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Δ^9-Tetrahydrocannabinol Facilitates Striatal Dopaminergic Transmission

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SAKURAI-YAMASHITA, Y., Y. KATAOKA, M. FUJIWARA, K. MINE AND S. UEKI. *Δ^9-Tetrahydrocannabinol facilitates striatal dopaminergic transmission.* PHARMACOL BIOCHEM BEHAV 33(2) 397–400, 1989.—We examined the effects of Δ^9-tetrahydrocannabinol (THC) on striatal dopaminergic neurons in rats. THC inhibited the uptake of ^{3}H-dopamine (DA) into striatal synaptosomes. THC facilitated the release of endogenous DA but not dihydroxyphenylacetic acid (DOPAC) from striatal slices. The concentration of DA in the dorsolateral striatum was reduced by THC. We propose that THC may stimulate nigrostriatal dopaminergic neurotransmission mainly by inhibiting uptake of DA and by facilitating release of DA.

Δ^9-Tetrahydrocannabinol ^{3}H-Dopamine Striatum Striatal dopamine release

Δ^9-TETRAHYDROCANNABINOL (THC) in a relatively narrow dose range can produce various types of bizarre behavior such as catalepsy, walking backwards, pivoting, irritable aggression and muricide depending on species, strains and experimental conditions (3–5, 8, 24, 25).

The remarkable hyperirritability observed in Wistar King A (WKA) rats given THC 5–6 mg/kg IP was significantly enhanced by the pretreatment with 6-hydroxydopamine ICV or the coadministration of methamphetamine (MAP). This would suggest that the catecholaminergic (CA-ergic) transmission may be facilitated in eliciting the aggression induced by THC 5–6 mg/kg (5, 10, 16, 17). Our previous study in which THC 5 mg/kg elicited ipsilateral circling in rats with unilateral nigral lesion suggests that THC 5 mg/kg may activate nigrostriatal dopaminergic (DA-ergic) neurotransmission at the presynaptic site (18).

The present study was designed to obtain support for the view that THC produced hyperirritability in WKA rats by enhancing CA-ergic (particularly DA-ergic) transmission at the presynaptic site. We examined the effects of THC on the release of endogenous DA, the uptake of ^{3}H-DA and the concentration of DA in dorsolateral and ventromedial striatum after injecting THC 5 mg/kg IP into WKA rats.

METHOD

Animals

WKA female rats weighing 180–200 g (8 weeks old) were obtained from Kyushu University Institute of Laboratory Animals. These rats were housed in groups of 5 per plastic cage (30 × 35 × 17 cm) and were maintained on a 12-hr light-dark schedule at 22 ± 2°C. Food and water were provided ad lib.

Drugs

THC was isolated from cannabis extract by Prof. I. Nishioka and Dr. Y. Shoyama, Department of Pharmacognosy, Faculty of Pharmaceutical Sciences, Kyushu University, as described (19). THC was emulsified in 1% tween 80, at a concentration of THC 10 mg/ml. For the release and uptake experiments, THC was suspended in Ringer solution. The maximal concentration of THC used here, 10^{-4} M, contained 0.003% tween 80. The concentration of tween 80 used as the vehicle was equivalent to that contained in each dose of THC suspension.

Nomifensine tartrate (Hoechst) and methamphetamine hydrochloride (Dainippon Pharmaceutical Company) were dissolved in Ringer solution. ^{3}H-DA (28.0 Ci/mmol) was from New England Nuclear.

^{3}H-DA Uptake

The striatum was rapidly excised from the decapitated rats and was homogenized in 10 vol. of ice-cold 0.32 M sucrose followed by centrifugation at 1,000 × g for 10 min at 4°C. The supernatant

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was recentrifuged at 10,000 × g for 20 min and the resulting pellet (crude P_2 fraction) was suspended in Krebs Ringer bicarbonate solution at pH 7.4, of the following composition: NaCl, 118.0; KCl, 4.7; $CaCl_2$, 1.3; $MgCl_2$, 1.2; NaH_2PO_4, 1.0; $NaHCO_3$, 25.0; glucose, 11.0; disodium EDTA, 0.04 and ascorbic acid, 0.11 mM.

