Biochemical Pharmacology, Vol. 19, pp. 2579–2584. Pergamon Press. 1970. Printed in Great Britain

BINDING OF Δ1-TETRAHYDROCANNABINOL TO HUMAN PLASMA PROTEINS*

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(*Received* 24 *February* 1970; *accepted* 25 *March* 1970)

Abstract—The binding of tritium-labelled Δ1-tetrahydrocannabinol to human plasma proteins *in vitro* was studied with electrophoretic techniques. Δ1-Tetrahydrocannabinol was found to be associated to 80–95 per cent with lipoproteins.

Δ1-TETRAHYDROCANNABINOL (Δ1-THC) is the major psychotomimetically active compound of Cannabis.[1, 2] However, the metabolic fate and pharmacological actions of Δ1-THC and other cannabinoid constituents of Cannabis are still relatively unknown.[3, 4] In previous papers of the present series we have studied the metabolism of Δ1-THC-^{3}H in animals.[3–5] These studies indicated that Δ1-THC might be bound to plasma proteins.[4]

The interaction between drugs and proteins are known to greatly influence the behaviour of drugs in the body. In particular the binding of drugs to plasma proteins has been extensively investigated as discussed in a recent review by Meyer and Guttman.[6] These authors also summarized the methods available to determine protein binding of drugs.

As briefly described previously,[4] ultra filtration was tested as a method to determine the protein binding of Δ1-THC-^{3}H.

The data obtained clearly indicated that Δ1-THC to a large extent was bound to plasma proteins, but the quantitative significance of the results might be uncertain, due to the low solubility of Δ1-THC in buffer solutions used as controls.

In the present paper, we have studied the protein binding of Δ1-THC-^{3}H using different electrophoretic techniques.

EXPERIMENTAL

Human blood was collected in heparinized tubes and centrifuged to obtain the plasma. The preparation of tritium-labelled Δ1-tetrahydrocannabinol (Δ1-THC-^{3}H) has been described[7] and a solution in petroleum ether was dried under a stream of

* Part VI of Metabolism of Cannabis. Part IV: *Acta Chem. Scand.* **23**, 2207 (1969). Part V: *Bull. on Narcotics* in press. Part VII: *Science* in press.

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nitrogen and redissolved in 50–100 μl of propylene glycol.[8] Δ1-THC-^{3}H (0·03–0·08 μM) was incubated with 1 ml human plasma at 37° for 1 hr. Δ1-THC-^{3}H incubated with potassium phosphate buffer, pH 7·4, 0·1 M was used as a control.

Determination of radioactivity

The liquid scintillator was prepared by dissolving 2,5-diphenyloxazole (PPO, 10g), 1,4-bis-2-(4-methyl-5-phenyloxazolyl)-benzene (Dimetyl-POPOP, 0·5 g) and naphthalene (80 g) in xylene (150 ml), 2-ethoxyethanol (450 ml) and *p*-dioxan (450 ml). Determination of radioactivity was carried out with a Packard Tricarb liquid-scintillation counter Model 3375, equipped with external standardisation for determination of counting efficiency.

Disc electrophoresis. The gel was cut in 2 mm slices with a razor which was rinsed in methanol and dried between each slice. Each segment was ground with 1 ml of water before adding 10 ml of scintillation liquid.

Agarose-gel electrophoresis. The agarose gel was cut in 2 mm slices and the radioactivity of each segment was determined by liquid scintillation counting as described above.

Agar-gel electrophoresis. The radioactivity of the agar-gel plate was determined with a Packard Radiochromatogram scanner Model 7201 using a 5 mm slit.

Disc electrophoresis. Polyacrylamide-gel electrophoresis was carried out as described for human serum proteins by Davis[9] with the modifications given by Sjöholm and Yman.[10] Samples of 10–20 μl of the incubation mixture (0·03 μM Δ1-THC-^{3}H/ml) was applied to the gel. The electrophoretic runs were carried out at 4°. When the light blue albumin front had migrated about 40 mm the electrophoresis was stopped. Several gels were run parallel. Two gels from each run were cut for determination of radioactivity as described above. One gel was stained for proteins with Coomassie Brilliant as described by Chrambach *et al.*[11] A few experiments were also made where the gel was cut longitudinally. One half of the gel was stained for proteins and the other was cut for determination of radioactivity.

