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Acute Effects of δ-9-Tetrahydrocannabinol on Dopaminergic Activity in Several Rat Brain Areas

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RODRÍGUEZ DE FONSECA, F., J. J. FERNÁNDEZ-RUIZ, L. L. MURPHY, M. CEBEIRA, R. W. STEGER, A. BARTKE AND J. A. RAMOS *Acute effects of δ-9-tetrahydrocannabinol on dopaminergic activity in several rat brain areas.* PHARMACOL BIOCHEM BEHAV 42(2) 269–275, 1992. – In this work, we examined the acute effects of two doses of δ-9-tetrahydrocannabinol (THC) on several pre- and postsynaptic biochemical measures of dopaminergic activity in the striatum, limbic forebrain, and hypothalamic-anterior pituitary area of adult male rats. The exposure to a low dose of THC (0.5 mg/kg bw) decreased the number of striatal D_2 dopaminergic binding sites, but did not affect their affinity. Treatment with a higher dose of THC was ineffective. In addition, both doses decreased the number of D_1 dopaminergic binding sites in the limbic forebrain without changing their affinity. We did not find any changes in the dopamine (DA) or L-3,4-dihydroxyphenylacetic acid (DOPAC) content, or in the DOPAC/DA ratio, in either the striatum or limbic forebrain. THC treatment produced a dose-related decline in plasma prolactin (PRL) levels. Furthermore, both the basal and DA-inhibited in vitro release of PRL were reduced in animals exposed to THC in a dose-dependent manner. This inhibitory effect of THC on PRL release was accompanied by a decreased DOPAC/DA ratio in the medial basal hypothalamus that, in turn, may be a result of the fall in PRL levels rather than a direct action of the drug. These data show that acute exposure to THC can alter brain dopaminergic neurotransmission. Our results suggest that the reduction of PRL release following THC exposure, both in vivo and in vitro, might be elicited by a direct action of THC on the pituitary.

δ-9-Tetrahydrocannabinol Striatum Limbic forebrain Hypothalamic-anterior pituitary area Dopamine DOPAC Dopaminergic receptors Prolactin

δ-9-TETRAHYDROCANNABINOL (THC) is the predominant psychoactive constituent of marijuana (16). It is well established that this psychoactive constituent of marijuana is able to alter the activity of several brain neurotransmitter systems, including dopamine (DA) (3,5,7,26), noradrenaline (28), serotonin (36), GABA (27), and acetylcholine (24). Moreover, THC exposure alters a variety of behavioral indices and neuroendocrine functions [for review, see (11)]. At the moment, experimental results suggest the existence of a complex interaction of this drug with brain mechanisms.

There is strong evidence that dopaminergic neurons in the brain mediate the actions of THC. Exposure to this cannabinoid has been reported to decrease prolactin (PRL) secretion (21,30), produce extrapyramidal effects (8,9), facilitate brain stimulation reward (14), and exacerbate or precipitate psychiatric disorders (2) via alterations in the dopaminergic systems involved in these processes.

The site of the action of THC on brain dopaminergic neurons has not yet been defined. Some authors have demonstrated that THC facilitates striatal DA release and suggested a presynaptic effect of THC (7,26). In vitro studies have shown that THC also stimulates nigrostriatal dopaminergic neurotransmission, perhaps by inhibiting DA uptake (34). In contrast to these studies, there is little information about the effects of THC on postsynaptic dopaminergic sensitivity (4,20).

Recently, specific cannabinoid receptors have been identified in the brain (10). These receptors appear in several brain areas, with the high density of receptors localized within the basal ganglia – caudate-putamen, substantia nigra, and globus pallidum – and also in limbic and hypothalamic areas (18). Moreover, cannabinoid receptors in the basal ganglia are neuronally located on striatal neurons, but not on dopaminergic nigrostriatal cell bodies or terminals (17).