Synaptosomes (0.3–0.4 mg protein) were preincubated for 5 min at 37°C in 900 μl of oxygenated (95% O_2 and 5% CO_2) Ringer containing THC, tween 80 or nomifensine (NMF). One hundred μl of ^{3}H-DA (final concentration of 1×10^{-7} M) were added to the synaptosomes and the mixture (1 ml) was incubated for 5 min at 37°C. The reaction was terminated by adding 2 ml of ice-cold Ringer. The mixture was passed through GF/B filters under vacuum. Each filter was transferred to a scintillation vial containing 10 ml ACS-II (Amersham) and the radioactivity was measured using a standard liquid scintillation counting procedure. Nonspecific uptake was determined in the same manner as described above, except that the reaction was carried out on ice.

Five experiments were done in triplicate. Results were expressed as a percentage of the inhibition of ^{3}H-DA uptake obtained in the presence of each vehicle.

DA Release

Rat striatal slices (0.5 mm in thickness, about 10 mg in wet weight) were superfused with oxygenated (95% O_2 and 5% CO_2) Krebs Ringer, as described above, at a flow rate of 0.1 ml/min at 37°C. Twenty minutes after commencing the superfusion experiment, the superfusate was collected every 2.5 min into tube containing 10 μl of 0.1 N perchloric acid (PCA) with 0.01% disodium EDTA and 0.1% $Na_2S_2O_5$ on ice. Superfusion with drugs was initiated just after collection of the 8th fraction serving as the control (basal spontaneous release) for each slice and was maintained during the collection of the 9th and 10th fractions.

Release was expressed as a ratio of the mean value of DA or DOPAC released in the 9th and 10th fractions to the value of basal DA or DOPAC release in the 8th fraction. Results are shown as a percentage of DA or DOPAC release, expressed as a ratio in case of superfusion of each vehicle.

Measurement of DA and DOPAC Concentration

The concentration of DA and DOPAC in each superfusate fraction was measured by high performance liquid chromatography (HPLC) coupled with an electrochemical detector (ECD). The HPLC system (Waters Assoc.) was compressed of an ODS-reverse phased column (7 μm, 4.6×250 mm, Yanapack-ODS-A, Yanagimoto) coupled with a glassy carbon ECD (VMD 501, Yanagimoto) at the applied potential of 0.7 V versus the reference electrode. The mobile phase consisted of 1.5 mM sodium octyl sulfate, 11% methanol and 20 μM disodium EDTA and was adjusted to pH 2.9 with orthophosphoric acid.

Each superfusate of the release experiment was directly injected into HPLC. The amounts of DA and DOPAC were calculated by external standard quantitation.

An Exo Vivo Experiment

Thirty rats were divided into two groups. Fifteen rats were given THC 5 mg/kg IP and the others were given 0.5% tween 80 IP. All these rats were decapitated 60 min later. The excised brain was immediately frozen at −80°C and cut into slices 0.8 mm thick by microtome (Histostat). The dorsolateral and ventromedial parts were punched out (1 mm in diameter) from the striatal slice (anterior 7.5–8.3 mm from lambda according to König and Klippel) (9). The tissue was homogenized in 400 μl of 0.5 M PCA containing 2 ng of dihydroxybenzylamine as an internal standard. Thirty μl (10 μl × 3) was separated for the assay of protein by the method of Lowry (11). The homogenate was centrifuged at 14,500 × g and the catechols in the supernatant were separated by alumina extraction, as described (7). The condition of the measurement of DA and DOPAC concentration by HPLC was as described above, except that the amounts of DA and DOPAC were determined using internal standard quantitation.

TABLE 1

INHIBITORY EFFECTS OF THC AND NMF (NOMIFENSINE) ON ^{3}H-DA UPTAKE INTO RAT STRIATAL SYNAPTOSOMES

Drug (M)	Inhibition (%) THC	(N)	NMF	(N)
10^{-5}	58.11 ± 6.0	(6)	92.94 ± 3.1	(5)
5×10^{-6}	35.40 ± 6.2	(6)		
10^{-6}	0	(6)	81.12 ± 3.4	(5)
10^{-7}			37.61 ± 5.2	(5)
10^{-8}			4.79 ± 1.7	(5)

(N) represents the number of separate experiments done in triplicate. Values were expressed as the inhibition (%) of [^{3}H]-DA uptake obtained in the presence of each vehicle (mean ± S.E.M.).

Statistics

The statistical analysis of the data from uptake and release experiments was performed by a one-factor ANOVA followed by the Duncan test to make individual comparisons. Data from exo vivo experiment were evaluated using Student's *t*-test.

