrahydrocannabinol (THC) in rats.* PHARMACOL BIOCHEM BEHAV **40**(3) 677–682, 1991.—THC is the major psychoactive constituent of marijuana and is known to produce psychopharmacological effects in humans. These studies were designed to determine whether acute or chronic exposure to marijuana smoke or THC produces in vitro or in vivo neurochemical alterations in rat or monkey brain. For the in vitro study, THC was added (1–100 nM) to membranes prepared from different regions of the rat brain and muscarinic cholinergic (MCh) receptor binding was measured. For the acute in vivo study, rats were injected IP with vehicle, 1, 3, 10, or 30 mg THC/kg and sacrificed 2 h later. For the chronic study, rats were gavaged with vehicle or 10 or 20 mg THC/kg daily, 5 days/week for 90 days and sacrificed either 24 h or 2 months later. Rhesus monkeys were exposed to the smoke of a single 2.6% THC cigarette once a day, 2 or 7 days a week for 1 year. Approximately 7 months after the last exposure, animals were sacrificed by overdose with pentobarbital for neurochemical analyses. In vitro exposure to THC produced a dose-dependent inhibition of MCh receptor binding in several brain areas. This inhibition of MCh receptor binding, however, was also observed with two other nonpsychoactive derivatives of marijuana, cannabidiol and cannabinol. In the rat in vivo study, we found no significant changes in MCh or other neurotransmitter receptor binding in hippocampus, frontal cortex or caudate nucleus after acute or chronic exposure to THC. In the monkey brain, we found no alterations in the concentration of neurotransmitters in caudate nucleus, frontal cortex, hypothalamus or brain stem. These data indicate that the in vitro effects of THC on MCh receptor binding are not observed in vivo in the rat. Neither chronic THC exposure in the rat nor chronic marijuana smoke exposure in the monkey resulted in any neurochemical alterations in the systems examined.

Marijuana smoke Delta-9-tetrahydrocannabinol Rat brain Monkey brain Muscarinic cholinergic receptor

THC is one of the primary components of marijuana known to produce psychopharmacological effects in animals and humans. However, the mechanism of action of THC is still unclear. It seems to affect all major neurotransmitter systems including the cholinergic (16), dopaminergic and adrenergic (1, 8, 12, 25, 34, 36, 39, 43), serotonergic (31,39), and GABAergic (33) systems when applied in vitro. THC has been shown to decrease dopamine release (35) and synthesis (8) and dopamine, norepinephrine and serotonin uptake sites in mouse (4, 21, 22) and rat (3) brain synaptosomes. Recently, Chen et al. (11) reported that low doses of THC (0.5–1.0 mg/kg) produced significant increases of dopamine efflux in nucleus accumbens of conscious, freely moving rats as measured by intracerebral microdialysis.

In contrast to the abundant data concerning presynaptic effects, there are few reports on the effects of THC on neurotransmitter receptors or on the neurotransmitter system in general after chronic exposure to THC or marijuana. Bloom and associates have reported that THC produces neurochemical changes in receptor-binding characteristics in vitro (5, 7, 23). In the present study, we evaluated whether acute or chronic exposure to THC produced any selective in vitro or in vivo alterations in cholinergic receptor binding in the rat brain. We also measured concentrations of dopamine, serotonin and their metabolites in several brain regions of monkeys after chronic exposure to marijuana smoke.

METHOD

Rat Treatment

Male Sprague-Dawley rats, 7–10 weeks old, were used in these studies. For the acute study, animals (n = 8–10) were dosed

[1]Requests for reprints should be addressed to Syed F. Ali, Ph.D., Division of Reproductive and Developmental Toxicology, National Center for Toxicological Research, N.C.T.R. Drive, HFT-132, Jefferson, AR 72079-9502.

