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BRAIN LEVELS OF Δ^1-TETRAHYDROCANNABINOL AND ITS METABOLITES IN MICE—CORRELATION WITH BEHAVIOUR, AND THE EFFECT OF THE METABOLIC INHIBITORS SKF 525A AND PIPERONYL BUTOXIDE

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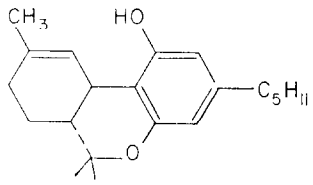
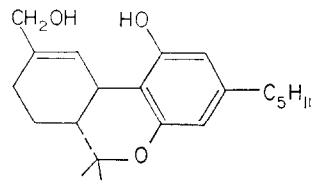
Abstract—Brain levels of Δ^1-tetrahydrocannabinol (Δ^1-THC) and its major metabolite (7-hydroxy-Δ^1-THC) following intravenous injection of tritium-labelled Δ^1-THC into mice were measured and correlated with inhibition of spontaneous motor activity. Metabolism of Δ^1-THC to 7-hydroxy-Δ^1-THC was fast and both substances rapidly penetrated the C.N.S. Peak brain levels of both compounds occurred within 20 min of injection and the correlation between pharmacological response and brain concentration of either compound was equally good. Twenty min after injection more than 50 per cent of the radioactivity in blood was irreversibly bound, whereas only 12 per cent of the activity in brain was unextractable. SKF 525A (25 mg/kg i.p.) produced only a slight increase in the brain level of Δ^1-THC but the level of 7-hydroxy-Δ^1-THC was increased nearly three-fold, presumably due to the inhibition of subsequent metabolic reactions. Piperonyl butoxide (100 mg/kg i.p.) produced an increase in the brain level of Δ^1-THC but no change in the level of 7-hydroxy-Δ^1-THC. Higher doses of either inhibitor produced behavioural changes in the absence of Δ^1-THC. It is concluded that 7-hydroxy-Δ^1-THC is centrally active, but that it has not yet been demonstrated that the effects of injected Δ^1-THC are due solely to its primary metabolite.

MUCH progress has been made recently in elucidating the pharmacology of the tetrahydrocannabinols, the main psychoactive constituents of cannabis, and the development of syntheses of radioactively labelled forms of these compounds was quickly followed by the study of their metabolism and tissue distribution.[1–3] However, there are few reliable and simple pharmacological assays based on the central effects of cannabinoids and, consequently, it is difficult to interpret some of the early work since the time-course of the distribution of these compounds was not correlated with measurements of their pharmacological effects. Another important factor in the study of the pharmacokinetics of cannabinoids was the demonstration that Δ^1-tetrahydrocannabinol (Δ^1-THC, I) is very rapidly metabolized[4] and that the primary metabolite, 7-hydroxy-Δ^1-THC (II), is pharmacologically active.[5,6] On the basis of similar observations on the metabolism of $\Delta^{1(6)}$-THC, Mechoulam[7,8] made the interesting suggestion that the C.N.S. effects of cannabis might be due to 7-hydroxy-Δ^1-THC rather than to Δ^1-THC itself.

Our investigation of the pharmacokinetics of cannabis was dependent upon the solution of two initial problems. Firstly it was necessary to devise a synthesis of tritium-labelled Δ^1-THC of high specific activity so that it would be possible to



I



II

measure the concentrations of Δ^1-THC and its metabolites in small quantities of tissue. Secondly, a reliable and convenient bioassay for Δ^1-THC was required. The preparation of high activity ^{3}H-Δ^1-THC has been achieved,[9] and the development by Pertwee of the mouse catalepsy test provided a simple method for measuring the time course of a behavioural effect of Δ^1-THC. In this paper we present observations on the correlation of brain levels of Δ^1-THC and its metabolites with drug-induced catalepsy in mice.

MATERIALS AND METHODS

Drugs and reagents

The synthesis of ^{3}H-Δ^1-THC (598 mc/mmole; $4{\cdot}23 \times 10^6$ dis./min/μg), specifically labelled in the pentyl side-chain, has been described elsewhere.[9] Unlabelled Δ^1-THC was isolated from the natural material;[10] 7-hydroxy-Δ^1-THC was prepared* by *in vitro* oxidation of Δ^1-THC using a liver microsome preparation.[5] SKF 525A was generously donated by Smith, Kline and French Laboratories Ltd. Piperonyl butoxide, obtained from Koch–Light Laboratories Ltd., was redistilled before use: $b.p._{0{\cdot}1\ mm}$ 180–2°, $n_{25}^{D} = 1{\cdot}4985$. Ethyl acetate was purified by shaking with 2 N sodium carbonate solution and distilled water; after preliminary drying with saturated brine and magnesium sulphate, it was refluxed with calcium hydride for 1 hr and distilled through a Widmer column. The chloroform used as a solvent for thin-layer chromatography was B.D.H. reagent grade, containing *ca.* 2% v/v ethanol as a preservative.

