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Is Tolerance to Delta-9-THC Cellular or Metabolic?

The Subcellular Distribution of Delta-9-Tetrahydrocannabinol and Its Metabolites in Brains of Tolerant and Non-Tolerant Rats

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Abstract. Rats were injected intraperitoneally once every 12 h with 10 mg/kg delta-9-THC and the time course of the depressant effect was determined after one and nine injections. The motor activity of naive rats was maximally depressed between 1 and 4 h and returned to control levels 8 h after treatment. After nine injections, the maximum intensity of the depressant effect was not different from that after one injection but had completely disappeared at an earlier time point (4 h p.i.) indicating the development of tolerance to the duration of effect on motor activity. The subcellular distribution studies in brains of tolerant and non-tolerant rats indicated that an accelarated shift in the concentrations of delta-9-THC and 11-OH-delta-9-THC towards highly polar metabolites in the brains, rather than an increased elimination of these cannabinoids or decreased sensitivity of the brain may be responsible for the development of tolerance to delta-9-THC.

Key words: Delta-9-THC and metabolites – Brain subcellular distribution – Tolerance development.

INTRODUCTION

Tolerance to a great number of pharmacological and behavioural effects of delta-9-THC develops in both animal and man (for review see Paton, 1975). The question as to whether the mechanism of tolerance to this drug is metabolic or cellular in origin has not been clearly answered. The possible involvement of a metabolic factor has been suggested by Lemberger et al. (1971), who found that half-life of delta-9-THC in the blood of chronic marihuana users is shorter than in non-users. Other investigators, however, have failed to detect any difference between concentrations of ^{3}H-delta-9-THC or metabolites in blood and brain of tolerant and non-tolerant pigeons and assumed tolerance to delta-9-THC to be cellular (McMillan et al., 1973; Dewey et al., 1973).

Data on the subcellular distribution of delta-9-THC and its metabolites in the brain of naive and tolerant animals may provide a more suitable interpretation of tolerance to this drug. We have recently reported that the diminution of the effect of delta-9-THC and 11-OH-delta-9-THC on locomotor activity of rats may be related to the formation of highly polar, water soluble metabolites within the brain (Magour et al., 1976). One possible mechanism of the development of tolerance to delta-9-THC could be that this formation would occur faster in tolerant than in non-tolerant animals.

In the present study the time course of the depressant effect on motor activity during daily treatment with 10 mg/kg delta-9-THC has been recorded until tolerance was apparent. In parallel experiments the concentrations of ^{3}H-delta-9-THC and its metabolites in brain subcellular fractions at different time intervals has been determined, in an attempt to correlate the development of tolerance with changes in the distribution of these cannabinoids in the brain.

MATERIALS AND METHODS

Unlabelled delta-9-THC was synthesized by Dr. Petrzilka at the Eidgenössische Technische Hochschule, Zürich, ^{3}H-delta-9-THC (spec. act. 31.81 mCi/mmol, 101.3 μCi/mg) was generously supplied by Dr. M. Braude of the National Institute of Mental Health, Center of Studies of Narcotics and Drug Abuse, U.S.A.

Female Wistar rats weighing 150–170 g were kept at constant temperature of 23 ± 2°C and 12 h light-dark illumination cycle (dark from 04.00 p.m. to 04.00 a.m.). Food and water were available ad libitum. Delta-9-THC was dissolved in 10% Cremophor EL/physiological saline and injected intraperitoneally (0.5 ml per 100 g of body weight).

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Motor Activity Studies. Six groups (3 controls and 3 experimentals) of 3 rats each were allowed to acclimatize to the experimental cages (42 × 24 cm) for at least 5 days before the start of the experiment. They were injected with 10 mg/kg delta-9-THC (or the vehicle in the case of controls) twice daily at 09.00 a.m. and at 09.00 p.m., and the locomotor activity was recorded daily every 15 min during the dark period from 09.30 p.m. to 04.00 a.m. in an animex motility apparatus (Farad Electronics AB). Only the data obtained after the first and ninth injection when tolerance was apparent, will be discussed in this communication.