Agarose-gel electrophoresis

Agarose, purchased from L'Industrie Biologique Francaise S.A., Gennevilliers (Seine) France. Buffer: Veronal buffer pH 8·6, ionic strength 0·06. For preparation of agarose plates, agarose (1 %) was melted in Veronal buffer on a boiling water bath and poured between two plane glass-plates (160 × 240 × 1 mm), separated by a U-shaped plastic frame (10 mm wide and 1 mm thick). The mould was held together tightly by paper clips.[12]

Samples of 10 μl (0·08 μM Δ1-THC-^{3}H/ml) were applied with a Hamilton syringe to 20 mm broad slits in the gel. An equipment for high-voltage electrophoresis was used (Analysteknik, Vallentuna). The agarose-gel was connected with the electrode vessels by paper bridges. The electrophoresis was run with 10–15 V/cm for 3–4 hr at 20°.

For fixation the agarose plate was placed for 10 min in a solution of protein fixative (750 ml water saturated with picric acid, 150 ml glacial acetic acid). The agarose plate was covered with filter paper, a 2-cm thick layer of cellulose wadding and a weighted glass plate. When the agarose layer was very thin the cellulose wadding was removed and the agarose plate, covered with the filterpaper, was dried in a warm stream of air. When the plate was dry, the proteins were stained for 10 min with Amido-Black

(Amido-Black 10B 10 g, methanol 800 ml, glacial acetic acid 200 ml and distilled water 1000 ml). It was rinsed in a wash-solution (methanol 1200 ml, distilled water 700 ml, glacial acetic acid 100 ml) and dried with air. From each run, one sample was stained for proteins and another was cut in 2 mm slices for determination of radioactivity.

Immunoelectrophoresis was carried out in 1% agarose according to Grabar.[13] Anti-human serum (Anti-Human-Pferde serum lyofilisiert für immunoelektrophorese) was purchased from Impfstoffproduktions und Forschungsinstitut, Budapest.

Agar-gel electrophoresis

Special agar-noble, Difco Laboratories. Buffer: Veronal buffer pH 8·6, ionic strength 0·056. For preparation of agar-plates, 8 g agar was melted in 200 ml Veronal buffer on a boiling water bath and another 200 ml of Veronal buffer was added. The solution was kept in a cold room as stockagar. This solution was melted and 20 ml was poured on a glassplate (4 × 30 cm) and cooled (4°).

For the sample application a piece of Whatman No. 3 MM filter paper (5 × 20 mm) was placed on the agar about 10 mm from the edge. The filter paper was removed after 5–10 min and a 10 μl sample (0·08 μM Δ1-THC-^{3}H/ml) was applied with a Hamilton syringe followed by two applications of 10 μl of 0·9% NaCl solution. Buffered agar blocks were used as bridges. The electrophoresis was carried out at 20° for 14–17 hr at 2·5V/cm. The radioactivity on the agar-gel plates was determined prior to fixation and staining for proteins or lipoproteins.

The agar plates were placed in a protein fixative (5% acetic acid) for 1 hr and another hour in distilled water. Drying and staining for proteins were performed as described for agarose electrophoresis. Lipoprotein staining was carried out with Sudan-Black as described by Uriel.[14]

Agar-gel electrophoresis was also carried out with samples from Δ1-THC-^{3}H (0·08 μM) incubated with 1 ml of 4·5% albumin solution (Kabi human albumin RdP 94, purified by preparative electrophoresis).