Animals

Male albino mice, Tuck strain No. 1, weighing 20–25 g, were obtained from Tuck, Rayleigh, Essex. They were kept for several days before use in cages containing pine sawdust bedding and were given Oxoid diet 41 and water *ad lib.* Mice weighing 24–27 g were used in the experiments.

Radioactivity measurements. Tritium was measured by liquid scintillation counting using a Beckman LS 200B instrument. The scintillator solution consisted of butyl PBD (7·0 g/l.) and naphthalene (50 g/l.) in purified dioxan; 5 ml aliquots were used in each counting vial. Counting efficiencies, obtained using a calibrant solution of standard ^{3}H-hexadecane (Radiochemical Centre, Amersham) in dioxan, were about 40 per cent.

Behavioural test. Δ^1-THC induces periods of prolonged inactivity in mice, and this "cataleptic" effect has been made the basis of an assay by Pertwee. A full account

* Preparation by Mr. D. K. Lawrence.

of the procedure will be published elsewhere* but the method, briefly, is as follows. The mouse is placed on a horizontal wire ring, 2 inches in diameter and observed for a period of 5 min. After the mouse has been injected with an appropriate dose of Δ^1-THC, its spontaneous activity on the ring is considerably reduced and it spends much of its time completely motionless except for respiratory movements. The total absence of whisker movement is a useful indication of inactivity. The immobility index is defined as the fraction of the 5 min period (expressed as a percentage) during which the mouse is completely inactive; the index is dose- and time-dependent. If the mouse falls or escapes from the ring before the conclusion of the test, it is immediately replaced, the timing being discontinued during the interval. In these experiments the mice were tested twice: 1·5 hr before injection of Δ^1-THC, in order to accustom them to the ring, and at various times after injection. The test was conducted at room temperature (20°).

Time course. Each mouse was injected, via a cannula inserted into a tail vein, with ^{3}H-Δ^1-THC (2 mg/kg body wt.) dispersed with Tween 80 (10 mg/kg) in physiological saline (about 0·2 ml). Fifteen min, 1·5 hr or 4 hr after injection, the mouse was tested and, immediately after measurement of the immobility index, was killed in a chamber containing carbon monoxide. A blood sample was obtained in an heparinized syringe by intracardiac puncture. The brain was removed, weighed, rinsed rapidly in distilled water (*ca.* 5 ml), and homogenised in distilled water (3 ml) at 20°. The radioactivity in the blood and brain was then analysed. Mice used as controls in the behavioural test were injected with Tween 80 (10 mg/kg) in saline (*ca.* 0·2 ml), and were tested at the same times after injection as the mice which had received Δ^1-THC.

Total brain activity. 0·3 ml of the brain homogenate was digested with 0·3 ml hyamine hydroxide (1·0 M in methanol) at 50° for 16 hr. The resulting solution was diluted with dioxan (4·2 ml), acidified with glacial acetic acid (0·2 ml) and an 0·2 ml aliquot was counted. The blood content of the mouse brain is small[11] and no correction was made for radioactivity present in the blood vessels of the brain.

Extractable brain activity. 2·0 ml of the brain homogenate was extracted with ethyl acetate (3 × 3 ml). The extract was dried with magnesium sulphate and an 0·1 ml aliquot was counted. A larger aliquot (1·5–4 ml, depending on the total activity present) was concentrated to a volume of about 0·1 ml under a stream of nitrogen (oxygen-free) and the concentrate was chromatographed.

Total blood activity. An 0·1 ml aliquot of whole blood was pipetted on to filter paper. After drying overnight at room temperature, the sample was combusted over 10 ml distilled water in a Schöniger flask[12] and an 0·2 ml aliquot of the water was counted.

Extractable blood activity. An 0·1 ml aliquot of whole blood was haemolysed in 2 ml distilled water immediately after withdrawal. The haemolysate was extracted with ethyl acetate (3 × 2 ml) and, after drying with magnesium sulphate, 0·1 ml of the extract was counted. An aliquot of the extract (2·5–3 ml) was concentrated and chromatographed.