Subcellular Distribution. Rats injected intraperitoneally with 10 mg/kg ^{3}H-delta-9-THC were decapitated 1, 4 or 12 h afterwards. Brain subcellular fractions were prepared as described before (Magour et al., 1976). From each fraction ^{3}H-delta-9-THC and its metabolites were extracted into heptan of various polarities according to Kreuz and Axelrod (1973). The extracts were combined and analysed by TLC using chloroform : aceton (4 : 1) as solvent (Magour et al., 1976). The residual radioactivity in the aqueous phase was designated "polar metabolites". The radioactive spots, corresponding to delta-9-THC, 11-OH-delta-9-THC and non-mobile material at the origin were scraped from the plate and counted in 15 ml Bray's solution (Bray, 1960) in a Packard liquid scintillation spectrometer model 3380. The concentrations of the non-mobile material from each fraction varied considerably and will not be discussed further. Aliquots (0.2 ml) of the remaining aqueous phase containing the polar metabolites were also counted. Protein content was assayed according to Lowry et al. (1951) using bovine serum albumin as the standard.

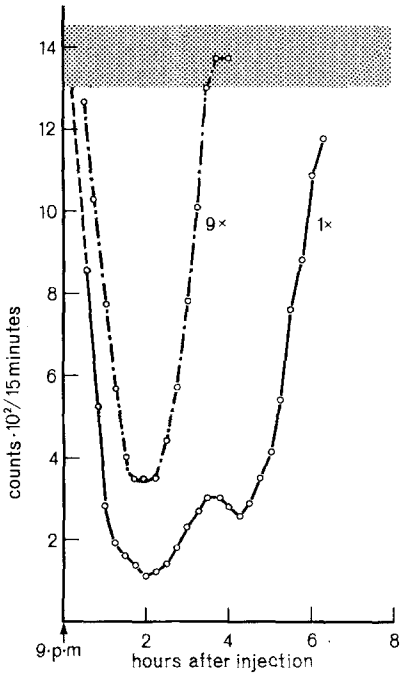

Fig. 1. Time course of the effect of one or nine injections of 10 mg/kg delta-9-THC on locomotor activity. The counts from groups of 3 rats each were summed and each point is the average of three such groups. The shadowed area represents the mean of the controls ± S.D. Measurements after periods longer than 7 h could not be made, due to onset of the light cycle after this time

RESULTS

Rats were injected with 10 mg/kg delta-9-THC (once every 12 h) and the time course of the changes in locomotor activity was recorded after the first and ninth injection. As shown in Figure 1 the motor activity of naive rats was maximally depressed between 1 and 4 h and returned to normal by ca. the eigth hour of treatment (cf. Magour et al., 1976). After 9 injections the maximum intensity of the depressant effect on motor activity (approximately 2 h p.i.) was slightly lower than that after one injection, but had completely disappeared at an earlier time point (4 h p.i.) indicating the development of tolerance to the duration of effect on motor activity.

In parallel experiments we injected naive rats with 10 mg/kg ^{3}H-delta-9-THC and determined the concentrations of delta-9-THC and its metabolites in brain subcellular fractions by thin layer chromatography. As depicted in Table 1 there were no substantial differences in the concentrations of delta-9-THC and 11-OH-delta-9-THC among all particulate fractions at 1, 4 and 12 h after injection which is probably due to the highly lipophilic characteristics of these substances. It is interesting to note that the concentrations of these cannabinoids successively declined during 12 h after injection with a correspondingly marked increase in the concentrations of the polar metabolites in all fractions, especially cytosol. These data indicate a shift in the concentrations of delta-9-THC and 11-OH-delta-9-THC towards the highly polar metabolites within the brain fractions during the 12-h period. Preliminary ultrafiltration and lyophilization experiments have shown that about 80% of the residual radioactivity in cytosol consists of unbound water soluble materials.