The present study was designed to investigate the effects of acute exposure to THC on dopaminergic postsynaptic sensitiv-

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ity in the striatum, limbic forebrain, and hypothalamic-anterior pituitary area. Postsynaptic sensitivity was evaluated by measuring the number (B_{max}) and affinity (K_d) of D_1 and D_2 dopaminergic receptors, as well as the in vitro basal and DA-inhibited PRL release from incubated hemipituitaries. For each dopaminergic pathway, DA and L-3,4-dihydroxyphenylacetic acid (DOPAC) contents and the DOPAC/DA ratio were also measured as an index of presynaptic activity. In the rat, doses of THC greater than 5.0 mg/kg body weight have been shown to have a brain depressor effect (11). Thus, we chose a low oral dose, 0.5 mg THC/kg body weight, and a high oral dose, 5.0 mg THC/kg body weight, and examined their effects 1 h after treatment. The choice of this route of administration is based on the fact that it produces less stress compared to other methods. Its major inconvenience—the existence of individual differences in the degree of intestinal absorption, which would lead to a broader interval of plasma levels—has been significantly reduced by monitoring plasma THC levels for each animal and studying individual correlations if required.

METHOD

Animals

Adult, male Sprague-Dawley rats (>8 weeks old; 240–250 g) were purchased from Harlan Sprague-Dawley, Inc. (Indianapolis, IN). Animals were housed in a room with controlled photoperiod (lights on 0800–2000) and temperature (22 ± 1°C). They had free access to standard food (TekLab) and water.

Cannabinoid Treatment

THC of greater than 95% purity was provided in an ethanol solution by the National Institute on Drug Abuse. Immediately before use, the alcohol was evaporated and the residue was emulsified in sesame oil as vehicle. Animals received a single oral dose of THC (0.5 or 5.0 mg/kg bw) or vehicle in a volume of 0.1 ml.

Sampling

One hour after treatment, animals were weighed and decapitated. Trunk blood, collected in tubes containing 6% EDTA, was centrifuged (2500 × *g*) at 4°C and the plasma separated and stored frozen at −40°C until assayed for PRL and THC levels. Brains were removed, immediately frozen, and stored at −70°C until assay. On the day of analysis, each brain was thawed and the striatum, limbic forebrain, and medial basal hypothalamus were dissected using methods described previously (15). Pituitaries were removed at autopsy and the posterior lobe was separated and discarded. Anterior pituitaries were hemisected and each half was placed in separate culture tubes for in vitro release studies.

Dopamine and DOPAC Determinations

DA and DOPAC contents were analyzed using high-performance liquid chromatography (HPLC) with electrochemical detection. Tissues were homogenized in ice-cold 0.2 N perchloric acid with 0.5 mM sodium bisulfite and 0.45 mM EDTA (100–200 μl/10 mg tissue). Dihydroxybenzylamine was added as an internal standard. The homogenates were centrifuged and the supernatants injected into the HPLC system. Details on the HPLC system have been previously published (12). Values are expressed as ng or pg/mg tissue weight.

D_1 and D_2 Dopaminergic Binding Site Analysis

Measurements of D_1 and D_2 binding sites were performed according to the procedures described by Reader et al. (29) and Leysen et al. (22), respectively, with slight modifications (13,32). The radioactive ligands, [^{3}H]SCH 23390 (60.4 Ci/mmol) for D_1 and [^{3}H]spiroperidol (27.5 Ci/mmol) for D_2, were purchased from New England Nuclear (Boston, MA). The range of concentrations was 0.125–3.0 and 0.05–0.80 nM, respectively. Protein concentration in the incubated membrane fractions, as measured by the Lowry et al. method (23), was 0.2–0.3 mg/ml for D_1 measurements in the striatum, 0.4–0.6 mg/ml for D_1 in limbic forebrain, and 0.15–0.20 mg/ml for D_2 measurements. For measurement of nonspecific binding, 30 μM (±)-SKF 38393 and 1 μM (+)-butaclamol (RBI, Natick, MA), respectively were used. The final volume of incubation medium was 0.5 ml. A Scatchard analysis of the data, using linear regression, was performed to evaluate the dissociation constant (K_d), expressed as nM units, and the number of binding sites (B_{max}), expressed as fmol/mg protein.

Pituitary Incubations

In vitro release of PRL was examined according to the method described by Steger et al. (37). Hemipituitaries were placed in culture tubes with 1 ml Medium 199 (GIBCO, M199) and preincubated at 37°C in a Dubnoff metabolic incubator in an atmosphere of 5% CO_2–95% O_2. After 60 min of incubation, media was aspirated and discarded and fresh medium was added. The hemipituitaries were incubated for another 60 min, after which time the media were collected for the determination of basal PRL release. Fresh media containing 10^{-7} M DA was then added and the tubes incubated an additional 60 min to determine DA-inhibited PRL release. All media were frozen at −70°C until assayed for PRL concentrations. Releases were expressed as ng/mg tissue weight · 60 min incubation.

