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^{3}H-Δ^9-Tetrahydrocannabinol, ^{3}H-Cannabinol and ^{3}H-Cannabidiol: Penetration and Regional Distribution in Rat Brain

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ALOZIE, S. O., B. R. MARTIN, L. S. HARRIS AND W. L. DEWEY. *^{3}H-Δ^9-Tetrahydrocannabinol, ^{3}H-cannabinol and ^{3}H-cannabidiol: Penetration and regional distribution in rat brain.* PHARMAC. BIOCHEM. BEHAV. 12(2) 217–221, 1980.—^{3}H-Δ^9-Tetrahydrocannabinol (^{3}H-Δ^9-THC), ^{3}H-cannabidiol (^{3}H-CBD) and ^{3}H-cannabinol (^{3}H-CBN) were administered (1 mg/kg) to male rats which were decapitated either 0.5, 1, 15, 30 or 90 min later. The plasma concentration was similar for all cannabinoids throughout the time course. After 5 min greater than 80% of the plasma radioactivity in each treatment was due to metabolites. Radioactivity rapidly entered brain after the administration of ^{3}H-CBD, ^{3}H-CBN, and ^{3}H-Δ^9-THC. The concentrations of unchanged ^{3}H-CBD and ^{3}H-CBN in whole brain were higher than that of ^{3}H-Δ^9-THC 5 min after administration. Regional distribution of radioactivity in the brain after 5 min was similar for all three cannabinoids, the only significant difference being in hypothalamus. Coadministration of ^{3}H-Δ^9-THC with a five-fold excess of either CBD or Δ^9-THC did not produce any significant alteration in the levels of radioactivity in brain or plasma 5 min after their injection. The difference in behavioral activity of Δ^9-THC, CBD and CBN cannot be explained by penetrability or regional distribution in the brain.

^{3}H-Δ^9-Tetrahydrocannabinol ^{3}H-cannabidiol ^{3}H-cannabinol Brain penetrability Brain distribution Pharmacokinetics

CANNABIDIOL (CBD) and cannabinol (CBN) are considered to be less active than Δ^9-tetrahydrocannabinol (Δ^9-THC), although they have been shown to produce pharmacological effects in humans and other species [15,25]. Differences in potency of these cannabinoids may depend upon their penetrability and disposition in the brain, as well as the pharmacokinetic interactions among the cannabinoids. Intraventricular injection (IVT) of the cannabinoids has shown CBN to be active in mice [8], and both CBD and CBN were found to be active in monkeys [7]. It has also been shown that CBN has anticonvulsant activity but at doses higher than those of Δ^9-THC necessary to produce pharmacological effects [17]. The IVT route of administration provides a means of comparing the potency of drugs at their site of action without certain dispositional and metabolic factors which contribute to their activity when peripheral route of administration is employed. The possibility exists that the activity of cannabinoids may be dependent upon their ability to penetrate the brain. Carney *et al.* [7] have suggested that the order of accessibility to the brain of the cannabinoids and their metabolites following IP administration is 11-OH-Δ^9-THC, Δ^9-THC, CBN and CBD and have suggested that these differences reflect possible selective permeability of the blood-brain barrier.

Additionally, it is possible that all the cannabinoids readily penetrate the brain but the inactive cannabinoids may not be reaching the site(s) of action. Studies on tolerance to the behavioral effects of Δ^9-THC in pigeons have shown that neither metabolism [10] nor uptake of Δ^9-THC into brain [23] was altered. Also experiments in dogs [21] have demonstrated that tolerance to Δ^9-THC could not be accounted for on the basis of altered distribution in peripheral organs or into different areas of the brain. However, it remains to be established whether or not differences among the potencies of the cannabinoids are due to differences in their disposition in brain.

CBD and CBN have been shown to interact with other centrally active drugs and can potentiate [3, 12, 19] or reduce [3,9] a number of THC-induced responses. The presence of CBN in administered Δ^9-THC has been shown to increase the rate of disappearance of Δ^9-THC from the blood [22]. CBD is a known inhibitor of hepatic drug metabolism [4, 11, 16] and it has been suggested that the pharmacological interactions of CBD are due to interference with the metabolism [5] rather than the plasma disappearance rate of Δ^9-THC [2].

