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Biochemical Pharmacology, Vol. 29, pp. 462–464 0006-2952/80/0201-0462 $02.00/0

Characterization of blood disappearance and tissue distribution of [^{3}H]cannabidiol

(*Received* 8 *January* 1979; *accepted* 14 *August* 1979)

Although cannabidiol (CBD), which is generally present in cannabis, does not possess the psychoactivity of Δ^9-tetrahydrocannabinol (THC), it is not devoid of pharmacological activity. For example, CBD can inhibit hepatic drug metabolism [1–4] and also possesses anticonvulsant properties [5, 6]. In view of the common use of marihuana, it is important to establish the time course during which an acute dose of CBD may be expected to exert its effects. Since the pharmacokinetics of CBD have not been examined, these studies have evaluated the kinetics of cannabidiol-1′,2′-[^{3}H] ([^{3}H]-CBD) disappearance from the blood of rats following intravenous (i.v.) and intragastric (i.g.) administration. The i.v. route of injection permitted an assessment of the rate of drug distribution, an observation not directly afforded by i.g. treatment. Since our previous studies [7] had demonstrated that tritiated water was an apparent product of the metabolism of Δ^9-tetrahydrocannabinol-1′,2′-[^{3}H] ([^{3}H]THC), which could modify significantly the estimate of the rate of disappearance of THC metabolites, these experiments also evaluated the possibility that tritiated water was produced from [^{3}H]CBD.

Furthermore, in a previous study [3] we observed that pentobarbital-induced sleeping time was prolonged significantly in rats at 21.5, 40 and 63 hr following an acute dose of CBD (23.4 mg/kg) administered i.g. in a marihuana extract. Our experiments suggested that this effect resulted from an inhibition of hepatic pentobarbital metabolism by CBD. This conclusion was consistent with reports by Paton and Pertwee [1] and Fernandes *et al.* [2] that CBD inhibited the metabolism of phenazone and hexobarbital, respectively. However, a direct relationship between the long-lasting inhibition of pentobarbital metabolism and a prolonged presence of unchanged CBD and/or its metabolites in the liver had not been established. We determined [3] that the CBD-induced prolongation of sleep was not due to an increase in the sensitivity of the brain to pentobarbital. In fact, animals which had received CBD, 21.5 hr before pentobarbital injection, recovered the righting reflex at higher blood pentobarbital concentrations compared to vehicle-treated controls, suggesting that CBD decreased the sensitivity of the brain to pentobarbital. Whether the latter effect was associated with the brain levels of CBD and/or its metabolites had also not been explored. Accordingly, the experiments described below determined the blood and organ concentrations of [^{3}H]CBD and its metabolites at the time points studies previously [3].

Male Wistar rats, 215–235 g, were housed individually in stainless steel hanging cages, with Teklad pellets and water available *ad lib.* throughout all experiments. [^{3}H]CBD and unlabeled CBD, each having a purity > 98 per cent, were supplied by NIDA through the courtesy of Dr. M. C. Braude.

The first experiment, initiated at 9:00 a.m., was carried out to characterize the disappearance of [^{3}H]CBD and total ^{3}H from the blood during the first 24 hr following drug administration. In one group of rats (N = 8), [^{3}H]CBD sp. act. 3.1 μCi/mg) was administered i.g. in olive oil solution (4 ml/kg) at a dose of 23.4 mg/kg. This dose had been used in the earlier CBD/pentobarbital interaction study [3]. Another group (N = 8) received [^{3}H]CBD (sp. act. 7.5 μCi/mg) in a 2% Tween-80–saline suspension (1 ml/kg) at a dose of 4 mg/kg as an i.v. bolus injection [3]. The 4 mg/kg dose of CBD was selected to be consistent with other cannabinoid interaction studies to be published elsewhere.