Prolactin Determination

Plasma and incubation media PRL concentrations were measured by a double antibody radioimmunoassay using materials kindly supplied by the National Hormone and Pituitary Program (NIH, Bethesda, MD). The intraassay coefficient of variation was 3.8%, the interassay coefficient of variation 8%, and the sensitivity 0.05 ng/tube when rPRL-RP3 was used as standard. Plasma PRL levels are expressed as ng/ml rPRL-RP3.

TABLE 1

PLASMA δ-9-THC LEVELS

Parameters	Treatments		
	+Oil	+Low THC	+High THC
THC (ng/ml)	ND	25.24 ± 3.23*	73.73 ± 13.68†

Plasma δ-9-THC levels in male rats 1 h after oral administration of either a low dose (0.5 mg/kg bw) or a high dose (5.0 mg/kg bw) of THC or vehicle (sesame oil). Details in the text. Values are means ± SEM of six to eight determinations per group. Statistical differences were assessed by ANOVA. Values with a different superscript are statistically different ($p < 0.05$).

TABLE 2

DOPAMINERGIC PARAMETERS IN STRIATUM

Parameters	Treatments		
	+ Oil	+ Low THC	+ High THC
DOPAC (ng/mg)	1.07 ± 0.11	1.05 ± 0.12	1.09 ± 0.11
DA (ng/mg)	5.85 ± 0.54	5.66 ± 0.48	6.69 ± 0.58
DOPAC/DA	0.18 ± 0.01	0.19 ± 0.02	0.16 ± 0.01
D_1 B_{max} (fmol/mg)	1370.3 ± 113.1	1147.0 ± 97.3	1418.2 ± 124.9
D_1 K_d (nM)	0.65 ± 0.06	0.62 ± 0.07	0.67 ± 0.02
D_2 B_{max} (fmol/mg)	537.0 ± 79.6	391.4 ± 21.2*	467.5 ± 35.4
D_2 K_d (nM)	0.41 ± 0.06	0.30 ± 0.03	0.35 ± 0.03

DA and DOPAC content and the number (B_{max}) and affinity (K_d) of D_1 and D_2 dopaminergic receptors in the striatum of male rats 1 h after oral administration of either a low dose (0.5 mg/kg bw) or a high dose (5.0 mg/kg bw) of THC or vehicle (sesame oil). Details in the text. Values are means ± SEM of six to eight determinations per group. Statistical differences were assessed by ANOVA.

*$p < 0.05$.

THC Levels

Plasma THC levels were kindly determined by Dr. J. Charles Eldridge using a specific radioimmunoassay kit prepared at the Research Triangle Institute (Research Triangle Park, NC) and furnished by NIDA (33). They are expressed as ng/ml. Sample volumes of 100 μl were extracted with methanol. The antiserum was generated against δ-9-THC; the radioligand was [^{125}I]δ-8-THC. The reference preparation was δ-9-THC in human plasma.

Statistics

Data were assessed by one-way analysis of variance (ANOVA). Differences were considered significant if the probability of error was less than 5%.

RESULTS

Monitoring Plasma THC Levels

Efficiency of the oral administration of THC was validated by measuring plasma THC levels. We observed the presence of significant concentrations of this cannabinoid in both THC-treated groups (Table 1). Plasma levels of THC were dose related, being approximately threefold greater in animals fed the high dose of THC than in those given the low dose of this compound (Table 1).

Cannabinoid Effects on Striatal Dopaminergic Neurons

Treatment with the low dose of THC elicited a significant decrease in the number of D_2 dopaminergic binding sites, but caused no changes in their binding affinity (Table 2). The high THC dose was ineffective, and neither dose altered the number of D_1 dopaminergic binding sites, the contents of DA or DOPAC, or the DOPAC/DA ratio (Table 2).

Cannabinoid Effects on Limbic Dopaminergic Neurons

Both the high and the low dose of THC decreased the number of D_1 dopaminergic receptors without affecting their affinity (Table 3). There were no changes in the contents of either DOPAC or DA or in the DOPAC/DA ratio (Table 3).

