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Motor Disturbances Induced by an Acute Dose of Δ^9-Tetrahydrocannabinol: Possible Involvement of Nigrostriatal Dopaminergic Alterations

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NAVARRO, M., J. J. FERNÁNDEZ-RUIZ, R. DE MIGUEL, M. L. HERNÁNDEZ, M. CEBEIRA AND J. A. RAMOS. *Motor disturbances induced by an acute dose of Δ^9-tetrahydrocannabinol: Possible involvement of nigrostriatal dopaminergic alterations.* PHARMACOL BIOCHEM BEHAV **45**(2) 291–298, 1993.—Exposure to cannabinoids has been reported to affect several neurotransmitter systems and their related behaviors. The present study has been designed to further explore the effects of cannabinoids on motor behavior and test the involvement of nigrostriatal dopaminergic neurotransmission and other neurotransmitters as possible neurochemical targets for these cannabinoid effects. Male rats treated with an oral dose of Δ^9-tetrahydrocannabinol (THC), the main psychoactive ingredient of cannabinoid derivatives, or vehicle were used 1 h after treatment for analyses of spontaneous motor and stereotypic activities together with neurochemical analyses of the nigrostriatal dopaminergic activity. Treatments and analyses were performed in the dark phase of photoperiod because it corresponds to the maximum behavioral expression in the rat. Neurochemical analyses were measurements of presynaptic activity—dopamine (DA) and L-3,4-dihydroxyphenylacetic acid (DOPAC) contents, tyrosine hydroxylase (TH) activity, and in vitro DA release—and postsynaptic sensitivity—number and affinity of D_1 and D_2 receptors—in the striatum. In addition, measurements of 5-hydroxytryptamine (5-HT) and 5-hydroxyindolacetic acid (5-HIAA) contents were also performed to evaluate serotoninergic activity in the striatum. An oral dose of THC produced a loss of spontaneous motor activity, measured in both actimeter and open-field test, and a decrease in the frequency of several stereotypic behaviors, such as rearing and self-grooming. This decrease was correlated to a low number of D_1-dopaminergic receptors in the striatum, although neither DA and DOPAC contents nor TH activity and D_2 receptors were altered. In addition, K^+-evoked DA release in vitro from perifused striatal fragments was slightly reduced, although nonsignificantly, in animals fed with THC, with no changes in the basal release. No significant changes were observed in striatal contents of 5-HT and 5-HIAA. In summary, these results allow us to conclude that acute THC exposure produces motor deficiencies, which might be originated, at least partially, as a consequence of THC-induced decreases in nigrostriatal dopaminergic sensitivity. However, the small magnitude of the dopaminergic changes following THC exposure suggests possible involvement of other neurotransmitters.

Δ^9-Tetrahydrocannabinol Cannabinoids Dopamine DOPAC Tyrosine hydroxylase
D_1 and D_2 dopaminergic receptors Motor activity Stereotypic behavior Nigrostriatal neurons

PREPARATIONS of *Cannabis sativa* (hashish, marihuana) are one of the most widely used psychoactive drugs (22). Their abuse produces multiple physiological effects, mainly at behavioral and neuroendocrine levels, during both developmental and mature ages [for review, see (11)]. For instance, exposure to marihuana or Δ^9-tetrahydrocannabinol (THC), its main psychoactive principle (7), have been reported to produce: a) decrease in the release of prolactin (PRL) (27,35), growth hormone (5), and gonadotropins (27); b) an increase in adrenocorticotrophic hormone secretion (16); c) alterations of the extrapyramidal motor activity (26); d) exacerbation of psychotic disorders (1); and e) potentiation of brain-stimulation reward (12).

