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SUBCELLULAR DISTRIBUTION OF Δ^9-TETRAHYDROCANNABINOL IN RAT BRAIN

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Abstract Δ^9-Tetrahydrocannabinol-^{3}H (Δ^9-THC-^{3}H), administered intracisternally, is distributed almost equally among particulate fractions of rat brain homogenates with only a small proportion in the supernatant fraction. Similar distribution of Δ^9-THC-^{3}H is found when the drug is added directly to brain homogenates. Most of the metabolites of Δ^9-THC-^{3}H, however, are found in the supernatant fraction, but synaptosomes retain significantly greater amounts of polar metabolites than do other particulate fractions. After incubation with synaptosomes *in vitro*, exceptionally high tissue/medium ratios were found for Δ^9-THC-^{3}H accumulation. This accumulation was not prevented by metabolic inhibitors and was not temperature dependent. The results indicate that significant amounts of Δ^9-THC and its polar metabolites can be concentrated in synaptosomes by nature of the drug's lipophilic properties.

Δ^9-TETRAHYDROCANNABINOL has been shown to be a principal psychoactive component of marijuana.[1–4] The availability of radiolabeled Δ^9-THC of relatively high specific activity has made possible studies of its metabolism and physiologic disposition,[5–8] as well as its regional distribution in brain after parenteral administration in animals.[9–11] We wish now to report studies on its subcellular distribution and the characteristics of its accumulation by particulate fractions of rat brain.

METHODS

Tritiated Δ^9-THC (20 μg, sp. act. 8·8 mCi/m-mole) suspended in 40 μl of a mixture of rat serum and polyethylene glycol (3:1 by volume) was administered to rats (150–200 g, male Sprague-Dawley) by intracisternal injection as described by Schanberg *et al.*[12] Two and one-half or 21 hr after injection, brains were rapidly removed, cooled on ice and homogenized in 9 vol. of cold 0·32 M sucrose for subcellular fractionation essentially by the method of Whittaker *et al.*[13] The brain homogenate was first centrifuged at 900 *g* for 10 min to remove broken cells (P1). The supernatant (S1) was then centrifuged at 22,000 *g* for 20 min, giving microsomes in the supernatant (S2) and myelin, mitochondria and synaptosomes in the pellet (P2). The S2 fraction was further centrifuged at 100,000 *g* for 30 min to give a microsomal pellet (P3) and supernatant (S3). The pellet P2 was resuspended in 10 ml of 0·32 M sucrose and its various components were fractionated further by centrifugation at 50,000 *g* for 1 hr on a discontinuous sucrose density gradient. Fractions containing myelin (A), synaptosomes (B) and mitochondria (C) were obtained. Aliquots of isolated tissue fractions were dissolved in soluene 100 (Packard) 16 hr prior to the addition of the

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counting mixture (18% ethanol in toluene with 2·7% liquifluor) (New England Nuclear) and assayed for radioactivity by liquid scintillation spectrometry. In some cases, a small hole was made at the bottom of the cellulose nitrate tube after sucrose gradient centrifugation and fractions were collected dropwise, consisting of 14 drops drops/fraction. These fractions were assayed for radioactivity as described above.

Δ^9-THC and its metabolites in the different subcellular fractions were separated by previously described extraction procedures.[7,8] The unchanged Δ^9-THC was measured by extraction at pH 6·5 to 7·5 into 4 vol. of heptane containing 1·5% isoamyl alcohol. Concurrent standards showed a recovery of 95 ± 5 per cent of Δ^9-THC extracted into the heptane. The radioactivity in the organic phase was assayed by liquid scintillation spectrometry. After extraction of Δ^5-THC with heptane, relatively polar metabolites which remained in the aqueous phase were extracted with ether. Aliquots of the ether extract (containing principally 11-hydroxy and 8,11-dihydroxy compounds) and the residual aqueous phase (containing the most polar metabolites) were also assayed for radioactivity. The identity of the Δ9-THC-^{3}H measured in the heptane extract was established by cochromatography with authentic Δ^9-THC on Eastman Silica gel chromatograms in a hexane–acetone (3:1) system.

