Pharmacology Biochemistry & Behavior, Vol. 40, pp. 587–591. © Pergamon Press plc, 1991. Printed in the U.S.A. 0091-3057/91 $3.00 + .00

THC Does Not Affect Striatal Dopamine Release: Microdialysis in Freely Moving Rats

EDWARD CASTAÑEDA,[1] D. E. MOSS,*[2] SCOTT D. ODDIE AND IAN Q. WHISHAW

Departments of Psychology, University of Lethbridge, Lethbridge, Alberta, Canada, T1K 3M4
**Laboratory of Psychobiochemistry, Department of Psychology, University of Texas at El Paso, El Paso, TX 79968*

CASTAÑEDA, E., D. E. MOSS, S. D. ODDIE AND I. Q. WHISHAW. *THC does not affect striatal dopamine release: Microdialysis in freely moving rats.* PHARMACOL BIOCHEM BEHAV **40**(3) 587–591, 1991.—The hypothesis that cannabinoids potentiate the motor effects of neuroleptics and produce their abuse potential by stimulating dopaminergic activity was tested by measuring the ability of THC to increase extracellular dopamine concentrations. Male Long-Evans rats were implanted with guide cannulae for the striatum or nucleus accumbens. Fifteen hours prior to testing, removable microdialysis probes were inserted through the guide cannulae. Dialysis samples were collected during resting baseline, after 1.0 mg/kg, 10 mg/kg THC, or vehicle of olive oil with 5% ETOH (by gavage) followed by amphetamine (1.5 mg/kg) or fluphenazine (0.3 mg/kg). THC produced no change in the extracellular concentrations of DA, DOPAC, and HVA, nor in 5-HIAA. THC also had no effect on the enhancement of extracellular DA produced by amphetamine nor on the transient increase in DA, DOPAC, and HVA produced by fluphenazine. There were also no behavioral differences between groups during any of these treatments.

Microdialysis THC Dopamine release Homovanillic acid 5-Hydroxyindoleacetic acid Striatum

A high affinity, stereoselective "cannabinoid" receptor (4) coupled to adenylate cyclase (7) has been identified and localized within the CNS (6). The high concentration of this receptor within the globus pallidus and substantia nigra (6) suggests that it may interact with dopamine (DA) and other neurotransmitters within the extrapyramidal system.

It has been reported that in vivo microdialysis shows that delta-9-tetrahydrocannabinol (THC) increases dopamine release from the corpus striatum up to 12 fold (14,15), and augments basal dopamine efflux from the nucleus accumbens (3). Such interactions between cannabinoids and dopamine suggest that dopamine may mediate some of the actions of THC (15). The actions of THC on dopaminergic activity may explain how cannabinoids potentiate the motor effects of neuroleptics (8–10) and why cannabinoids have abuse potential which may involve reward-relevant dopamine circuits in the brain (5).

In order to further evaluate the significance of the effect of THC on extracellular dopamine in the neostriatum or nucleus accumbens, particularly with regard to extrapyramidal motor functions, we studied THC-induced changes in extracellular concentrations of dopamine (DA), 3,4-dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA), and 5-hydroxyindoleacetic acid (5-HIAA). The experiments used in vivo microdialysis in freely moving rats and included the effect of THC on the augmentation of DA release which is produced by stimulants such as amphetamine and DA antagonists such as fluphenazine.

METHOD

Subjects

Subjects were 22 adult male Long-Evans rats weighing between 300–400 g. They were housed singly in wire hanging cages on a 14:10 h light:dark cycle (lights on at 0800) with free access to food and water.

Apparatus

The rats were tested in a clear Plexiglas box 31 cm by 31 cm by 35 cm high. During the experiments, behavior was filmed using a Sony camera and Betamax recorder for subsequent behavioral quantification at 2 times normal playback speed (see below for behavioral measures).

Dialysis Probes

Microdialysis probes (2, 11, 12) were modified to be removable for multiple insertions (1,11). The dialysis probes were of a concentric design and had an outside diameter of 250 μm. Probes for the striatum had a 4.0 mm dialysis fiber length and probes for the nucleus accumbens had a 2.0 mm dialysis fiber length. The probes were tested for recovery in vitro at 37°C prior to implantation, and dialysate values were later corrected for probe recovery (2,13).