Distribution studies of very low doses of CBN and CBD have not been investigated. The purpose of the present investigation was to compare the penetrability and distribution of ^{3}H-Δ^9-THC, ^{3}H-CBD and ^{3}H-CBN in the brain and to determine whether or not CBD altered the pharmacokinetics

of Δ^9-THC with the hope of elucidating the role of these factors with regard to the range in behavioral potencies of the cannabinoids.

METHOD

Administration of Cannabinoids

^{3}H-Δ^9-THC (43 mCi/mmole), ^{3}H-CBD (28 mCi/mmole), ^{3}H-CBN (38 mCi/mmole), all labeled in the side chain (C-1' and C-2'), were obtained from the National Institutes on Drug Abuse. The specific activities of all the cannabinoids were adjusted to 3.14 mCi/mmole by the addition of unlabeled drug. An ethanol solution of the cannabinoids was mixed with an equal volume of emulphor (GAF Corp., Linden, NJ) and appropriate dilutions made with saline [24]. Male Sprague-Dawley rats, 175–200 g, were administered a dose of 1 mg/kg of either ^{3}H-Δ^9-THC, ^{3}H-CBD or ^{3}H-CBN (10 μCi/mg/kg) via the tail vein and were decapitated either 0.5, 1, 5, 15, 30 or 90 min later.

Quantitation of Radioactivity

Blood from the cervical wound was collected in heparinized glass tubes and centrifuged at 1000 G for 20 min. Plasma was pipetted off and 50 μl aliquots were added to a mixture of 2 parts of toluene containing 0.4% diphenyloxazole, and 0.01% 1,4-bis-[2-(4-methyl-5-pheyloxazolyl)] benzene and 1 part of Triton X-100 (TPP-TX). Brain was homogenized in seven volumes of water and aliquots added directly to TPP-TX. Radioactivity was quantitated by liquid scintillation spectrometry with external standarization.

Regional distribution of radioactivity was studied in the brain areas (medulla, cerebellum, hypothalamus, corpus striatum, midbrain, hippocampus and cortex) as defined by Glowinski and Iversen [13]. Animals were injected IV with the radiolabeled cannabinoid (1 mg/kg), and decapitated 5 min later. The brain areas (75–150 mg) were oxidized in a Packard Tri-Carb sample oxidizer (recovery > 95%) and radioactivity counted by liquid scintillation spectrometry.

Extraction and TLC Analysis of Radioactivity

Radioactivity was extracted from aliquots (6 ml) of the brain homogenate and from 1 ml of plasma using a modified procedure of the method of Schoolar *et al.* [27]. Approximately 90% of the radioactivity was extracted from plasma and brain from all treatment groups. Modifications of the methodology included extracting twice in 100% methanol and the combined extracts, after centrifugation, were stored overnight at −15°. The cold extracts were centrifuged, and the methanol was transferred to a flask and evaporated to dryness in a rotatory evaporator (Büchi Rotavapor). Five ml of chloroform were added to the brain extracts before evaporation to minimized frothing of the samples. The residue was dissolved in 1.5 ml of chloroform-methanol (2:1), followed by 2.0 ml methanol-water (1:1). Aliquots (100 μl) of the extracts were applied as a band on 5×10 cm silica gel F-60 plates (Merck, 0.25 mm thickness) along with 5 μg of the appropriate cannabinoid standard, and the plates were developed in a petroleum ether (40–60°)-diethyl ether (70:30, v/v) solvent system. The reference compounds were visualized by spraying with 0.1% Fast Blue B salt in 1 N sodium hydroxide. The plates were divided into 5 bands with band 1 as the origin and band 3 corresponding to the unchanged cannabinoids. Band 5 was above the solvent front which served as the blank for each plate. The bands were scraped into scintillation vials containing 1.0 ml ethanol and allowed to stand 10 minutes before addition of scintillation fluid (TPP-TX).

