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Plasma protein binding of 7-hydroxy-Δ^1-tetrahydrocannabinol: an active Δ^1-tetrahydrocannabinol metabolite*

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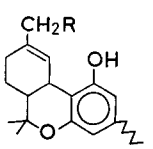
7-Hydroxy-Δ^1-tetrahydrocannabinol, which is a pharmacologically and psychologically active metabolite of Δ^1-tetrahydrocannabinol in man, has been shown by equilibrium dialysis and ultrafiltration to be bound 94–99% to plasma proteins. Further experiments, using the [^{14}C]labelled compound, with agarose and polyacrylamide gel electrophoresis and ultracentrifugation suggest that albumin, α_1-lipoprotein and, to a minor degree, also β-lipoprotein are involved in the protein binding of 7-hydroxy-Δ^1-tetrahydrocannabinol in blood plasma.

Δ^1-Tetrahydrocannabinol (Δ^1-THC; Ia) is the major psychotomimetically active compound of cannabis (Mechoulam, Shani & others, 1970).

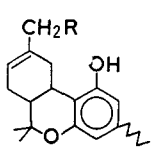
It has been shown by us (Nilsson, Agurell & others, 1970) and others (Wall, Brine & others, 1970; Foltz, Fentiman & others, 1970; Burstein, Menezes & others, 1970) that both Δ^1-THC† and its $\Delta^{1(6)}$-isomer‡ (II a) are primarily metabolized by oxidation to the corresponding 7-hydroxy derivatives (Ib and IIb). These metabolites are then further transformed (Agurell, Nilsson & others, 1970; Burstein, Rosenfeld & Wittstruck, 1972). Indications that the metabolite 7-hydroxy-Δ^1-THC (I b),§ which occurs at detectable levels in man (Lemberger, Axelrod & Kopin, 1971), contributes substantially to the pharmacological and psychological effects of Δ^1-THC have been verified by intravenous injection of 7-hydroxy-Δ^1-THC in man (Lemberger, Crabtree & Rowe, 1972; Perez-Reyes, Timmons & others, 1972).

Earlier we have shown that Δ^1-THC is extensively bound to the lipoproteins of human blood plasma (Wahlqvist, Nilsson & others, 1970) and this conclusion is supported by the work of Klausner, Wilcox & Dingell (1971).

In the present paper we have studied the binding of [^{14}C]7-hydroxy-Δ^1-THC to human plasma proteins *in vitro* using different electrophoretic techniques, ultracentrifugation, equilibrium dialysis and ultrafiltration.



Ia. R=H Δ^1-THC
Ib. R=OH



IIa. R=H $\Delta^{1(6)}$-THC
IIb. R=OH

* Metabolism of cannabis. Part X

Also known as † Δ^9-THC,‡ Δ^8-THC and §11-hydroxy-Δ^9-THC when using the dibenzopyran numbering system.

MATERIALS AND METHODS

[^{14}C]7-Hydroxy-Δ^1-tetrahydrocannabinol (specific activity 3 μCi mg^{-1}) was prepared by microsomal oxidation of [^{14}C]Δ^1-THC. The metabolite was purified by column and thin-layer chromatography (Nilsson & others, 1970) and was according to its specific activity over 95% pure.

[^{14}C]7-Hydroxy-Δ^1-THC was incubated with fresh heparinized human plasma and purified human serum albumin (Kabi Human albumin) at 37° for 1 h. 7-Hydroxy-Δ^1-THC dissolved in a small amount of propylene glycol incubated with phosphate buffer pH 7·4, 0·1M was used for control experiments.

Radioactivity determinations were made with a Packard Tricarb liquid scintillation counter Model 3375 (external standardization). Instagel (Packard) was used as liquid scintillator. Agarose and polyacrylamide gels were cut in 2 mm slices and each slice was homogenized in 1 ml of water and allowed to stand for 24 h before adding 10 ml of Instagel.

Equilibrium dialysis and ultrafiltration. The extent of binding of [^{14}C]7-hydroxy-Δ^1-THC to blood plasma proteins was determined by equilibrium dialysis at 37° (Ehrnebo, Agurell & others, 1971) and by ultrafiltration at 23° using Amicon Centriflo Ultrafiltration Membrane cones (Amicon Corp., Lexington, Mass., USA). The radioactive compound (1·5–2 μg ml^{-1}) was incubated with human plasma and as a control, with a phosphate buffer as described above. Equilibrium dialysis of 7-hydroxy-Δ^1-THC (1·5 μg ml^{-1}) in a 4·5% solution of purified human albumin in a buffer (Ehrnebo & others, 1971) was also carried out.