RESULTS

Effects of THC on the uptake of ^{3}H-DA into rat striatal synaptosomes are shown in Table 1. THC at a concentration of 5×10^{-6} and 10^{-5} M inhibited the uptake of ^{3}H-DA by 35 and 60%, respectively. THC induced no change of the ^{3}H-DA uptake at doses less than 10^{-6} M. THC 10^{-4} M inhibited the uptake of ^{3}H-DA by about 90% when compared with 0.003% of tween 80.

The vehicle, that is, 0.003% of tween 80 at the concentration equivalent to 10^{-4} M THC inhibited by about 15% ^{3}H-DA uptake, when compared with findings obtained when Ringer was used as the vehicle. Tween 80 at a concentration less than 0.003% had no influence on the uptake of ^{3}H-DA. A potent DA uptake inhibitor, NMF 10^{-5} M, almost completely inhibited ^{3}H-DA uptake.

Figure 1 shows the effect of THC on the release of endogenous DA and DOPAC from striatal slices. THC 10^{-5} M significantly facilitated the release of DA in comparison with findings with tween 80. At a dose of 10^{-4} M, THC did not significantly stimulate DA release and at doses less than 10^{-6} M, THC did not alter the release of DA. MAP 10^{-6} M enhanced to a great extent the release of DA. Neither THC nor MAP altered the release of DOPAC.

In an exo vivo experiment, the concentration of DA in the dorsolateral striatum was reduced by THC 5 mg/kg IP [$t(24) = 1.73$, $0.05 < p < 0.01$, Fig. 2]. THC had no influence on the concentration of DA and DOPAC in the ventromedial striatum.

DISCUSSION

THC was found to inhibit the uptake of ^{3}H-DA into striatal

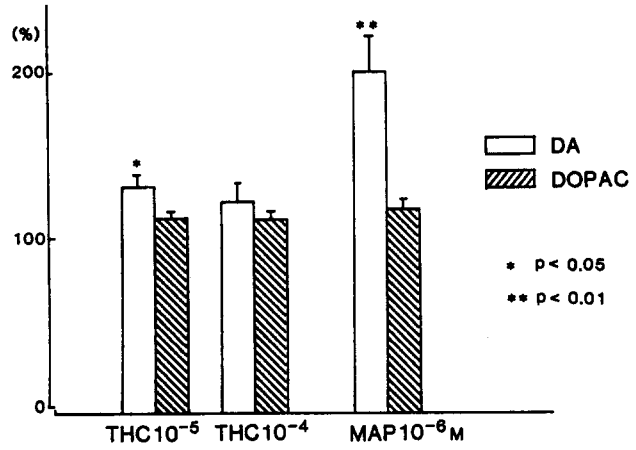

FIG. 1. Effects of THC and methamphetamine (MAP) on DA release from rat striatal slices. Data are mean ± S.E.M. of 5–8 separate experiments. The ordinate indicates a percentage of vehicle; DA or DOPAC released under the presence of drugs / DA or DOPAC released under that of each vehicle × 100% *p<0.05, **p<0.01 when compared with the vehicle.

synaptosomes and facilitate the release of DA from striatal slices. Together, the findings suggest that THC stimulates DA neurotransmission at the presynaptic site by inhibiting uptake of DA and facilitating its release. Such effects may contribute to the irritable aggression in WKA rats induced by THC (5, 17, 18). THC, NMF and MAP have been reported to induce ipsilateral circling in rats with a unilateral nigrostriatal lesion (18). The moderate ipsilateral circling evoked by THC appears to be supported by the present observations that effects of THC on the release and the uptake of DA were not so pronounced.

There are reports concerning neurochemical studies of THC, as related CA systems (1, 2, 12, 14). The most consistently observed effect is an increase in CA synthesis. The behavioral and biochemical effects of THC are dependent on animal strains and species. We examined the effect of THC on the uptake of ^{3}H-DA into striatal synaptosomes of WKA rats used in our studies. The uptake of ^{3}H-DA in WKA rat striatal synaptosomes was inhibited by THC in a dose-dependent manner. Howes and Osgood found that THC produced a concentration-related decrease in the uptake of ^{14}C-DA into striatal synaptosomes of Charles-River mice (6). The IC_{50} of THC on the uptake of ^{14}C-DA was 5.4×10^{-6} M, which was close to our results. THC increased the release of the preloaded ^{14}C-DA. Poddar and Dewey noted biphasic effects of THC on the uptake and the release of ^{3}H-DA (14). In these experiments, the tissue was preloaded with radiolabeled DA. The effect of THC on the release of labeled DA previously taken up may differ from that of THC on the release of endogenous DA (15, 22, 23).