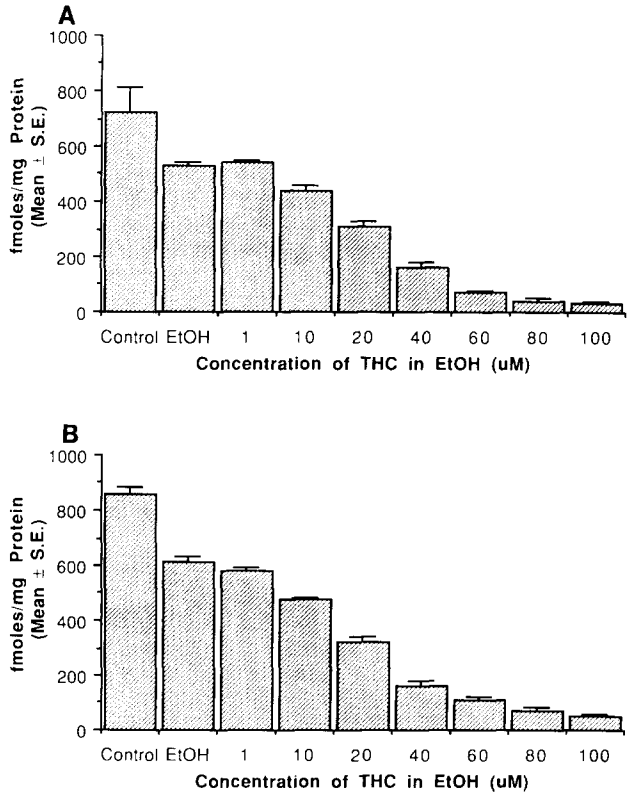

FIG. 1. Muscarinic cholinergic receptor binding after in vitro exposure to THC in (A) frontal cortex and (B) hippocampus of the rat brain.

with 1, 3, 10 or 30 mg/kg THC intraperitoneally (IP) and sacrificed 2 hours later. For the chronic study, animals (n = 6–8) were dosed by gavage (PO) with 10 or 20 mg/kg THC daily for five days/week for 90 days. Control animals received vehicle (Triton X-100/ethanol/saline; 1:10:89). After 90 days of treatment, animals were sacrificed by decapitation, brains were quickly dissected into several brain regions following the guidelines of Glowinski and Iversen (20), frozen over dry ice and stored at −70°C until analysis.

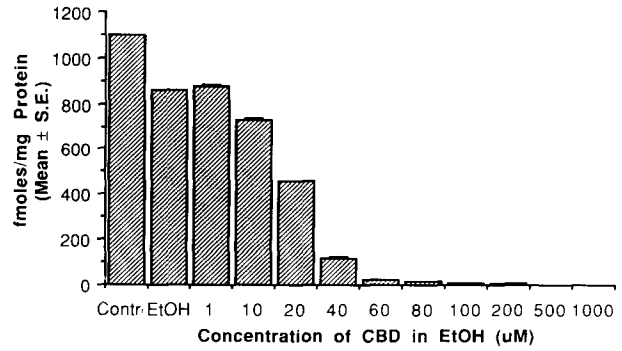

FIG. 2. Muscarinic cholinergic receptor binding in the rat frontal cortex after in vitro exposure to cannabidiol (CBD).

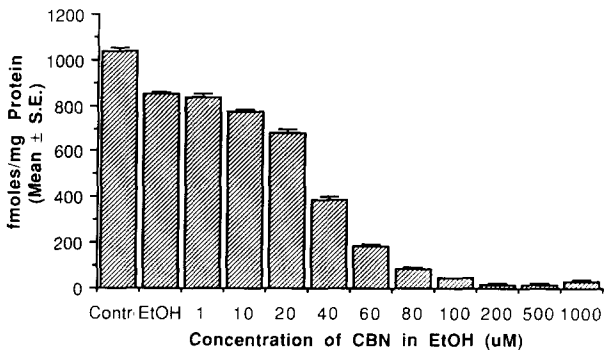

FIG. 3. Muscarinic cholinergic receptor binding in the rat frontal cortex after in vitro exposure to cannabinol (CBN).