Cannabinoid Effects on Hypothalamic Dopaminergic Neurons

Exposure to THC caused a dose-dependent fall in plasma PRL levels, although only the high dose elicited a statistically

TABLE 3

DOPAMINERGIC PARAMETERS IN LIMBIC FOREBRAIN

Parameters	Treatments		
	+ Oil	+ Low THC	+ High THC
DOPAC (ng/mg)	0.58 ± 0.08	0.60 ± 0.06	0.52 ± 0.05
DA (ng/mg)	2.12 ± 0.21	1.85 ± 0.13	2.14 ± 0.15
DOPAC/DA	0.27 ± 0.02	0.36 ± 0.05	0.25 ± 0.02
D_1 B_{max} (fmol/mg)	786.2 ± 38.1	672.5 ± 40.4*	682.6 ± 27.1*
D_1 K_d (nM)	0.61 ± 0.05	0.51 ± 0.03	0.56 ± 0.03

DA and DOPAC content and the number (B_{max}) and affinity (K_d) of D_1 dopaminergic receptors in the limbic forebrain of male rats 1 h after oral administration of either a low dose (0.5 mg/kg bw) or a high dose (5.0 mg/kg bw) of THC or vehicle (sesame oil). Details in the text. Values are means ± SEM of six to eight determinations per group. Statistical differences were assessed by ANOVA.

*$p < 0.05$.

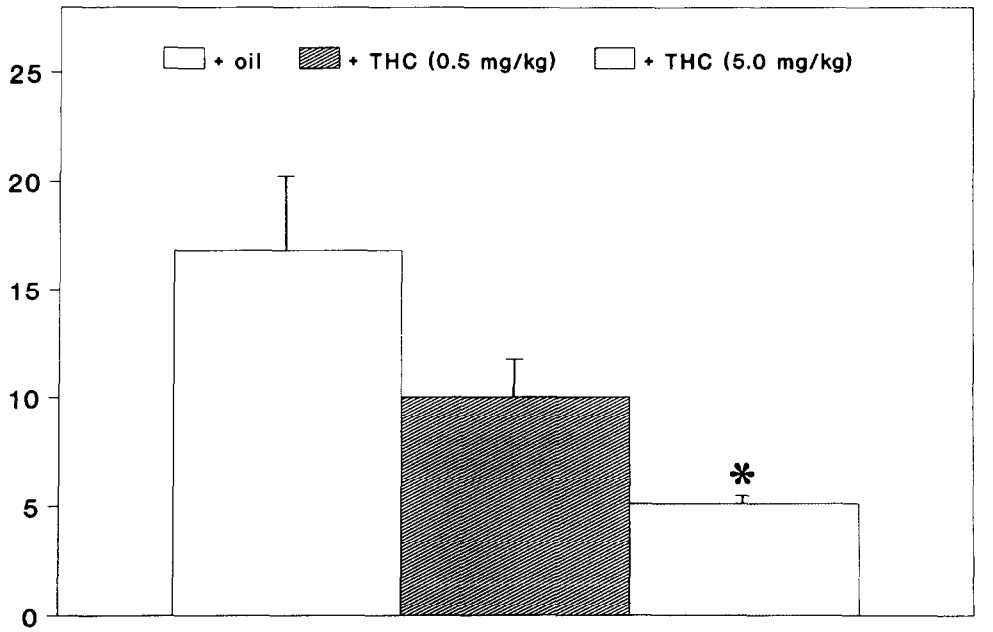

FIG. 1. Plasma PRL levels in male rats 1 h after oral administration of either a low dose (0.5 mg/kg bw) or a high dose (5.0 mg/kg bw) of THC or vehicle (sesame oil). Details in the text. Values are means ± SEM of six to eight determinations per group. Statistical differences were asessed by ANOVA. *$p < 0.05$.

significant decrease (Fig. 1). Moreover, treatment with the low dose of THC produced a decrease in both basal and DA-inhibited release of PRL in vitro (Fig. 2). The percentage of PRL inhibition in response to DA was significantly greater in low-dose THC-exposed pituitaries (47.46%) when compared to controls (27.83%) or high-dose THC-exposed pituitaries (36.18%). THC-induced decreases in PRL levels, both in vivo and in vitro, were accompanied by a dose-related reduction in the DOPAC/DA ratio in the medial basal hypothalamus (Table 4), although only the decrease caused by the high dose was statistically significant. These changes in the DOPAC/DA ratios were caused by a significant increase in the DA content, with no change in DOPAC after exposure to a low dose of THC and a significant decrease in the amount of DOPAC with no change in DA content after exposure to the high dose of the cannabinoid (Table 4).