In a second series of experiments we injected rats twice daily (once every 12 h) with 10 mg/kg of unlabelled delta-9-THC for 4 days. Twelve hours after the last injection they received a single dose of 10 mg/kg ^{3}H-delta-9-THC and were decapitated either 1 or 4 h later. The data in Table 2 indicate that pretreatment of rats with delta-9-THC did not alter the overall pattern of distribution of either this substance or its 11-OH-metabolite among all brain particulate fractions. The initial concentrations of these cannabinoids in the various fractions were rather lower than the corresponding quantities in the naive rats but dropped within 3 h (4 h p.i.) to a low level similar to that observed after 12 h in the non-tolerant animals. Concomitantly the concentration of the polar metabolites were increased over the same period of time in all fractions, especially cytosol.

Figure 2 shows the time course of the change in the concentration of delta-9-THC and 11-OH-delta-9-THC summed together and the corresponding change

Table 1

	Δ^9-THC	11-OH-Δ^9-THC	Non-mobile	Polar metabolites	Total (brain)
One hour p.i.					
Crude nuclei	68	57	33	5	
Myelin	110	79	41	9	
Synaptosomes	143	118	92	28	
Mitochondria	137	98	80	11	
Microsomes	89	84	45	11	
Cytosol	36	14	16	109	
Total (fractions)	583 = 38%	450 = 29%		173 = 11%	1513 = 100%
Four hours p.i.					
Crude nuclei	30	29	5	16	
Myelin	62	53	9	10	
Synaptosomes	47	52	52	38	
Mitochondria	34	13	19	76	
Microsomes	30	38	4	15	
Cytosol	58	48	39	313	
Total (fractions)	261 = 23%	233 = 21%		468 = 43%	1090 = 100%
Twelve hours p.i.					
Crude nuclei	12	16	10	65	
Myelin	18	35	37	86	
Synaptosomes	22	39	19	120	
Mitochondria	29	28	28	159	
Microsomes	13	20	12	74	
Cytosol	33	5	19	419	
Total (fractions)	127 = 10%	143 = 11%		923 = 70%	1318 = 100%

The concentrations of delta-9-THC and metabolites in brain subcellular fractions at 1, 4 and 12 h after an i.p. injection of 10 mg/kg ^{3}H-delta-9-THC. Data are expressed as pmoles/g brain. For clarity, individual values of S.D. are not included in the table; they lay between 6 and 12%. $n = 3$

Table 2

	Δ^9-THC	11-OH-Δ^9-THC	Non-mobile	Polar metabolites	Total (brain)
One hour p.i.					
Crude nuclei	38	26	9	12	
Myelin	77	61	12	20	
Synaptosomes	81	76	24	50	
Mitochondria	61	70	10	31	
Microsomes	51	43	7	27	
Cytosol	26	13	13	111	
Total (fractions)	334 = 35%	289 = 30%		251 = 26%	949 = 100%
Four hours p.i.					
Crude nuclei	10	7	13	51	
Myelin	22	15	13	40	
Synaptosomes	19	13	11	68	
Mitochondria	28	18	25	71	
Microsomes	11	9	5	86	
Cytosol	15	16	14	641	
Total (fractions)	105 = 9%	78 = 6%		957 = 78%	1221 = 100%

The subcellular distribution of delta-9-THC and metabolites in brains of tolerant rats 1 and 4 h after a single i.p. injection of 10 mg/kg ^{3}H-delta-9-THC. Data are expressed as pmoles/g brain. For clarity individual values of S.D. are not included in the table; they all lay between 5 and 10%. $n = 3$