Chromatography. Each concentrated extract was chromatographed on Whatman SG 81 silica-impregnated paper (solvent: 1% v/v methanol in chloroform). The distance between the origin and solvent front in each chromatogram was 14 cm and authentic Δ^1-THC and 7-hydroxy-Δ^1-THC were chromatographed on the same

* R. G. Pertwee, *Br. J. Pharmac.*, submitted for publication.

paper. The length of paper containing these marker compounds was separated from the radioactive area and sprayed with 1% w/v aqueous Fast Blue B salt. The radioactive strip was cut into fifteen 1 cm wide pieces which were placed in counting vials, eluted directly with scintillator solution, and counted *in situ*.

Use of metabolic inhibitors. Mice were pre-injected intraperitoneally either with SKF 525A (25 mg/kg) in saline (*ca.* 0·2 ml), or with piperonyl butoxide (100 or 640 mg/kg), dispersed by Tween 80 (100 or 640 mg/kg) in saline (*ca.* 0·2 ml), 25 min before intravenous injection of labelled (2 mg/kg) or unlabelled (1 mg/kg) Δ^1-THC. Control groups of mice were pre-injected with *ca.* 0·2 ml saline i.p. Fifteen min after injection of Δ^1-THC, the immobility index was determined, and, where labelled Δ^1-THC had been used, brain levels of radioactivity were measured as before.

Octanol–water partition. 25 μg of ^{3}H-Δ^1-THC in 0·1 ml benzene was added to physiological saline (3 ml) and *n*-octanol (3 ml), and the mixture was shaken for 1 min. After standing in a thermoregulated water bath at 25° for 15 min, aliquots were removed from each layer and centrifuged. The octanol layer was diluted with dioxan and the concentrations of ^{3}H-Δ^1-THC in this dilute solution and in the aqueous layer were then measured by liquid scintillation counting.

RESULTS

About 88 per cent of the radioactivity present in the brain 20 min after intravenous injection of ^{3}H-Δ^1-THC (2 mg/kg) was extractable into ethyl acetate. The amount of extractable radioactivity declined rapidly during the 4 hr period covered by these experiments, whereas the amount of non-extractable activity remained more or less constant at the level present 20 min after injection. In general, three distinct peaks of radioactivity could be seen in the chromatograms of the extractable material (Fig. 1). The fastest running component was identified as Δ^1-THC and a second, less mobile, component as 7-hydroxy-Δ^1-THC on the basis of R_f values obtained using authentic

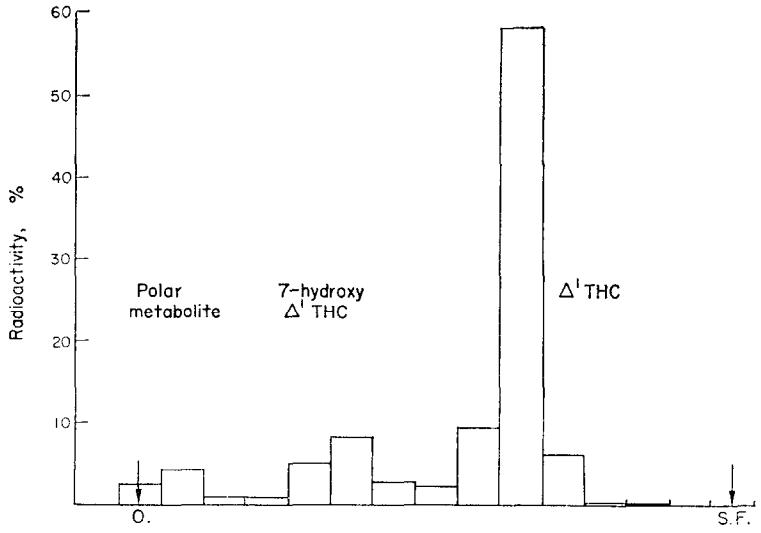


FIG. 1. Chromatogram of mouse brain extract 20 min after injection of ^{3}H-Δ^1-THC (2 mg/kg i.v.). The radioactivity in each strip is expressed as a percentage of the total extractable activity.

samples of these two compounds. The resolving power of the chromatographic system was not high, and the possibility cannot be excluded that each of these well resolved peaks might have been due to mixtures of similar substances. For example, 6-hydroxy-Δ^1-THC has been identified as a minor metabolite of Δ^1-THC in rabbits but not in rats,[13] and this metabolite has been shown to be pharmacologically active;[14] the chromatographic peak identified as 7-hydroxy-Δ^1-THC might also have contained some of this isomeric substance. The third, much less mobile, peak of activity was almost certainly inhomogeneous and is referred to as the "polar metabolite". No attempt was made to resolve this fraction by further chromatography. Figure 2