DISCUSSION

Plasma THC levels were significantly increased in a dose-related fashion 1 h after oral THC administration. However, plasma THC levels were not in the same proportion as the amount administered. This may be due to the fact that orally administered THC is absorbed slowly and erratically, resulting in a lower systemic availability than via other routes of administration (1). Plasma THC levels in this study were within the range of those reported to cause psychological and physiological effects in animal models (1,11).

The presence of this cannabinoid in the blood was associated with measurable changes in brain dopaminergic neurotransmission at the postsynaptic level. Postsynaptic sensitivity in both the striatum and limbic forebrain was significantly decreased. This was reflected by decreases in the number of D_2 receptors in the striatum after the low dose of THC and in the number of D_1 binding sites in the limbic forebrain after both doses. No changes in affinity were observed in either brain areas after exposure to low or high doses of THC. These decreases could be explained on the basis of a possible specific interaction of this cannabinoid with the dopaminergic receptor, thus modifying the binding parameters. A priori, these results could also indicate that THC treatment reduces the ability to evaluate the neurotransmitter signal postsynaptically. It has been shown (4) that THC is able to decrease the binding of D_2 antagonists, spiperone and haloperidol, to mouse striatal membranes in vitro in a dose-dependent fashion, whereas it increased the affinity for D_2 agonists, DA and apomorphine. This may suggest a shift of the D_2 receptors from a low- to high-affinity state for receptor agonists and a corresponding association with a G-protein into a ternary complex (4). We observed a similar interaction of THC with the D_1 receptor in ovine brain striatal membranes (Rodríguez de Fonseca et al., unpublished observations). THC is also capable of interacting with rat brain opioid receptors in a similar way. This interaction seems to be direct with the receptor protein complex or with the specific protein–lipid complex and not merely the result of a perturbation of the lipid bilayer of the membrane (38). The regional differences in THC-induced changes in postsynaptic dopaminergic sensitivity may be explained by the different properties of dopaminergic receptors depending on the brain area (31).

However, cannabinoid and dopaminergic receptors are colocalized in basal ganglia intrinsic neurons (17). This allows a possible interaction between these receptors, which use the same transduction mechanism, which may compete for the same pool of G-proteins.

In the case of the tuberoinfundibular system, our data also support the hypothesis of a facilitatory role of THC and its action at the postreceptor level. Thus, THC produced a dose-