It is likely that these neurobiological effects of cannabinoids are caused through changes in the activity of neurotransmitter systems involved in the control of those processes in specific brain areas. Thus, various neuronal groups, whose cell bodies are usually located in the mesencephalic reticular

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formation, innervate, playing a regulatory action, the forebrain structures composed of executive neurons that carry out the integrative and output processes of behavioral response (39). Dopamine (DA) is probably one of the most important among these regulatory neurotransmitters. In general, the different dopaminergic neuronal systems regulate and enable integrative functions in the neuronal systems onto which they project (4,18). There exists much information that suggests that nigrostriatal dopaminergic neurons, one of the most important extrahypothalamic dopaminergic pathways, play a primary facilitatory role in the elaboration of patterns of locomotor and stereotyped behavior (4). A number of experimental studies have demonstrated the existence of changes in the activity of this dopaminergic pathway after cannabinoid exposure, which might explain the extrapyramidal effects associated with cannabinoid consumption. Thus, both acute or chronic THC exposures result in inhibition of neurotransmitter uptake (37) and stimulation of its release (40), and modified the binding of D_2 receptors to spiroperidol in striatal membranes (3,33,35). Other important support of the possibility that nigrostriatal dopaminergic neurons might be one of the most important targets for the extrapyramidal actions of cannabinoids is the fact that a high density of the recently described cannabinoid binding sites in the brain (6,15,21) has been found in areas closely related to extrahypothalamic dopaminergic neurotransmission (13), such as the nuclei of the basal ganglia.

However, despite the great amount of information about the extrapyramidal effects of cannabinoids, some aspects have been scarcely studied. In particular, there are only a few reports studying correlations between behavioral and neurochemical effects following cannabinoid exposure, and most of them have been almost exclusively carried out during the light phase of photoperiod, which does not correspond to the period of maximum expression of the motor behavior in rats. Both aspects have been considered in the design of the present study. To this end, male rats treated with an oral dose of THC or vehicle were used 1 h after treatment for behavioral and neurochemical analyses. Treatments and behavioral analyses were performed in the dark phase of the photoperiod. Behavioral parameters analyzed were: a) spontaneous locomotor activity, measured in actimeter and open-field test; and b) frequency of spontaneous stereotypic behaviors (rearing and grooming), measured in the open-field test. Neurochemical analyses were measurements of presynaptic activity—contents of DA and L-3,4-dihydroxyphenylacetic acid (DOPAC) (its main intraneuronal metabolite), activity of tyrosine hydroxylase (TH) (the rate-limiting enzyme in DA synthesis), and in vitro DA release—and postsynaptic sensitivity—number (B_{max}) and affinity (K_d) of D_1 and D_2 receptors—in the striatum. In addition, measurements of 5-hydroxytryptamine (5-HT) and 5-hydroxyindolacetic acid (5-HIAA) contents were also performed to evaluate serotoninergic activity in the striatum.

METHOD

Animals, Treatments, and Sampling

Animals. Male rats of the Wistar strain were housed from birth in a room with a controlled photoperiod (light 1500–0300; + 1 h vs. Greenwich time) and temperature (23 ± 1°C). They had free access to standard food (Panlab, Barcelona, Spain) and water. They were used for the experiments at adult age (>8 weeks of life; 150–200 g). All studies were made during the period between 0900 and 1200 h.

Cannabinoid treatment. THC was obtained from Sigma Chemical Co. (St. Louis, MO). For treatment purposes, it was emulsified in a sesame oil solution after dilution in ethanol and subsequent evaporation under a nitrogen flow. Animals received a single oral dose of THC (5 mg/kg body weight). This dose was chosen on the basis of the results obtained in a previous dose-response study (35). Control rats were fed with vehicle alone. One hour after treatment, animals were used for either behavioral or neurochemical studies. Both treatments and tests were always conducted during the dark phase of the photoperiod (1000–1200 h) under red light.

Sampling. Animals used for the purpose of neurochemical studies were killed by rapid decapitation. Trunk blood was collected in tubes containing 0.4 ml 6% EDTA, immediately centrifuged, and the plasma removed, aliquoted, and stored frozen at −70°C. Their brains were quickly removed and the striatum dissected and immediately frozen at −70°C. For perifusion experiments, dissected striatum was used immediately after sacrifice.