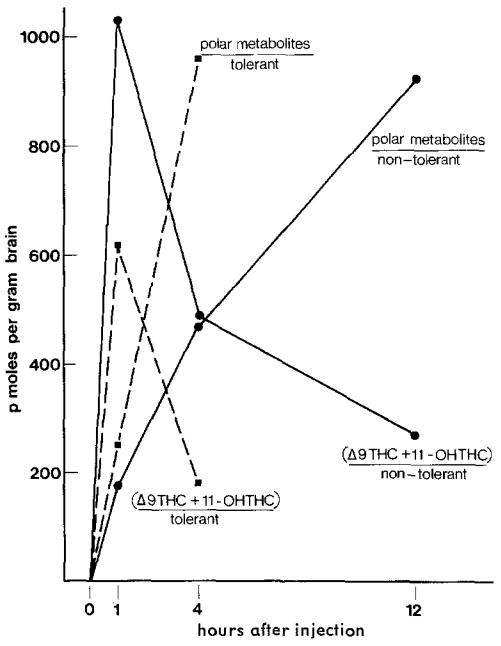

Fig. 2. The concentration of delta-9-THC and 11-OH-delta-9-THC and the polar metabolites in brain homogenate of tolerant and non-tolerant rats. Rats were injected (i.p.) with 10 mg/kg ^{3}H-delta-9-THC and decapitated either 1, 4 or 12 h later. $n = 4$

in concentration of the polar metabolites in brain homogenates of both tolerant and non-tolerant rats. The level of the polar metabolites in tolerant animals 4 h after injection was as high as that observed after 12 h in non-tolerant animals. Further, from Figure 2 the concentrations of delta-9-THC and 11-OH-delta-9-THC at any given point after injection can be predicted. For example the predicted value 2 h after injection in tolerant rats is similar to that observed after 4 h in non-tolerant animals, which agrees well with the observed similarity in motor activity counts at both of these time points (Fig. 1).

DISCUSSION

The purpose of this study is to correlate the time course of the effect of delta-9-THC on motor activity with the subcellular distribution of ^{3}H-delta-9-THC and its metabolites in brains of tolerant and non-tolerant rats which may provide a suitable explanation for the mechanism of tolerance to this drug. Nine injections of 10 mg/kg delta-9-THC (once every 12 h) markedly reduced the duration of the depressant effect from 8 to 4 h indicating the development of tolerance to the duration of effect on motor activity.

Davis et al. (1972) and Anderson et al. (1975) reported the development of tolerance to the depressant effect of delta-9-THC on motor activity of rats and mice. These authors made their measurements at a fixed time point (either at 1 or 2 h) after injection. The data in Figure 1 clearly show that the difference in motor activity counts between tolerant and non-tolerant rats changes considerably at different time points after injection. This stresses the importance of measuring the time course of the effect of delta-9-THC when assessing the development of tolerance to this drug.

As shown in Table 1 there were no substantial differences in the concentration of total cannabinoids at 1, 4 and 12 h after injection, but there was a marked shift in the concentrations of delta-9-THC and 11-OH-delta-9-THC towards the highly polar metabolites which parallels the diminution of the acute effect on motor activity.

The data in Table 2 show how a dose of radioactive labelled delta-9-THC is distributed in brain subcellular fractions of tolerant rats but do not provide information about the possible accumulation of unlabelled delta-9-THC from the previous injections. It is evident that under our experimental conditions, pretreatment of rats with delta-9-THC accelerates the above observed shift in the concentration of ^{3}H-delta-9-THC in the brain which again accompanies the early disappearance of the depressant effect in tolerant animals (Fig. 1). Thus repeated administration of delta-9-THC to rats not only develops tolerance to the duration of effect on motor activity but also enhances the formation of highly polar metabolites in the brain.

The chemical nature and the mechanism of formation of these polar substances are as yet unknown, but certain points should be discussed. Although the brains used in our study may have been contaminated to a small degree with metabolites originated from the blood, this cannot only account for the observed increase in the concentrations of polar metabolites in tolerant rats. It is well established that repeated intraperitoneal injections with delta-9-THC or marihuana extracts do not stimulate the hepatic metabolism of ^{14}C-delta-9-THC in rats (Kupfer et al., 1973; Siemens and Kalant, 1974). It is generally known that polar substances do not easily pass through brain cell membranes in either direction. Therefore, it can be speculated that the observed highly polar metabolites may be formed, at least in part, inside rather than outside the brain. It is likely that certain hydroxylated metabolites of delta-9-THC, which may have been formed in the liver, are further conjugated or esterified in the brain giving rise to highly polar water soluble compounds which do not easily migrate out of the cells. A more definitive identification of these compounds will be necessary to clarify this point.