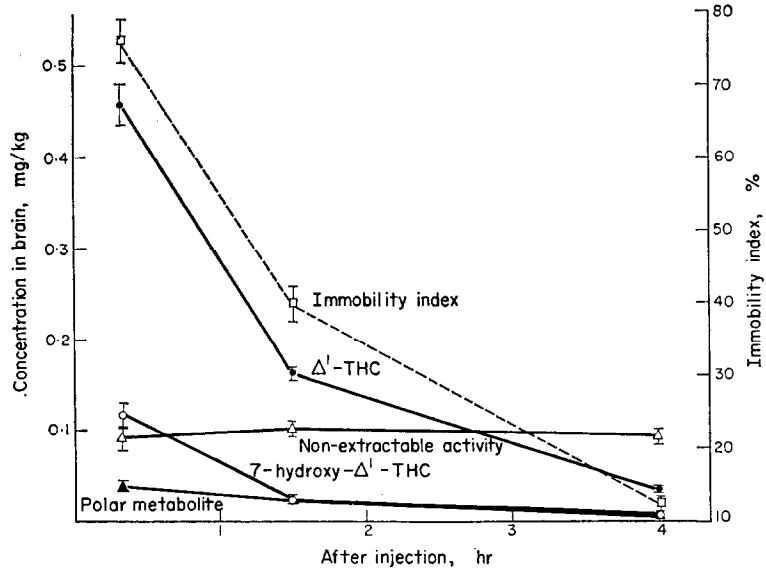


FIG. 2. Mouse brain concentrations of Δ^1-THC and its metabolites (left-hand ordinate), and immobility index (right-hand ordinate), after injection of ^{3}H-Δ^1-THC (2 mg/kg i.v.). The concentrations of the uncharacterized non-extractable component and the polar metabolite are expressed as their Δ^1-THC equivalents. Each point represents the mean of six determinations $\pm$ the standard error of the mean (S.E.M.).

shows the variation with time of brain levels of the four components: Δ^1-THC, 7-hydroxy-Δ^1-THC, polar metabolite and non-extractable activity. It will be seen that initially Δ^1-THC and 7-hydroxy-Δ^1-THC were the major cannabinoids in the brain, and that the levels of these two compounds were at a maximum within 20 min of injection. After an intravenous dose of 2 mg/kg total body weight, the peak concentration of Δ^1-THC in the brain was about 0·5 mg/kg of brain tissue. Of the total injected dose of Δ^1-THC, only about 0·6 per cent reached the brain, and of this about 65 per cent was present as the unmetabolized compound.

The variation during the same period of levels of Δ^1-THC and its metabolites in whole blood is shown in Fig. 3. In contrast to brain, rather more than 50 per cent of the radioactivity in blood 20 min after injection was so firmly bound that it was not extractable with ethyl acetate. Similar observations have been made by other workers using rabbits[4] and human subjects,[15,16] and evidence has been presented that plasma

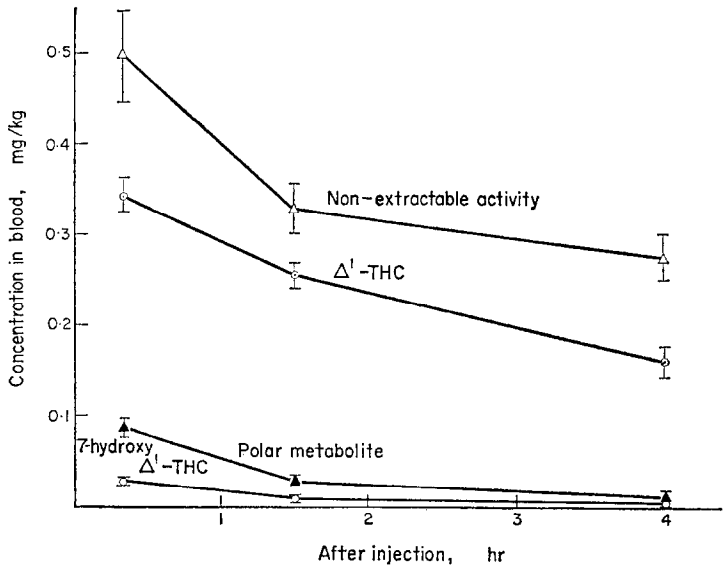


FIG. 3. Concentrations in the haemolysed whole blood of mice of Δ^1-THC and its metabolites after injection of ^{3}H-Δ^1-THC (2 mg/kg i.v.).

