The partitioning of Δ^1-tetrahydrocannabinol into erythrocyte membranes in vivo and its effect on membrane fluidity[1]

J.T.A. Leuschner, D.R. Wing, D.J. Harvey,[2] G.A. Brent, C.E. Dempsey, A. Watts and W.D.M. Paton

University Departments of Pharmacology and Biochemistry, South Parks Road, Oxford (England), 21 September 1983

Summary. Δ^1-Tetrahydrocannabinol (Δ^1-THC) has been quantified directly in erythrocyte membranes from drug-treated mice using gas chromatography/mass spectrometry. Concentrations of approximately 6 ng Δ^1-THC/mg membrane protein (10^{-5} M) were found when effects of the drug on behavior were prevalent. At these concentrations the drug produced a decrease in membrane order as measured by ESR.

Cannabis is one of the oldest and most widely used illicit drugs in the world, well-known for its psychoactive properties that are mainly attributed to the constituent Δ^1-tetrahydrocannabinol (Δ^1-THC). Despite recent advances in our knowledge of the chemistry and metabolism of the cannabinoids, including Δ^1-THC[3], the mechanism of action of this compound has not been elucidated. Some studies have suggested a possible interaction of Δ^1-THC with a specific receptor or, at least, with a hydrophobic site of a specific conformation. Thus minor structural alterations may produce marked variations in psychoactivity[4–7]. However, it is also known that Δ^1-THC is extremely lipophilic with a partition coefficient between octanol and water of approximately 6000 : 1[8]. On the basis of this high lipophilicity, it has been suggested that some of the actions of Δ^1-THC might be similar to the mode of action of general anaesthetics. This is compatible with the ability of Δ^1-THC to potentiate classical anesthesia with its sedative action.

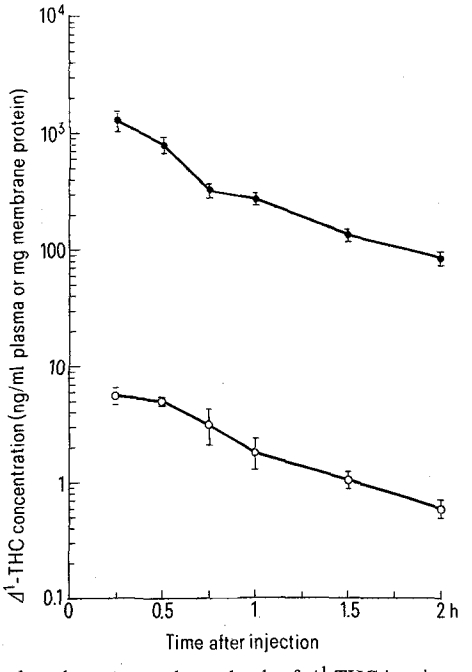
In vitro studies have shown that Δ^1-THC partitions into biological membranes[9,10] and liposomes[11] and that, in contrast to psychotomimetically inactive cannabinoids such as cannabinol, it produces an increase in fluidity of liposomes at high doses[11]. The drug, in common with lipid-soluble anesthetics[12], is also capable of protecting erythrocytes against osmotic hemolysis[13,14] again suggesting that the cell membrane is a primary site for its action. In early studies, the partitioning of Δ^1-THC was assessed using methods such as radiolabeling techniques[9,10] which often measured total cannabinoid content. The present study uses a sensitive, direct method of quantification based on gas chromatography/mass spectrometry (GC/MS) with metastable-ion monitoring[15] to measure Δ^1-THC concentrations directly in the readily-available and well-characterized erythrocyte membrane. Unlike earlier studies, this has enabled measurement of membrane levels of the drug to be made from in vivo situations such as those involving drug-induced alterations in behavior. At the low concentrations found in vivo the drug was found to increase membrane fluidity, as measured by ESR, in a manner similar to that previously found in vitro.

Methods. Male Charles-River CD-1 mice (25–30 g), maintained on a standard laboratory diet [No.41B, E. Dixon & Sons (Ware) Ltd.] were kept in a warm cage, before and after drug administration, to avoid drug-induced hypothermia. The dose of Δ^1-THC, given by tail-vein injection in human plasma (John Radcliffe Hospital, Oxford) was 10 mg/kg b.wt. Δ^1-THC was suspended in plasma by shaking the drug and carrier, in darkness, for 6 h in a 37 °C water-bath. The amount of Δ^1-THC in the plasma solution was determined by GC/MS[15] and the solution was diluted, as appropriate, with more plasma. Doses were administered in a volume of 0.01 ml/g b.wt. Control mice were injected with an equivalent volume of plasma.