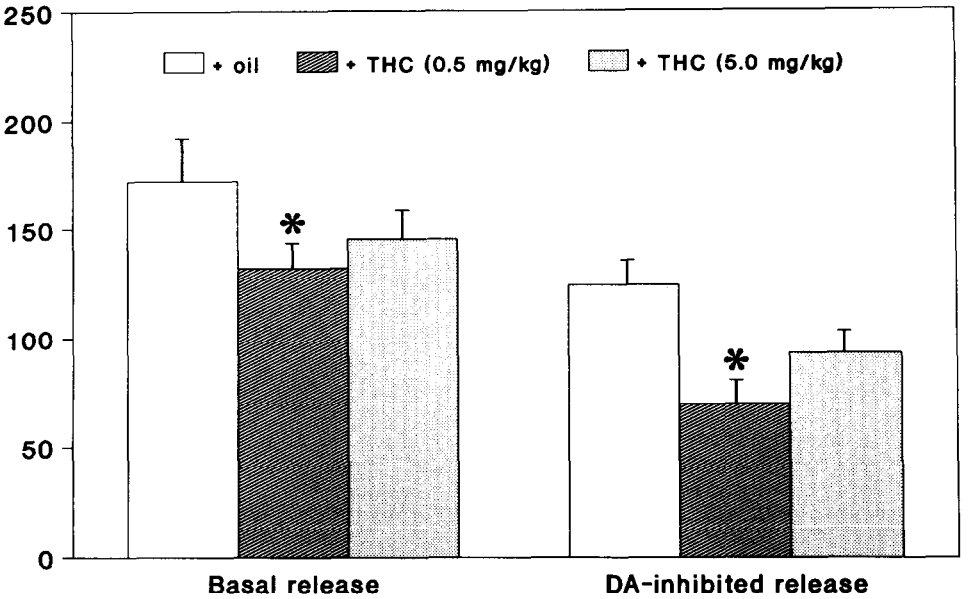

FIG. 2. PRL release (basal or DA 10^{-7} M inhibited) from incubated hemipituitaries of male rats 1 h after oral administration of either a low dose (0.5 mg/kg bw) or a high dose (5.0 mg/kg bw) of THC or vehicle (sesame oil). Details in the text. Valued are means ± SEM of six to eight determinations per group. Statistical differences were assessed by ANOVA. *$p < 0.05$.

related fall in plasma PRL levels that was accompanied by an unexpected decrease of the DOPAC/DA ratio in the medial basal hypothalamus. These results suggest that THC may exert either a direct pituitary effect by increasing the ability of DA to inhibit PRL release and/or a hypothalamic effect by modifying the activity of neurotransmitters other than DA, which are also involved in the neuroendocrine control of PRL release. A decrease in the activity of hypothalamic dopaminergic neurons could also be a result of the rapid desensitization response of the dopaminergic neurons to the decline in plasma PRL levels via a well-described feedback mechanism (35). Although the possibility of a hypothalamic site of THC action to inhibit PRL release cannot be excluded (19,30), our data support the existence of a pituitary site of action. This is based on our findings that exposure to low THC doses caused an inhibition of basal PRL release concomitantly with increased responsiveness to DA from incubated anterior pituitaries. In a recent study, THC was shown to prevent estradiol-induced desensitization of lactotrophs to the inhibitory influence of DA by a direct THC effect at the adenohypophyseal level (25).

In contrast, neither DOPAC or DA content nor their ratio were altered in limbic or striatal areas after acute exposure to THC. This could reflect absence of changes at the presynaptic level in both dopaminergic neuronal systems, as is the case for hypothalamic dopaminergic neurons, where decreases in DOPAC/DA ratio could be related primarily to a desensitization of the feedback mechanism induced by the fall in peripheral PRL levels. There are several studies reporting contradictory findings on the ability of THC to elevate extracellular DA levels in striatum and nucleus accumbens (6,7,26). As recently demonstrated, there are no cannabinoid receptors on nigrostriatal dopaminergic terminals (17). It has been proposed that the presynaptic effects of THC on dopaminergic neurons may be indirect since they can be blocked by naloxone (7,17).

TABLE 4

DOPAMINERGIC PARAMETERS IN MEDIAL BASAL HYPOTHALAMUS

Parameters	Treatments		
	+ Oil	+ Low THC	+ High THC
DOPAC (pg/mg)	36.1 ± 5.9*	25.9 ± 8.2*†	17.3 ± 4.8†
DA (ng/mg)	115.2 ± 18.1*	168.5 ± 15.7†	150.1 ± 26.5*†
DOPAC/DA	0.34 ± 0.08*	0.19 ± 0.06*†	0.13 ± 0.02†

DA and DOPAC content and the medial basal hypothalamus of male rats 1 h after oral administration of either a low dose (0.5 mg/kg bw) or a high dose (5.0 mg/kg bw) of THC or vehicle (sesame oil). Details in the text. Values are means ± SEM of six to eight determinations per group. Statistical differences were assessed by ANOVA. Values with a different superscript are statistically different ($p < 0.05$).

However, the absence of presynaptic effects could also be related to the dose of THC utilized in the present study. It has been shown that THC increases DA metabolism in limbic areas in a dose-dependent manner at doses greater than 5 mg/kg body weight, whereas the striatum was less sensitive, showing this effect only at doses greater than 10 mg/kg body weight (5). Thus, in both cases these effects were observed with very high doses of the cannabinoid, which also caused a marked depressor effect on the brain (11).

In summary, the present data suggest that acute exposure to THC produces several effects on dopaminergic neurotransmission, manifested by changes in postsynaptic sensitivity to DA. Thus, THC reduced the number of dopaminergic receptors in both the striatum and limbic forebrain, whereas it decreased PRL release by acting at the pituitary level. The molecular basis of these effects of THC will be the subject of further investigation.

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