Cannula Implants

Two weeks prior to experimentation, standard stereotaxic procedures were used to implant a unilateral 21 gauge cannula aimed to lie just above the corpus of the striatum, or just above the nucleus accumbens on the contralateral side, for later insertion of dialysis probes. Coordinates from bregma and the skull surface were: striatum, anterior 0.5 mm, lateral 3.0 mm, ventral

[1]Current address: Department of Psychology, Arizona State University, Tempe, AZ 85287.
[2]Requests for reprints should be addressed to Dr. D. E. Moss.

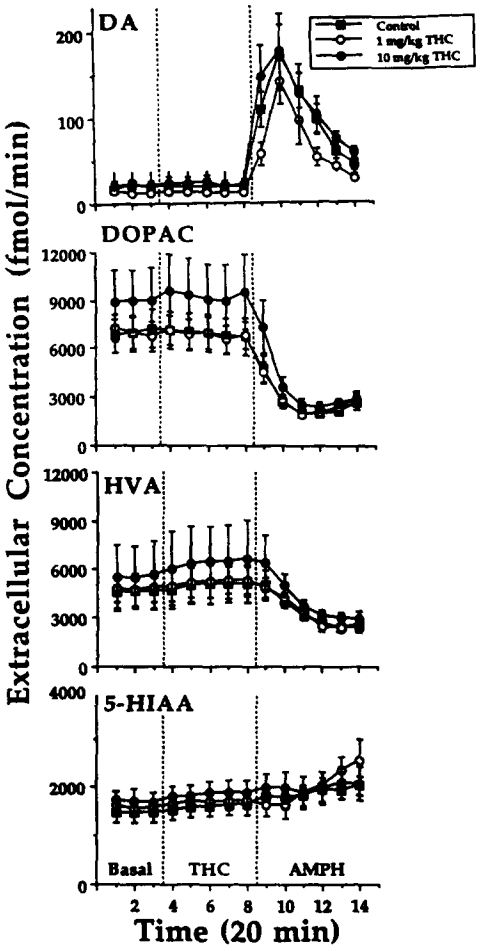

FIG. 1. Time course of striatal concentrations (mean ± S.E.M.) of extracellular dopamine (DA), 3,4-dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA), and 5-hydroxyindoleacetic acid (5-HIAA). During rest (Basal, 60 min) the rats received no treatment. In the THC condition groups received vehicle, 1.0 mg/kg, or 10 mg/kg THC (THC, 100 min). In the amphetamine condition all groups received 1.5 mg/kg amphetamine administration (AMPH, 120 min). Note that there were no differences in basal and THC conditions between the three groups, but that after amphetamine, DA increased and DOPAC and HVA decreased, as is typical.

1.0 mm; nucleus accumbens, anterior 1.8 mm, lateral 1.3 mm, ventral 1.0 mm; with bregma and lambda horizontal. A stainless steel insect pin, cut to size, was inserted into the cannula as an obturator. Striatal probes penetrated 7.0 mm and the nucleus accumbens probes penetrated 8.5 mm ventral from the skull surface.

Drugs

(−)-Trans-delta-9-tetrahydrocannabinol (THC; provided by NIDA) was prepared for gavage administration from vials containing 50 mg in 0.25 ml dehydrated ethanol. This stock was prepared for gavage administration by diluting it 1:20 in olive oil with 5% ethanol under nitrogen so that the final concentration was 10 mg THC/ml (10). When required, this preparation of THC was further diluted in vehicle to reach a concentration of 1.0 mg THC/ml.