Behavioral Studies

Both THC-fed and control rats were submitted to two kinds of behavioral tests: actimeter and open-field test. Different groups of rats were used for each kind of test. Spontaneous locomotor activity was evaluated in both actimeter and open-field test, whereas the frequency of several stereotypic behaviors, rearing and self-grooming, was evaluated in the open-field test. All the behavioral tests were carried out by an investigator who had no knowledge of the treatment of each rat.

Actimeter. A standard actimeter was used to measure spontaneous motor activity, as previously described (29). Animals were placed in motility cages (26 × 21 × 9.5 cm each) with photocell motility meters (Actimeter Photoelectrique, Apelab, France). The apparatus was located in a sound-isolated cubicle and the number of crossings was recorded every 5 min. Rats were always placed in the motility cages for a period of 10 min before the onset of the test to become acclimated. Values are expressed as accumulated scores at 5, 10, and 15 min.

Open-field test. This test allowed the simultaneous measurement of spontaneous locomotor and stereotypic activities. It was carried out simultaneously with the measurement of sexual motivation in a sociosexual approach behavior test (data not shown). It is based upon the method described by Meyerson (23). The structure consisted of a circular arena composed of a hardboard (diameter 90 cm) with a surrounding wall (height 30 cm), both made of transparent polyvinyl chloride. The floor was divided into five inner and eight outer parts using circles and radial segments. For 2 consecutive days before the test day, each experimental animal was adapted to the apparatus by placing it in the arena for 15 min. On the day of the test, the experimental animal was placed in the center of the arena and its spontaneous activity was recorded in a TV-video system to allow rescoring and reanalyzing of the results. The duration of the test was 15 min. The apparatus was washed with an odoriferous solution after each rat had been tested. The following parameters were analyzed in three periods of 5 min: a) spontaneous locomotor activity—number of sector crossings (a single line-crossing was defined as the rat placing two front paws into an adjacent quadrant); and