lipoproteins constitute the major binding site.[17] Whereas the chromatograms of material extracted from the brain exhibited a clear peak attributable to 7-hydroxy-Δ^1-THC, the chromatograms of blood extracts did not show such a peak. In these, the small amount of radioactivity found in the position expected for 7-hydroxy-Δ^1-THC has been attributed to that compound. Thus the values for 7-hydroxy-Δ^1-THC shown in Fig. 3 must be taken as maximum values.

Δ^1-THC is a very fat-soluble substance, with an octanol–water partition ratio of about 6000 : 1, and it would be expected to equilibrate rapidly between blood and brain. Corresponding blood and brain levels of Δ^1-THC and 7-hydroxy-Δ^1-THC are shown in Figs. 2 and 3. The half-lives of Δ^1-THC and its primary metabolite appeared to differ in the two compartments: brain levels of both compounds declined faster than blood levels. However, ethyl acetate is an effective polar solvent which is likely to extract weakly bound as well as free cannabinoids from tissue homogenates. The extent of this binding probably differs in blood and brain so that the concentrations measured in these two compartments may not have been precise measures of the equilibrating pools. It is evident, however, that Δ^1-THC and its primary metabolite rapidly penetrated the C.N.S. and that there was no effective blood–brain barrier to these substances. 7-hydroxy-Δ^1-THC could be detected in the brain up to 4 hr after injection of Δ^1-THC, whereas at no time could clear evidence be obtained for its presence in blood. It has been shown[18,19] that Δ^1-THC is metabolized in the liver, (and also in the lungs, spleen and blood) but not in the brain. Consequently, 7-hydroxy-Δ^1-THC must be formed outside the brain and transported there in the bloodstream; its relatively high concentration in the brain suggests that it partitions selectively into the C.N.S. However, it is clear that blood levels are not necessarily good indicators of brain levels, even for such rapidly equilibrating substances.

The immobility indices measured immediately before determination of brain levels of Δ^1-THC and its metabolites appear in Fig. 2. The dose of Δ^1-THC chosen (2 mg/kg i.v.) produced a peak (near-maximal) cataleptic effect about 15 min after injection. The effect declined quite rapidly and the behavioural pattern of the mice returned to normal within 4 hr.* The immobility index of mice which had received only Tween 80 in saline did not vary significantly throughout this 4 hr period and was about 12 $\pm$ 4 per cent (mean of six mice $\pm$ S.E.M.). In the mice which had received Δ^1-THC, the

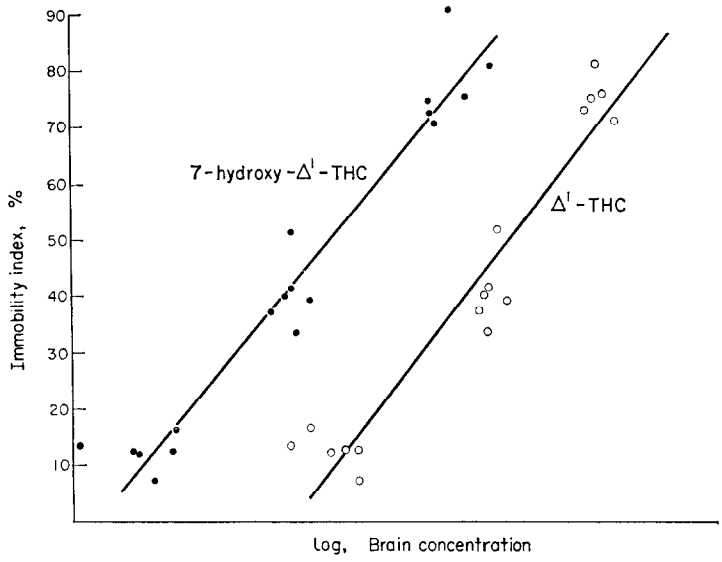
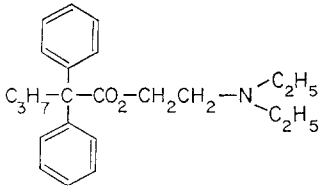


FIG. 4. Correlation of immobility index, measured at various times after injection of ^{3}H-Δ^1-THC (2 mg/kg i.v.), with whole brain concentrations of Δ^1-THC (○) and 7-hydroxy-Δ^1-THC (●). Each point represents measurements on a single mouse.