RESULTS AND DISCUSSION

Δ1-Tetrahydrocannabinol, being highly lipophilic, has very low water solubility. This was found to interfere with a determination of the protein binding by ultrafiltration.[4] Hence, we have utilized some experimental methods previously used to study the plasma protein binding of the lipophilic vitamin D.[8, 15, 16]

Δ1-THC-^{3}H was dissolved in propylene glycol[8] and incubated with human plasma (see Experimental). A suitable amount (10–20 μl) of the incubate was separated by disc electrophoresis and the distribution of radioactivity and proteins in the gel was determined. The results (Fig. 1, Table 1) show that in the control (no plasma) Δ1-THC-^{3}H is located close to the starting point. In the plasma samples there occur two additional radioactive peaks, one in the albumin area (II) and one broad peak (I) located around transferrin. These two peaks which must be attributed to protein bound Δ1-THC-^{3}H amount to 81–87 per cent of the total radioactivity.

To be able to more definitely correlate the protein bound Δ1-THC-^{3}H with certain plasma protein fractions, two other electrophoretic systems were used. Agarose electrophoresis showed (Fig. 2, Table 1), somewhat surprisingly that the major radioactive peak II, did not coincide with the albumin band. Otherwise both peak I and II were comparable in size with the disc electrophoretic results. The location of

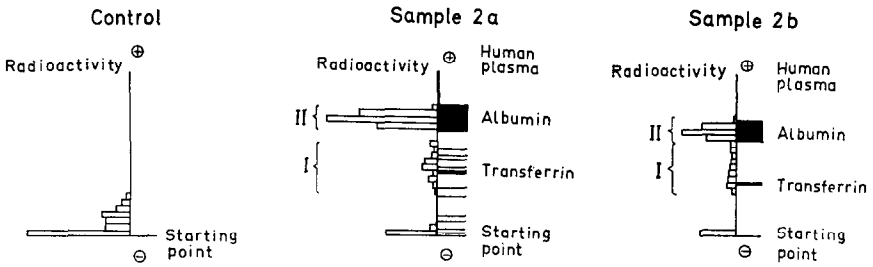

FIG. 1. Disc electrophoresis of Δ1-THC-^{3}H incubated with plasma (Sample) and with potassium phosphate buffer (Control). Sample 2a shows the protein distribution after staining. Distribution of radioactivity shown as dpm/2mm gel.

TABLE 1. DISTRIBUTION OF RADIOACTIVITY (Δ1-THC-^{3}H) IN PLASMA AFTER INCUBATION AND ELECTROPHORESIS

Electrophoretic method	Experiment no.	Radioactivity (%) Starting point	Peak I	Peak II
Disc electrophoresis	1a	19	21	60
	1b	14	28	58
	2a	13	21	66
	2b	19	28	53
Agarose electrophoresis	1	7	31	62
	2	6	29	65

the radioactive peaks in agarose electrophoresis suggested that Δ1-THC-^{3}H might be associated with lipoproteins.[17] Immunoelectrophoretic identification supported the assumption that the location of the lipoproteins coincided with the radioactive peaks I and II.

This assumption was verified by agar electrophoresis (Fig. 3). Plasma incubated with Δ1-THC-^{3}H was separated by agar electrophoresis followed by determination of radioactivity and staining of lipoproteins. The radioactivity determination was carried out by radiochromatogram scanning. Although the relatively low amount of tritium necessitated the use of a broad slit in the scanner and consequently gave a poor resolution, it is evident from Fig. 3, that the major part of the radioactivity is connected with two lipoprotein bands. Incubation of Δ1-THC-^{3}H with purified human albumin revealed that there is no radioactivity associated with the albumin band after electrophoretic separation. Thus, albumin is actually no carrier of Δ1-THC-^{3}H.

The propylene glycol used to dissolve Δ1-THC-^{3}H did not cause any noticeable change in the distribution pattern of Δ1-THC in human plasma as shown by the following experiment. Δ1-THC-^{3}H was applied as a thin film over the surface of a 2 ml beaker and incubated with human plasma at 37° for 2 hr. After electrophoretic separation in an agarose-gel a similar pattern as shown in Fig. 2 was obtained.