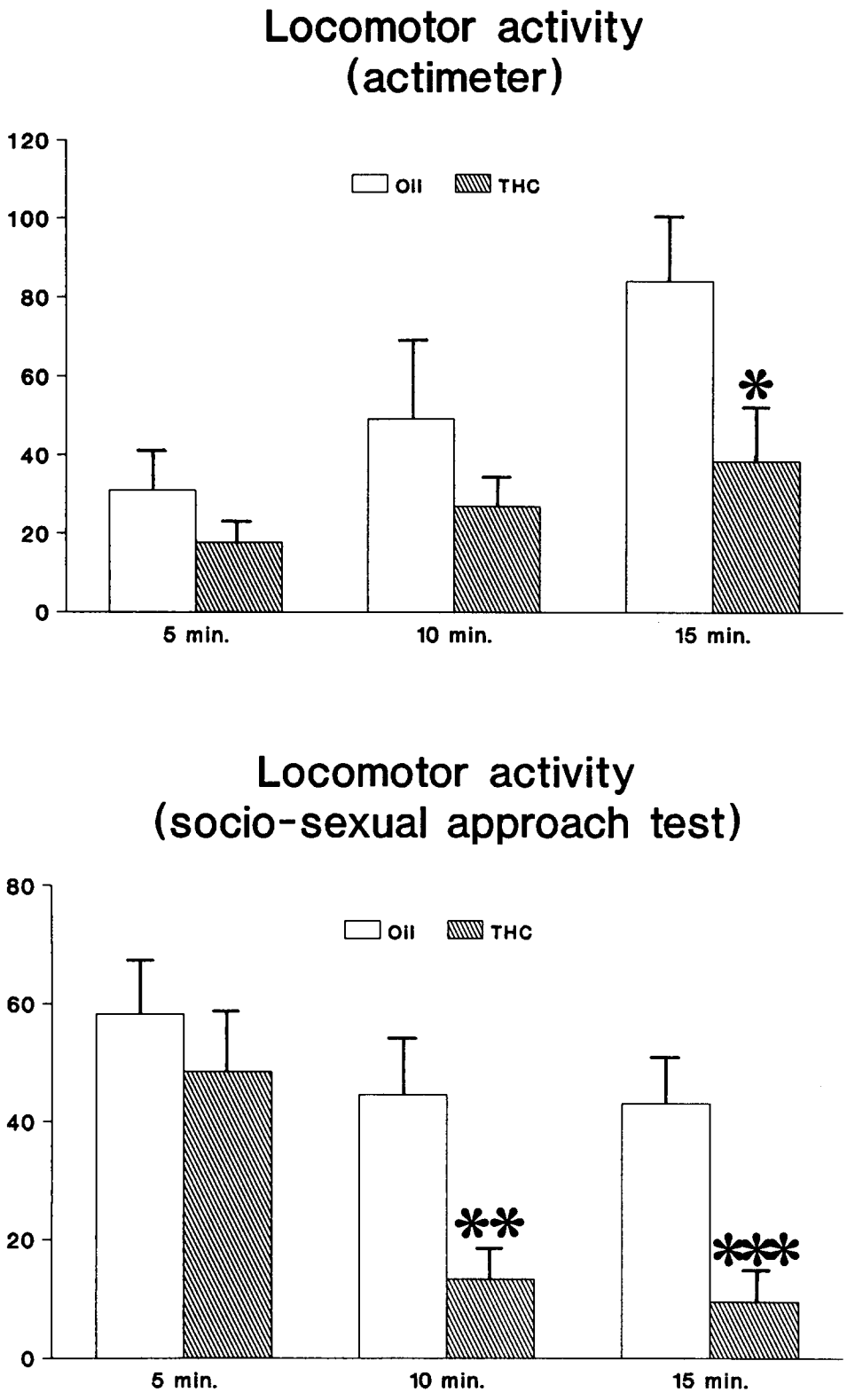

FIG. 1. Spontaneous locomotor activity, measured by actimeter (upper panel) and sociosexual approach behavior test (lower panel), in adult males acutely treated with Δ^9-tetrahydrocannabinol (THC) or vehicle (oil). In both cases, values are expressed as scores at 5, 10, and 15 min after the beginning of the test. Details in the text. Values are means ± SEM of eight determinations per group. Statistical differences were obtained by one-way analysis of variance (*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$).