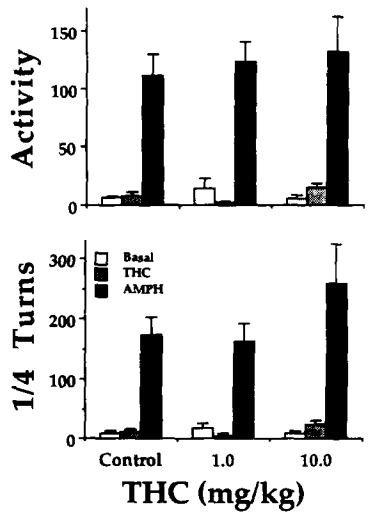

FIG. 2. Mean (± S.E.M.) locomotor activity (top), calculated as the sum of the total number of crossovers from one side of the test chamber to the other plus the total number of times the animal reared with both its front paws in the air; and the average (± S.E.M.) number of ¼ turns (bottom) exhibited during microdialysis testing in groups that received vehicle injection (control), 1 mg/kg or 10 mg/kg THC. Note that all groups were markedly more active after amphetamine (1.5 mg/kg) than they were in basal or THC only conditions.

D-Amphetamine SO_4 (1.5 mg/kg, Sigma Chemical, St. Louis, MO) was administered subcutaneously in a concentration of 3.0 mg/ml (salt) in 0.9% physiological saline. Fluphenazine 2 HCl (0.3 mg/kg, Squibb & Sons, Inc., Princeton, NJ) was injected intraperitoneally in a concentration of 0.3 mg/ml (salt).

The monoamines, used as standards for HPLC, dopamine HCl (DA), 3,4-dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA), and the serotonin metabolite 5-hydroxy-indoleacetic acid (5-HIAA) were obtained from Sigma Chemical Co. (St. Louis, MO). Standards were made from 1 mg/ml 0.05 N $HClO_4$ stock solutions (free base) in the Ringers solution described below.

Dialysis Recovery and Assay

Fifteen h prior to testing, the animals were anesthetized lightly with ether, and the dialysis probes were inserted. Testing began the next day between 0800 and 1000 h. A Ringers buffer medium (128.3 mM NaCl, 268 mM KCl, 1.35 mM $CaCl_2$, 2.0 mM $MgCl_2$, pH 7.3) was perfused through the probes at a rate of 1.5 μl/min, and dialysis samples were collected in 20-min fractions. Dialysate was analyzed within 30 min of collection for DA, DOPAC, HVA, and 5-HIAA by high performance liquid chromatography and series oxidative-reductive electrochemical detection (2,12). The amount of each compound was determined by comparison with the peak height of standards run with each assay (4 pg/μl of DA and 200 pg/μl of DOPAC, HVA and 5-HIAA).

Behavioral Analysis

During microdialysis testing, the rats were videotaped. The tapes were analyzed for: 1) *activity*; the sum of the number of crossovers from one half of the test box to the other half plus the number of times the animal reared with its front paws in the

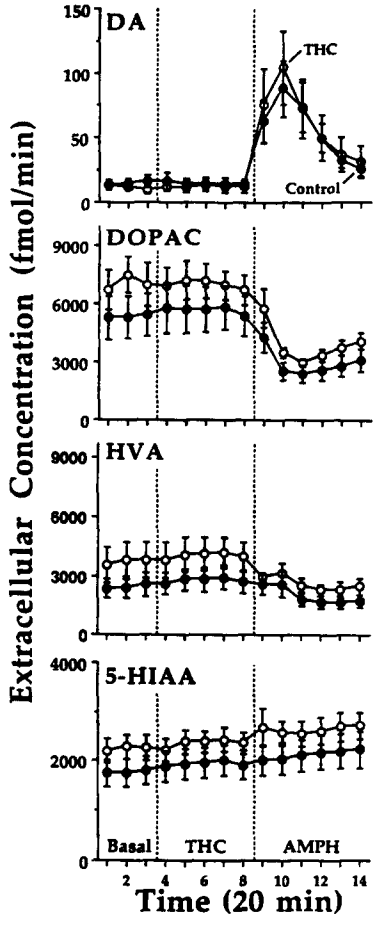

FIG. 3. Time course of nucleus accumbens concentrations (mean ± S.E.M.) of extracellular DA, DOPAC, HVA, and 5-HIAA during rest (Basal, 60 min), after vehicle or 1.0 mg/kg THC (THC, 100 min) and following an additional treatment of 1.5 mg/kg amphetamine (AMPH, 120 min). Note that there were no differences in basal and THC conditions, but that after amphetamine, DA increased and DOPAC and HVA decreased, as is typical.