Tail tip blood samples were collected serially with heparinized micropipets beginning at 1 hr following i.g. administration, and at 1 min after i.v. injection of [^{3}H]CBD, and continuing for 24 hr. Two 50 μl blood samples were transferred to paper Combusto-Cones (Packard Instrument Co., Downers Grove, IL) for oxidation in a Packard, model 306, Sample Oxidizer in preparation for liquid scintillation counting (Searle Analytic, Isocap 300). One of these blood samples was oxidized immediately, but the other was air dried before analysis. The third blood sample (100 μl) was immediately mixed with 100 μl of sodium citrate solution (3.8%,w/v) and was extracted with 2 ml *n*-heptane to remove unchanged [^{3}H]CBD for liquid scintillation spectrometry, as described previously [8]. In pilot experiments, thin-layer chromatography and gas chromatography-mass spectrometry (Finnigan, 1015D, 6100 MS Data System) confirmed that > 95 per cent of the radioactivity present in *n*-heptane extracts of blood collected from rats 1–6 hr after i.v. [^{3}H]CBD treatment was unchanged [^{3}H]CBD; that is, *n*-heptane extracted only trace amounts of [^{3}H]CBD metabolites. Also in preliminary experiments, 97–99 per cent of [^{3}H]CBD added to blood was extracted by the procedure. Therefore, all [^{3}H]CBD concentration data were corrected to 100 per cent, using a 98 per cent recovery factor.

The second experiment was designed to evaluate the disappearance of [^{3}H]CBD and total ^{3}H from the blood, liver and brain from 21.5 to 84 hr after [^{3}H]CBD (23.4 mg/kg, i.g.) administration, a time period which coincided with that studied previously [3]. The time of drug treatment was selected so that all animals were killed between 9:00 and 10:00 a.m. Upon decapitation at 21.5, 40, 63 and 84 hr (N = 8/time), three neck blood samples were collected and analyzed, as described above. The brain and liver were removed immediately and placed on ice. Two small segments (approximately 100–150 mg) were dissected at random sites from each organ. Fresh weights were taken, and one segment of each organ was oxidized immediately. The other was lyophilized before analysis for ^{3}H.

The remainder of the fresh brain and approximately 1 g of each liver were assayed for unchanged [^{3}H]CBD. The tissues were homogenized in 2 vol. of 0.10 M phosphate buffer, pH 7.0, and were extracted twice with 3 ml of *n*-heptane each time. The extracts were assayed for ^{3}H [8]. Pilot experiments showed that > 90 per cent of the radioactivity present in *n*-heptane extracts of organs of rats that had been treated i.g. with [^{3}H]CBD, 24 hr earlier, was unchanged [^{3}H]CBD. Furthermore, the procedure

extracted 85–90 and 87–91 per cent of the [^{3}H]CBD which was added to brain and liver tissue respectively. The [^{3}H]CBD concentrations in each of the organs were corrected to 100 per cent using the respective median extractability values.

The organization and statistical analyses of the drug concentration data were carried out using the PROPHET System, a national computer resource sponsored by the National Institutes of Health. Pharmacokinetic analyses of the disappearance of unchanged [^{3}H]CBD and total ^{3}H from the blood and organs were carried out according to Gibaldi and Perrier [9].

Intragastric administration of [^{3}H]CBD resulted in a rapid appearance of unchanged [^{3}H]CBD in the blood, with a maximum concentration occuring at 2 hr (Fig. 1). The $T_{1/2}$ ($0.693/\beta$) for the disappearance of unchanged [^{3}H]CBD, based on the terminal portion of the calculated curve, was 11.2 hr.

Maximum concentrations of total ^{3}H (expressed as μg of [^{3}H]CBD equivalents/ml) were not reached until 4–6 hr whether the blood was analyzed fresh or after drying (Fig. 1). Thereafter, the rate of disappearance of ^{3}H was much greater when based upon the analysis of dried blood ($T_{1/2}$ = 5.3 hr relative to fresh blood ($T_{1/2}$ = 12.3 hr). These results are closely comparable to those observed previously for [^{3}H]THC administered i.g. [7,10].

The disappearance of unchanged [^{3}H]CBD from the blood following i.v. injection was described by a multiexponential function (Fig. 2). The initial distribution phase was very rapid ($T_{1/2}$ = 2.0 min), whereas the terminal disappearance from the blood occurred much more slowly ($T_{1/2}$ = 10.9 hr), in agreement with the rate observed after i.g. treatment. Thus, the rate of dispositon for CBD was about two times greater than that reported previously for THC in the rat [11].

The apparent volume of distribution of unchanged [^{3}H]CBD [dose/AUCβ, where AUC is the total area under the drug concentration–time (extrapolated to ∞) curve] was 41.1 l/kg, and the volume of the central compartment [dose/Co, where Co is the calculated zero time blood concentration] was 0.4 l/kg, suggesting that CBD was concentrated in tissues other than the blood. This is consonant with the well-known lipid solubility of the cannabinoids and the previous observation that THC accumulates in fat [12].