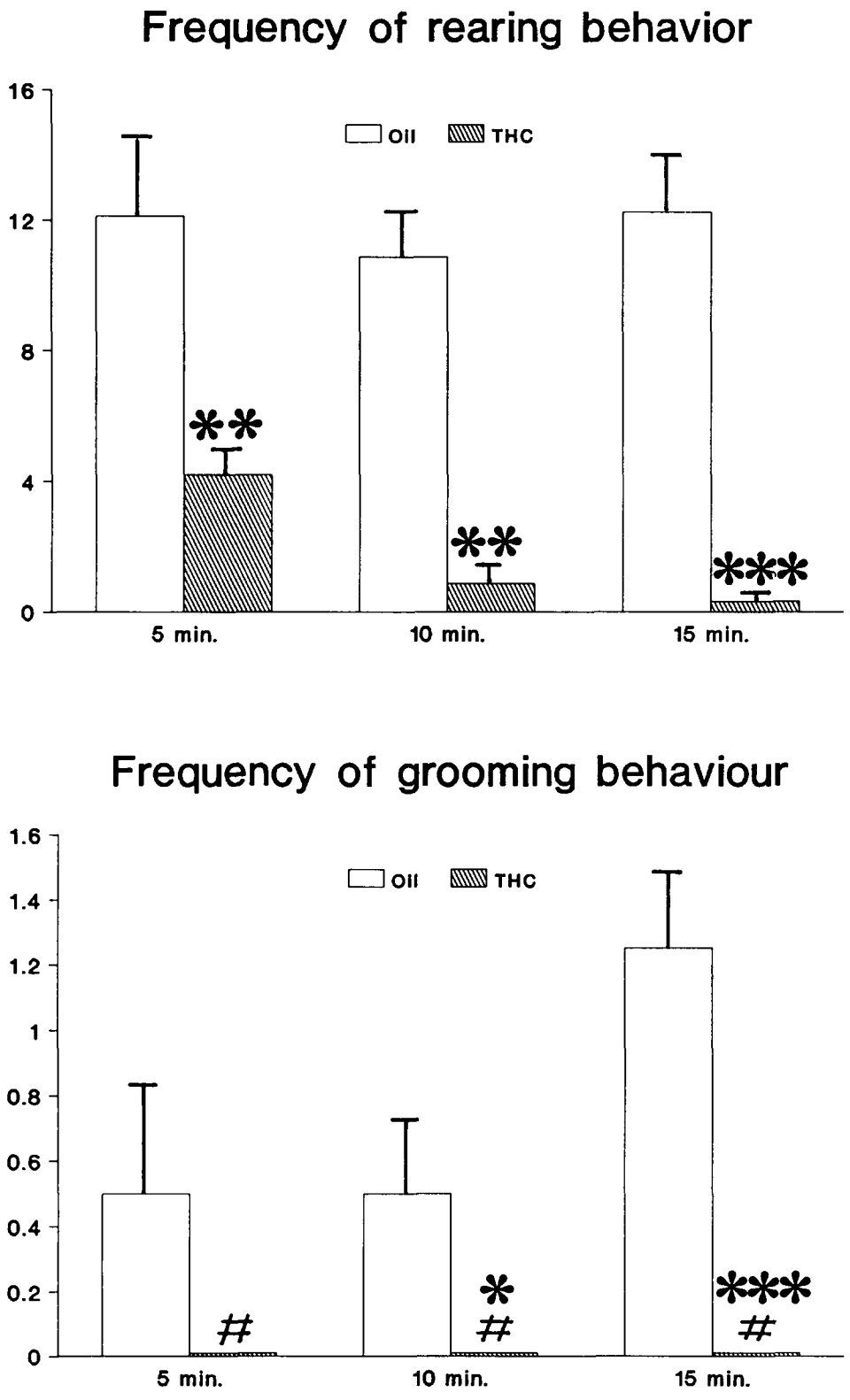

FIG. 2. Stereotypic behavior: Rearing (upper panel) and self-grooming (lower panel), both measured in a sociosexual approach behavior test in adult males acutely treated with Δ^9-tetrahydrocannabinol (THC) or vehicle (oil). In both cases, measurements were made at 5, 10, and 15 min after the beginning of the test. Details in the text. Values are means ± SEM of eight determinations per group. Statistical differences were obtained by one-way analysis of variance (*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$; #nonobservable).

b) stereotypic behavior—frequencies of rearing and self-grooming.

Brain Fragment Perifusion

In vitro basal and K^+-evoked DA release from small fragments (<1 mm each) obtained from striatal areas were analyzed using a perifusion system. This system was developed according to previously described procedures (17,24). Briefly, it consisted of two thermostatized twin chambers, each coupled to a four-way valve, a multichanneled peristaltic pump, and a fraction collector. The volume between the valve and the chamber was 1 ml. Appropriate corrections were made for this dead volume. The basal perifusion medium consisted of a Krebs-Ringer bicarbonate buffer (pH 7.4) with the following ionic composition: 118.5 mM NaCl, 4.7 mM KCl, 2.5 mM $CaCl_2$, 1.2 mM $MgCl_2$, and 24.9 mM $NaHCO_3$.

It was supplemented with 0.1% (w/v) bovine serum albumin, 10 mM glucose, 5.7 mM ascorbic acid, and 0.35 mM pargyline. When a 50 mM K^+ (high K^+) medium was used, NaCl concentration was lowered accordingly to maintain osmolarity. Media were equilibrated against a mixture of O_2/CO_2 (95%/5%) and perifused to a flow of 0.5 ml/min. After 45 min of perifusion with basal medium (restabilization period), where no samples were taken, effluent samples were collected every 3 min. They corresponded to 15 min of perifusion with basal medium, followed by 30 min of perifusion with high K^+ medium. Fractions were immediately placed on ice. At the end of the perifusion, tissue fragments were removed and weighed. Fractions and tissues were frozen at −40°C to measure DA release and remaining DA content, respectively.