Monkey Treatment

Details of the experimental design, clinical chemistry data, and plasma THC levels are reported in full elsewhere (37). Briefly, male rhesus monkeys (n = 6) were exposed to the smoke

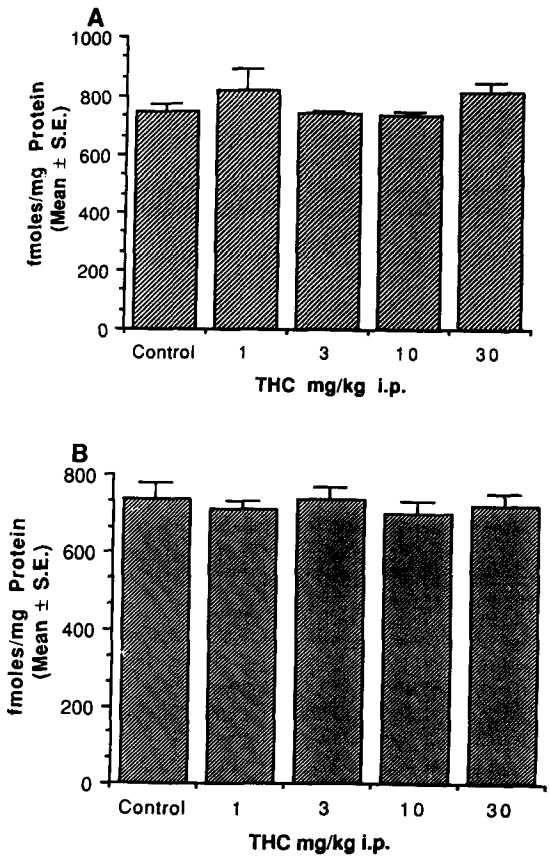

FIG. 4. Muscarinic cholinergic receptor binding in the rat frontal cortex and hippocampus after acute in vivo exposure to THC. Animals were sacrificed 2 h after dosing. (A) Frontal cortex, (B) hippocampus.

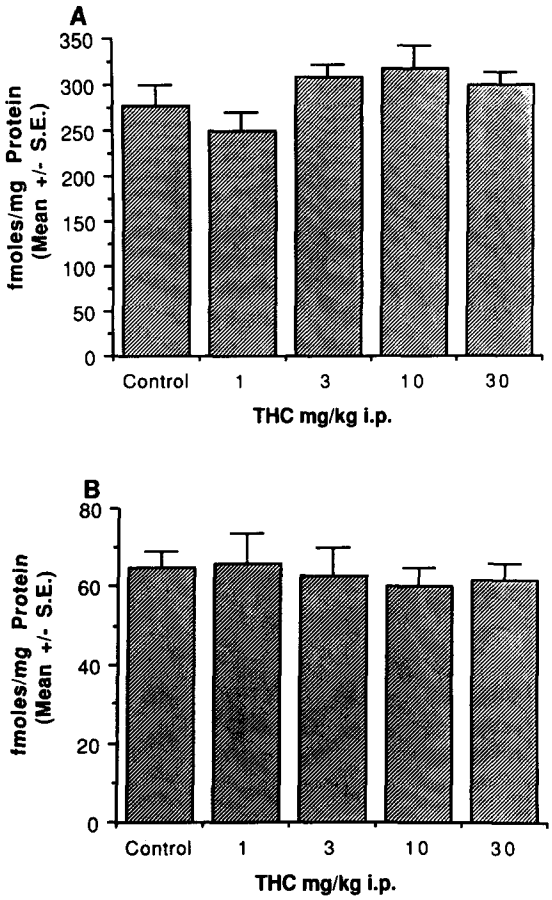

FIG. 5. Dopamine receptor binding in the rat caudate nucleus and frontal cortex after acute in vivo exposure to THC. Animals were sacrificed 2 h after dosing. (A) Caudate nucleus, (B) frontal cortex.

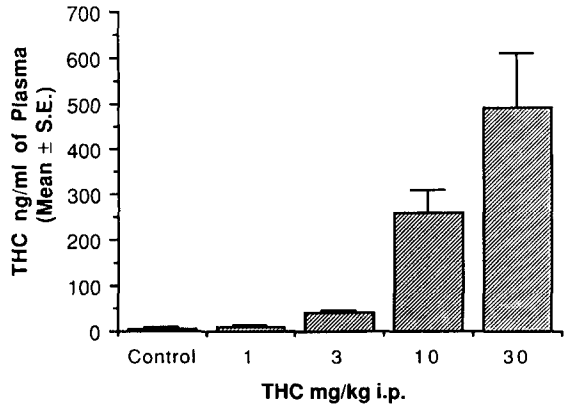

FIG. 6. THC levels in rat plasma 2 h after acute exposure to a single dose of THC.