At several times from 15 min to 2 h after injection, mice were killed by cervical dislocation and immediately bled from the neck. Blood was combined from at least 4 mice and was collected in heparinized tubes at 4 °C. The plasma was separated for analysis of Δ^1-THC, and erythrocyte membranes were isolated from the pellet of red blood cells by the method of Hanahan and Ekholm[16]. Erythrocyte membrane protein was assayed according to the method of Lowry et al.[17]. Membrane lipids were extracted by the method of Folch et al.[18] and their phospholipid content was estimated by assay of phosphorus according to the method of Morrison[19]. Δ^1-THC was measured in plasma by GC/MS as described earlier[15], and the same method was used for Δ^1-THC measurement in erythrocyte membranes. Samples of membrane containing approximately 2.5 mg protein were used in the assay.

Erythrocyte membranes were obtained as above from groups of 8 mice for measurements of the spin-label order parameter (S). ΔS was obtained by comparisons between control and drug-treated animals. Membranes were labelled with approximately 1 mole % of either 5- or 12-doxyl-stearic acid[20] and ESR spectra were recorded at various temperatures (table) using a nitrogen gas flow controler fitted to the cavity of a Varian E-109 ESR spectrometer. Calculations of the order parameter were based on the method of Gaffney.[21] The nature of the signal with 12-doxylstearic acid above 30 °C did not allow accurate values for S to be measured. Δ^1-THC was obtained from the National Institute on Drug Abuse (Rockville, MD., U.S.A.) and was found by GC/MS to be 98% pure, the remainder being cannabinol. All solvents were distilled twice and all glassware was silanized with a 5% solution of dichlorodimethylsilane followed by methanol.

Results and discussion. Control tests showed that the concentration of Δ^1-THC in the erythrocyte membrane did not de-



Plasma and erythrocyte membrane levels of Δ^1-THC in mice at various times after i.v. administration of 10 mg Δ^1-THC/kg in plasma. ●, ng Δ^1-THC/ml plasma; o, ng Δ^1-THC/mg membrane protein; mean values ± SEM; n = 4–7 groups of mice, with 4–8 mice/group from at least 4 separate experiments.

Concentration of Δ^1-THC and change in order parameter (ΔS) in mouse erythrocyte membranes

Exp. No.	Dose Δ^1-THC mg/kg	Time of blood sample (min)	Membrane concentration of Δ^1-THC (ng/mg protein)	ΔS Temperature of membrane 21.5°C	26.5°C	31.0°C	37.0°C
1[a] (n = 2)	3.2	30	1.57 (1.51, 1.63)	−0.0050 (−0.0040 −0.0060)			
2[a] (n = 3)	10.0	60	1.78[b] ±0.51	−0.0067 ±0.0003	−0.0174 ±0.0117	−0.0098 ±0.0030	−0.0085 ±0.0036
3[a] (n = 4)	10.0	60	2.19 ±0.12				−0.0118 ±0.0070
4[c] (n = 4)	16.0	30	5.25 ±0.12		−0.0128 ±0.0084	–	–

[a] 5-Doxyl-stearic acid probe, n refers to groups of 8 mice; [b] Mean ± SEM; [c] 12-Doxyl-stearic acid probe. No measurements of ΔS were made at the blank positions of the table.

crease with the repeated washings used in the isolation procedure. The observed values therefore represent Δ^1-THC that was strongly associated with the membranes. Plasma and erythrocyte membrane concentrations of Δ^1-THC at intervals after the i.v. injection of the drug at 10 mg/kg are shown in the figure. The earliest measurements show plasma concentrations (of free and bound drug) of approximately 1500 ng Δ^1-THC/ml at which time the mice showed marked behavioral effects, such as increased aggression. At 2 h after the injection, plasma drug concentrations had declined by 90–95%. These concentrations may be compared with those of 50–100 ng Δ^1-THC/ml in man at early times after smoking a few mg of Δ^1-THC[22]. The figure shows that the decline in Δ^1-THC concentration in the erythrocyte membrane followed a similar pattern to that in plasma, falling from approximately 6 ng Δ^1-THC/mg membrane protein to about 0.6 ng/mg after 2 h. This indicates an early equilibrium of the drug between plasma and the erythrocyte membrane.