Neurochemical Determinations

Determination of DA, 5-HT, and related metabolites. DA, DOPAC, 5-HT, and 5-HIAA contents in striatal tissues and DA concentrations in perifusion samples were analyzed using high-performance liquid chromatagrophy (HPLC) with electrochemical detection. Tissues were homogenized in 50–100 vol ice-cold 0.2 N perchloric acid with 0.5 mM sodium bisulfite and 0.45 mM EDTA, whereas perifusion samples were extracted using activated alumina previous to their preparation in the perchloric solution. Dihydroxybenzylamine and *N*-methyl-5-hydroxytryptamine were added as internal standards for the measurement of dopaminergic and serotoninergic metabolites, respectively. The homogenates and extracted perifusates were then centrifuged and the supernatants injected into the HPLC system. Details on the HPLC system have been previously published (8). Values are expressed as ng/mg of tissue and pg/mg of tissue/min for tissue contents and DA release, respectively.

Tyrosine hydroxylase determination. Tissues were weighed and homogenized in 5 vol 0.25 M sucrose and processed according to the method described by Nagatsu et al. (28). The evaluation of the amounts of L-dopa formed was carried out by HPLC according to our previously reported method (10). Values are expressed as ng/mg of tissue/h of incubation.

D_1 and D_2 dopamine binding site analysis. Measurements of D_1 and D_2 binding sites were performed according to the procedures described by Reader et al. (32) and Leysen et al. (19), respectively, with slight modifications. Radioactive ligands were [^{3}H]SCH23390 (60.4 Ci/mmol) for D_1 and [^{3}H]spiroperidol (27.5 Ci/mmol) for D_2, both purchased from New England Nuclear [(NEN), Boston, MA]. The concentration range was 0.125–3.0 nM and 0.05–0.80 nM, respectively. Protein concentration, measured by the Lowry method (20) in the incubated membrane fractions, was 0.2–0.3 mg/ml of incubation for D_1 and 0.15–0.20 mg/ml for D_2. Thirty micromolars (+)-SK&F38393 and 1 μM (+)-butaclamol, both purchased from Research Biochemicals Inc. [(RBI), Natick, MA], were respectively used for measurement of nonspecific binding. The final volume of incubation media was 0.5 ml. Details of the methods have been previously reported (9,34). 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 of protein.

Plasma THC Determination

Plasma THC concentrations were determined by using a specific radioimmunoassay (RIA) kit prepared at the Research Triangle Institute (Research Triangle Park, NC) and provided by the NIDA. Details of this method have been previously published (36). Plasma THC levels are expressed as ng/ml.

Statistics

For data analysis, when appropriate, Student's *t*-test or analysis of variance were used. Differences were considered significant if the probability of error was less than 5%.

RESULTS

An oral dose of THC (5 mg/kg body weight) led to the presence of significant amounts of this cannabinoid 1 h after administration in the plasma of these animals [THC-fed rats 20.17 ± 3.28 ng/ml vs. oil-fed rats nondetectable (<2.5 ng/ml)]. This administration produced a clear loss of spontaneous locomotor activity, measured in both actimeter (Fig. 1 upper panel) and open-field test (Fig. 1 lower panel), which was especially relevant at longer test times. Moreover, it produced a decrease in various spontaneous stereotypic behaviors (Fig.

TABLE 1

NEUROCHEMICAL INDICES OF DOPAMINERGIC AND SEROTONERGIC NEUROTRANSMISSION IN THE STRIATUM

Parameters	+Oil	+THC
DOPAC (ng/mg tissue)	0.88 ± 0.14	0.89 ± 0.09
DA (ng/mg tissue)	6.01 ± 0.74	5.99 ± 0.29
TH (ng/mg tissue/h)	69.46 ± 13.64	54.78 ± 8.42
5-HT (ng/mg tissue)	1.90 ± 0.18	2.01 ± 0.16
5-HIAA (ng/mg tissue)	1.20 ± 0.14	1.50 ± 0.11
D_1 binding sites:		
B_{max} (fmol/mg protein)	1362.85 ± 216.77	923.39 ± 100.43*
K_d (nM)	1.28 ± 0.21	1.03 ± 0.08
D_2 binding sites:		
B_{max} (fmol/mg protein)	727.31 ± 75.42	640.72 ± 56.46
K_d (nM)	0.19 ± 0.03	0.16 ± 0.02