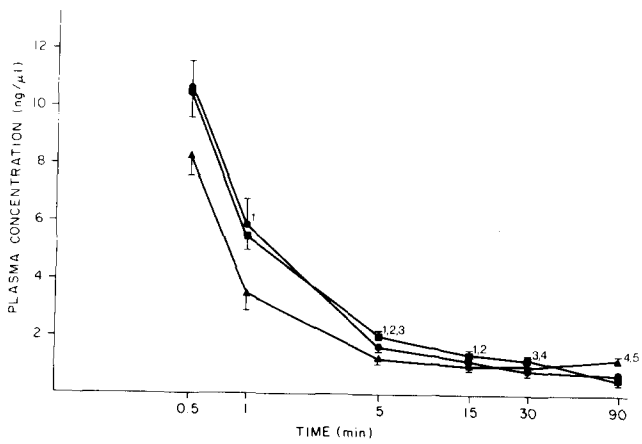

FIG. 1. Time-course of radioactivity in rat plasma following IV injection of ^{3}H-Δ^9-THC, ^{3}H-CBD or ^{3}H-CBN (1 mg/kg). Radioactivity is expressed as ng/μl THC (●), CBD (▲), CBN (■) and/or metabolites (mean ± SE, n=4).
(1) THC>CBD, $p<0.05$; (2) CBN>CBD, $p<0.05$; (3) CBN>THC, $p<0.05$; (4) CBD>THC, $p<0.05$; (5) CBD>CBN ($p<0.05$); Analysis by Duncan's Multirange Test

Pharmacokinetic Interaction of ^{3}H-Δ^9-THC and CBD

Five groups of male rats, 4 animals per group, were administered ^{3}H-Δ^9-THC (10 μCi/mg/kg, IV) that had been mixed with either Δ^9-THC (1 or 5 mg/kg), CBD (1 or 5 mg/kg) or saline. Five minutes following the administration of the drug-drug or drug-saline mixture, the animals were sacrificed by decapitation and blood collected. Radioactivity was quantitated in plasma and in brain, as described above. The Dunnett's *t*-test was used to analyze the data.

RESULTS

The time course of radioactivity in plasma, presented in Fig. 1, was similar for all three cannabinoids. In all cases, radioactivity declined rapidly for the first 5 min and much more slowly for the next 85 min. However, there were some significant differences among the plasma concentrations of cannabinoids at the various time intervals indicated in Fig. 1. The initial concentration of H-CBD radioactivity were somewhat less than those of ^{3}H-CBN and ^{3}H-Δ^9-THC, but after 90 min ^{3}H-CBD concentrations of radioactivity were significantly higher than those of ^{3}H-CBN and ^{3}H-Δ^9-THC. Generally, the ^{3}H-Δ^9-THC and ^{3}H-CBN radioactivity was similar at all time-points investigated.

In order to distinguish between parent compound and metabolites, radioactivity was extracted from plasma samples and analyzed by co-chromatography with cannabinoid standards on thin-layer plates. The data in Fig. 2 show that the sojourn in plasma was similar for all parent compounds. After 30 sec 75–80% of the radioactivity was due to parent compound, but by 5 min approximately 80% of the radioactivity in all treatment groups had been converted to metabolites (TLC bands 1 and 2). The plasma concentration of the

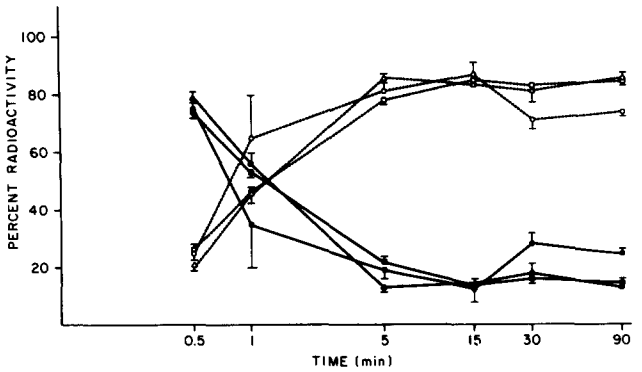

FIG. 2. Percentage of radioactivity due to parent compounds and their metabolites (TLC bands 1 and 2) in plasma after IV injection of 1 mg/kg or ^{3}H-Δ⁹-THC, ^{3}H-CBD or ^{3}H-CBN to male rats. Results expressed as mean ± SE, n=3. ●——● Δ⁹-THC; ▲——▲ CBD; ■——■ CBN; ○——○ Δ⁹-THC metabolites; △——△ CBD metabolites; □——□ CBN metapolites.