Agarose gel electrophoresis. Fifteen μl samples of human plasma incubated with [^{14}C]7-hydroxy-Δ^1-THC (20 μg ml^{-1}) were applied to the agarose gel. The radioactive compound was also incubated with purified human albumin (15–20 μg ml^{-1}) and samples of 15 μl were run. From each run one sample was stained for proteins and another was cut in slices for determination of radioactivity. Further experimental details including procedures for lipoprotein staining were published earlier (Wahlqvist & others, 1970).

Polyacrylamide gel electrophoresis. The electrophoresis was described by Andersson, Borg & Mikaelsson (1972). The polyacrylamide gel (Universal Scientific Ltd., London, U.K.) had a continuous concentration gradient from 4–26% (Margolis & Kenrick, 1968). The buffer consisted of 0·09 M tris, 0·003 M EDTA, 0·08 M borate, pH 8·3. Samples of 10 μl of the incubation mixture (15 μg 7-hydroxy-Δ^1-THC ml^{-1}) were applied to the gel. Three samples were run simultaneously on one plate at a temperature of 6° at 100 V for 18 h. Two samples were cut for determination of radioactivity and one sample was stained for proteins with 0·5% amido black in methanol–water–acetic acid (5:5:1) for 5 h and destained electrophoretically in 7% acetic acid for 1·5h at 6–12 V.

Electrophoresis was also carried out in a polyacrylamide gel having a concentration gradient from 15–35%.

Ultracentrifugation. Ultracentrifugation was in a Spinco model L2 ultracentrifuge with a Type 50 titanium rotor. Samples of plasma incubated with 7-hydroxy-Δ^1-THC (1·5 μg ml^{-1}) were adjusted to a density of 1·063 g ml^{-1} with a NaCl/KBr solution of density 1·354 g ml^{-1} and centrifuged at 40 000 rev min^{-1} for 20 h at 17° (Havel, Eder & Bragdon, 1955). The lipoproteins of density less than 1·063 g ml^{-1} floated and were removed—the so called low density lipoproteins or LDL.

The LDL fraction was resuspended in a NaCl/KBr solution of density 1·063 g ml^{-1} and recentrifuged. The density of the remaining plasma was adjusted to 1·21 g ml^{-1} and the high density lipoprotein fraction (HDL) was collected from the top after centrifugation at 40 000 rev min^{-1} for 24 h at 17°. The HDL was recentrifuged in a NaCl/KBr solution of density 1·21 g ml^{-1} to remove traces of albumin and other plasma proteins.

The washed LDL and HDL were tested for purity by immunoelectrophoresis (Horse anti-human serum, horse anti-human α_1-lipoprotein and horse anti-human β-lipoprotein, Behringwerke, Marburg, DBR) on agar (Scheidegger, 1955).

RESULTS AND DISCUSSION

[^{14}C]7-Hydroxy-Δ¹-THC incubated with blood plasma and dialysed against a buffer was found to be bound to 96–99% (four determinations) by equilibrium dialysis at 37°. Equilibrium dialysis using a 4·5% albumin solution at 37° showed a protein binding of 82–83% (four determinations) at the same 7-hydroxy-Δ¹-THC concentrations (1·5 μg ml^{-1}). By ultrafiltration, 94% of the compound appeared to be bound to the protein fraction. These results show that 7-hydroxy-Δ¹-THC, as previously shown for Δ¹-THC (Wahlqvist & others, 1970), is extensively bound to plasma proteins—although not necessarily only bound to albumin—and that at most a few per cent of the total amount in blood plasma is unbound and freely diffusable.

Electrophoresis in agarose and polyacrylamide gel were used to elucidate which proteins that were carriers of 7-hydroxy-Δ¹-THC. The results (Fig. 1) from the

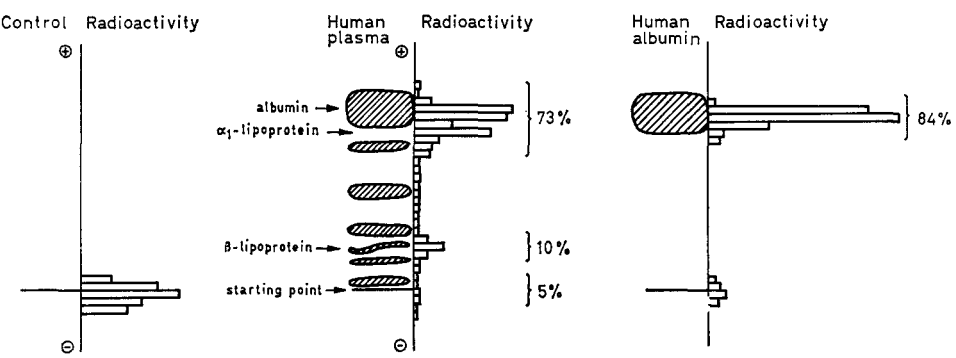


FIG. 1. Agarose gel electrophoresis of [^{14}C]7-hydroxy-Δ¹-THC incubated with plasma, albumin and phosphate buffer (control). Distribution of radioactivity shown as d min^{-1} per 2 mm gel.