brain levels of Δ^1-THC and 7-hydroxy-Δ^1-THC declined markedly over the period from 15 min to 4 hr after injection, and there was an equally satisfactory regression of immobility index against the logarithm of the brain concentration of either compound (Fig. 4). The regression lines were approximately parallel and the concentration of Δ^1-THC in the brain was about 6 times the concentration of 7-hydroxy-Δ^1-THC. Over the time scale of the experiment, the level of the non-extractable material



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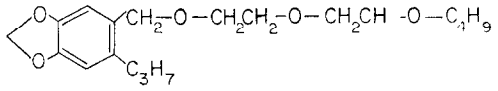

IV

* This time-course closely parallels that observed for the effects of marihuana in human subjects.[20]

remained virtually constant and there was, therefore, no reason to associate any of the short-term pharmacological effects of Δ^1-THC with the presence of this component. The concentration of the polar metabolite declined in a similar manner to that of Δ^1-THC, but the overall levels were too low compared with those of the other cannabinoids present in the brain for any significant conclusions to be drawn.

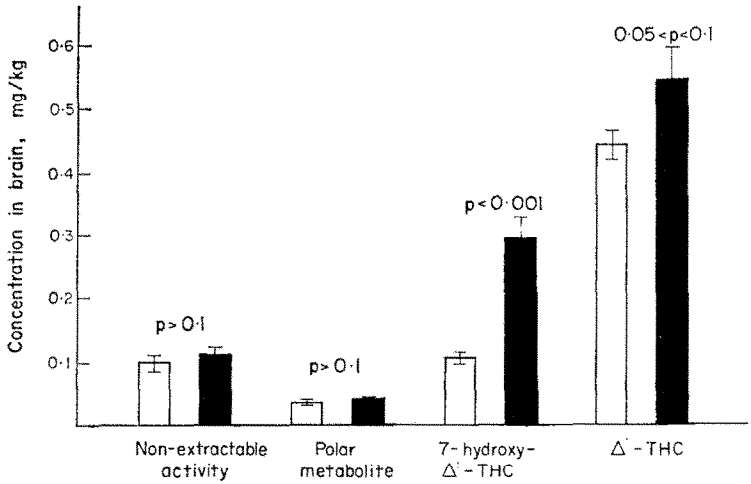

FIG. 5. The effect of pre-treatment with SKF 525A (25 mg/kg i.p.) 25 min before injection of ^{3}H-Δ^1-THC (2 mg/kg i.v.). Brain concentrations (mean of six mice $\pm$ S.E.M.) were determined 20 min after injection of Δ^1-THC. Control, □; SKF 525A, ■.

The effects of pretreating mice with the metabolic inhibitor SKF 525A (III, 25 mg/kg i.p.) are shown in Fig. 5. There was a small increase in the concentration of Δ^1-THC in the brain, but this was not significant ($0{\cdot}05 < P < 0{\cdot}1$). In contrast, however, the brain concentration of 7-hydroxy-Δ^1-THC was increased nearly three-fold ($P < 0{\cdot}001$). The levels of unextractable material and of the polar metabolite were not changed significantly ($P > 0{\cdot}1$). The high level of 7-hydroxy-Δ^1-THC in the brain was not depressed by pretreatment with 50 mg/kg i.p. of SKF 525A. Doses of 100 mg/kg or greater produced marked behavioural changes in the absence of Δ^1-THC, and experiments with such high doses of the inhibitor were not pursued since it would be impossible to distinguish behavioural effects due to Δ^1-THC and its metabolites and those due to SKF 525A. The effect of pretreatment with SKF 525A (25 mg/kg) on the immobility index induced by an intravenous dose of 1 mg/kg (sub-maximal) of unlabelled Δ^1-THC was observed. The 15-min immobility index of the pre-treated group of mice was 50 ± 4 per cent (mean of 10 mice $\pm$ S.E.M.) whereas that of the control group was 46 ± 5 per cent. The difference was not significant ($0{\cdot}5 < P < 0{\cdot}6$). This was an interesting result, since, if 7-hydroxy-Δ^1-THC were the only pharmacologically active component, from Fig. 4 it would appear that a three-fold increase in the brain concentration should result in an increase in the mean immobility index of about 22 per cent.