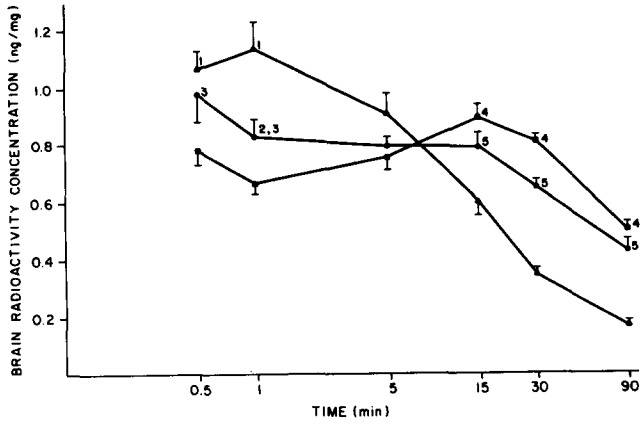

FIG. 3. Time-course of radioactivity in rat brain after IV injection of 1 mg/kg ^{3}H-Δ⁹-THC, ^{3}H-CBD and ^{3}H-CBN. Radioactivity is expressed a ng/g THC (●), CBD (▲), CBN (■) and/or metabolites (mean ± SE, n=4).
(1) CBD >CBN, $p<0.01$; (2) CBD>Δ⁹-THC, $p<0.05$; (3) Δ⁹-THC>CBN, $p<0.05$; (4) CBN>CBD, $p<0.01$; (5) Δ⁹-THC>CBD; $p<0.05$; Analysis by Duncan's Multirange Test

three cannabinoids remained relatively constant between 5 and 90 min.

All the cannabinoids penetrated the brain rapidly after IV administration, with the maximum concentration of radioactivity occurring by 0.5 min (Fig. 3). Brain radioactivity in the ^{3}H-CBD group was significantly higher than those in the ^{3}H-Δ⁹-THC and ^{3}H-CBN groups at both 0.5 and 1.0 min.; however, the ^{3}H-CBD radioactivity declined more rapidly than those of ^{3}H-Δ⁹-THC and ^{3}H-CBN. The initial brain levels of radioactivity in the ^{3}H-Δ⁹-THC and ^{3}H-CBN treated animals were sustained up to 15 min after which they dropped constantly with time. The ease with which ^{3}H-CBD and its metabolites penetrated brain is illustrated by the brain-plasma ratios of radioactivity. The brain-plasma ratios for ^{3}H-CBD radioactivity were significantly higher ($p<0.01$) than those for ^{3}H-Δ⁹-THC and ^{3}H-CBN at 0.5, 1 and 5 min. The ^{3}H-CBD brain-plasma ratios (0.7) peaked at 5 min;

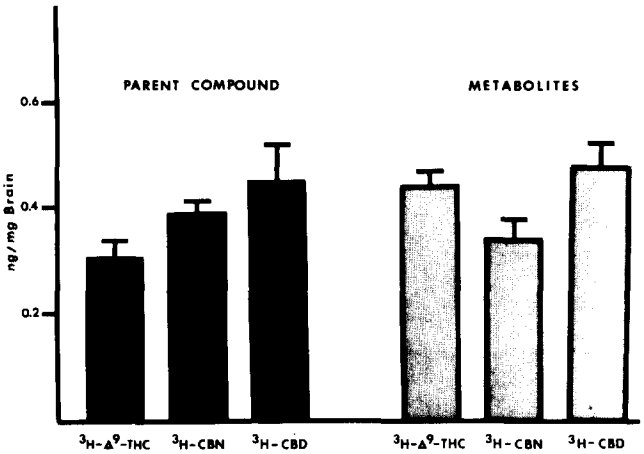

FIG. 4. The concentration of ^{3}H-Δ⁹-THC, ^{3}H-CBN, ^{3}H-CBD and their metabolites in brain 5 min after drug administration. The results are presented as means ± SE, n=3.