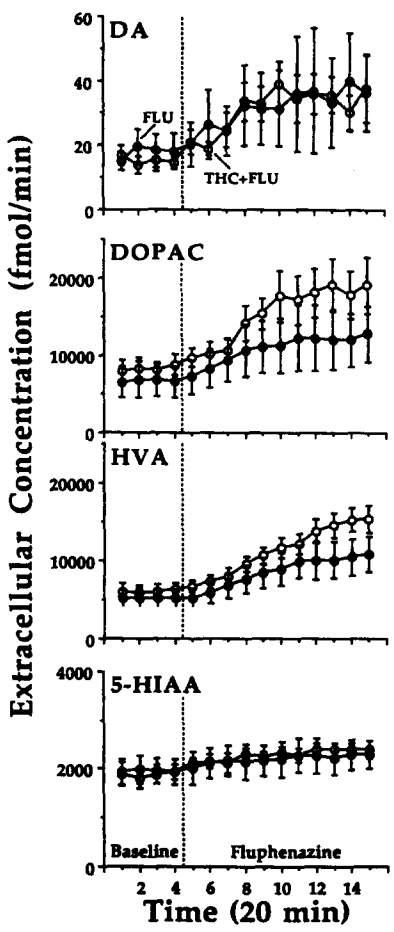

FIG. 4. Time course of striatal concentrations (mean ± S.E.M.) of extracellular DA, DOPAC, HVA and 5-HIAA during rest (Baseline, 80 min) and after either 10.0 mg/kg THC followed by fluphenazine (THC + FLU) or vehicle plus fluphenazine (FLU). Note that although concentrations of DA, DOPAC and HVA increased after fluphenazine, the changes were equivalent in the vehicle and THC-treated groups.

air, and 2) *turns*; the number of 90° turns made. These behaviors were quantified for a 20-min interval of baseline testing, for the last 60 min of the THC condition and for the entire 120 min following amphetamine administration.

Statistical Analysis

Analysis of variance was used to compare drug dosage (between subjects), samples, and treatments (within subjects). Significant differences ($p<0.05$) were subjected to Scheffe's follow-up tests (16).

Procedure

THC and amphetamine. Animals were randomly assigned to THC and vehicle treatment groups, which were counterbalanced such that at least one week after animals had received one treatment, they received the other treatment. Rats with striatal probes received 1.0 mg/kg THC (n = 6), 10 mg/kg THC (n = 6) or vehicle (n = 5). Rats with nucleus accumbens probes received either 1.0 mg/kg THC (n = 6) or vehicle (n = 6).

Baseline dialysis samples were collected for 60 to 80 min. Then the rats received either THC, or vehicle of olive oil with 5% ethanol, 1.0 ml/kg. Samples were collected for 100 min. The rats then received 1.5 mg/kg amphetamine and dialysis samples were collected for 120 min.

THC and fluphenazine. Animals with striatal probes were randomly assigned to a 10 mg/kg THC group (n = 6) or a vehicle-treated (n = 5) group. Samples were collected during baseline (80 min), and following 10 mg/kg THC or 1.0 ml/kg vehicle combined with 0.3 mg/kg fluphenazine 2 HCl. Dialysis samples were collected for 240 min.

RESULTS

Histology

Histological examination showed that striatal and nucleus accumbens probes were placed in the intended rostrocaudal and dorsoventral locations (2,12).

THC and Amphetamine

Figure 1 illustrates the average extracellular concentrations of striatal DA, DOPAC, HVA and 5-HIAA, as estimated by the

levels of these compounds in dialysate. There were no significant differences between the control, 1 mg/kg, and 10 mg/kg THC groups. However, comparisons between basal, THC and amphetamine conditions showed significant differences in concentrations of DA, DOPAC, HVA, and 5-HIAA, $F(2,38) = 68.86$, 59.42, 63.56, and 41.86, respectively, $ps < 0.001$. Follow-up Scheffe's tests indicated that there were no significant differences between the basal and THC conditions, but in the amphetamine condition, DA was significantly elevated and DOPAC and HVA were significantly depressed.