Dopamine (DA), L-3,4-dihydroxyphenylacetic acid (DOPAC), 5-hydroxytryptamine (5-HT) and 5-hydroxyindolacetic acid (5-HIAA) contents, tyrosine hydroxylase (TH) activity, and D_1 and D_2 binding site number (B_{max}) and affinity (K_d) in the striatum of adult males acutely treated with Δ^9-tetrahydrocannabinol (THC) or vehicle (oil). Details in the text. Values are means + SEM of eight determinations per group. Statistical differences were obtained by Student's *t*-test (*$p < 0.05$).

2). Thus, self-grooming behavior was completely abolished in THC-fed animals during the whole test period (Fig. 2 lower panel), whereas the number of rears, which can be used as an index of both exploratory behavior and motor activity, was dramatically decreased (Fig. 2 upper panel), especially during the last two thirds of the test.

These motor deficiencies that followed acute THC exposure were correlated to a small, although significant, decrease in the number of D_1-dopaminergic receptors in the striatum (Table 1), although neither DA and DOPAC contents nor TH activity and D_2 receptors were altered (Table 1). In addition, the pattern of K^+-evoked DA release in vitro from perifused striatal fragments was always lower in animals fed with THC (Fig. 3), although the differences were nonsignificant, whereas basal release and postperifusion tissue contents were not altered. No differences were observed in striatal contents of 5-HT and 5-HIAA (Table 1).

DISCUSSION

Acute administration of THC caused a marked alteration in motor activity. Spontaneous locomotor activity, measured as number of crossings in actimeter and in open-field test, was clearly decreased after THC administration. Moreover, some stereotypic behaviors, which are partially indicative of a motor expression, such as rearing and self-grooming, also decreased significantly after THC, in particular self-grooming behavior, which was completely abolished in THC-fed animals.

The observation of decreased motor activity after THC is concordant with previous reports [for review see (7,11)] showing decreased spontaneous activity (14) and catalepsy (31) in mice and potentiation of reserpine-induced hypokinesia in rats (26) after THC. Similar results have been shown after perinatal cannabinoid exposure (11). However, it contrasts with the results of other authors, who found an enhanced motor expression after cannabinoid exposure. For instance, Sakurai and coworkers (38) have shown that THC is able to increase aggression behavior, which was interpreted by these authors as a consequence of a THC-induced facilitation of striatal dopaminergic transmission (37). Both physiological and methodological reasons can be argued to explain these discrepancies: First, aggression behavior has an important limbic component, which precludes an exclusive interpretation as a motor behavior; second, most of these studies were performed during the light phase of the photoperiod, which represents the period of lowest motor activity in the rat, whereas our studies were done during the dark phase, corresponding to the maximum of behavioral expression. In this respect, Wirz-Justice (41) and Le Moal and Simon (18) recently reviewed the published literature about the existence of circadian variations in brain dopaminergic neurotransmission—receptor density and