TABLE 1
REGIONAL LOCALIZATION OF RADIOACTIVITY THROUGHOUT THE BRAIN OF RATS*†

Brain parts	^{3}H-Δ⁹-THC	^{3}H-CBD	^{3}H-CBN
Hypothalamus	0.96 ± 0.09‡	0.92 ± 0.14‡	0.62 ± 0.06
Cortex	0.95 ± 0.10	1.06 ± 0.13	0.85 ± 0.07
Cerebellum	0.91 ± 0.09	1.05 ± 0.14	0.75 ± 0.07
Midbrain	0.89 ± 0.06	1.05 ± 0.15	0.76 ± 0.05
Corpus striatum	0.87 ± 0.06	0.96 ± 0.12	0.69 ± 0.05
Medulla	0.83 ± 0.03	0.99 ± 0.13	0.70 ± 0.06
Hippocampus	0.72 ± 0.05	0.89 ± 0.14	0.64 ± 0.04

*Results are expressed as mean ± SE of the parent compound plus metabolites (ng/mg tissue).
†Four rats received 1 mg/kg (IV) of ^{3}H-Δ⁹-THC, ^{3}H-CBD or ^{3}H-CBN and were sacrificed after 5 min.
‡Significantly greater than ^{3}H-CBN ($p<0.05$) by Duncan's Multirange Test.

whereas, those of ^{3}H-Δ⁹-THC (0.8) and ^{3}H-CBN (0.7) peaked at 30 min.

To determine the quantity of parent compound present in brain, the radioactivity in the brain homogenates at 5 min (the time when brain levels of radioactivity did not differ significantly among the groups) was extracted and chromatographed on TLC. Approximately 85% of the radioactivity was removed from the brain homogenates of all treatment groups. The brain concentration of the parent compounds, as well as their metabolites, are presented in Fig. 4. Both ^{3}H-CBD and ^{3}H-CBN were present in higher concentrations than ^{3}H-Δ⁹-THC. The total quantity of metabolites of ^{3}H-CBN was somewhat less than that of either ^{3}H-Δ⁹-THC or ^{3}H-CBD. At this 5 min time point, the brain-to-plasma ratio for ^{3}H-Δ⁹-THC, ^{3}H-CBM and ^{3}H-CBD were 0.96, 0.88 and 2.61, respectively.

The regional localization of total radioactivity in brain areas 5 min after the IV administration of ^{3}H-Δ⁹-THC, ^{3}H-CBD and ^{3}H-CBN is presented in Table 1. The radioactivity in the ^{3}H-Δ⁹-THC-treated group was rather evenly distributed throughout the brain, although somewhat lower levels were found in medulla and hippocampus. The brain

TABLE 2

BRAIN AND PLASMA CONCENTRATION OF RADIOACTIVITY 5 MIN AFTER THE COADMINISTRATION OF ^{3}H-Δ^9-THC WITH EITHER Δ^9-THC OR CBD

Tissues	Tissue concentration of radioactivity* Δ^9-THC†	Δ^9-THC‡	CBD†	CBD‡	Saline
Brain	±0.04	±0.05	±0.02	±0.07	±0.08
	0.80	0.87	0.81	0.79	0.81
Plasma	±0.09	±0.08	±0.04	±0.90	±0.08
	1.38	1.58	1.37	1.44	1.39

*Data calculated as ^{3}H-Δ^9-THC plus metabolites (ng/ml or μl) by dividing the radioactivity by the specific activity of ^{3}H-Δ^9-THC in the saline group. Means ± SE (N=4) are presented.

All treatment groups were compared to the saline group using the Dunnett's *t*-test.