Accumulation of labeled Δ^9-THC by different tissue fractions was studied by a method similar to that previously described.[14] Each subcellular fraction was prepared as described above and resuspended in buffer (made up of 118 mM NaCl, 4·7 mM KCl, 2·2 mM $CaCl_2$, 1·18 mM $MgSO_4$, 11 mM dextrose and 25 mM sodium phosphate at pH 7·0) so that each 5-ml aliquot of tissue suspension gave an equivalent of tissue derived from 70 mg of initial whole brain. For studies on uptake, ^{14}C-Δ^9-THC (sp. act. 6·0 mCi/m-mole) at the required concentration was added to the tissue suspension at 4° with constant mixing. Effect of drugs on uptake of ^{14}C-Δ^9-THC was examined by addition of the drug to the tissue suspension prior to addition of the labeled Δ^9-THC. After addition of ^{14}C-Δ^9-THC, tissue aliquots (5 ml) were then transferred to cellulose nitrate centrifuge tubes and incubated at 4° or 37° for 20 min. After incubation, all tubes were cooled to 4° and centrifuged at 16,000 *g* for 10 min at 4°. An aliquot (100 μl) of the supernatant fluid was transferred to a vial and its radioactivity assayed by liquid scintillation spectrometry. The remainder of the supernatant fluid was discarded and the tissue pellet was rinsed once with 1 ml of ice-cold buffer. The radioactivity accumulated in the pellet was then extracted by homogenization with 250 μl of 0·4 N perchloric acid in 50% ethanol. The extracted pellets were centrifuged at 900 *g* for 2 min at 4° and an aliquot (200 μl) of the extract was transferred to a counting vial and the radioactivity determined by liquid scintillation spectrometry. In each experiment, the weights of the tissue in each 5-ml aliquot of the tissue suspensions were also determined. Tissue/medium ratios were calculated from the cpm/g of tissue divided by the cpm/ml of supernatant fluid.

RESULTS

Two and one-half hr after intracisternal injection of Δ^9-THC-^{3}H, both Δ^9-THC and its metabolites were present in the subcellular fractions of brain homogenates. Slightly less than half of the total radioactivity was recovered as metabolites (Table 1). Δ^9-THC-^{3}H was present mostly in the particulate fractions, whereas polar metabolites (residual fraction) were predominantly found in the supernatant fraction.

TABLE 1. SUBCELLULAR DISTRIBUTION OF Δ^9-THC-^{3}H AND METABOLITES AFTER INTRACISTERNAL ADMINISTRATION*

	Δ^9-THC-^{3}H	^{3}H-metabolites	
	Heptane extract	Ether extract	Residual
Broken cells (P1)	4816 ± 186	1353 ± 218	238 ± 15
Microsomes (P3)	358 ± 29	23 ± 11	107 ± 74
Supernatant (S3)	293 ± 58	27 ± 1	2197 ± 388
Myelin (A)	756 ± 71	191 ± 35	204 ± 6
Synaptosomes (B)	666 ± 78	73 ± 21	653 ± 136
Mitochondria (C)	477 ± 51	26 ± 7	145 ± 15

* Results are expressed in cpm recovered in each fraction from 1·7 g of whole brain and are he means ± S.E.M. of three experiments.

About 10 per cent of the unchanged Δ^9-THC fraction and about 15 per cent of the total metabolites were present in the synaptosomal fraction.

In some experiments, Δ^9-THC-^{3}H was added directly to the whole rat brain *in vitro* prior to homogenization and its subcellular localization compared with that after intracisternal administration *in vivo*. As seen in Fig. 1, the subcellular distribution of the radiolabeled Δ^9-THC was similar whether the isotope was given *in vivo* or added to the whole brain during homogenization.