Expression of these results as a partition coefficient, defined as the number of moles of Δ^1-THC kg^{-1} dry membrane/No. of moles of free Δ^1-THC l^{-1} plasma gave a mean value over the time period studied of 118.5 ± 13.9 (± SEM, N = 26). A slight increase with time was found, from a mean of 95.7 ± 14.0 (± SEM, n = 7) for the combined 15 min and 30 min values to a mean of 135.4 ± 24.1 (± SEM, n = 9) for the combined 1½ h and 2 h values. These calculations assume that protein constituted 49% of erythrocyte membrane dry weight[23] and that 3% of total Δ^1-THC in plasma was free[24]. The lower values for the partition coefficient seen at early times suggested that equilibration is attained some 30–60 min after dosing, the partitioning being dependent on both the equilibrium between the bound drug (largely lipoprotein-bound)[25] and the 3% that was free in solution as well as that between the unbound drug and Δ^1-THC associated with the membrane.

It is difficult to compare the results of this in vivo study with earlier observations on Δ^1-THC partitioning in vitro[9,10] because of the different conditions. The relatively small differences in partition coefficients seen over the 10-fold range in plasma concentrations in this study indicated that the membrane levels were not saturating ones. This was confirmed by calculations showing that membrane concentrations of Δ^1-THC were in the range 10^{-6} to 10^{-5} M (moles /kg dry membrane), much lower than calculated levels of approximately 10^{-4} to 10^{-3} M obtained from earlier in vitro studies[10]. Roth and Williams[10] point out, however, that from the in vivo tissue data of Gill and Lawrence[26] it may be estimated that brain membrane concentrations, at effective doses for behavioral effects, would be only about 2×10^{-5} M, in reasonable agreement with values from the present in vivo work. It seems unlikely that a saturating membrane concentration of Δ^1-THC could be achieved in vivo after i.v. injection of the drug since the dose required would be fatal. Since the mouse erythrocyte membranes in this study were found to contain 0.36 mg ± 0.01 (mean ± SEM) phospholipid/mg membrane protein, it may be calculated that for every molecule of Δ^1-THC in the membrane there would be of the order of at least 2×10^4 phospholipid molecules. However, some variation in partitioning between different membrane types might arise from differences in membrane composition, particularly in cholesterol content, as has been shown for thiopental[27]. In vitro studies, however, indicate that the partitioning of Δ^1-THC into erythrocyte and into excitable membranes is of the same order[9].

The table shows that at the low concentrations of Δ^1-THC observed in the erythrocyte membrane, a disordering effect of the lipid chain motion was consistently seen as shown by the decrease in order parameter over a wide range of temperatures. This may be contrasted to the much higher drug concentrations of 30 mM required of general anesthetics for anesthesia[28] and which produce similar changes in the order parameter. It has been suggested that the volume occupied by the general anesthetics alone, rather than any additional disordering influence, accounts for the associated membrane expansion[29]. In the present study, the volume occupied by the few Δ^1-THC molecules appears to be an insufficient explanation, suggesting that the disordering of the lipid phase was initiated by a more specific interaction. Although the nature of the interaction is unknown, either conformational changes of protein within the bilayer could cause changes in the bulk properties of the membrane that could correlate with a decreased order parameter or the overall lipid-protein interactions could be modified[30]. Such interactions could also be of relevance in brain tissue since membrane proteins immunologically related to erythrocyte spectrin[31] and ankyrin[32], for example, have been found in that tissue. It is not known, however, how closely related changes in the fluidity of brain membranes may be to the behavioral effects of Δ^1-THC.

The present work does not eliminate the possibility that the observed effects on fluidity could have been produced by an in vivo metabolite of Δ^1-THC rather than Δ^1-THC itself. Suitable analytical techniques have not yet been developed for measuring these compounds in membranes. However, as the concentrations of the major psychoactive metabolite, 7-hydroxy-Δ^1-THC, in plasma are some 10–40 times lower than those of Δ^1-THC itself[33] after i.v. injection, it is thought unlikely that metabolites would contribute singnificantly to the observed effects.