†1 mg/kg.

‡5 mg/kg.

distribution of radioactivity in the ^{3}H-CBD and ^{3}H-CBN groups were similar to that of ^{3}H-Δ^9-THC, the only exception being the ^{3}H-CBN group that contained a significantly lower concentration of radioactivity in the hypothalamus than did the other groups.

Effect of CBD on the tissue concentration of radioactivity when administered jointly with ^{3}H-Δ^9-THC (1 mg/kg) is shown in Table 2. Δ^9-THC (1 mg/kg and 5 mg/kg) was administered jointly with the ^{3}H-Δ^9-THC to serve as an internal standard. The results did not show any difference in the concentration of radioactivity in plasma when either unlabeled Δ^9-THC or CBD was administered with ^{3}H-Δ^9-THC. Also, the quantity of radioactivity that penetrated brain was not diminished by the presence of Δ^9-THC or CBD.

DISCUSSION

The primary objective of this study was to provide pharmacokinetic evidence for some of the differences in potency observed for behavioral activity among the cannabinoids. It has been reported that the onset and duration of the biological effects of the cannabinoids were determined by the route of administration [1,14]. Carney *et al.* [7] have suggested that the increases in the relative potency between the IVT and IP routes of administration in the squirrel monkey may be due to differences in the penetrability of the cannabinoids into the brain.

In the present study rats were given a behaviorally inactive dose of CBD or CBN and a behaviorally effective dose of Δ^9-THC [26] to compare their penetration into the brain. The concentrations of radioactivity in plasma were similar for all three cannabinoids throughout the time course, although the initial levels of radioactivity after ^{3}H-CBD were somewhat lower than those after ^{3}H-CBN and ^{3}H-Δ^9-THC. The plasma concentration of the parent compounds (Fig. 2) were similar for the entire time course suggesting little difference in the pharmacokinetics of the cannabinoids.

The brain levels of radioactivity did differ somewhat depending upon the cannabinoid, but there were no indications that ^{3}H-CBD, ^{3}H-CBN and their metabolites did not readily enter brain. Actually, ^{3}H-CBD radioactivity penetrated brain to a greater extent than did that of ^{3}H-Δ^9-THC. The brain concentration of unchanged ^{3}H-Δ^9-THC, ^{3}H-CBN and ^{3}H-CBD were measured at 5 min, a time when the total brain radioactivity did not differ significantly among the groups. Both ^{3}H-CBD and ^{3}H-CBN levels were higher than that of ^{3}H-Δ^9-THC. At this time point ^{3}H-Δ^9-THC has behavioral activity in rats (gross observation), mice [8] and dogs [2]; whereas, CBN and CBD produce little or no effect.

Even though all the cannabinoids penetrate the brain easily, they may have different potencies as a result of differences in their distribution throughout the brain. Therefore, the regional localization of the cannabinoids was studied 5 min after their administration. In all the brain areas analyzed for radioactivity, there were few differences in the concentrations among the treatment groups. It is evident from this study that the cannabinoids, irrespective of their activity, do penetrate the brain and are almost equally distributed to the various brain areas.

Several authors have suggested that the activity of Δ^9-THC may be altered by the presence of CBD [6,18]. Several mechanisms of CBD interaction with Δ^9-THC have been postulated, including acting as a partial agonist or competitive antagonist of Δ^9-THC at a common site of action in the CNS [5]. The suggestion that the mechanism of interaction could be attributed either to a functional origin within the CNS [19] or an alteration in their disposition [12] has not been corroborated by the results of the present study. Coadministration of two dosage levels of CBD with ^{3}H-Δ^9-THC did not influence the penetration of radioactivity into the brain or alter plasma levels. It is possible, therefore, that the dose and the route of administration might be a factor in the interaction of CBD and Δ^9-THC, as shown by previous studies pointing to such interactions.

On the basis of the data presented in this manuscript, it does not appear that pharmacokinetic factors are responsible for the potency differences of the cannabinoids. It remains to be established whether or not there is a correlation between pharmacodynamic factors and behavioral activity of cannabinoids.

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