We found that the effect of THC on the release of DA was less than its effect on ^{3}H-DA uptake into synaptosomes (10^{-5} M THC induced only a 20–25% enhancement of spontaneous DA release, compared to a 60% inhibition of uptake). Because "release" from superfused slices is a composite of the actual release, uptake, metabolism and diffusion of transmitter, it can be argued that slight enhancement of the release of DA was merely due to blockade of the uptake. The lack of increase of DOPAC in the superfusing fluid lends support to this notion because part of the DA released by stimulation is normally taken up and converted into DOPAC, which in turn diffuses out of the neuron (13). Our results suggest that THC facilitated DA transmission related to the inhibition of DA uptake. MAP 10^{-6} M remarkably increased the release of DA but not DOPAC in the present study. The lack of any stimulatory action of MAP on DOPAC release may relate to the inhibition of monoamine oxidase. THC 10^{-4} M produced the inhibition of DA uptake by 90%, more potently than THC 10^{-5} M, yet did not stimulate the release of DA. Therefore, we cannot rule out possible actions of THC on the process of the release of DA as a cause of increase in DA release. Taken together, the facilitatory action of THC on DA transmission appears to result from mainly the inhibition of DA uptake and also the stimulation of DA release.

THC 5 mg/kg IP produced a 25% decrease of the DA level in the dorsolateral striatum, and a decrease presumably related to the stimulation of DA release induced by THC. Part of this effect of THC may be counteracted by an increase in DA synthesis. In previous experiments, DA reduced the release of ^{3}H-acetylcholine evoked by high K^+ from rat dorsolateral striatum slices but not from ventromedial ones (20,21). Thus, THC may predominantly affect DA-ergic neurons in the dorsolateral region functionally linked to cholinergic systems in the striatum.

In conclusion, THC (5 mg/kg) facilitated DA neurotransmission by inhibiting DA uptake and stimulating its release, and this effect may contribute to irritable aggression in WKA rats induced by this compound.

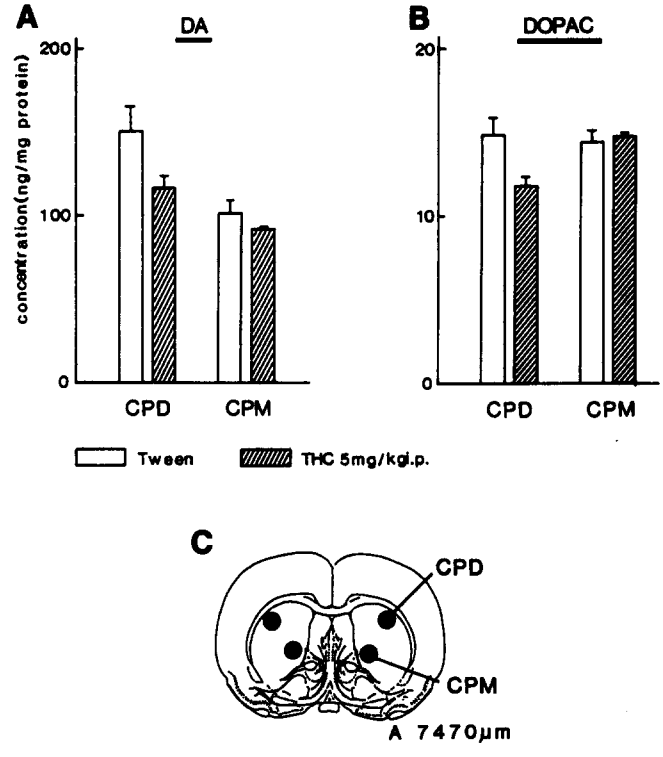

FIG. 2. (A–B) Effect of THC on concentration of dopamine (DA) and dihydroxyphenylacetic acid (DOPAC) in dorsolateral caudate putamen (CPD) and ventromedial caudate putamen (CPM). (C) Brain region punched out. CPD: dorsolateral caudate putamen; CPM: ventromedial caudate putamen. Data are mean ± S.E.M. of 15 rats.

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