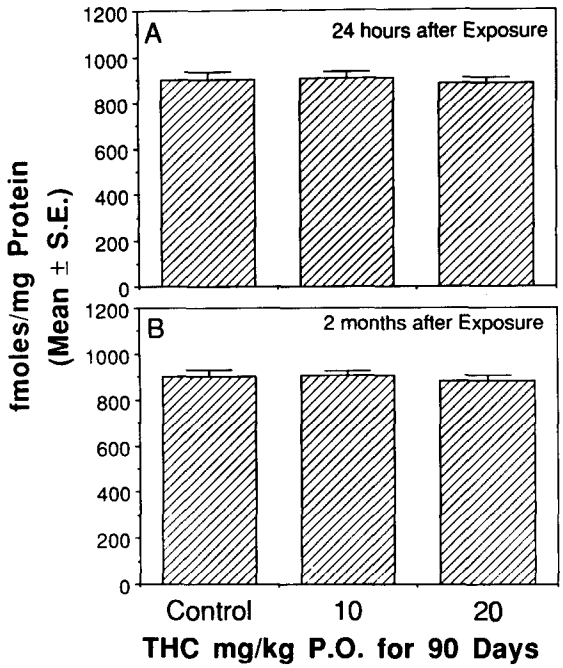

FIG. 7. Muscarinic cholinergic receptor binding in different regions of the rat brain after chronic (90-day) exposure to THC. Animals were sacrificed 24 hours (A) or two months (B) after the last dose.

of a single marijuana cigarette approximately (2.6% THC) once a day, 2 or 7 days a week for one year. Control animals were exposed to the smoke of one ethanol-extracted marijuana cigarette 7 days/week for one year, and sham control animals underwent the same exposure conditions except that no cigarettes were placed in the smoking machines. Approximately seven months after the last exposure, animals were sacrificed by overdose with pentobarbital, and the brains were removed and dissected on an ice-cold plate according to the method of Brown et al. (10) using the rhesus monkey atlas of Szebenyi (41).

Neurochemical Analyses

Receptor binding.

Membrane preparation. A crude membrane fraction was prepared from the frontal cortex, hippocampus and caudate nucleus by homogenizing in 20 volumes of 0.32 M sucrose followed by centrifugation (50,000 × g, 10 min). The pellet from this step was then homogenized in deionized distilled water (pH adjusted to 7.4), centrifuged (50,000 × g for 10 min), resuspended in 50 mM Tris-HCl (pH 7.4) buffer, and centrifuged again. The buffer-rinsing step was repeated one more time. The final pellet was suspended in the 50 mM Tris-HCl buffer containing 2.5 mM $CaCl_2$, 1 mM $MgCl_2$; 5 mM KCl, 120 mM NaCl, 0.1% ascorbate and 10 μM pargyline at a concentration of 50 mg (original weight equivalent)/ml.

[^{3}H]-quinuclidinyl benzilate (QNB) binding. Muscarinic cholinergic receptor binding was assayed by incubating 100 μl of the membrane preparation with 1.0 nM [^{3}H]-QNB (33.2 Ci/mmol; NEN Research Products, Boston, MA) following the method of Ali et al. (2). For the in vitro experiment, membranes were prepared from different regions of untreated rat brains. The effects of the addition of THC (1–100 μM), cannabidiol (1–1000 μM) and cannabinol (1–1000 μM) on [^{3}H]-QNB binding was evalu-