The accumulation of ^{14}C-Δ^9-THC by brain subcellular fractions was studied *in vitro*. Accumulation of labeled Δ^9-THC was not significantly different at 4° or 37°

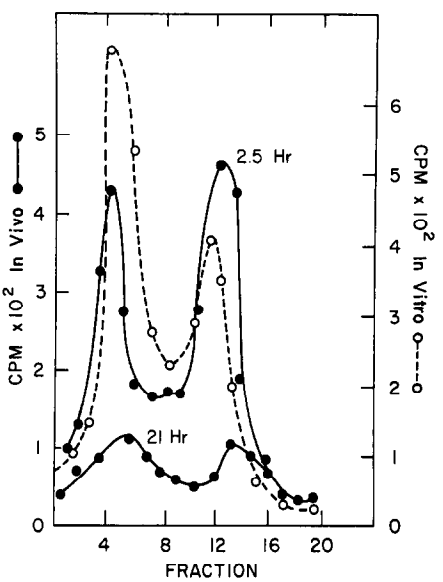

FIG. 1. Sedimentation characteristics of Δ^9-THC-^{3}H in homogenates of rat brain on discontinuous sucrose gradients. Δ^9-THC-^{3}H (20 μg) was injected intracisternally to rats *in vivo* and their brains were homogenized 2·5 or 21 hr later (●). In other experiments, equivalent isotope was added to rat brains *in vitro* prior to homogenization (○). Fractions were obtained by piercing the bottom of the centrifuge tube and 15-drop samples were collected and assayed. Each result represents the average of three experiments.

TABLE 2. ACCUMULATION OF ^{14}C-Δ^9-THC *in vitro* BY BRAIN SUBCELLULAR FRACTIONS*

	Drug added			
	4°	37°		
Tissue	Control	Control	Ouabain†	DNP‡
Myelin	319 ± 30	238 ± 21	217 ± 15	243 ± 29
Synaptosomes	591 ± 99	557 ± 39	626 ± 51	546 ± 24
Mitochondria	618 ± 33	750 ± 80	787 ± 82	771 ± 56

* Accumulation is expressed as the tissue/medium ratio (cpm/g tissue divided by cpm/ml incubation medium). Each result is the mean value (± S.E.M.) for four determinations. ^{14}C-Δ^9-THC concentration, 1×10^{-6} M (6·0 mCi/m-mole).

† Ouabain concentration, 1×10^{-5} M.

‡ DNP concentration, 1×10^{-4} M.

and was not affected by metabolic inhibitors such as ouabain or dinitrophenol (DNP) (Table 2). The tissue/medium ratios in all cases were found to be exceedingly high (Table 3).

The characteristics of Δ^9-THC-^{3}H accumulation by synaptosomes were further examined at different Δ^9-THC concentrations. The amount of Δ^9-THC accumulation in tissues relative to the Δ^9-THC concentration in the incubation medium was very high in all cases and remained unchanged with increasing concentrations of Δ^9-THC at both 4° or 37° (Table 3).

DISCUSSION

Our findings provide direct evidence that Δ^9-THC, as well as its metabolites, are concentrated, presumably as a consequence of physical properties rather than physiologic processes, by particulate fractions of brain with exceptionally high affinity. Tissue concentration of Δ^9-THC and metabolites exceeds by several hundred-fold the concentration in the medium. In all cases the accumulation process is not temperature or energy dependent and is non-saturable over the concentration range examined. If there is a specific localization *in vivo* which is not reflected in these studies, such localization must be rapidly reversible since the distribution of Δ^9-THC added to brain homogenates in the cold paralleled that seen after administration *in vivo*. Such characteristics of accumulation most likely reflect the partition coefficient of the drug which has a reported octanol–water partition higher than most of the barbiturates.[15]

TABLE 3. ACCUMULATION OF Δ^9-THC BY RAT BRAIN SYNAPTOSOMES*

^{14}C-Δ^9-THC	4°	37°
3×10^{-7}	650 ± 72	541 ± 42
1×10^{-6}	632 ± 101	633 ± 50
1×10^{-5}	575 ± 82	562 ± 13

* Accumulation is expressed as the tissue/medium ratio (cpm/g tissue divided by cpm/ml incubation medium). Each result is the mean value (± S.E.M.) for four determinations.

The results of these studies are consistent with the lipophilic nature of Δ^9-THC as shown previously by other investigators.[16] The lipophilic properties of Δ^9-THC may explain in part its persistence in brain after acute[9,11,17] or chronic[16] administration. The high affinity of this drug for membrane components of brain may be of potential significance in the long-term effects of this drug. Whether its accumulation at synaptosomal sites could contribute to its psychoactive effects after acute administration remains to be elucidated.

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