The mechanism underlying the development of tolerance to delta-9-THC is still controversial. There are some indications that enhanced microsomal enzyme systems may be involved. Lemberger et al. (1971) reported that chronic marihuana users may metabolize delta-9-THC more rapidly than non-users. This conclusion is based on determination of the half-life time of delta-9-THC and metabolites in the blood and not on the actual activity of liver microsomal enzymes. In contrast, other workers suggested that tolerance to delta-9-THC does not involve any metabolic changes, and postulated that it could be due to adaptation of the central nervous system. Siemens and Kalant (1974) reported that the hepatic metabolism of delta-9-THC was not influenced by the development of tolerance to marihuana-induced enhancement of ethanol sleeping time. Martin et al. (1976) found no significant difference in the level of total radioactivity in brain subcellular fractions of tolerant and non-tolerant dogs after a single injection of ^{3}H-delta-9-THC. These latter authors, however, did not separate the various metabolites of delta-9-THC, before making their radioactive measurements.

Our subcellular distribution studies clearly indicate that an accelerated shift in the concentration of delta-9-THC and 11-OH-delta-9-THC towards highly polar metabolites in the brain, rather than an increased elimination of these cannabinoids or decreased sensitivity of the brain, may have caused the rapid disappearance of their effect on motor activity of tolerant rats. However, the possible simultaneous involvement of various other processes in the development of tolerance to delta-9-THC cannot be ruled out.

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REFERENCES

Anderson, P. F., Jackson, D. M., Chesher, G. B., Malor, R.: Tolerance to the effects of delta-9-tetrahydrocannabinol in mice on intestinal motility, temperature and locomotor activity. Psychopharmacologia (Berl.) **43**, 31–36 (1975)

Bray, G. A.: A simple efficient liquid scintillator for counting aqueous solutions in a liquid scintillation counter. Analyt. Biochem. **1**, 279–385 (1960)

Davis, W. M., Moreton, J. E., King, W. T., Pace, H. B.: Marihuana on locomotor activity: Biphasic effect and tolerance development. Res. Comm. Chem. Path. Pharmacol. **3**, 29–35 (1972)

Dewey, W. L., McMillan, D. E., Harris, L. S., Turk, R. F.: Distribution of radioactivity in brain of tolerant and nontolerant pigeons treated with ^{3}H-delta-9-tetrahydrocannabinol. Biochem. Pharmacol. **22**, 399–405 (1973)

Kreuz, D. S., Axelrod, J.: Delta-9-tetrahydrocannabinol: Localization in body fat. Science **179**, 391–392 (1973)

Kupfer, D., Levin, E., Burstein, S. H.: Studies on the effects of delta-1-tetrahydrocannabinol (delta-1-THC) and DDT on the hepatic microsomal metabolism of delta-1-THC and other compounds in the rat. Chem.-Biol. Interact. **6**, 59–66 (1973)

Lemberger, L., Tamarkin, N. R., Axelrod, J., Kopin, I. J.: Delta-9-tetrahydrocannabinol: metabolism and disposition in long-term marihuana smokers. Science **173**, 72–74 (1971)

Lowry, O. H., Rosenbrough, N. J., Farr, A. L., Ranall, R. I.: Protein measurement with the folin phenol reagent. J. biol. Chem. **193**, 265–275 (1951)

Magour, S., Coper, H., Fähndrich, Ch., Hill, R.: Relationship between the subcellular distribution of delta-9-tetrahydrocannabinol and its metabolites in rat brain and the duration of the effect on motor activity. Life Sci. **18**, 575–584 (1976)

Martin, B. R., Dewey, W. L., Harris, L. S., Beckner, J. C.: ^{3}H-delta-9-tetrahydrocannabinol tissue and subcellular distribution in the central nervous system and tissue distribution in peripheral organs of tolerant and non-tolerant dogs. J. Pharmacol. exp. Ther. **196**, 128–144 (1976)

Paton, W. D. M.: Pharmacology of marihuana. Ann. Rev. Pharmacol. **15**, 191–220 (1975)

Siemens, A. J., Kalant, H.: Metabolism of delta-1-tetrahydrocannabinol by rats tolerant to cannabis. Canad. J. Physiol. Pharmacol. **52**, 1154–1166 (1974)

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