agarose gel electrophoresis show that in the control experiments the radioactivity is located close to the starting point. In the plasma sample, there occur two radioactive peaks. The broad peak in the albumin area—also containing e.g. α_1-lipoprotein—contains 73–80% (four experiments) of the total radioactivity present in the gel. The small peak which coincides with the β-lipoprotein band contains 6–10% of the radioactivity and at the starting point about 5% of the activity could be recovered.

Incubation of 7-hydroxy-Δ¹-THC with purified human albumin (Fig. 1) suggested—in agreement with the dialysis experiments—that albumin is a carrier of this compound (80–85% bound in four experiments). However, the results from the agarose electrophoresis do not differentiate between how much of the radioactivity that is associated with albumin and how much that is bound to the other proteins, e.g. α_1-lipoprotein,

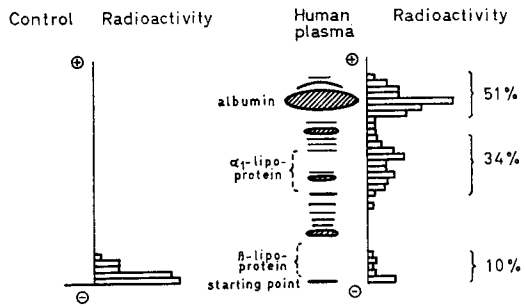


FIG. 2. Electrophoresis in polyacrylamide gel of graded porosity of [^{14}C]7-hydroxy-Δ^1-THC incubated with plasma and phosphate buffer (control). Distribution of radioactivity shown as d min^{-1} per 2 mm gel.

which are located close to the albumin band. By electrophoresis in polyacrylamide gel of graded porosity, albumin could be separated and the radioactivity associated with this protein could be determined (Fig. 2).

The results of the polyacrylamide gel electrophoresis are shown in Fig. 2. The albumin band contains about (two experiments) 50% of the total radioactivity in the gel. The broad radioactive peak present in the middle of the gel contains some 35% of the activity. Proteins located in this latter area have a molecular weight of 150 000–250 000 (Andersson & others, 1972) and α_1-lipoprotein belongs to them. Thus, α_1 lipoprotein might be another carrier of 7-hydroxy-Δ^1-THC, and this was indicated by the results of the agarose electrophoresis. About 5% of the activity is located at the starting point (Fig. 2) and is probably due to precipitated proteins. Some radioactivity also seems to be associated with β-lipoprotein.

When polyacrylamide gel electrophoresis was performed in a continuous concentration gradient from 15–35%, no activity was found ahead of the albumin band. Thus, proteins with a molecular weight less than albumin are not associated with 7-hydroxy-Δ^1-THC.

Ultracentrifugation was used to verify the indications obtained by electrophoresis that lipoproteins are carriers of 7-hydroxy-Δ^1-THC. In the LDL fraction, which contains β-lipoprotein, about 7% (two experiments) of the radioactivity in the plasma incubate was present. This supports the interpretation from the electrophoretic results.

The HDL fraction, containing α_1-lipoprotein, contained about 20% of the radioactivity. Both LDL and HDL fractions were washed and then checked immunologically for purity before determination of radioactivity. No contaminants were seen in the two fractions.

Thus, the results of the electrophoretic and ultracentrifugation experiments support the view that 7-hydroxy-Δ^1-THC is bound extensively to plasma proteins and that both albumin, α_1-lipoprotein, and to a minor extent also β-lipoprotein, are carriers of 7-hydroxy-Δ^1-THC.

Some of these experiments (equilibrium dialysis, ultracentrifugation and agarose electrophoresis) have been repeated using the isomeric [^{3}H]7-hydroxy-$\Delta^{1(6)}$-THC and with essentially identical results.

It is generally assumed that only the proportion of a compound which is in equilibrium with the unbound fraction in blood plasma, is pharmacologically active.

Since the peak levels of Δ¹-THC in man appears to be 25–50 ng ml^{-1} (Galanter, Wyatt & others, 1972) and the levels of 7-hydroxy-Δ¹-THC are even lower (*e.g.* Lemberger, Silberstein & others, 1970; Lemberger & others, 1971) the activity of the drug, on a weight basis, must indeed be remarkable. Besides depots of Δ¹-THC in *e.g.* lung, spleen and fatty tissues also the plasma proteins of the blood may serve as a depot for Δ¹-THC and its hydroxylated metabolite.

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