Locomotor activity and turning behavior are illustrated in Fig. 2. There were no differences between the control, 1 mg/kg and 10 mg/kg THC groups. However, there was an effect of drug treatment for both behaviors, $F's(2,38) > 50.00$, $p < 0.001$. Follow-up Scheffe's tests indicated that there were no significant differences between the basal and THC conditions, but locomotor activity and turning were significantly elevated in the amphetamine condition.

Similar results were obtained with the extracellular concentrations of compounds in the dialysate obtained from the nucleus accumbens (Fig. 3). There were no differences between the control and THC groups. However, comparison of DA, DOPAC, HVA, and 5-HIAA concentrations between basal, THC and amphetamine conditions did give significant effects, $F(2,18) = 22.83$, 28.73, 8.54, and 17.41, respectively, $ps < 0.003$. Follow-up Scheffe's tests also indicated that although the basal and THC conditions did not differ, they were significantly less than the amphetamine condition.

THC and Fluphenazine

Figure 4 shows a comparison of the effects of fluphenazine only and fluphenazine combined with 10 mg/kg of THC. There were no differences in concentrations of DA, DOPAC, HVA or 5-HIAA between the group that received 10 mg/kg THC and the group that received THC combined with fluphenazine. There were, however, differences between the concentrations obtained during baseline compared with the concentrations obtained after fluphenazine treatment, $F(1,9) = 6.72$, 14.76, 30.94, and 16.49 for DA, DOPAC, HVA, and 5-HIAA, respectively, $ps < 0.03$. There were significant increases in DA, DOPAC and HVA after fluphenazine treatment.

DISCUSSION

The microdialysis procedures used in these experiments allowed for removable probes to be inserted into guide cannulae several hours prior to the experiment without confounding effects of anesthesia except for the brief exposure to ether required to prevent damage to the delicate dialysis probes. Furthermore, rates of recovery could be confirmed immediately before and after each experiment. The ability to conduct microdialysis in freely moving animals allows the correlation between changes in neurotransmitters, metabolites, and ongoing behaviors within a time scale of twenty minutes. Such measurements clearly showed the effect of amphetamine on dialyzed compounds and behaviors.

In view of other reports that THC increases the endogenous release of dopamine (3, 5, 14, 15), it was a surprise to find no effect of THC in these experiments. The failure to find such an effect does not appear to be due to insensitivity of the technique, insofar as the expected changes in response to amphetamine and fluphenazine were robust.

There are, however, several other possible causes for a failure to observe a THC effect on dopamine release or metabolism. For example, Taylor et al. (14,15) conducted microdialysis while the animals were under the direct effect of halothane anesthesia. Insofar as THC has mild analgesic effects, it may be that Taylor et al. (14,15) have discovered an interaction between THC and an anesthetic that may be useful in elucidating this effect of THC. In addition, however, our experiments differ from others in routes of administration (gavage versus IV) and strains of rats (Long-Evans versus Sprague-Dawley and Lewis) used. The importance of strain differences is clearly shown in the work of Gardner and colleagues wherein a small increase in dopamine lasting only a few minutes is only observed in Lewis rats (3,5).

Discovery of a cannabinoid receptor suggests that some endogenous cannabinoid(s) may account for the CNS effects of these compounds. Although receptors are present in high concentrations in the extrapyramidal system, our findings show that THC-stimulated dopamine release is not a general phenomenon. Potentiation of motor effects of neuroleptics, and the abuse potential of cannabinoids in general, may involve nondopaminergic mechanisms.