In conclusion, this study has indicated relatively low concentrations of Δ^1-THC in mouse erythrocyte membranes following injection of psychoactive doses of the drug. Neither membrane nor membrane binding sites appeared to be saturated and an early equilibrium was established for the drug between plasma and membrane. The observed changes in membrane fluidity associated with the low concentration of the drug in the membrane and the previously reported absence of a fluidizing effect for non-psychoactive cannabinoids, suggests some type of structurally specific interaction with the membrane.

1 This work was supported by grants from the Medical Research Council, the Wellcome Trust and the E.P. Abraham Cephalosporin Trust.
2 Reprint requests to Dr D.J. Harvey, University Department of Pharmacology, South Parks Road, Oxford, OXl 3QT, UK.
3 Harvey, D.J., Martin, B.R., and Paton, W.D.M., in: Marihuana: Biological Effects, Adv. Biosci. 22/23, p.45. Eds G.G. Nahas and W.D.M. Paton. Pergamon, Oxford 1979.
4 Mechoulam, R., and Edery, H., in: Marihuana: Chemistry, Pharmacology, Metabolism and Clinical Effects, p.101. Ed. R. Mechoulam. Academic Press, New York 1973.
5 Ohlsson, A., Widman, M., Carlsson, S., Ryman, T., and Strid, C., Acta pharmac. tox. *47* (1980) 308.
6 Jones, G., Pertwee, R.G., Gill, E.W., Paton, W.D.M., Nilsson, I.M., Widman, M., and Agurell, S., Biochem. Pharmac. *23* (1974) 439.
7 Lawrence, D.K., Pertwee, R.G., Gill, E.W., and Piper, J.M., Biochem. Pharmac. *23* (1974) 3017.
8 Gill, E.W., and Jones, G., Biochem. Pharmac. *21* (1972) 2237.
9 Seeman, P., Chau-wong, M., and Moyyen, S., Can. J. Physiol. Pharmac. *50* (1972) 1193.
10 Roth, S.H., and Williams, P.J., J. Pharm. Pharmac. *31* (1979) 224.
11 Lawrence, D.K., and Gill, E.W., Molec. Pharmac. *11* (1975) 595.
12 Roth, S., and Seeman, P., Nature New Biol. *231* (1971) 284.
13 Chari-Bitron, A., Life Sci. *10* (1971) 1273.
14 Raz, A., Schurr, A., and Livne, A., Biochim. biophys. Acta *274* (1972) 269.
15 Harvey, D.J., Leuschner, J.T.A., and Paton, W.D.M., J. Chromat. *202* (1980) 83.
16 Hanahan, D.J., and Ekholm, J.E., Meth. enzym. *31* (1974) 168.
17 Lowry, O.H., Rosebrough, N.J., Farr, A.L., and Randall, R.J., J. biol. Chem. *193* (1951) 265.
18 Folch, J., Lees, M., and Sloane-Stanley, G.H., J. biol. Chem. *226* (1957) 497.
19 Morrison, W.R., Analyt. Biochem. *7* (1964) 218.
20 Watts, A., Volotovski, I.D., and Marsh, D., Biochemistry *18* (1979) 5006.
21 Gaffney, B.J., in: Spin Labeling, appendix 4, p.567. Ed. L.J. Berliner. Academic Press, New York 1976.
22 Agurell, S., Lindgren, J.-E., and Ohlsson, A., in: Marihuana: Biological Effects, Adv. Biosci. 22/23, p.3. Eds G.G. Nahas and W.D.M. Paton. Pergamon, Oxford 1979.
23 Guidotti, G., A. Rev. Biochem. *41* (1972) 731.
24 Garrett, E.R., and Hunt, C.A., J. pharm. Sci. *63* (1974) 1056.
25 Wahlqvist, M., Nilsson, I.M., Sandberg, F., and Agurell, S., Biochem. Pharmac. *19* (1970) 2579.
26 Gill, E.W., and Lawrence, D.K., Biochem. Pharmac. *23* (1974) 1140.
27 Korton, K., Sommer, T.J., and Miller, K.W., Biochim. biophys. Acta *599* (1980) 271.
28 Pang, K.-Y., Braswell, L.M., Chang, L., Sommer, T.J., and Miller, K.W., Molec. Pharmac. *18* (1980) 84.
29 Franks, N.P., and Lieb, W.R., Nature, Lond. *292* (1981) 248.
30 Marsh, D., and Watts, A., in: Lipid-protein Interactions, vol.2, p.53. Eds P.C. Jost and D.H. Griffiths. Wiley Interscience, New York 1982.
31 Bennett, V., Davis, J., and Fowler, W.E., Nature, Lond. *299* (1982) 126.
32 Bennett, V., Nature, Lond. *281* (1979) 597.
33 Wall, M.E., Harvey, T.M., Bursey, J.T., Brine, D.R., and Rosenthal, D., in: Cannabinoid Analysis in Humans, NIDA Res. Monogr.7, p.107. Ed. R.E. Willette. Rockville, Maryland 1976.