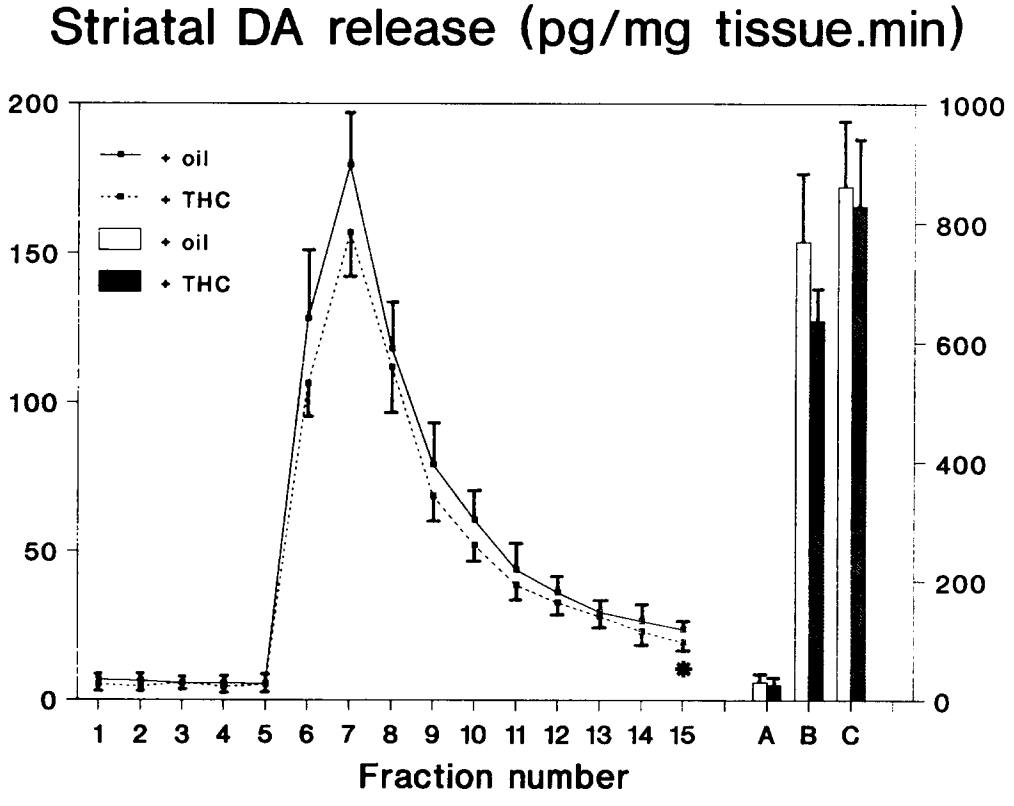

FIG. 3. Basal (fractions 1-5) and K^+-evoked (fractions 6-15) dopamine (DA) release from perifused striatal fragments of adult males acutely treated with Δ^9-tetrahydrocannabinol (THC) or vehicle (oil). Details in the text. Each value represents the release, expressed as pg/mg of tissue/min, corresponding to a period of 3 min. Right: (A) accumulated basal release in both groups; (B) accumulated K^+-evoked release; (C) postperifusion tissue content. Values are means ± SEM of eight determinations per group. Statistical differences at each time interval were obtained by Student's *t*-test $^*p < 0.05$).

DA utilization. These latter authors suggested that this has a relevant importance for the understanding of the dopaminergic transmission reactivity at the pharmacological and behavioral levels. Additional studies will be necessary to clarify this issue.

Despite these marked motor disturbances, the affectation of nigrostriatal dopaminergic activity, which plays a facilitatory role in the control of motor behavior (4), was small. Concretely, we found a slight, although significant, decrease in the number of D_1 receptors and a nonsignificant decrease in the K^+-evoked DA release in vitro from striatal fragments. The meaning of these neurochemical modifications was concordant with our behavioral observations and also with previous studies (11,36). For instance, it has been demonstrated that activation of D_1 receptors has a pronounced and specific effect in increasing grooming behavior (2,25). However, the absence of marked changes in other parameters, such as DA and DOPAC contents, TH activity, and basal DA release in vitro, concordant with the results of other authors (30) using in vivo microdialysis, and the small magnitude of the receptor changes also suggests the possible affectation of other neurotransmitters. In this regard, we also examined striatal seroninergic neurotransmission, which plays a minor role in the control of motor activity, but failed to find statistically significant effects in 5-HT and 5-HIAA contents after THC exposure. Alternatively, the possibility that only small modifications at the neurochemical level might produce marked alterations at the behavioral level could also be argued.

In summary, these results allow us to conclude that acute THC produces a marked motor deficiency, which seems to be originated, at least in part, as a consequence of THC-induced changes in nigrostriatal dopaminergic activity. However, the small magnitude of some of the dopaminergic changes following THC exposure suggests a possible involvement of other neurotransmitters.

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