Piperonyl butoxide (IV) is a general inhibitor of metabolizing enzymes and is widely used as a synergist in insecticides.[21,22] It is a non-polar, lipid-soluble substance

and would be expected to have similar distribution properties *in vivo* to those of Δ^1-THC. Like SKF 525A, this inhibitor produced behavioural changes when administered to mice in large doses. Pre-treatment with 640 mg/kg i.p. before intravenous injection of ^{3}H-Δ^1-THC raised the brain level of Δ^1-THC and reduced the level of 7-hydroxy-Δ^1-THC. However, at this high dose level, the inhibitor and its vehicle had such a profound effect on the immobility index of control mice that a correlation of behaviour with brain levels of Δ^1-THC and its metabolites in treated mice was impossible. Pre-treatment with the highest doses of piperonyl butoxide that did not produce any noticeable change in the behaviour of control mice (100 mg/kg i.p.) resulted in an increase in the brain level of Δ^1-THC but little change in the level of 7-hydroxy-Δ^1-THC; the level of polar metabolite was reduced whereas that of the non-extractable component was unchanged. However, at this dose level, the inhibitor-induced changes in the concentrations of Δ^1-THC and 7-hydroxy-Δ^1-THC were not sufficiently large to cause a detectable change in the immobility index and experiments with this compound were discontinued.

DISCUSSION

The early work on the chemistry and pharmacology of cannabis culminated in the demonstration that the main psycho-active constituent is Δ^1-THC.[23,24] Studies *in vivo* and *in vitro* of the metabolism of Δ^1-THC have shown that hydroxylation is the primary step, and that metabolism occurs mainly in the liver, but also in the lungs, spleen and blood.[18,19] The major primary metabolite of Δ^1-THC appears to be 7-hydroxy-Δ^1-THC (II) in most species, although other primary metabolites have been detected[19] and characterized.[13] 7-hydroxy-Δ^1-THC has been isolated in a pure state and has been shown to be pharmacologically more active than Δ^1-THC in mice.[5,6,18] Mechoulam has suggested that the main C.N.S. effects of cannabis might be due to the primary metabolite rather than to Δ^1-THC itself,[7,8] basing his argument on the fact that the metabolite appears to be indistinguishable from Δ^1-THC in its pharmacological effects; the fact that it is apparently more potent than Δ^1-THC; and the frequent reports that the central effects of cannabis are experienced by some human subjects only after repeated exposure. This last observation suggests that the induction of hydroxylating enzymes is a necessary precondition for the production of central effects by cannabis. However, in this context, it is pertinent to note that after injecting ^{14}C-Δ^1-THC, Lemberger *et al.* found 7-hydroxy-Δ^1-THC in the blood of three human volunteers who had not previously taken cannabis,[15] although the same workers certainly found that habitués had a greater capacity for metabolizing Δ^1-THC than naïve subjects.[16] It is possible that other factors may be more important in achieving a "high" than the induction of metabolizing enzymes.[16]

During the development of the mouse catalepsy test in this laboratory we had been impressed by the speed of onset of catalepsy following intravenous administration of either crude cannabis or Δ^1-THC. This led us to doubt that sufficient quantities of the metabolite could have been formed within the short period between injection and the production of the maximum cataleptic effect, and we hoped to resolve the question of the active compound by direct correlation of the brain levels of Δ^1-THC and its primary metabolite with the catalepsy produced in mice.

In the event, this simple experiment proved inconclusive. The nature of the catalepsy

test makes it difficult to obtain a meaningful measure of the pharmacological response less than 15 min after injection, since ideally, the mouse must be left for a short period to recover from the stress of the injection before being placed on the ring. Within this period, brain levels of both Δ^1-THC and 7-hydroxy-Δ^1-THC have reached a maximum, both then decline over the same period and the correlation between the immobility index and the logarithm of the brain concentration of either compound is equally satisfactory. Δ^1-THC and its primary metabolite are present in a molar ratio of about 6 : 1 throughout the time course of the cataleptic effect and, since the metabolite has been shown to be active, some or all of the central effects following administration of Δ^1-THC could be due to this compound. Whilst we cannot find clear evidence for the existence of unbound 7-hydroxy-Δ^1-THC in the blood of mice after injection of ^{3}H-Δ^1-THC, Lemberger *et al.*[15,16] have demonstrated its presence in the blood of human volunteers injected with ^{14}C-Δ^1-THC. Our observations on mice suggest that the rapidly-formed primary metabolite is selectively absorbed by the C.N.S. from the blood. If a similar effect occurs in man, and if the blood–brain partition ratio is about the same in both species, the peak concentration of primary metabolite in the human brain might be as high as 70 per cent of the peak concentration of Δ^1-THC. A brain concentration of this magnitude would support the theory that the metabolite is largely responsible for the pharmacological action of cannabis in man.