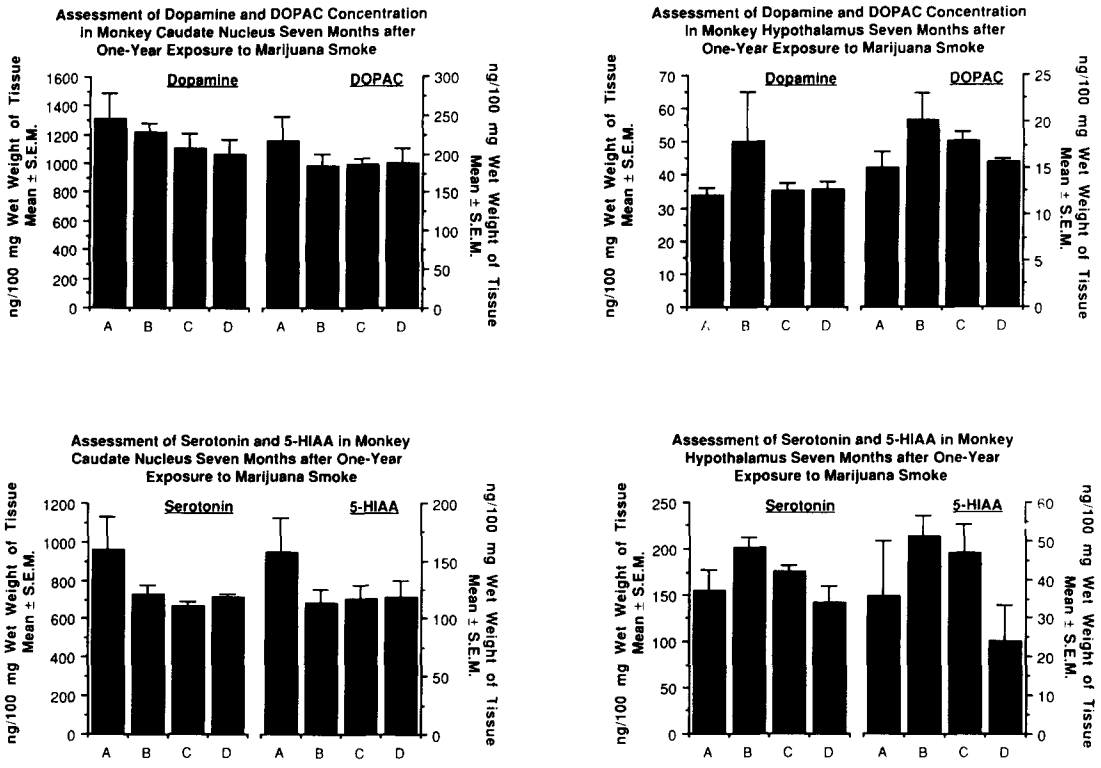

FIG. 8. Effect of chronic marijuana cigarette smoke exposure on neurotransmitter concentrations in caudate nucleus and hypothalamus of monkey brain (n=6 animal per group). Animals were exposed to the smoke from marijuana cigarettes (2.6% THC) for 1 year and sacrificed seven months after the cessation of exposure. (A) Sham controls; (B) extracted marijuana cigarette controls; (C) low-dose group (weekend-only exposure group); (D) high-dose group (daily exposure group).

ated following the same procedure as described for the in vivo experiments (1,2).

Protein determination. Aliquots of the membrane preparations were used for determination of protein content by the method of Lowry et al. (28), using bovine serum albumin as the standard.

Neurotransmitter concentration determination. Concentrations of dopamine, serotonin and their metabolites [3,4-dihydroxyphenylacetic acetic (DOPAC) and 5-hydroxyindoleacetic acid (5-HIAA)] were resolved by high-performance liquid chromatography (HPLC) and quantified by electrochemical detection following the method of Ali et al. (1,2).

Statistical analysis. Data were evaluated by a one-way analysis of variance, followed, where appropriate, by Student's *t*-test. A value of $p<0.05$ was taken as significant.

RESULTS

In the in vitro study, THC produced a dose-dependent inhibition of muscarinic cholinergic receptor binding in the frontal cortex and hippocampus of the rat (Fig. 1). In a follow-up in vitro study, we used two nonpsychoactive derivatives of THC, cannabidiol (CBD) and cannabinol (CBN), and found that these agents also produced a dose-dependent inhibition of muscarinic cholinergic receptor binding in the frontal cortex of the rat (Figs. 2 and 3).

In vivo, acute exposure to THC in the rat (1, 3, 10 or 30 mg/kg), however, did not produce any significant alterations in muscarinic cholinergic receptor binding in the frontal cortex or hippocampus (Fig. 4) or in dopamine receptor binding in either the caudate nucleus or frontal cortex (Fig. 5) as measured two hours after the IP administration of single doses. The levels of THC two hours after dosing were measured by RIA in the plasma of treated rats and ranged up to about 500 ng/ml two hours after the 30-mg/kg dose (Fig. 6). Chronic exposure to THC (10 or 20 mg/kg, PO, for 90 days) in the rat did not produce any significant alterations in muscarinic cholinergic receptor binding in the hippocampus 24 hours or 2 months after exposure (Fig. 7).