Consistent with the i.g. experiment, the disappearance

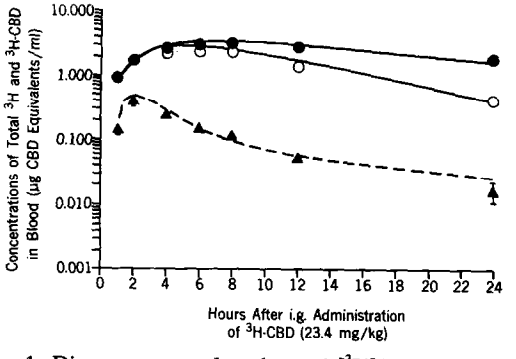

Fig. 1. Disappearance of unchanged [^{3}H]CBD and total ^{3}H based on the analysis of fresh and dried blood during the first 24 hr after intragastric [^{3}H]CBD administration. Key: (▲) unchanged [^{3}H]CBD in fresh blood; (●) total ^{3}H in fresh blood; and (○) total ^{3}H in dried blood. The lines represent the weighted (1/variance) least squares best fits and are described by the following functions:

(▲): $y = -4.72e^{-1.554x} + 1.70e^{-0.52x} + 0.12e^{-0.062x}$

(●): $y = -7.61e^{-0.284x} + 6.79e^{-0.056x}$

(○): $y = -11.51e^{-0.321x} + 10.31e^{-0.134x}$.

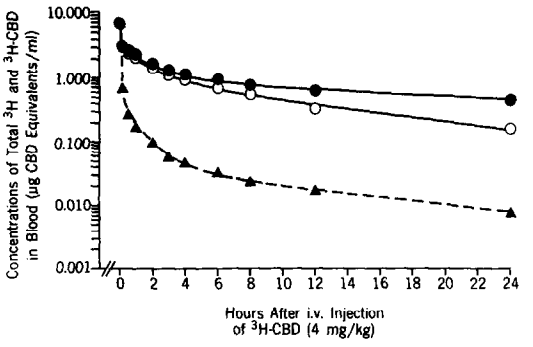

Fig. 2. Disappearance of unchanged [^{3}H]CBD and total ^{3}H based on the analysis of fresh and dried blood during the first 24 hr after intravenous [^{3}H]CBD administration. Symbols are defined in Fig. 1. The lines represent the weighted (1/variance) least squares best fits and are described by the following functions:

(▲): $y = 9.17e^{-20.975x} + 0.29e^{-0.982x} + 0.07e^{-0.518x} + 0.04e^{-0.064x}$

(●): $y = 5.44e^{-16.995x} + 1.96e^{-0.505x} + 0.94e^{-0.028x}$

(○): $y = 5.20e^{-13.444x} + 1.83e^{-0563x} + 0.96e^{-0.074x}$.

of total ^{3}H was more rapid when based on the analysis of dried blood ($T_{1/2}$ = 9.4 hr) in comparison to fresh blood ($T_{1/2}$ = 25.0 hr). The reason for the slower disappearance of total ^{3}H from both dried and fresh blood after i.v. compared to i.g. treatment is not clear, but could be related to a more prominent first pass effect in the liver and/or metabolism in the gut following i.g. administration.

In the second experiment, in which the disappearance of [^{3}H]CBD was studied over a period of 84 hr after i.g. treatment, unchanged [^{3}H]CBD was present in neck blood at a concentration of 20.2 ± 5.0 (S.E.) ng/ml at 21.5 hr but

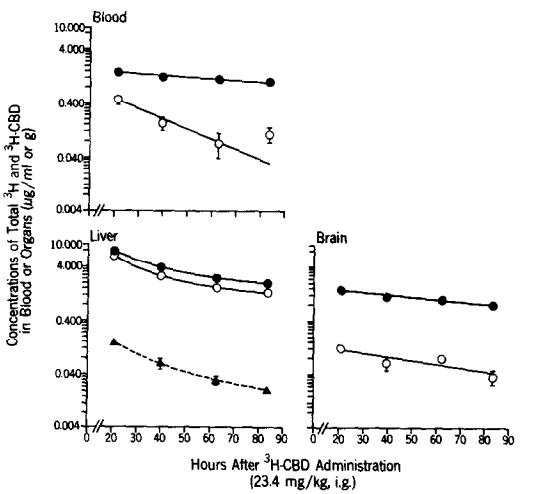

Fig. 3. Disappearance of unchanged [^{3}H]CBD and total ^{3}H (assayed in fresh and dried tissues) from the blood and organs after intragastric administration of [^{3}H]CBD. Key: (▲) unchanged [^{3}H]CBD; (●) total ^{3}H in fresh blood or organs; and (○) total ^{3}H in dried blood or organs. The lines represent the unweighted least squares best fits and are described by the following functions:

Blood, (●): $y = 1.78e^{-0.007x}$

(○): $y = 1.18e^{-0.043x}$

Brain, (●): $y = 1.85e^{-0.010x}$

(○): $y = 0.16e^{-0.016x}$

Liver, (▲): $y = 0.55e^{-0.082x} + 0.07e^{-0.015x}$

(●): $y = 17.80e^{-0.059x} + 2.37e^{0.004x}$

(○): $y = 23.55e^{-0.086x} + 2.55e^{-0.008x}$

could not be detected at 40–84 hr. This observation is consistent with the previously determined 11-hr half-life for this cannabinoid. The concentration of total ^3H in the blood, which was analyzed without initial drying, declined very slowly from 1.6 μg/ml at 21.5 hr to 1.1 μg/ml at 84 hr (Fig. 3). In contrast, ^3H levels in dried blood samples were only about one-third of the concentration present in fresh samples at 21.5 hr and decreased to negligible levels at 63 and 84 hr. ^3H could not be detected in the dry blood of four of the eight animals studied at 63 hr and in three of the eight rats at 84 hr.

Although a low level (6.4 ± 1.1 ng/g) of unchanged [^3H]CBD was detected in the brain at 21.5 hr, the unchanged drug had disappeared by 40 hr. However, unchanged [^3H]CBD was still present in the liver (20.8 ± 2.9 ng/g) at 84 hr, the liver having much higher drug levels than the blood and brain at all time points (Fig. 3). Drying the tissue samples before analysis clearly decreased the concentrations of ^3H in the brain and liver throughout the 84-hr experiment. However, metabolites were present in both organs up to 84 hr.

It is likely that the hepatic concentrations of CBD plus its metabolites, which were 50- to 30-fold higher than those in the brain from 21.5 to 63 hr, were sufficient to cause the previously reported [3] inhibition of pentobarbital metabolism. In support of this conclusion, the inhibition of pentobarbital metabolism *in vitro* was 2.5 times greater at 21.5 hr than at 63 hr [3], in close correlation with a 3-fold decrease in the hepatic concentration of CBD metabolites over the same time interval in the present study. Since the K_i of CBD for the inhibition of hepatic pentobarbital metabolism was found to be about 90 μM [3], it is not likely that the concentrations of unchanged CBD in the liver (<0.5 μM), as determined in this study, could have accounted for the inhibitory effect alone. Karler *et al.* [13] have also concluded recently that metabolites of CBD, rather than the parent cannabinoid, were responsible for prolonging hexobarbital-induced sleep in mice by means of a metabolic mechanism.

Furthermore, since [^3H]CBD was not detected in the brain from 40 to 84 hr, the previously observed [3] prolongation of pentobarbital-induced sleep (up to 63 hr following CBD treatment) could not have been dependent upon the presence of the unchanged cannabinoid in the brain. However, it is possible that the amounts of CBD and/or its metabolites present in the brain at 21.5 hr could have been responsible for an apparent decrease in the brain sensitivity to pentobarbital [3]. Alternatively, acute tolerance to pentobarbital may have developed, irrespective of cannabinoid brain levels, in the 21.5-hr, CBD-treated animals during their prolonged duration of sleep (53 per cent longer than controls [3].

The more rapid disappearance of total ^3H from dried compared to fresh blood and organs is consistent with previously observed absorption and disappearance characteristics of [^3H]THC and its metabolites in the rat [7,10]. As determined for [^3H]THC, the higher ^3H concentrations in fresh relative to dried tissues were probably due to the formation of tritiated water during the metabolic hydroxylation of the pentyl side chain of CBD-1′,2′-[^3H]. Hydroxylation of CBD at the 1′ and 2′ positions has been observed in rat [14] and mouse [15] liver. Since the rate of body water turnover in the rat is about 7–10 per cent of the total body water per day [16], the formation of tritiated water from ^3H-labeled cannabinoids, such as [^3H]CBD and [^3H]THC, could contribute substantially to the apparent slow rate of disappearance of these compounds from the body.

Acknowledgements—The authors are grateful to Mrs. Olivia L. Doyle and Mr. Patrick George for their excellent technical assistance and to Dr. Hebe B Greizerstein for g.c.-m.s. analyses. The study was supported in part by USPHS/NIDA Grant 2R01 DA01136.

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