0014-4754/84/080866-03$1.50 + 0.20/0

(^{3}H) Spiroperidol binding decreases in brains of rats infected with Venezuelan equine encephalomyelitis virus[1]

E. Bonilla, M. Salazar, J. Estevez, H. Hernández and P. Rangel

Instituto de Investigaciones Clínicas, Facultad de Medicina, Universidad del Zulia and Inbiomed – Fundacite, Apartado 1151, Maracaibo (Venezuela), 21 October 1983

Summary. After i.p. inoculation with the Guajira strain of Venezuelan equine encephalomyelitis virus a significant decrease in the density of (^{3}H) spiroperidol binding sites in the striatum, midbrain and frontal cortex was observed. No changes in the affinity of the receptors could be demonstrated. This finding is compatible with neuronal degeneration caused by the viral infection.

We have found that different central aminergic systems are affected by infection with the Venezuelan equine encephalomyelitis (VEE) virus. A significant decrease in the activities of the enzymes glutamate decarboxylase[2], tyrosine hydroxylase[3], and choline acetyltransferase[4] has been observed in several brain regions of mice and rats infected with VEE virus. However, the receptor components of these aminergic transmitter systems have not been studied in animals infected with VEE virus.

(^{3}H) Spiroperidol is at present the most commonly used dopamine antagonist radioligand and it has been widely employed to detect changes in striatal dopamine receptors in response to a variety of conditions. It has recently been shown that both (^{3}H) spiroperidol and (^{3}H) haloperidol bind to the same striatal receptors[5] which seem to be approximately evenly distributed among striatal interneurons and corticostriatal glutamate-containing neurons[6]. It has been reported that in the striatum, (^{3}H) spiroperidol binds mainly to dopamine (D_2) receptors, though in parts of the brain such as hippocampus and frontal cortex, it is known to bind with high affinity to serotonin-related sites also[7]. From studies with an in vivo (^{3}H) spiroperidol radioreceptor assay it has been concluded that (^{3}H) spiroperidol binds mainly to dopamine receptors in hippocampus as well as in striatum, whereas both serotonin and dopamine receptors are labeled in frontal cortex[8]. In order to get a better insight into the pathogenesis of this viral disease, we therefore analyzed the binding of (^{3}H) spiroperidol to its brain receptors, which seem to be located in pre and post synaptic membrane structures that have the characteristics of plasma membranes[9]. We now report evidence that the density of receptor sites (B_{max}) is reduced in VEE infected rats without alterations in the affinity of the ligand for its binding sites.

Sprague-Dawley male rats, weighing 200–300 g were inoculated i.p. with 0.3 ml of a suspension containing 100 LD_{50} of the Guajira strain of VEE virus in 0.4% bovine albumin borate-buffered saline solution (BABS), pH 7.4. To control animals we administered equivalent volumes of BABS. They were killed by decapitation simultaneously with the diseased animals, 6–8 days after the inoculation, when the latter presented signs of encephalitis. The brain was quickly removed and the striatum, frontal cortex, and midbrain were dissected out at 4 °C following the technique of Glowinski and Iversen[10], and stored at −80 °C until analyzed. Each brain region was homogenized and the incubation performed according to a previously reported procedure[11]. The concentrations of (^{3}H) spiroperidol used were: 0.10, 0.25, 0.375, 0.50, 1.00, 1.50, 2.00