REFERENCES

1. Camp, D. M.; Robinson, T. E. A method for repeated intracerebral microdialysis. Soc. Neurosci. Abstr. 15:559; 1989.
2. Castañeda, E.; Whishaw, I. Q.; Lermer, L.; Robinson, T. E. Dopamine depletion in neonatal rats: Effects on behavior and striatal dopamine release assessed by intracerebral microdialysis during adulthood. Brain Res. 508:30–39; 1990.
3. Chen, J.; Paredes, W.; Li, J.; Smith, D.; Gardner, E. L. In vivo brain microdialysis studies of delta-9-tetrahydrocannabinol on presynaptic dopamine efflux in nucleus accumbens of the Lewis rat. Soc. Neurosci. Abstr. 15:1096; 1989.
4. Devane, W. A.; Dysarz, F. A.; Johnson, M. R.; Melvin, L. S.; Howlett, L. S. Determination and characterization of a cannabinoid receptor in rat brain. Mol. Pharmacol. 34:605–613; 1988.
5. Gardner, E. L. Marijuana's interaction with brain reward systems: Update 1991. Pharmacol. Biochem. Behav. 40:571–580; 1991.
6. Herkenham M.; Lynn, A. B.; Little, M. D.; Johnson, M. R.; Melvin, L. S.; de Costa, B. R.; Rice, K. C. Cannabinoid receptor localization in the brain. Proc. Natl. Acad. Sci. USA 87:1932–1936; 1990.
7. Howlett, A. C.; Champion, T. M.; Wilken, G. H.; Mechoulam, R. Stereochemical effects of 11-OH-delta-8-tetrahydrocannabinol dimethylheptyl to inhibit adenylate cyclase and bind to the cannabinoid receptor. Neuropharmacology 29:161–165; 1990.
8. Moss, D. E.; Manderscheid, P. Z.; Montgomery, S. P.; Norman, A. B.; Sanberg, P. R. Nicotine and cannabinoids as adjuncts to neuroleptics in the treatment of Tourette syndrome and other motor disorders. Life Sci. 44:1521–1525; 1989.
9. Moss, D. E.; Manderscheid, P. Z.; Kobayashi, H.; Montgomery, S. P. Evidence for the nicotinic cholinergic hypothesis of cannabinoid action within the central nervous system: Extrapyramidal motor behaviors. In: Chesher, G.; Consroe, P.; Musty, R., eds. Marijuana: An international research report. Canberra: Australian Government Publishing Service; 1988:359–364.
10. Moss, D. E.; McMaster, S. B.; Rogers, J. Tetrahydrocannabinol potentiates reserpine-induced hypokinesia. Pharmacol. Biochem. Behav. 15:779–783; 1981.
11. Robinson, T. E., Camp, D. M. The feasibility of repeated microdialysis for within subjects design experiments: Studies on the mesostriatal dopamine system. In: Robinson, T. E.; Justice, J. B., Jr. eds. Microdialysis in the neurosciences. Amsterdam: Elsevier; 1991:in press.
12. Robinson, T. E.; Jurson, P. A.; Bennett, J. A.; Bentgen, K. M. Persistent sensitization of dopamine neurotransmission in ventral striatum (nucleus accumbens) produced by past experience with (+)-amphetamine: A dialysis study in freely moving rat. Brain Res. 462:211–222; 1988.

13. Robinson, T. E.; Whishaw, I. Q. Normalization of extracellular dopamine in striatum following recovery from a partial unilateral 6-OHDA lesion of the substantia nigra: A microdialysis study in freely moving rat. Brain Res. 450:209–224; 1988.
14. Taylor, D. A.; Sitaram, B. R.; Elliot-Baker, S. Effect of delta-9-tetrahydrocannabinol on release of dopamine in the corpus striatum of the rat. In: Chesher, G.; Consroe, P.; Musty, R., eds. Marijuana: An international research report. Canberra: Australian Government Publishing Service; 1988:405–408.
15. Taylor, D. A.; Sitaram, B.; Nyoni, E. Effect of repeated administration of delta-9-tetrahydrocannabinol on dopaminergic transmission and 5-HIAA levels in the corpus striatum of rats. Unpublished lecture presented at Marijuana '90 conference.
16. Winer, B. J. Statistical principles in experimental design. New York: McGraw-Hill; 1971.