Chronic marijuana smoke exposure in the monkey did not produce any alterations in the concentrations of dopamine, DOPAC, serotonin or 5-HIAA in caudate nucleus or hypothalamus (Fig. 8).

DISCUSSION

In vitro exposure to THC produced a dose-dependent inhibition of muscarinic cholinergic receptor binding in rat frontal cortex and hippocampus. However, similar inhibition of muscarinic cholinergic receptor binding was observed with two nonpsychoactive derivatives of THC, CBD and CBN. In in vivo studies in which rats were dosed with THC and the same brain regions were analyzed for muscarinic cholinergic or dopaminergic receptor binding, we did not identify any significant treatment-related changes after either acute or chronic administration. There are reports in the literature describing significant elevations of acetylcholine 30 min after an in vivo exposure to large doses of delta-9-THC (17). In follow-up studies, these same authors reported no changes in acetylcholine levels, but significant increases in utilization of acetylcholine in the hippocampus (16,18).

Our data suggest that in vitro exposure to THC, CBD and CBN produce dose-dependent inhibition of muscarinic cholinergic receptor binding. However, no significant changes were found after acute or chronic exposure to THC in vivo.

There are also reports in the literature describing the effects of THC on endogenous levels of catecholamines under different experimental conditions. These reports show increased (31), decreased (19) or no effects (9) on catecholamine levels. Bloom et al. (4) reported that THC increased the functional activity of dopaminergic and noradrenergic systems in mouse brain. They also demonstrated that behaviorally active cannabinoids can increase the synthesis of catecholamines in the rodent brain. These changes in synthesis rates did not alter the endogenous levels of the neurotransmitters, suggesting that the rate changes were the result of increased utilization (4,7). There are several reports suggesting a biphasic effect of THC on neurotransmitter systems including synaptosomal uptake and release (4,35), biogenic amine levels (24), cyclic AMP levels (15), Mg^{++}-dependent ATPase activity (6), electrical excitability of hippocampal slices (42), 2-deoxyglucose turnover (30) and even body temperature (38). These reports suggest that THC interacts with biological membranes in a biphasic manner.

In the present study, when monkeys were exposed to marijuana smoke for one year and sacrificed seven months after cessation of exposure, we did not find any significant alterations in monoamine levels in the caudate nucleus or hypothalamus. This lack of residual effect is consistent with other endpoints in these same monkeys, as indicated by our collaborative THC receptor studies, which demonstrated no alterations of THC receptor density (44). One explanation for this effect could be that animals developed a tolerance to chronic THC exposure, as has been demonstrated for many neuroreceptors after exposure to agonist ligands (29, 40, 45). One cellular mechanism for tolerance is the down-regulation of receptors (14), as has been shown for β-adrenergic, muscarinic cholinergic and opiate receptors (13, 14, 26, 27). Therefore, one would predict that chronic exposure to THC would result in a decreased number of cannabinoid receptors in the brain. In our monkey studies, however, we conducted the neurochemical evaluation seven months after the cessation of marijuana smoke exposure. This lag time was sufficient for normalization of behavioral parameters (32) and perhaps allowed for recovery of any receptor alterations or neurotransmitter changes that might have occurred during the treatment period. In our rat study, however, no neurotransmitter alterations were observed when animals were sacrificed 24 h and 60 days after chronic exposure to THC.

In conclusion, in vitro exposure to THC and other nonpsychoactive cannabinoids produces significant alterations in MCh receptor binding in several brain areas; however, in vivo exposure to THC does not mimic this effect. The in vitro effects of THC on MCh receptors are, therefore, apparently not linked to its psychoactive effects. Because there were also no effects on any of the biogenic amine levels measured seven months after chronic marijuana smoke exposure, there appear to be no residual neurochemical affects as assessed by these techniques. It is possible that neurochemical alterations occurred during marijuana smoke exposure but that these effects dissipated during the seven months after the last drug exposure. However, the lack of effects on these same neurochemical systems after either acute or chronic THC treatment in rats sacrificed soon after exposure indicates that THC or marijuana smoke has no significant effect on the assessed neurochemical systems.

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