Thus, it has been shown that Δ1-THC-^{3}H in human plasma *in vitro* under the conditions used (temperature, pH, concentration of Δ1-THC, buffer, etc.) is bound to 80–95 per cent to lipoprotein fractions. That the results are slightly higher for agarose

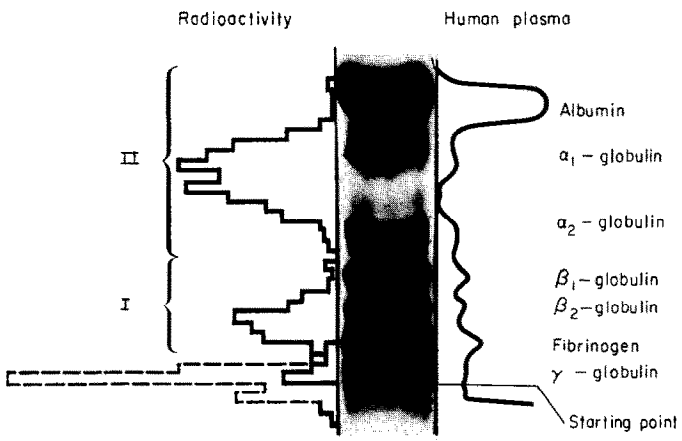

FIG. 2. Agarose-gel electrophoresis of Δ1-THC-^{3}H incubated with plasma (———) and with phosphate buffer (– – – –). To the right is shown the evaluation of colour density of gel after staining of the proteins (Analytrol provided with micro-zone scanning attachment, Beckman Instrument Inc. Palo Alto, Cal.).

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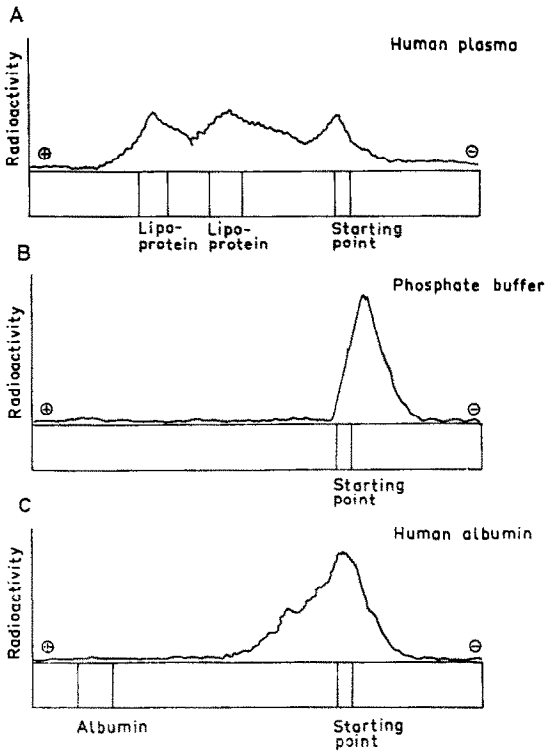

FIG. 3. Agar-gel electrophoresis of Δ1-THC-^{3}H incubated with A. human plasma, B. phosphate buffer, C. human albumin. A. stained for lipoproteins. Radioactivity determined with a radiochromatogram scanner.

than for disc electrophoresis may be due to the fact that a lipoprotein band is known to remain at the origin in disc electrophoresis.[9]

The *in vivo* situation is somewhat difficult to study, since Δ1-THC is very rapidly metabolized and metabolites of Δ1–THC are also protein bound.[3, 4] However, it is reasonable to assume that also *in vivo* in a Cannabis-smoker the psychotomimetical Δ1-THC is transported by the blood in lipoprotein bound form.

Acknowledgements—This investigation was supported by the Swedish Medical Research Council, Grant K69-13X-2724-01A/02B. For valuable discussions we are indebted to Drs. L. Yman, I. Sjöholm and F. Sjöqvist. We are grateful to Mrs. Berit Lind for her technical assistance.

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