The proposition that C.N.S. activity is due entirely to the metabolite and that Δ^1-THC is completely inactive could be substantiated if it were possible to measure the pharmacological response to Δ^1-THC under conditions in which its rapid metabolism had been inhibited. The experiments with SKF 525A and piperonyl butoxide were directed to that end.

SKF 525A has been shown to inhibit the microsomal metabolism of a wide range of compounds,[21,22] and some workers have used it as a diagnostic agent. For example, Sofia and Barry,[25] found that Δ^1-THC prolonged barbiturate sleeping time in mice, and that this effect was augmented by SKF 525A. On the assumption that administration of the metabolic inhibitor must have increased the brain concentration of Δ^1-THC and reduced that of 7-hydroxy-Δ^1-THC, they concluded that the prolongation of barbiturate sleeping time was due to Δ^1-THC itself and not to its metabolite. Burstein[26] has since demonstrated that SKF 525A inhibits the *in vitro* metabolism of Δ^1-THC by rat liver preparations. We find, however, that pre-treatment of mice with SKF 525A increases the brain concentration of Δ^1-THC by only about 23 per cent (not significant) but that the concentration of 7-hydroxy-Δ^1-THC is nearly trebled. A similar observation has been made by O'Brien[27] on the effect of SKF 525A on the metabolism of parathion in mice. This somewhat paradoxical effect, the production by a supposed metabolic inhibitor of a marked increase in the level of primary metabolite, becomes explicable when the range of enzymes inhibited by SKF 525A is considered. It is known that this compound is not very specific in its effects[21,22] and the overall effect of SKF 525A on the circulating amounts of 7-hydroxy-Δ^1-THC will depend on its effect on enzymes catalysing the removal of this compound as well as on the enzyme responsible for its production. If SKF 525A at a dose-level of 25 mg/kg i.p. only marginally inhibits the hydroxylation of Δ^1-THC but inhibits almost completely some subsequent oxidation or conjugation reaction, the net effect will be an increase in the concentrations of both the primary substrate, Δ^1-THC, and of its immediate metabo-

lite, 7-hydroxy-Δ^1-THC. Since SKF 525A produces a much greater proportional increase in the brain concentration of primary metabolite than it does of Δ^1-THC, the opposite conclusion to that of the original authors can be drawn from the observations of Sofia and Barry, namely that the potentiation of barbital induced sleeping time by Δ^1-THC can more plausibly be attributed to the metabolite than to the parent compound. From these results it is impossible to conclude that Δ^1-THC is definitely inactive, however.

Contrasting with the results of Sofia and Barry is our observation that after pretreatment with SKF 525A, there was no significant increase in the mean immobility index of mice receiving 1 mg/kg of Δ^1-THC. If the metabolite were the only active component, a significant increase in the immobility index would be expected, whereas if the two compounds were about equipotent at the site of action, little change in the immobility index would result, since the inhibitor-induced increase in the combined brain concentrations of Δ^1-THC and 7-hydroxy-Δ^1-THC is proportionately much smaller than the increase in the concentration of metabolite alone.

In these experiments the correlation of immobility index was made with the concentrations of Δ^1-THC and 7-hydroxy-Δ^1-THC in the whole mouse brain; no attempt was made to allow for unequal distribution between different regions of the brain. McIsaac *et al.*[28] have reported the disposition of ^{3}H-Δ^1-THC in the monkey brain but further investigation will be required to demonstrate whether apparent concentration differences in different areas are due to variations in vascularity or to selective absorption of the circulating cannabinoids. Of ultimate concern in the elucidation of the molecular pharmacology of Δ^1-THC and its metabolites is the concentration of these at their site of action; such local concentrations may well differ from the concentrations in the brain taken as a whole.

As yet no definite conclusions can be drawn about the nature of the molecular species responsible for the effects of cannabis. The use of SKF 525A and piperonyl butoxide has not resolved the question since neither inhibitor eliminates the primary metabolite from the C.N.S. Insofar as the choice lies between Δ^1-THC and 7-hydroxy-Δ^1-THC, it will probably be found that they are both active to some degree. However, a precise attribution will require either further analysis using more selective enzyme inhibitors, possibly in animals with a low capacity for hydroxylating Δ^1-THC (e.g. female rats) or further quantitative studies on the pharmacological properties of pure 7-hydroxy-Δ^1-THC.

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